OpenPlant Handbook

BBSRC-EPSRC Synthetic Biology Research Centre
UK Synthetic Biology for Growth programme

Sharing tools for a sustainable future.

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OpenPlant Handbook

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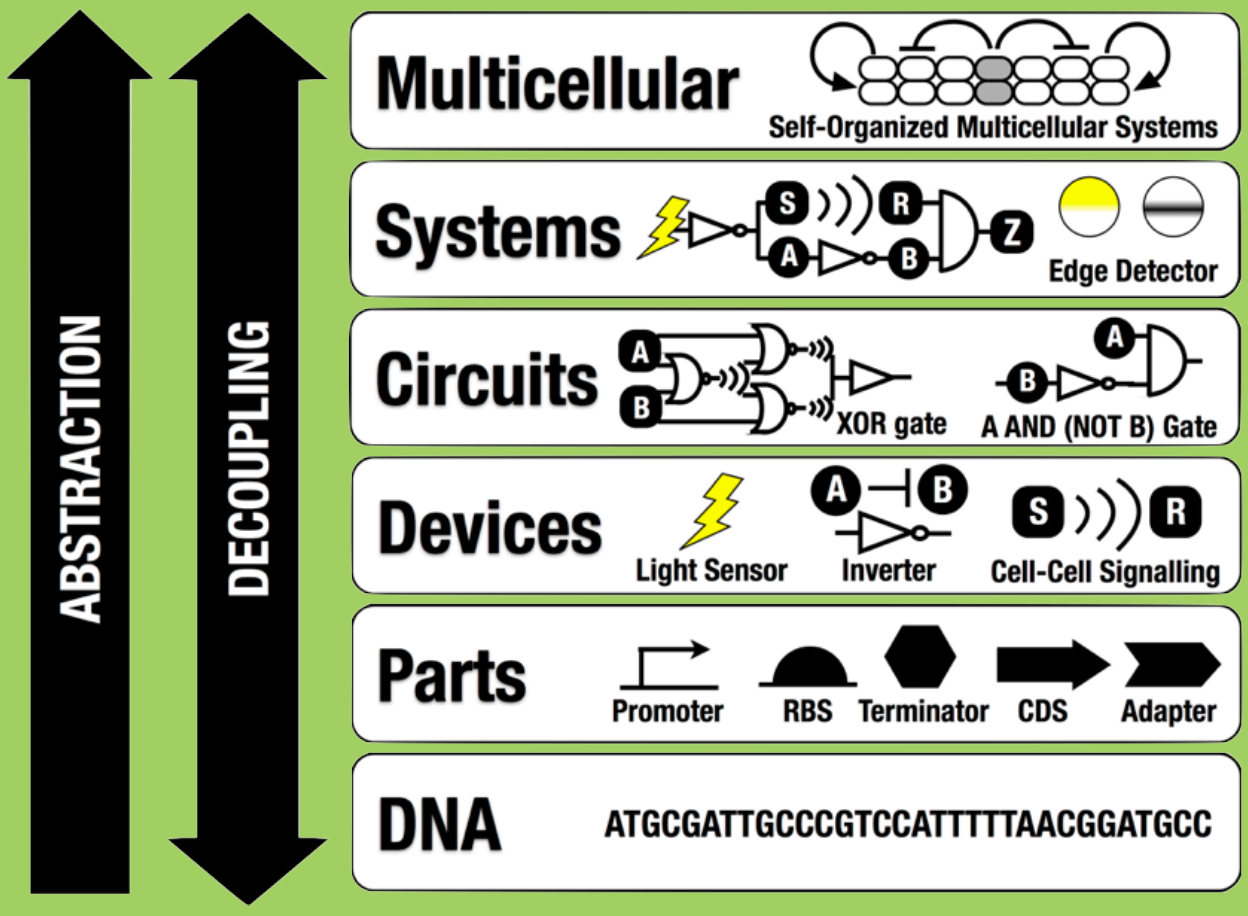
Plants and the New Biology:

Plants are attractive platforms for synthetic biology and metabolic engineering. The modular and plastic body plans, capacity for photosynthesis, extensive secondary metabolism, and agronomic systems for large scale production make plants ideal targets for genetic reprogramming. However, efforts in this area have been constrained by slow growth, long lifecycles, the requirement for specialized facilities, a paucity of efficient tools for genetic manipulation, and the complexity of multicellularity. There is a need for better experimental and theoretical frameworks to understand the way genetic networks, cellular populations and tissue-wide physical processes interact at different scales.

New biological techniques are offering radical approaches to the analysis and large-scale synthesis of genetic systems in plants. This is part of a technical revolution in our ability to construct DNA code and to edit existing genomes in order to reprogramme the growth and physiology of organisms. When applied to plant systems, these new synthetic biology technologies offer the long-term prospect of rational design and programming of new plant traits, with potentially revolutionary consequences for agriculture and bioproduction.

Over the last century, agriculture has become increasingly intensive and industrialised. Crop farming in the developed world is highly mechanised, with ever-increasing reliance on fertilisers and pesticides and highly tuned and efficient germplasm. Innovations in agriculture have led to an explosive growth in the extent of protected technologies and plant varieties at play in the field (see top right). At the same time, we are seeing an unparalleled consolidation of ownership, as large corporations merge and integrate their agrochemical and seed businesses. With the mergers of Dupont-Dow, Monsanto-Bayer and Adama-Syngenta, a handful of companies will share a 70-80% stake in global seed and agrochemical sales (see mid-lower right). The last century has seen a growing intensification of agricultural practices, with increased adoption of vertically integrated agribusiness models, closed technologies and restrictive licensing practices in the field.

New technologies offer the prospect of a breakout from the limitations of conventional breeding and the single-gene GM agronomic traits that currently dominate the market. However, the new approaches require access to multiple DNA elements, sophisticated design and assembly tools, multi-scale analyses for debugging, and above all, sharing of knowledge.



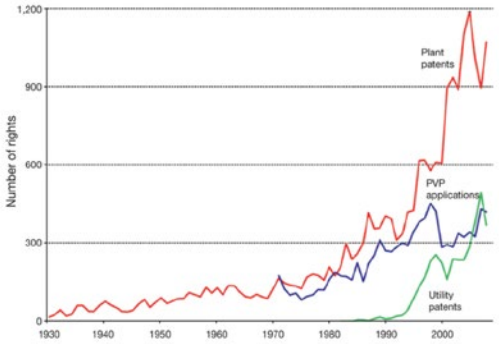
Federici F, Rudge TJ, Pollak B, Haseloff J, Gutiérrez RA. Biological Research 46:383-93, (2013)

Innovation in Agriculture

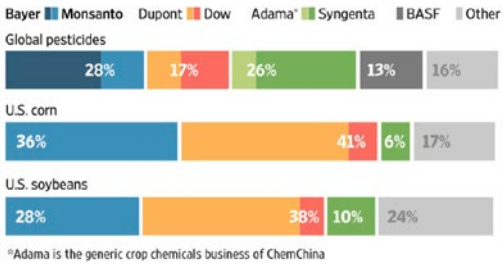
Investment in Synthetic Biology is rapidly increasing in all major economies. Synthetic Biology is seen to underpin major developments in sustainable, biology-based technologies, and is a key component of the growing global bioeconomy.

We believe that there is a clear opportunity and need to create open systems and free tools for biological engineering in plants. The availability of open systems will create a self-reinforcing ecosystem for exchange of scientific knowledge and resources, and will facilitate innovation in research laboratories. Frameworks for the easy commercial use of low-level tools will promote entrepreneurship and social enterprise.

Perhaps most importantly, the technology is not intrinsically expensive. Open frameworks for education and sharing would facilitate technical transfer to developing countries - which are often rich in biological resources, but lack the capacity to take full advantage of their potential. OpenPlant aims to create open frameworks for sharing that will facilitate equitable exchange of knowledge, innovation and enterprise for the new biotechnologies that will be crucial for global food security and future sustainable production.



Growth of IP protection in agriculture
Pardey *et al.* Nature Biotechnology



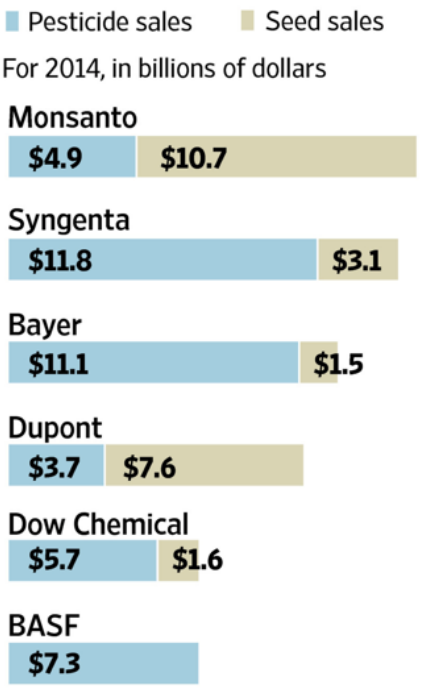
Mergers and consolidation in global agribusiness
Wall Street Journal

What is Synthetic Biology?

An essential concept in the Synthetic Biology approach is that genetic systems can be constructed using standardised, interchangeable parts. For example, microbial promoters, ribosome binding sites, coding sequences etc. have been collected as a set of standard DNA parts at MIT (BioBricks: http://parts.mit.edu), and this forms the basis of the annual iGEM competition. Similar approaches have been taken in research laboratories. These collections of parts are designed to be easily combined in the assembly of larger devices, and can be freely distributed for use by others. The aim is to facilitate the construction of systems with known parts and devices, so predictable behaviour and reliability become the norm.

The adoption of standardised DNA parts and modular assembly techniques allows the benefits of abstraction and decoupling of design from fabrication that are found in other forms of engineering.

We wish to extend this approach to the genetic reprogramming of plant systems. Liverworts, with their simple genomes and open forms of growth, make good testbeds for engineering.



Increasing market size for agricultural inputs
Economist

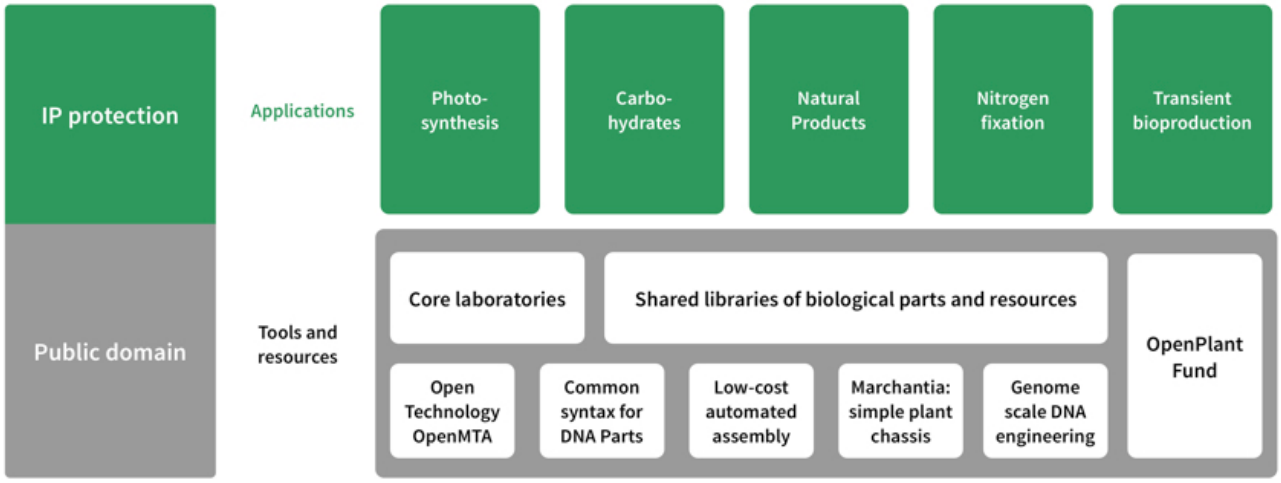
Our vision for OpenPlant:

Sharing tools for a sustainable future

What is OpenPlant?

Synthetic Biology offers the prospect of reprogrammed biological systems for improved and sustainable bioproduction. While early efforts in the field have been directed at microbes, the engineering of plant systems provides even greater potential benefits. In contrast to microbes, plants are already globally cultivated at extremely low cost, harvested on the giga-tonne scale, and routinely used to produce the widest range of biostuffs, from fibres, wood, oils, sugar, fine chemicals, drugs to food. Plants are genetically facile, and GM plants are currently grown on the >100 million hectare scale. Plant systems are ripe for synthetic biology, and any improvement in the ability to reprogram metabolic pathways or plant architecture will have far-reaching consequences.

Institutional strengths in plant sciences.
We believe that there is a crucial need to accelerate the development and open sharing of new tools and methods for plant synthetic biology. OpenPlant is a joint initiative between the University of Cambridge, the John Innes Centre and the Earlham Institute, funded by the BBSRC and EPSRC as part of the UK Synthetic Biology for Growth programme. The initiative promotes (i) interdisciplinary exchange, (ii) open technologies and (iii) responsible innovation for improvement of sustainable agriculture and conservation.



open technology underpins innovation

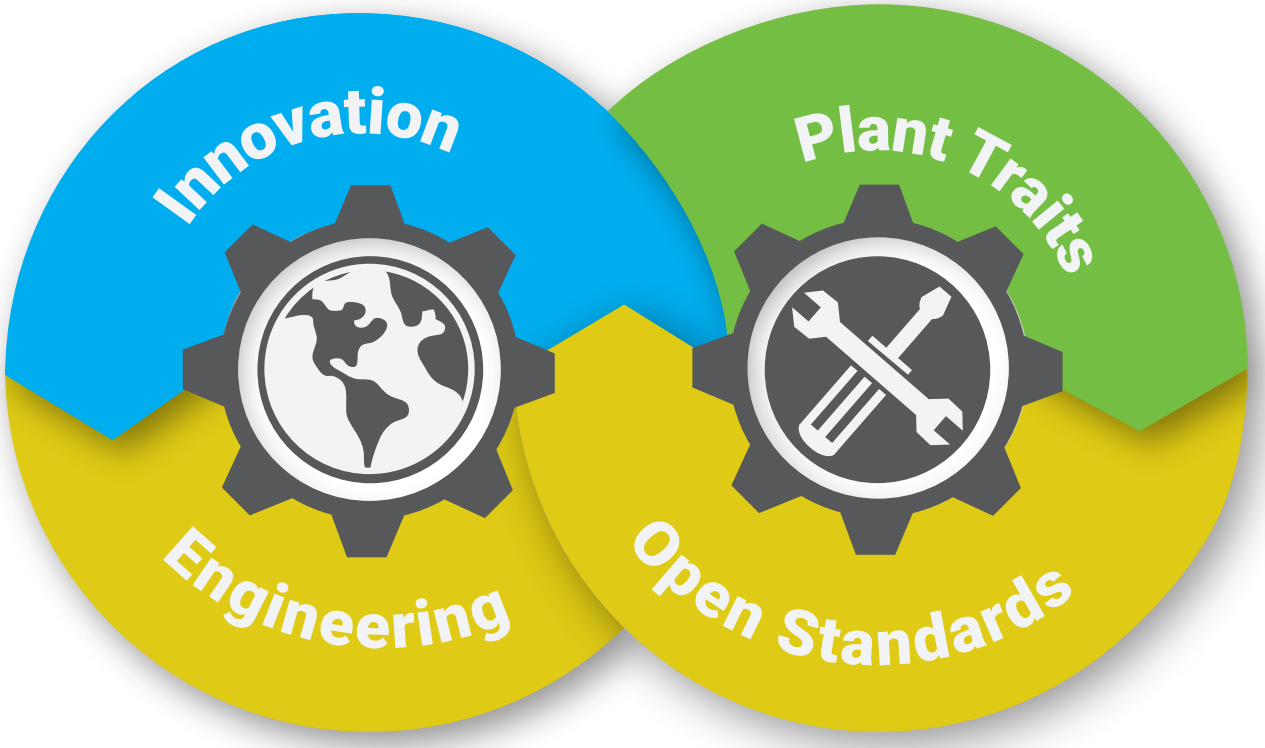
Interdisciplinary exchange
The UK provides an ideal hub for interdisciplinary exchange between foundational sciences like botany, agronomy, physics, chemistry, computer sciences and engineering. This exchange drives innovation for the engineering of biological systems. OpenPlant promotes and funds the development of novel foundational technologies, the creation of international standards for plant synthetic biology, and open tools for trait development. We believe that advances in plant synthetic biology will provide a key to securing and sustaining future food and materials production, and that there should be worldwide open access to these benefits.

Open technologies for innovation
Current IP practices and restrictive licensing threaten to restrict innovation as the scale of DNA systems increases. We believe that the field needs to explore new "two-tier" intellectual property models that will protect investment in applications, while promoting sharing of DNA components and freedom-to-operate for innovators in business and social enterprises. We are building new frameworks and collaborations for open innovation in plant synthetic biology.

A two-tier model for ownership of intellectual property in plant biotechnology

An overview of the work programmes implemented in OpenPlant is shown above. Work programmes are divided into two tiers, those aiming at the development of new plant traits and applications (green), which may require IP protection, and foundational work on the development of low level tools and resources (grey), which stay in the public domain.

OpenPlant aims to promote the adoption of open standards and technologies and better ways of these sharing tools. We believe that open ways of working are necessary for innovation and equitable access to the new biotechnologies that will underpin future sustainable practices and the global bioeconomy.



Outreach & Exchange

OpenPlant Fund
Supporting entrepreneurship and innovation. Promoting small scale interdisciplinary projects for research and new working practices in the plant synthetic biology community.

Biomaker Challenge
Promoting plant synthetic biology through international synthetic biology challenges. Working alongside large projects tackling grand challenges in crop traits such as nitrogen fixation and C4 photosynthesis.

Open Materials Transfer Agreement
Enabling innovation by establishing a common syntax for plant DNA parts, and a legal framework for open international exchange of biological parts.

Open hardware for science
Through funding (OpenPlant Fund, Biomaker Challenge), training (with TReND Africa and Science Makers) and community building, to enable low-cost automation for plant applications.

Research & Development

Novel traits and applications
Creating novel plant applications, and engineering traits including photosynthesis, leaf structure, carbohydrate content, nitrogen fixation and metabolic engineering.

Marchantia as a simple plant testbed
Developing the liverwort *Marchantia polymorpha* as a facile model system for plant synthetic biology, with simple genome, growth, transformation, open development and access for quantitative analysis at the cellular scale.

Open DNA part library
Sharing and characterising an open library of standardised DNA parts for plants, algae and cyanobacteria including useful regulators, markers and enzymes.

Research automation
Integrating automation and open source control systems in OpenPlant research labs, improving low-cost custom instrumentation through 3D printing, microcontrollers, optics and software.

OpenPlant Workpackages

1. Open Standards and Engineering:

Development of open standards, resources and technologies for DNA-based reprogramming of plants.

Workpackage A: Development of the lower plant Marchantia as a highly simplified and facile chassis for reprogramming plant form and physiology.

Workpackage B: A common syntax for plant DNA parts and assembly of genetic circuits. Establishment of a shared library of DNA parts for plant engineering.

Workpackage C: New DNA parts for the control and quantitative imaging of genetic circuits.

Workpackage D: Techniques for routine genome-scale engineering in plants.

Workpackage E: Software tools with improved performance for DNA part catalogues, automated assembly, and modelling of synthetic gene circuits and cellular morphogenesis.

2. Plant Trait Engineering:

Application of the new ways of working to engineer target traits in plants.

Workpackage F: Altered photosynthesis and leaf structure.

Workpackage G: Changes in plant carbohydrate content.

Workpackage H: Engineered pathways for the metabolic engineering of natural products.

Workpackage I: New forms of symbiosis and nitrogen fixation for crop plants.

Workpackage J: Methods for high level production of biomolecules by transient expression.

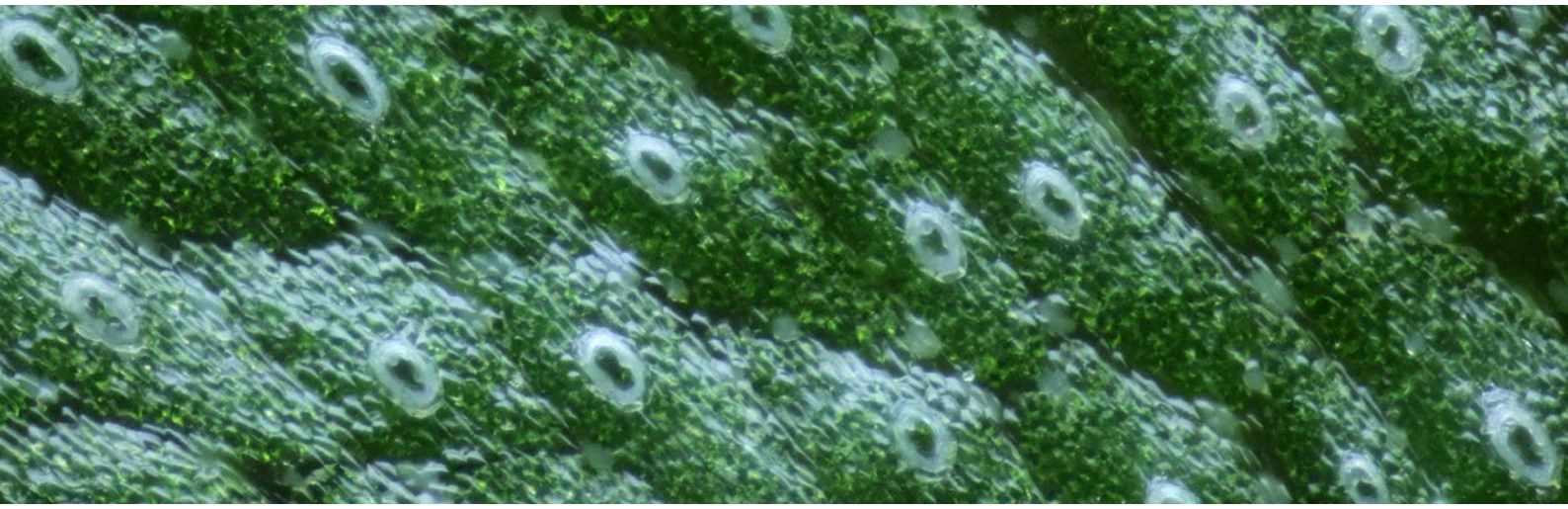
3. Shared Innovation:

Promotion of global outreach and interdisciplinary exchange to seed innovation and equitable access.

Workpackage K: Mini-funds to seed novel interdisciplinary exchange and innovation.

Workpackage L: Outreach activities, training and tools for open exchange of DNA parts and other reagents in biotechnology.

Workpackage M: Project management and communication



Workpackage A:
Simple Plant Chassis, Tools and Gene Delivery

The liverworts (or Marchantiophyta) are descendants of the earliest terrestrial plants. The group is characterised by morphological simplicity, and this is matched by simple underlying genome structures. Many lower plants, including liverworts, demonstrate a striking tolerance of extreme stresses, a trait that would be valuable in a production system. Liverworts have been a largely neglected area of plant biology, but show promise as new experimental systems after recent developments in transformation methods and genome characterisation. Marchantia polymorpha is the best characterised liverwort plant. It is a common weed, and can grow quickly and resiliently. The relative simplicity of genetic networks in Marchantia, combined with the growing set of genetic manipulation, culture and microscopy techniques, are set to make this primitive plant a major new system for analysis and engineering. We aim to establish Marchantia as a testbed for plant synthetic biology, which will provide a prototype for other OpenPlant initiatives in higher plants.

The aim of this workpackage is to exploit the extraordinary experimental properties of Marchantia, and produce systematic collections of (i) experimental protocols and (ii) shared DNA parts. This will include a comprehensive collection of promoters, selection markers, and fluorescent and biopigment reporter genes. In addition, (iii) we will produce and distribute Marchantia lines with integrated cell fate markers in order to track physiological and morphogenetic changes. We will distribute information using laboratory-based web sites and specialised DNA registries, and distribute DNAs and plant material via stock centres. The same methods for public distribution will be used widely in other OpenPlant workpackages.

Investigators
Jim Haseloff; Giles Oldroyd; Jim Ajioka; Pietro Cicuta; Lisa Hall

Staff Employed
Susana Sauret-Gueto (Research Manager; Haseloff lab). Started October 2015.
Linda Silvestri (Research technician, Haseloff lab). Started February 2016.
Fernan Federici (PDRA, Haseloff lab). July-Dec 2015; July-Dec 2016.
Tim Rudge (PDRA; Cicuta lab). August 2015 - 2016.
Tom Meany (PDRA; Haseloff and Hall labs). October 2015-2017.
Eftychis Frangedakis (PDRA; Haseloff lab). Started April 2017.
Marta Tomaselli (PhD student; Haseloff lab). Started October 2016

Partners
Bernardo Pollak; Christian Boehm; Mihails Delmans (Haseloff Lab)
Liam Dolan (Oxford)
John Bowman (Monash)
Takayuki Kohchi (Kyoto)
Kimitsune Ishizaki (Kobe)

A

Milestones:

A1: Establishment of laboratory for automated DNA assembly, measurement, and quantitative imaging.
Deliverable: Commissioning of the OpenPlant laboratory (month 9, Haseloff).

A2: Establishment of microfluidic platforms for high throughput single cell imaging.
Deliverable: Production of microfluidic devices for culture, sensing and time-lapse observations (month 24, Cicuta, Hall).

A3: Establishment of Marchantia spore transformation system.
Deliverable: Validation of Marchantia spore transformation using GFP expression (month 24, Haseloff, Oldroyd, Schornack, Osbourn, Smith).

A4: Distribution of new Marchantia vectors for quantitative imaging.
Deliverable: Public release* of DNA vectors with ratiometric fluorescent markers (month 36, Haseloff).

A5: Distribution of a collection of Marchantia transcription factor promoters.
Deliverable: Public release* of a collection of synthetic promoter DNA parts (month 60, Haseloff, Harrison).

A6.1: Cell and tissue type transcriptomes.
Deliverable: Analysis and publication* of Marchantia tissue-specific transcriptome data (month 60, Haseloff).

A6.2: Distribution of cell and tissue type specific expression vectors and lines.
Deliverable: Public release* of a collection of Marchantia promoter-GFP gene fusions with tissue-specific expression patterns (month 60, Haseloff).

Online resources:

Resource for information about Marchantia culture and manipulation
(www.marchantia.org)

Haseloff lab
(www.haseloff-lab.org)

Schornack lab
(www.slcu.cam.ac.uk/research/schornack-group)

Cicuta lab
(people.bss.phy.cam.ac.uk/~pc245/)

Hall lab
(www.ceb.cam.ac.uk/research/groups/rg-cab)

Imaging resources, including macrophotography techniques and Marchantia images
(haseloff.plantsci.cam.ac.uk/imaging/marchantia)

Progress to date:

Objective A1: **Establishment of laboratory for automated DNA assembly, measurement, and quantitative imaging**

The OpenPlant laboratory in Cambridge was created from a refurbished teaching laboratory at the Department of Plant Sciences, including the construction of new imaging suites, dedicated equipment room and office space, and commissioned in December 2014.

Following this, the tender process was completed and we installed advanced imaging equipment, including (i) a Leica SP8 confocal laser scanning microscope with hybrid detectors and white light laser, (ii) upgrade to a Leica SP5 confocal laser scanning microscope for routine work, and (iii) a Keyence VHX-5000 3D surface imaging microscope (iv) Leica area-scanning digital stereoscope, multiple microplate readers and robotics equipment, including a Labcyte Echo 550 acoustic focusing liquid handler.

Objective A2: Establishment of microfluidic platforms for high throughput single cell imaging.

Dr. Tim Rudge worked in the Cavendish Laboratory in Pietro Cicuta's Lab to finalise microscale approaches for quantitative measurement of gene expression in single cells. Using *E. coli* expressing GFP under the control of both constitutive and regulated promoters, he investigated correlations between the rate of growth and the rate of gene expression, and the spatial variations of both within the colony. It was possible to optimise confocal imaging of colonies growing from single cells up to a millimetre across. Initial results indicate changes in both growth and gene expression between the core and edges of the colony. Image analysis, including optical flow algorithms, was used to measure growth when cell segmentation became unfeasible. This work allows better understanding of how physical structure in a growing clonal population can lead to morphological differentiation even in a simple prokaryote system. It has led to several publications, and this biological understanding, and the experimental methods, is applicable to other cell types. This work was developed in algal cells through appointment of Dr. Lucia Parolini from November 2016, and through continuing collaboration with Dr Tim Rudge, now an Assistant Professor at the Universidad Católica de Chile in Santiago.

Dr. Tom Meany was appointed to a shared position in Jim Haseloff and Lisa Hall's laboratories, where the OpenPlant funded position was extended by the joint award of a Wellcome Trust Interdisciplinary Fellowship. Tom explored Raman spectroscopy based approaches for high throughput analysis of plant oil cells in Marchantia, using a combination of microfluidic and *in planta* approaches. He has used this non-invasive microscopy approach to detect terpene-like molecules in oil bodies, and validated these observations using micro sampling and mass spectroscopy. Tom is now CEO and founder of Cell-Free Technology (http://cell-free.tech), a company based in Cork, Ireland.

Objective A3: Establishment of Marchantia spore transformation system

Ahead of schedule, Bernardo Pollak and Linda Silvestri (Haseloff lab) established a reliable Marchantia spore transformation system, which is capable of high throughput production of transgenic plants. Further, Guru Radhakrishnan (Oldroyd lab) developed a transformation protocol for vegetative tissues, based on blender treatment and plantlet regeneration. This has primarily been applied to *Marchantia paleacea*. This is a useful technique for super-transformation experiments. The protocols are shared online (https://www.protocols.io/groups/openplant-project).

The spore transformation protocol is widely used by OpenPlant Marchantia groups, and is continuing to be refined, for example through miniaturisation and scale-up in number. We have created new families of transformation vectors compatible with Loop assembly, and are testing different promoters and herbicide resistance markers for *in planta* selection. Dr. Susana Sauret-Gueto (Haseloff lab) is coordinating this project and interacting with others in the Marchantia community, including Suvi Honaken from the University of Western Australia, and researchers in the Schornack lab. We use fluorescent markers to screen for successful transformants, which allows identification around 5 days after plating the transformed sporelings, and plant transfer by 2 weeks.

Dr. Eftychios Frangedakis is leading the establishment of thallus transformation techniques in the Cambridge OpenPlant Lab, as this allows transformation of mutants or marker lines without need for spore production (a 2-3 month process). Sauret-Gueto has compiled protocols for Marchantia work, being documented at protocols.io and the website www.marchantia.org. She has also established the ROC group (Researchers with OpenPlant in Cambridge), convening members from different Departments interested in sharing resources for Synthetic Biology. A Marchantia-ROC subgroup meets monthly and is a key group for sharing knowledge of Marchantia protocols, promoting type IIS assembly, and synthesis and characterisation of standardised DNA parts for Marchantia..

Objective A4: Distribution of new Marchantia vectors for quantitative imaging.

We have refactored spectral variants of fluorescent proteins for efficient expression in Marchantia, and these have been published (Mihails Delmans*, Bernardo Pollak* and Jim Haseloff. Plant Cell Physiol. 58: e5(1–9) 2016), and DNA vectors will be distributed via Addgene. These co-localised multispectral markers are suitable for use in our *in planta* quantitative cytometry method (Federici, et al. Nature Methods, 9:483-485 (2012). The vectors contain spectrally distinct fluorescent protein markers that allow autosegmentation of cell geometries and quantitative assignment of biological parameters on a cell-by-cell basis. The Haseloff lab is continuing to improve the system, testing multiple combinations of promoters, fluorescent proteins and signal peptides, and employing vectors compatible

with Loop assembly.

Objective A5: Distribution of a collection of Marchantia transcription factor promoters.

Bernardo Pollak (Haseloff lab) generated draft genome and transcriptome sequences for the CAM-1 (male) and Cam-2 (female) isolates of *Marchantia polymorpha*. He and Mihails Delmans have annotated the genome, and constructed and published an online, gene-centric database (www.MarpoDB.io) that allows facile access to gene features from the *M. polymorpha* genome. In Norwich, the Oldroyd lab has generated genome sequence for *M. paleacea* (which, unlike *M. polymorpha*, forms symbiotic fungal associations). MarpoDB provides features that allow mining of the genome dataset for synthetic promoters, genes and terminators that can be exported directly as sequence files for synthesis of standardised Phytobrick DNA parts.

The annotated genome sequence has provided a basis for comparison with the Tak-1 genome, which is best annotated and becoming the community standard. Polymorphisms between the Tak-1 and Cam-1/2 isolates are around 1/1000 bp within genes. The sequence comparison between the Cam-1/2 and Tak-1 isolates is a contribution to the community-driven Marchantia genome paper that was published recently (JL Bowman, T Kohchi, KT Yamato, J Jenkins, S Shu, K Ishizaki, et al. (including Pollack B., Delmans M, Boehm CR, Haseloff J) Cell 171 (2), 287-304. E15, 2017).

Delmans used MarpoDB to batch retrieve the proximal promoters of transcription factors (TF) in Marchantia (398) which were refactored as standard L0 parts for Loop assembly and chemically synthesised. Sauret-Gueto established and curates a registry for L0 parts using Benchling. Linda Silvestri is maintaining the stocks of L0 parts. In January 2018, we have 403 L0 parts corresponding to Promoters and 5UTR of 258 Mp TFs. We are continuing with synthesis of the 140 remaining TF promoters. In parallel with this effort, we have built Loop assembly compatible vectors that are compatible with the OpenMTA, and thus suitable for open distribution. All L0 parts have been cloned into these new vectors.

Objective A6.1: Cell and tissue type transcriptomes.

The assembly of an annotated Marchantia genome has allowed precise analysis of transcription dynamics, using RNAseq studies of germinated Marchantia spores. Changes in mRNA production were mapped over the crucial early stages of germination, cell enlargement, chloroplast differentiation, asymmetric cell division, rhizoid and thallus formation and cell differentiation. This data was published in the recent Marchantia genome paper (JL Bowman, T Kohchi, KT Yamato, J Jenkins, S Shu, K Ishizaki, et al. (including Pollack B., Delmans M, Boehm CR, Haseloff J, Cell 171 (2), 287-304. E15, 2017). The resultant data has been used to evaluate expression of particular transcription factors and metabolic markers.

Objective A6.2: Distribution of cell and tissue type specific expression vectors and lines.

We are screening the family of TF promoters for cell and tissue specific expression patterns. We will make those lines, as well as the L0 parts with the promoters, freely available to the community under the OpenMTA license.

Another source of cell and tissue type specific expression lines comes from the screening of enhancer trap lines being generated in the Haseloff Lab. This project was initiated by Pollak and continued by Silvestri and Sauret-Gueto. Marta Tomaselli is further characterising lines with air chamber related expression patterns and identifying genes and regulatory sequences that are responsible for key expression patterns.

Publications

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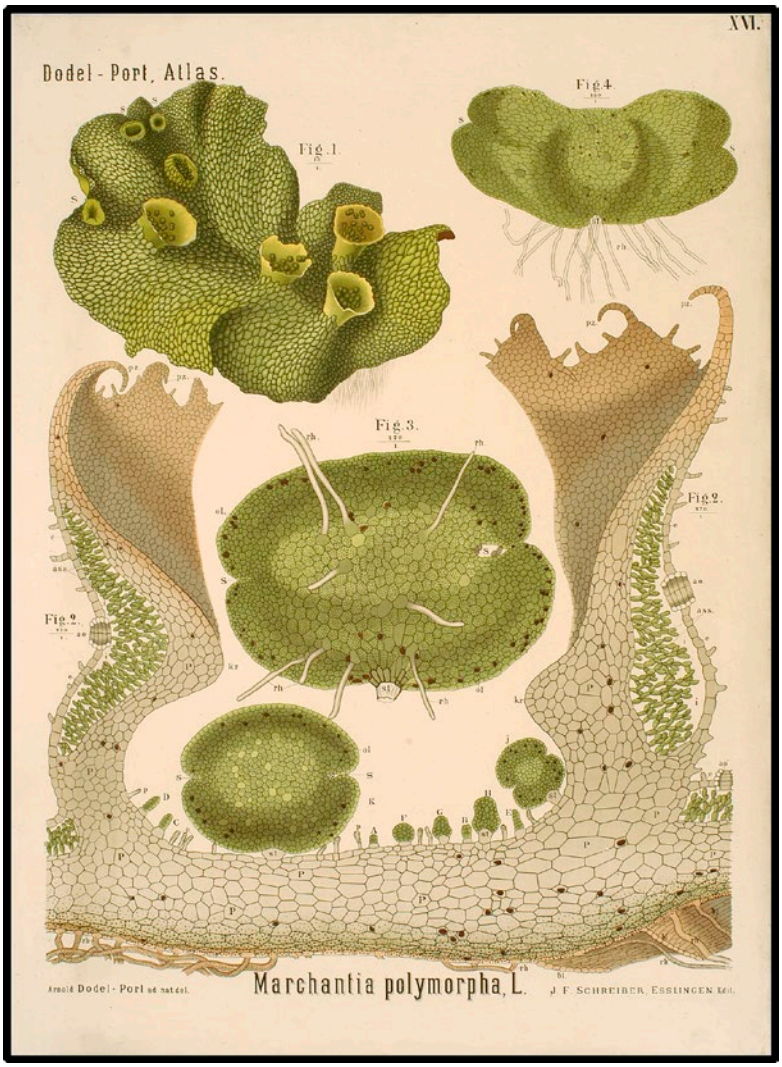
Marchantia

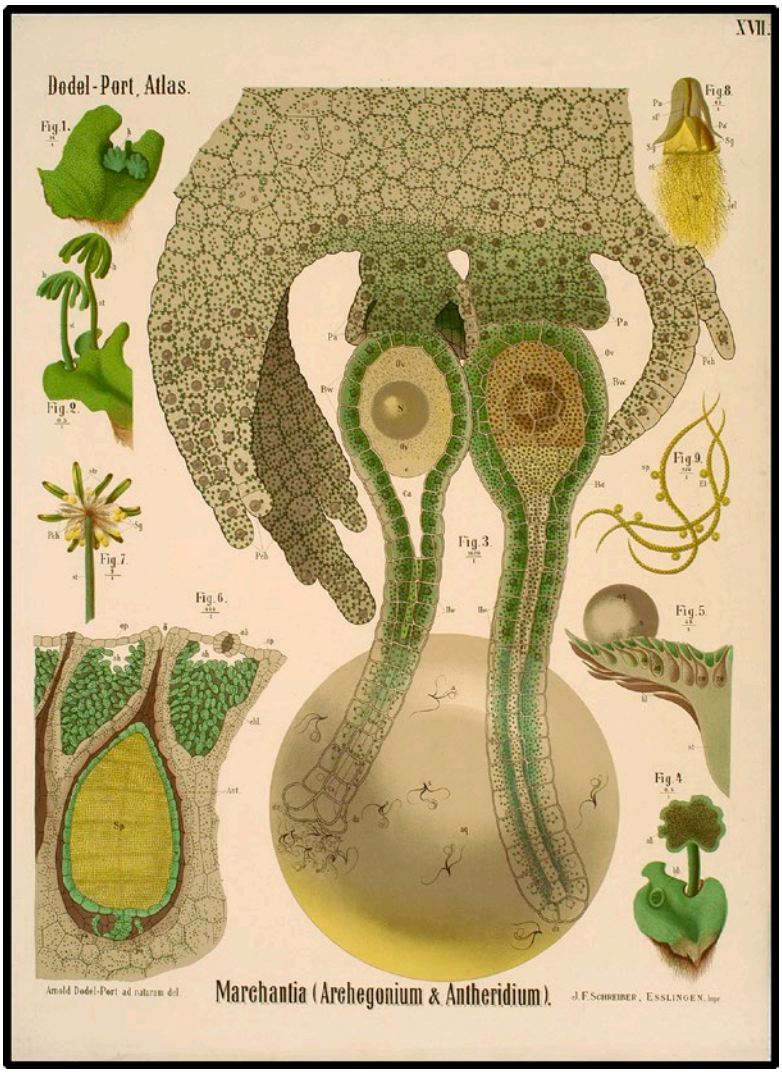
Marchantia polymorpha is the best known representative of the liverworts (or Marchantiophyta) and the species has been a subject of study for many years. These pages show copies of botanical teaching posters that feature *Marchantia polymorpha*, and are over a century old.

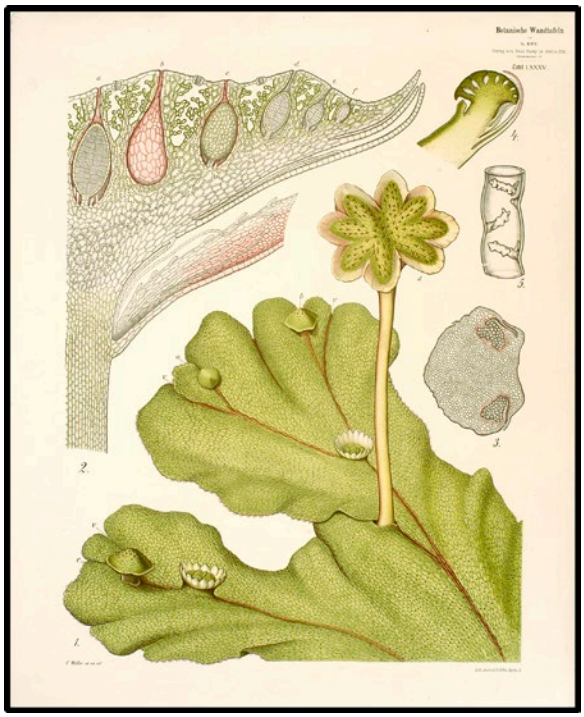
The liverworts are evolutionary relics. They are a sister clade to modern flowering plants that may have diverged almost 500M years ago. They appear to retain features of the earliest land plants including a profound morphological and genomic simplicity.

The liverworts have alternate haploid and diploid generations. Like other Bryophytes, the gametophyte or haploid generation is dominant phase of the life cycle. *M. polymorpha* has a global distribution and is often found as a weed in horticulture. M. polymorpha plants are easy to grow. They show distinctly weed-like properties, growing vigorously on soil or artificial media. The plants produce vegetative propagules. These form vegetatively inside conical splash cups. Superficial cells on the inside of a cup undergo cell proliferation to form a group of cells carried on a short stalk, the cells continue to proliferate in regular fashion to form a bi-lobed gemma. This is eventually detached from the stalk, and can be dispersed from the cup, typically by water splash. The propagules are robust and long-lived, even tolerating desiccation. Gemmae can be used for simple vegetative propagation and amplification of plantlets during experiments.

Liverworts have less efficient systems for water transport and retention than higher plants, and to compensate, show marked tolerance to desiccation. Many similar lower plants demonstrate a striking tolerance of extreme stresses, a trait that would be valuable in a crop system. Liverworts have been a largely neglected area of plant biology, but show promise as new experimental systems after recent developments in transformation methods and genome characterisation. The relative simplicity of genetic networks in liverworts, combined with the growing set of genetic manipulation, culture and microscopy techniques, are set to make these lower plants major new systems for analysis and engineering.



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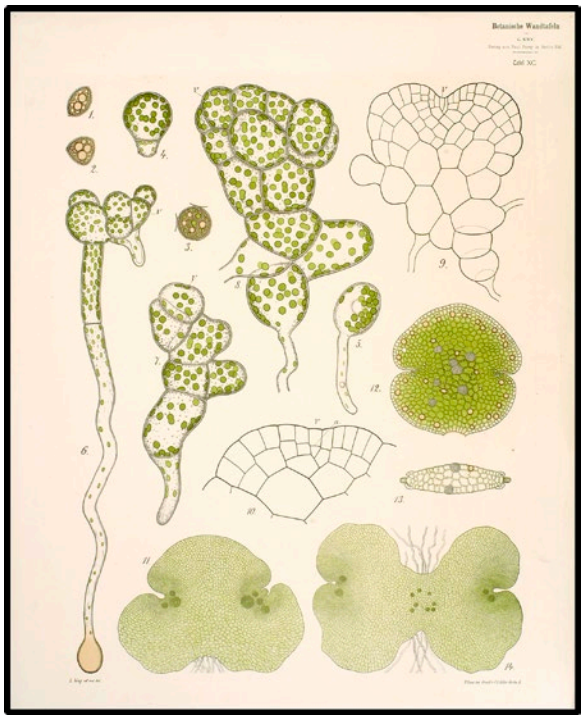
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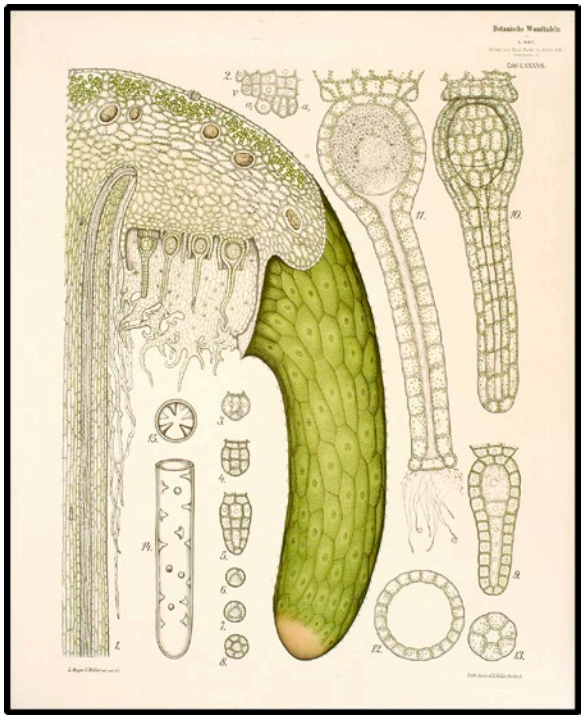
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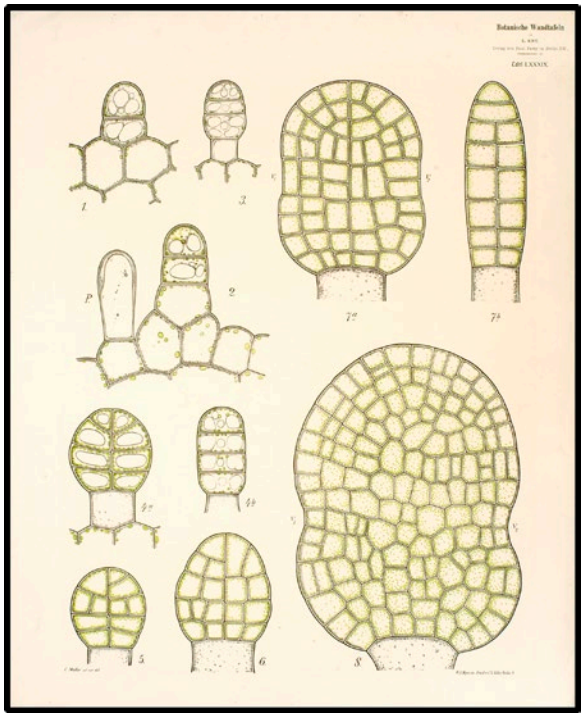
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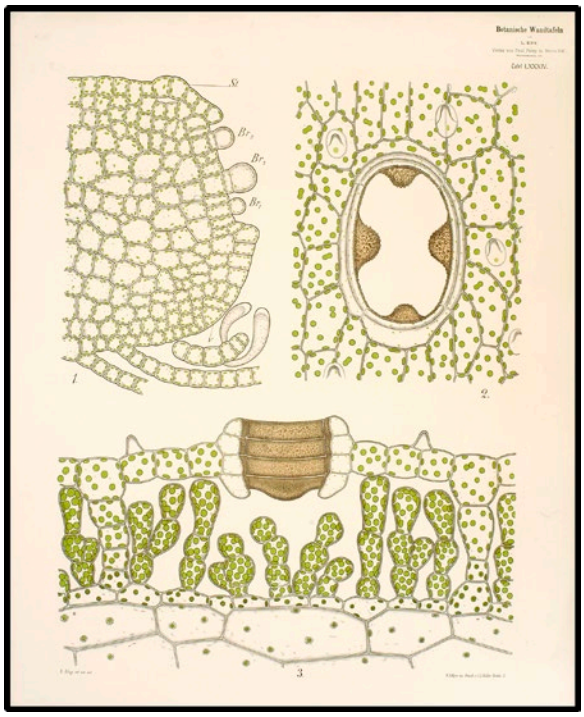
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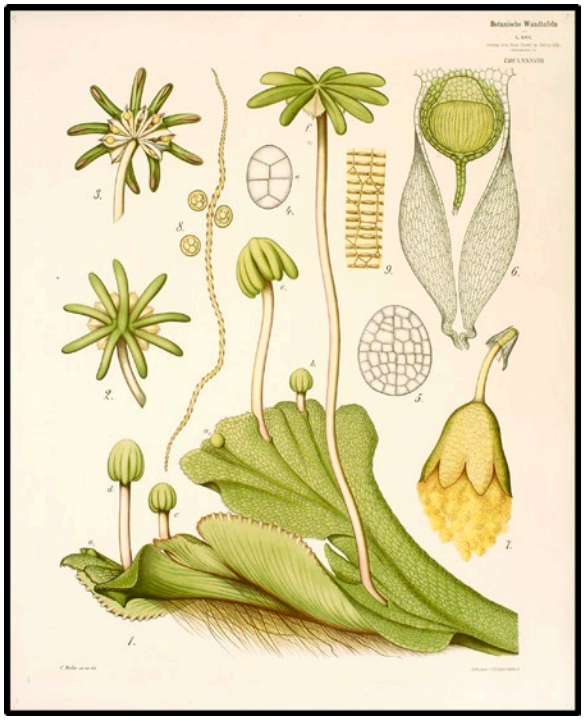
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Marchantia

Simple & Fast.

Simple morphology

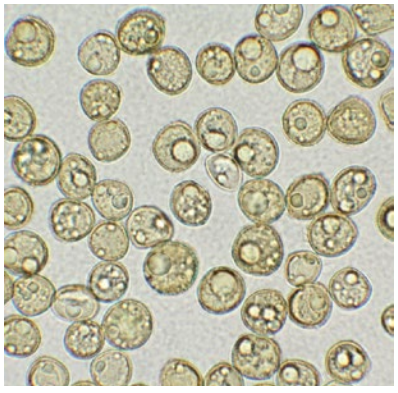
Marchantia polymorpha is the best-studied species of liverwort. Liverworts form a sister clade to modern flowering plants, thought to have emerged around 480M years ago.

Male and female plants

Marchantia plants are haploid with a simple prostrate forms. Male (right) and female (left) plants produce distinctive gamete-bearing structures.

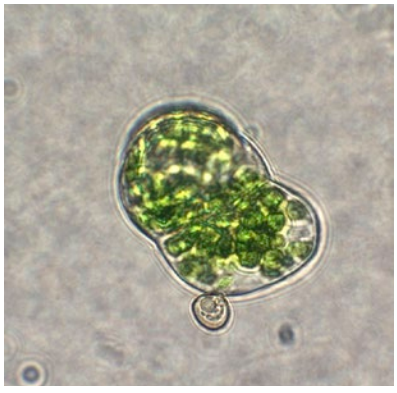
Sexual reproduction

Fertilisation results in the production of a short-live diploid phase (sporophyte), which terminates in the production of yellow sporangia.



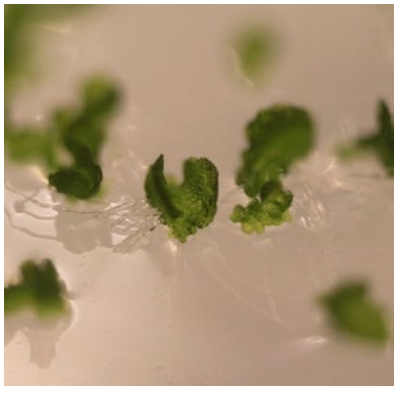
Millions of spores

Each sporangium contains 100,000's of spores, which can he stored cryogenically and used for propagation.



Spore germination

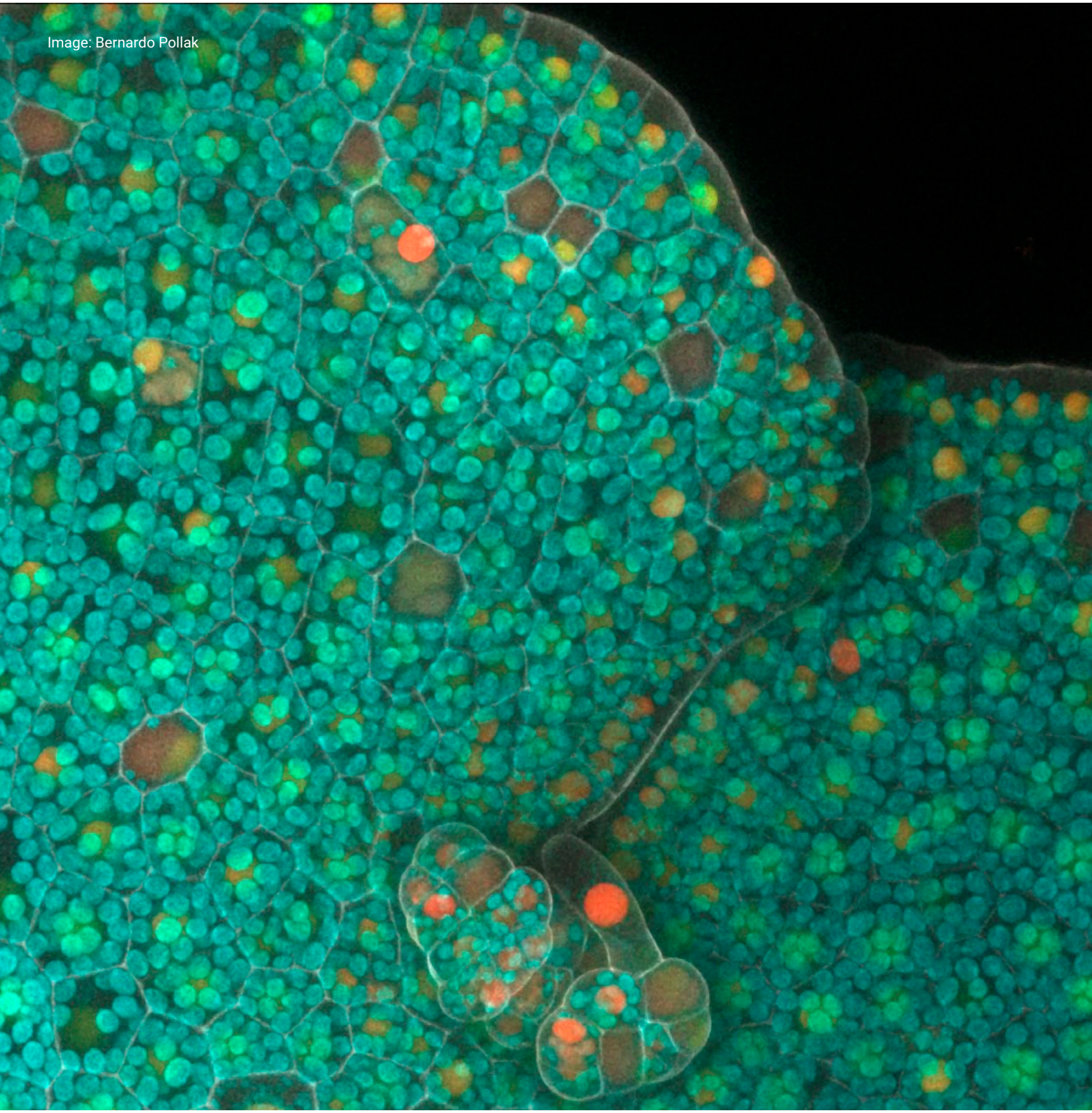
Spores germinate rapidly when transferred to suitable media, and the entire process of early development is exposed, and can be visualised directly.



Tiny plants in culture

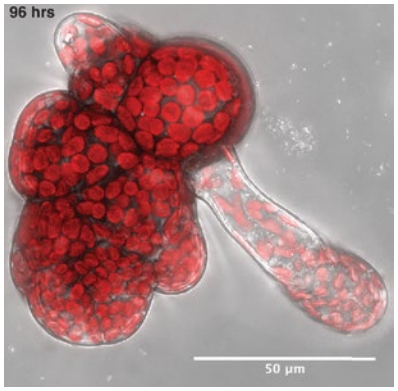
Germinating spores give rise to plantlets with recognisable body plans and anatomical features within a few days,

(images: Jim Haseloff)



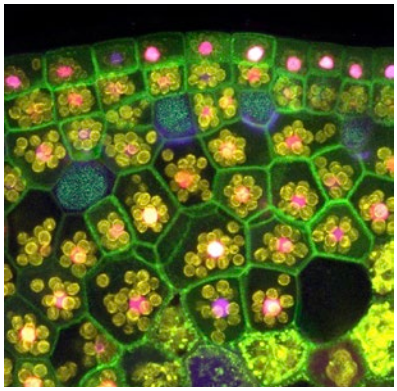
Visualising the plant

Marchantia plants can be easily transformed with fluorescent protein gene markers, and directly visualised using advanced fluorescence microscopy techniques. The example above shows cells in the meristematic notch of a Marchantia plant labelled with a nuclear-localised red fluorescent protein. The early stages of development in Marchantia are open and unobscured by surrounding tissues. This allows easy and direct observation of formative processes during morphogenesis.



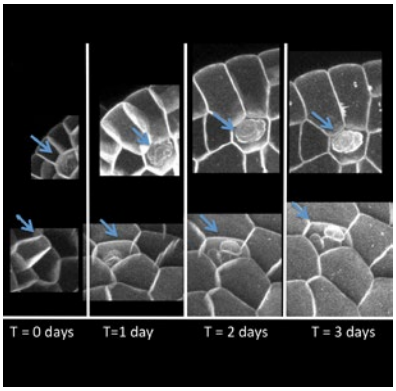
Chloroplast development

Chloroplast development can be sampled and visualised in a synchronised cohort of germinating spores (image: Bernardo Pollak).



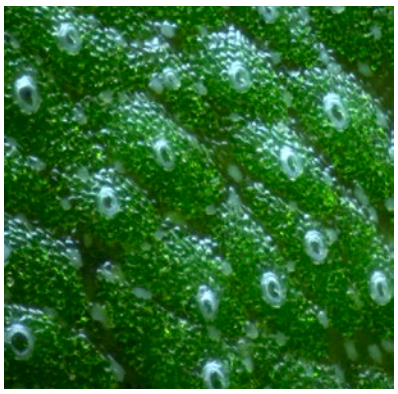
Microscopy

High resolution microscopy techniques allow the non invasive imaging of subcellular features and dynamics in intact plants (image: Fernan Federici).



Oil cells

Marchantia produces oil cells, which are devoted to the production of secondary compounds. The differentiation of these cells can be directly visualised *in situ* (image: Nuri Purswani).



Modular architecture

Cell proliferation, patterning and differentiation results in the formation of repeated air chamber structures across the surface of the plant.

Simple root system

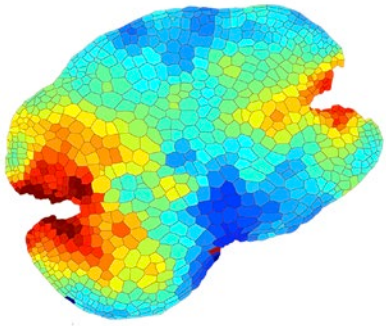
Marchantia lacks a proper root system, instead elongated rhizoid cells perform this role. Specification of rhizoids shows similarity to that of root hairs in higher plants.

Clonal propagation

Marchantia form cup-like organs that spontaneously produce clonal propagules called gemmae.

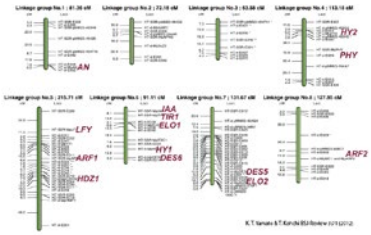
Asexual propagules

Gemmae have a regular size and morphology, and can be harvested and germinated for simple observation of engineered growth and development (image: Jim Haseloff)



Quantitative imaging

The dynamics of cellular growth and development can be quantitatively measured and parameterised using quantiative imaging techniques (image: Nuri Purswani).



Simple genome

The *Marchantia polymorpha* genome is relatively small (280 MB) and comprises 8 autosomes and 1 sex chromosome that make up the haploid genome.

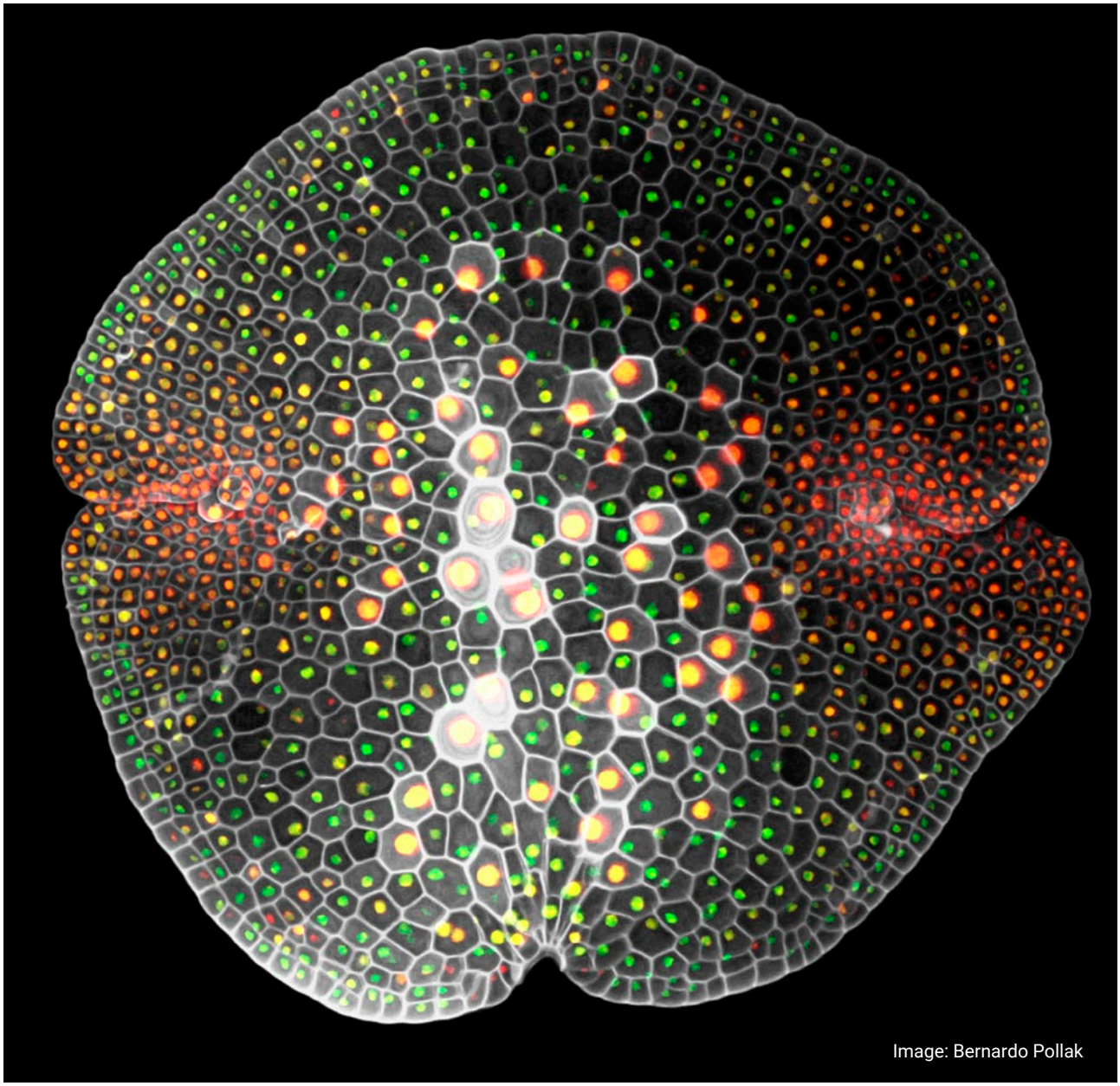


Image: Bernardo Pollak

A testbed for plant synthetic biology

Marchantia polymorpha is the best characterised liverwort. It is a thalloid liverwort, forming a body of sheet-like tissues that possess distinct upper and lower surfaces. The upper surface has a modular structure, with repeated cellular units that form simple cell complexes adapted for photosynthesis and gas exchange. Like other Bryophytes, the gametophyte or haploid generation is dominant phase of the life cycle. Marchantia has a global distribution, and is often found as a weed in horticulture. The plants grow vigorously on soil or artificial media. Marchantia plants spontaneously produce clonal vegetative propagules, or gametogenesis can be induced by exposure to far red light. Male and female plants can be sexually crossed to produce spores.

The plants are extraordinarily prolific. A single cross can produce millions of propagules in the form of single-cell spores. Spores can be harvested in huge numbers and stored indefinitely in a cold, desiccated state. Each spore can germinate to produce a new plant, and, unlike higher plants, can undergo the entire developmental sequence to produce an adult plant under direct microscopic observation.

Sequencing efforts have provided a draft of the ~280Mbp genome. Most of the major gene families present in more advanced plants are represented by a single or few orthologues in Marchantia, meaning that there is low genetic redundancy. The apparent simplicity of genetic networks in liverworts, combined with the growing set of techniques for genetic manipulation, culture and microscopy, are set to make this primitive plant a major new system for analysis and engineering.

OpenPlant has adopted Marchantia as a simple testbed for plant synthetic biology.

We have identified and sequenced a Cambridge isolate of *Marchantia polymorpha*, and are using the annotated genome to compile a novel library of DNA building blocks based on a common syntax for DNA parts and a technique for rapid assembly of DNA circuits. We aim to create an open system for reprogramming plant metabolism and form in a simple engineering testbed.

Image: Jim Haseloff

Workpackage B: Gene Assembly and Open Registries

As part of the OpenPlant initiative, we will (i) establish open-source DNA registries in the UK for sharing information, and join a web of registries with plant-chassis specific parts. (ii) We will explore new models for distributing plant DNAs and promoting quality control. There is a need to explore multi-tier strategies, as the field is moving quickly from physically defined BioBrick parts, to PCR-defined termini for end-linking, to software-defined parts for direct synthesis. We will establish a pilot scheme for open distribution within the OpenPlant project.

Standards for DNA parts and assembly underpin the development of other technologies and platforms and therefore the outcomes of workpackage B are relevant to other platforms and technologies, especially workpackage D (Genome assembly).

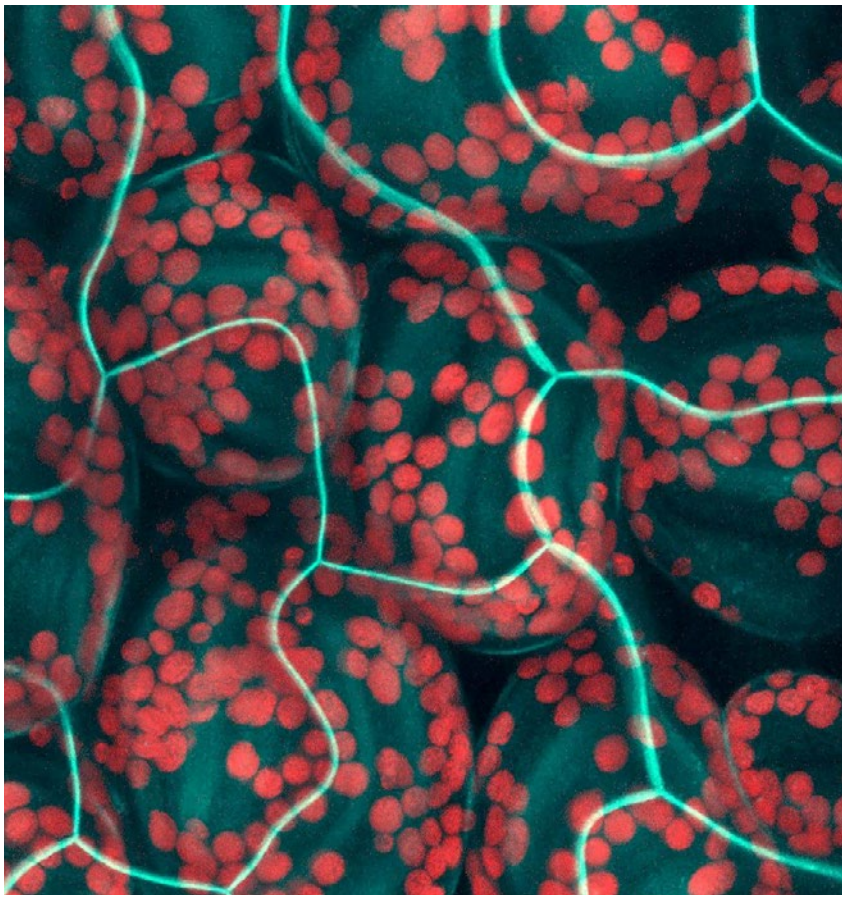
The genome editing tools and large-scale gene assembly technologies developed in workpackages B and D will be of direct benefit to all the trait-engineering work-packages (F, G, H, I and J). Specifically, vectors for chloroplast manipulation and methods to achieve homoplasty are being developed for use in workpackage F and molecular tools for targeted genome editing are being developed for use in workpackages G, H and I.

Investigators
Nicola Patron; Jim Haseloff; Jim Ajioka; Giles Oldroyd; James Locke; Christopher Howe

Staff Employed
Oleg Raitskin (PDRA; Patron lab). Started Jan 2015. Re-employed at EI Sep 2016
Douglas Griffith (PDRA, Locke lab). July 2015 - Nov 2016
David Willey (PDRA, Ajioka lab). Started Sep 2015, end Mar 2016
Susana Sauret-Gueto (Research Manager; Haseloff lab). Started Oct 2015.
Orr Yarkoni (PDRA, Ajioka lab). Started May 2016
Philip Carella (PDRA, Schornack lab). Started Sep 2016
Bruno Martins (PDRA, Locke lab). Started Jan 2017
Stephen Rowden (PDRA, Howe lab). Started Sep 2017

Partners
The CRISPR/Cas9 workshop held at JIC in September 2015 was co-funded by the Genomic Arabidopsis Resource Network (GARNet)
Through Workpackage I (N2 Fixation), OpenPlant interfaces with the Engineering Nitrogen Symbiosis for Africa (ENSA) project and other Gates-funded cereal engineering projects.
Henderson lab, University of Cambridge
Uauy lab, John Innes Centre
Wendy Harwood, John Innes Centre

B



Milestones:

B1: Simple, non-technical guide to installation of a DNA registry.
Deliverable: Online publication of a simple installation guide for use in OpenPlant laboratories (month 6, Haseloff).

B2: Installation of central database for sharing of published DNA details.
Deliverable: Installation of servers and publication of first records (month 24, Haseloff, Patron, Oldroyd).

B3.1: Characterised inducible cyanobacteria gene promoters.
Deliverable: Public release* of cyanobacterial DNA promoter part collection (month 30, Locke).

B3.2: A suite of biological parts that enable construction of synthetic circuits in Synechococcus elongatus.
Deliverable: Public release* of S. elongatus DNA circuit element collection (month 30, Locke).

B4: Parts for electrically regulated gene expression.
Deliverable: Public release* of voltage regulated cyanobacteria DNA parts (month 60, Howe).

B5: Comprehensive and integrated OpenPlant kit for distribution of IP-free DNA parts.
Deliverable: Establishment of a badged OpenPlant part collection for DNA distribution (month 60, Haseloff)

Online resources:

Patron lab
(nicolajpatron.com)

Locke lab
(www.slcu.cam.ac.uk/research/locke-group)

Howe lab
(www.bioc.cam.ac.uk/howe)

OpenMTA
(biobricks.org/openmta)
(www.openplant/openmta)

Addgene
(www.addgene.org)

Phytobricks
(2016.igem.org/Resources/Plant_Synthetic_Biology/PhytoBricks)

Plant DNA parts in iGEM
(parts.igem.org/Collections/Plants)

Progress to date:

Objective B1: Simple, non-technical guide to installation of a DNA registry.

A simple method for the one-click installation of a DNA database has been implemented. In collaboration with Nathan Hilson (JBEI), we have compiled a public OSX based version of the ICE DNA registry. The resulting IceApp is available and documented as an open source project on Github (https://github.com/fathomlabs/IceApp) and provides a platform for compilation and distribution of the ICE in app form, for individual and local use as a one-click installation for the DNA database. As the project has progressed, we have developed MarpoDB, a customised gene-centric database for Marchantia that allows description of the genome as a series of DNA encoded parts, and allows easy refactoring and extraction of parts in standardised format for synthesis and reuse. Further, we have found that Benchling provides a free web-based solution for an online lab notebook, sequence editor and registry of entries (www.benchling.com). In Benchling, members of an organisation can share a registry, and a registry of Marchantia DNA parts has been established. Sauret-Gueto is cordinating the use of Benchling with other labs to share registries and schemas. This combination of web-accessible databases is proving to be a more accessible and flexible solution as a DNA registry, and beginning to find wider use across and beyond the OpenPlant community.

Objective B2: Installation of central database for sharing of published DNA details.

We have established a public server in Cambridge with a central database for genome-wide DNA part mining and documentation at www.marpodb.io. In addition, we have documented synthetic DNA parts, and vectors and plasmids constructs on the Benchling web platform, paired with the use of Addgene as the main repository for DNAs.

Objective B3: Characterised inducible cyanobacteria gene promoters & a suite of biological parts that enable construction of synthetic circuits in *Synechococcus elongatus*.

The Locke lab has constructed rationally designed DNA parts for examining the circadian clock and its outputs at the single cell level in the model cyanobacteria *Synechococcus elongatus* 7942. They have developing improved fluorescent reporters to enable better quantification of the output of inducible promoters. The parts include codon optimised versions of newly available YFP and CFP fluorescent proteins as reporters for the key circadian (24 hour) clock output genes sigC and psbA1. The reporters contain a degradation tagged YFP to allow the analysis of fast changing gene expression dynamics. The reporters were used to examine psbA1 dynamics at the single cell level, and revealed that psbA1 displays 12 hour oscillations, and not 24 hour as previously thought. They went on to show that the frequency of the circadian clock in cyanobacteria can be doubled through an incoherent feedforward loop circuit involving sigC.

Objective B4: Parts for electrically regulated gene expression.

The biophotovoltaic system has the potential to form the basis of an innovative method for controlling the expression of biomolecules in modified micro-algae. Such a system would be of great scientific and commercial interest, and could be used for controlled expression of biopharmaceuticals, nutraceuticals and metabolic proteins. The identification of cis-regulatory elements is a prerequisite for the use of electropotential as an inducible system. Three promising cis-regulatory elements have been identified using RNA-Seq data previously obtained in the lab, comparing electricity-generating biofilms to non-electricity generating biofilms of the cyanobacterium Synechocystis. One of these three elements is conserved in many other species of cyanobacteria opening up the possibility that the element could be used as a universal BioBrick for synthetic biology in cyanobacterial species. The OpenPlant syntax will be used to construct variants of promoter regions that control a codon optimized eYFP gene. RNA samples from electricity generating, and non-electricity generating, biofilms have been taken and qPCR analysis is underway to validate the RNA-Seq data.

Objective B5: Low-cost license-free distribution of DNA parts. (month 60, All)

A primary aim of the OpenPlant initiative is to establish and promote better practices for producing and sharing standardised DNA parts for engineering plant systems. To this end, we have made major advances on four fronts:

(i) **Common syntax for the exchange of modular DNA parts**. The syntax is a regularised and consistent version of that used in Golden Gate, MoClo, Golden Braid and ENSA Type IIS based DNA assembly methods, and was published as a technical manifesto with wide support from the international plant science community (Patron et al. New Phytol. 2015). We have also promoted the common syntax for plant DNA parts as a new standard for the iGEM competition (RFC 106, Rutten et al. 2015; http://hdl.handle.net/1721.1/96069). This was a collaborative output from the Cambridge-JIC, Valencia and Norwich Research Park iGEM teams. Following this, the iGEM foundation agreed that parts can be submitted to the Registry of Biological Parts in RFC 106 (and are termed Phytobricks). This set the foundation for an inaugural Plant Prize at the 2016 iGEM competition (won by Cambridge-JIC in 2016).

(ii) **DNA assembly methods**. The Phytobrick standard is a consolidated and consistent standard for Type IIS restriction endonuclease-based assembly of DNA parts to make synthetic genes. We have been deliberately agnostic about higher level gene assembly, where Gibson assembly and higher order Mo-Clo and Golden Braid techniques are generally used. Work by Fernan Federici and Bernardo Pollak (Haseloff lab) has demonstrated the feasibility of using unique nucleotide sequences (UNS, first popularised in Pam Silver's laboratory) as landing pads for assembly of multigene constructions, using conserved sets of oligonucleotide adapters. This provides a flexible and low cost method of assembly in laboratories where

custom oligonucleotides are expensive or difficult to obtain. We have constructed a series of plant based vectors to exploit this method of assembly.

Further, we have developed a simple and flexible protocol for assembly of plant vectors, the Loop assembly technique. As part of a collaboration between the University of Cambridge and the Universidad Católica de Chile, Federici and Pollak (Haseloff Lab) have devised a new method for recursive gene assembly based on two Type IIS restriction endonucleases, BsaI and SapI. Loop Assembly allows rapid and efficient production of large DNA constructs, is compatible with widely used Level zero (L0) DNA parts such as Phytobricks, and has been automated through a collaboration with the DNA Foundry and Patron lab at the Earlham Institute.

(iii) **Automation**. Like other "Golden-gate"-based protocols, Loop Assembly does not require purification of individual DNA fragments, side products are recut during the ligation reaction to drive efficient formation of end-products. Loop Assembly is well suited to automation. OpenPlant researchers at the Earlham Institute (Patron Lab) and Cambridge (Haseloff Lab) are developing methods using acoustic-focusing non-contact liquid handling robots, which increases speed and scale of assembly, while reducing consumable costs and allowing reactions to be performed in nanolitre volumes. The Patron Lab has miniaturized and automated DNA Assembly reactions at the Earlham DNA foundry (Patron Lab) to provide assembles for Workpackage D and B. Oleg Raitskin (Patron Lab) with colleagues at the Earlham Institute have automated assembly in <500 nL in 384 well microplates using the Labcyte Echo. Transformed bacteria are dispensed by shaking and multiple droplet dispensing onto agar in square, 8-well plates. Colony picking is performed on the Hamilton platforms, which have an on-board camera and light table. The EasyPic software is used to select positive colonies. Assemblies are validated using a Miniaturised Nextera XT library construction on an Illumina MiSeq. Transfection of plant protoplasts using a modified isolation procedure with automated transformation on the Hamilton platform is ongoing.

(iv) **Open materials transfer**. Current IP practices and restrictive licensing threaten to restrict innovation as the scale of DNA systems increases. We believe that the field needs to explore new "two-tier" intellectual property models that will protect investment in applications, while promote sharing of DNA components and freedom-to-operate for small companies in commercial applications of Synthetic Biology. We are collaborating with Dr. Linda Kahl and colleagues at the Biobricks Foundation to draft and implement an Open Materials Transfer Agreement (OpenMTA). This is a simple, standardized legal tool that enables individuals and organizations to share their materials and associated data on an open basis. Materials can be freely used (even for commercial purposes) and further distributed by the recipients. The primary purpose of the OpenMTA is to effectively place low-level DNA parts into the public domain, and to eliminate or reduce transaction costs associated with access, use, modification, and redistribution of materials and associated data. This in turn will help minimize delay and redundancy in the scientific research process and promote access to materials and associated data for researchers in less privileged institutions and world regions. Details of the draft can be found at http://openmta.org, and a description has been submitted for publication.

(v) **Distribution**. In order to avoid unwanted restrictions on OpenMTA-based distribution, we have constructed, a "clean" plasmid vector (pUAP1) suitable for cloning of Level zero DNA parts. Further, we have constructed two families of Loop plant transformation vectors, based on pCambia and pGreen backbones, respectively. These differ in replication origins and copy number in bacterial hosts. We have now generated a growing library of Marchantia TF promoters, as well as a basic Marchantia DNA tool-kit for core promoters, resistance genes, fluorescent proteins, signal peptides, CRISPR-Cas9 and other tools for plant synthetic biology. We are in discussions with Addgene to explore the use of the OpenMTA as an alternative to the standard UB-MTA for routine, open distribution of DNAs intended for the public domain.

Publications

Patron NJ (2016). **Blueprints for Green Biotechnology: Development and Application of Standards for Plant Synthetic Biology.** *Biochem. Soc. Trans., 44, 702–708*

Patron NJ (2016) **Synthetic Plants:** in Synthetic Biology Handbook. Ed. Darren N. Nesbeth. CRC Press.

Patron NJ (2016). **Synthetic Biology and Gene Cloning** in Encyclopedia of Applied Plant Sciences 2nd Edition. Editor-in-Chiefs: Brian Thomas Denis J Murphy Brian G Murray. Academic Press. eBook ISBN: 9780123948083 Hardcover ISBN: 9780123948076

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Rutten V, Munabi A, Riche F, Lewy G, Wilson H, Pipan M, Bhate S, Nghiem T-A, Kaufhold W, Haseloff J, Rubert A, González A, Quijano A, Llopis I, Gavaldá J, Estellés L, Vásquez M, Orzáez D, Deal C, Gray J, Spiegel M, Monsey S, Middlemiss A, Day J, Patron NJ* (2015) **A Standard Type IIS Syntax for Plants.** *RFC #106 (http://hdl.handle.net/1721.1/96069)*

Pollak B, Cerda A, Delmans M, Álamos S, Moyano T, West A, Gutiérrez RA, Patron N, Federici F & Haseloff J **Loop Assembly: a simple and open system for recursive fabrication of DNA circuits** *in prep/review*

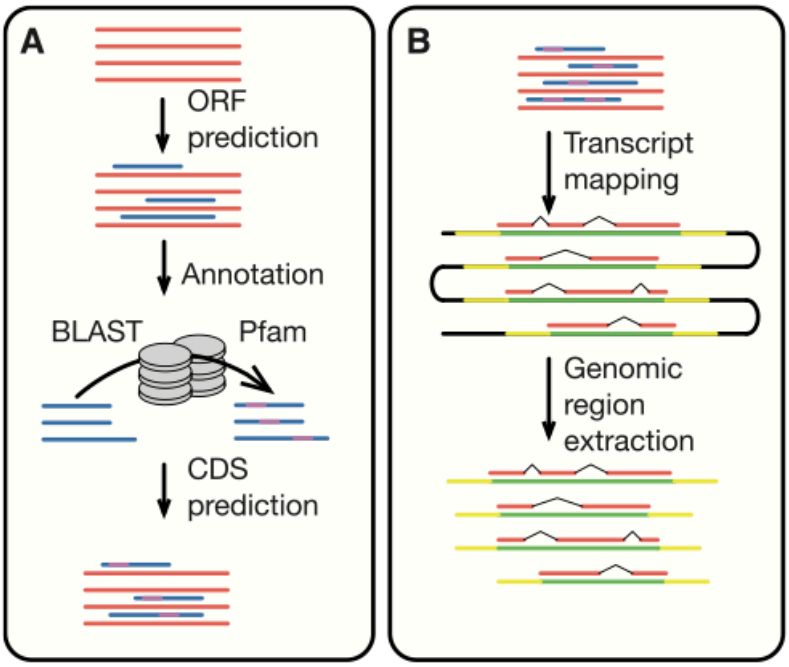
Vazquez-Vilar M, Orzaez D, and Patron, N. **DNA Assembly Standards: Setting the Low-Level Programing Code for Plant Biotechnology.** *In review*

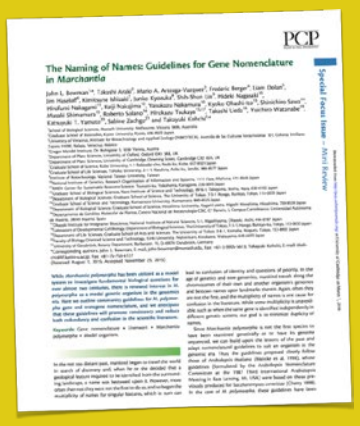
Workflow for standardised DNA assembly


Article

Insights into Land Plant Evolution Garnered from the *Marchantia polymorpha* Genome

Insights into Land Plant Evolution Garnered from the Marchantia polymorpha Genome. Bowman JL *et al.*, Cell, 171:287–304 (2017)




PCP

The Naming of Names: Guidelines for Gene Nomenclature in *Marchantia*

The Naming of Names: Guidelines for Gene Nomenclature in Marchantia. Bowman JL, Araki T, Arteaga-Vazquez MA, Berger F, Dolan L, Haseloff J, Ishizaki K, Kyozuka J, Lin SS, Nagasaki H, Nakagami H, Nakajima K, Nakamura Y, Ohashi-Ito K, Sawa S, Shimamura M, Solano R, Tsukaya H, Ueda T, Watanabe Y, Yamato KT, Zachgo S, Kohchi T. Plant Cell Physiol. 57:257-61, (2016).

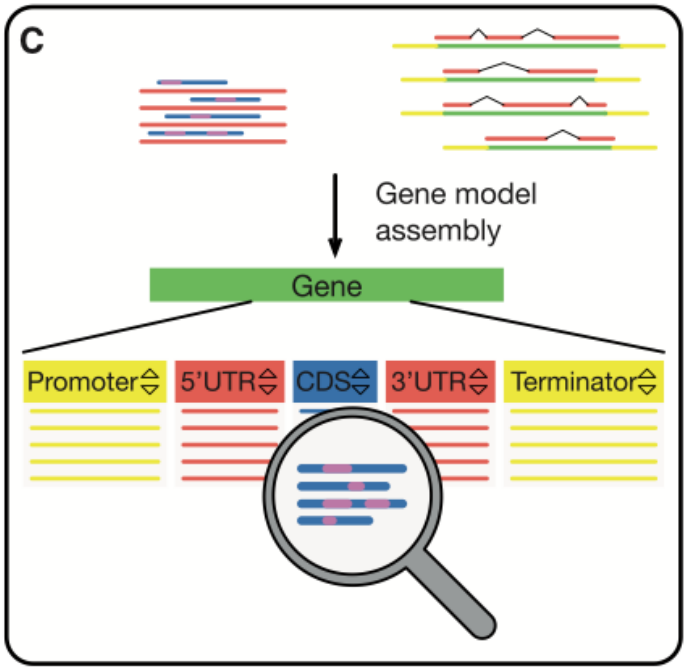
The Marchantia genome

MELBOURNE, KYOTO, JGI, CAMBRIDGE

The genome of *Marchantia polymorpha* has been sequenced as part of an effort by a multinational consortium, including OpenPlant. The type isolate (Tak-1) was sequenced, assembled and annotated as a collective effort with participation of many laboratories. Marchantia has a genome of around 280 Mbp with around 19,000 encoded genes. Many families of regulatory genes are found with highly reduced numbers, compared to higher plants. The reference genome has been published at the phytozome.org website.

OpenPlant has contributed the genome and transcriptome sequences for male (Cam-1) and female (Cam-2) isolates from Cambridge. In addition, we have produced a map of genome-wide transcription in germinating sporelings. We have compared the genomes, and used these to build a general resource for gene design and synthetic biology experiments in Marchantia.

Principal contact: Jim Haseloff



MarpoDB: An Open Registry for Marchantia Polymorpha Genetic Parts. Delmans M, Pollak B and Haseloff J, Plant Cell Physiol. 58: e5(1–9) (2016)

MarpoDB: a gene-centric database to mine for DNA parts

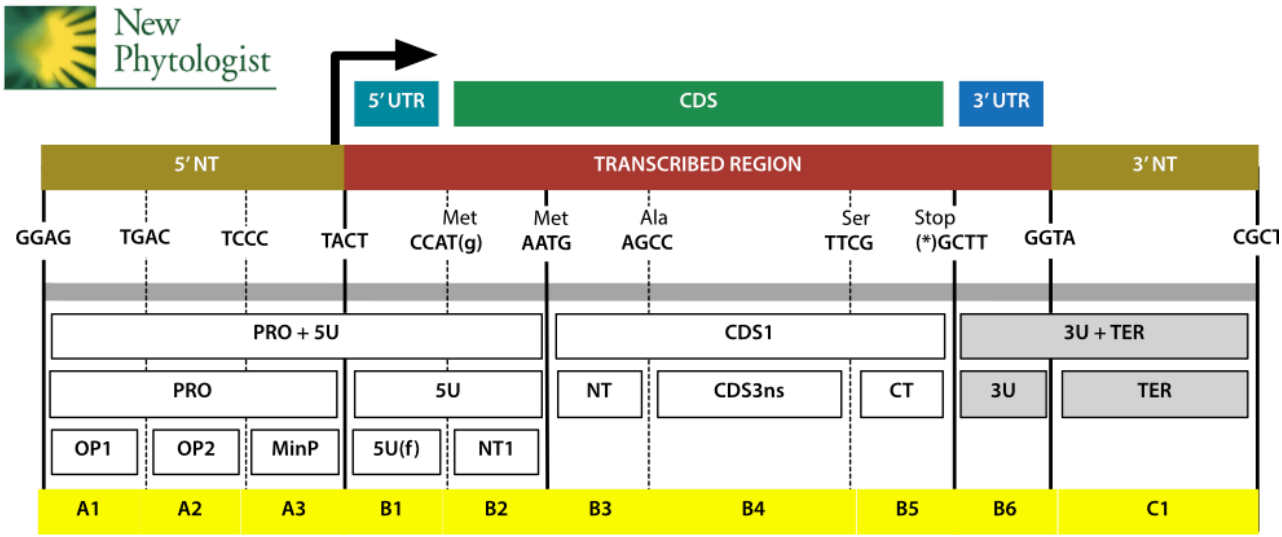
CAMBRIDGE

MarpoDB is an open source database that presents the Marchantia genome from an engineer's perspective, rather than a geneticist's. The database handles the Marchantia genome as a collection of parts. This is highly useful for automatically mining new parts, and managing part description, and part characterisation. We think that this break from standard genome database architecture is essential for tackling the refactoring of synthetic plant genomes. MarpoDB also provides a useful container for gene expression data, and integration of cellular features via Plant Ontology terms. (http://marpodb.io)

MarpoDB has been designed to facilitate the definition and extraction of synthetic DNA elements to be synthesised as standardised DNA parts. For example, we have identified core promoter candidates, and extracted these from the Marchantia genome.

Principal contacts: Mihails Delmans & Susana Sauret-Gueto

Workflow for standardised DNA assembly



Standards for plant synthetic biology: a common syntax for exchange of DNA parts
New Phytologist 208:13-9. (2015)
Patron, N; Orzaez, D; Marillonnet, S; Warzecha, H; Matthewman, C; Youles, M; Raitskin, O; Leveau, A; Farre-Martinez, G; Rogers, C; Smith, A; Hibberd, J; Webb, A; Locke, J; Schornack, S; Ajioka, J; Baulcombe, D; Zipfel, C; Kamoun, S; Jones, J; Kuhn, H; Robatzek, S; Van Esse, HP; Oldroyd, G; Sanders, D; Martin, C; Field, R; O'Connor, S; Fox, S; Wulff, B; Miller, B; Breakspear, A; Radhakrishnan, G; Delaux, PM; Loque, D ; Granell, A; Tissier, A; Shih, P; Brutnell, T; Quick, P; Rischer, H; Fraser, P; Aharoni, A; Raines, C; South, P; Ané, JM; Hamberger, B; Langdale, J; Stougaard, J; Bouwmeester, H; Udvardi, M; Murray, J; Ntoukakis, V; Schafer, P; Denby, K; Edwards, K; Osbourn, A & Haseloff, J

A common syntax for DNA parts

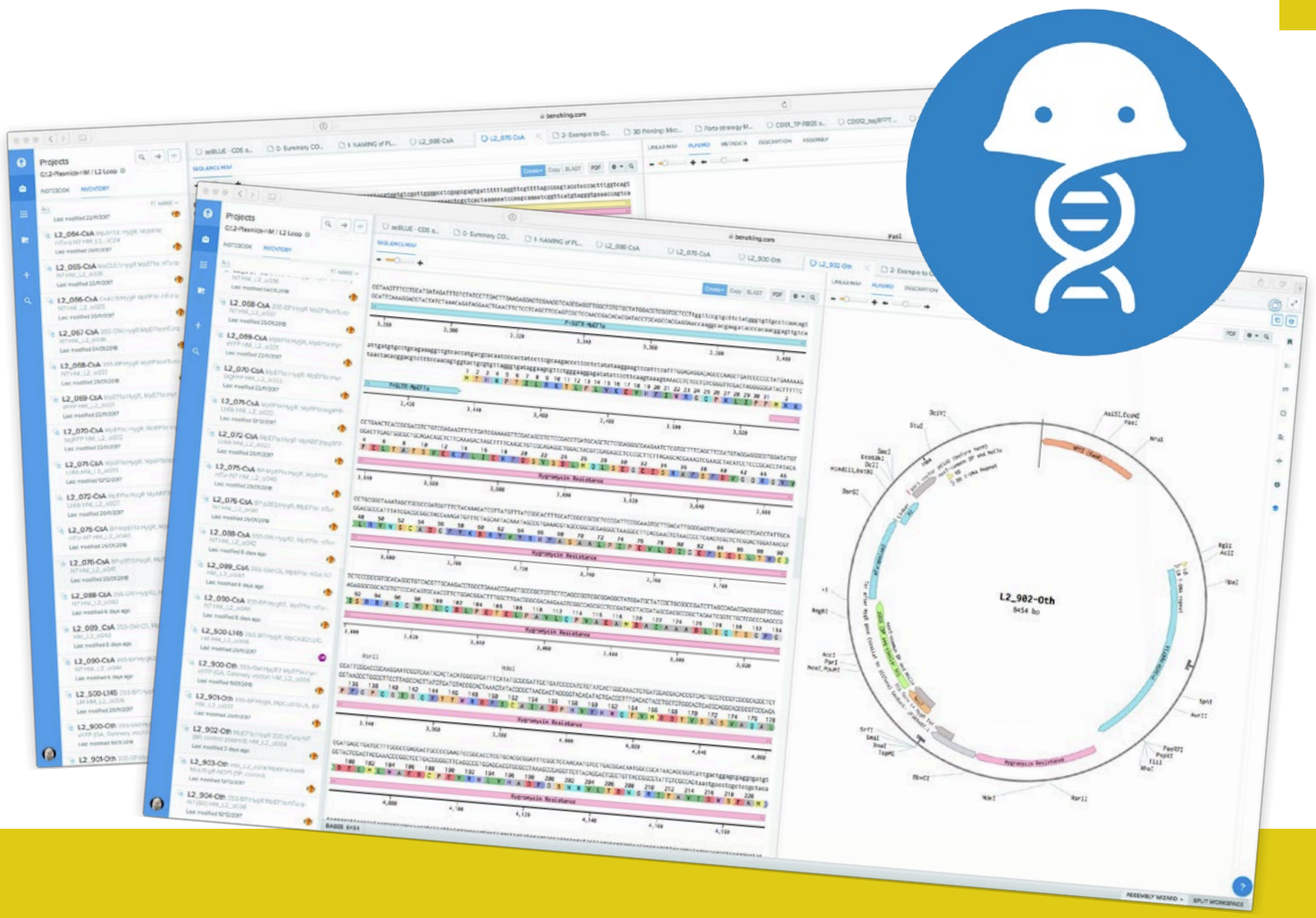
COMMUNITY STANDARD

With wide support from the international plant science community, we have established a common genetic syntax for exchange of DNA parts for plants, extensible to all eukaryotes (Patron et al. 2015). This common syntax for plant DNA parts is at the core of RFC 106, posted at OpenWetWare, and accepted as an official standard for DNA parts in the iGEM synthetic biology competition.

The Phytobrick standard is a consolidated and consistent standard for Type IIS restriction endonuclease based assembly of DNA parts to make synthetic genes. It is based on the widely used "Golden-Gate"-type standard, and allows highly efficient assembly of multiple standard parts into genes without the need to isolate DNA fragments. A range of existing techniques such as Gibson assembly, MoClo and Golden Braid can be used for higher order multiple-gene assemblies, however we have developed a simple and flexible protocol for assembly of plant vectors, the Loop Assembly technique.

The Phytobrick standard is general, and applicable to all plants, and other eukaryotes

Principal contacts: Nicola Patron & Jim Haseloff



Benchling as a DNA design platform and registry

CAMBRIDGE

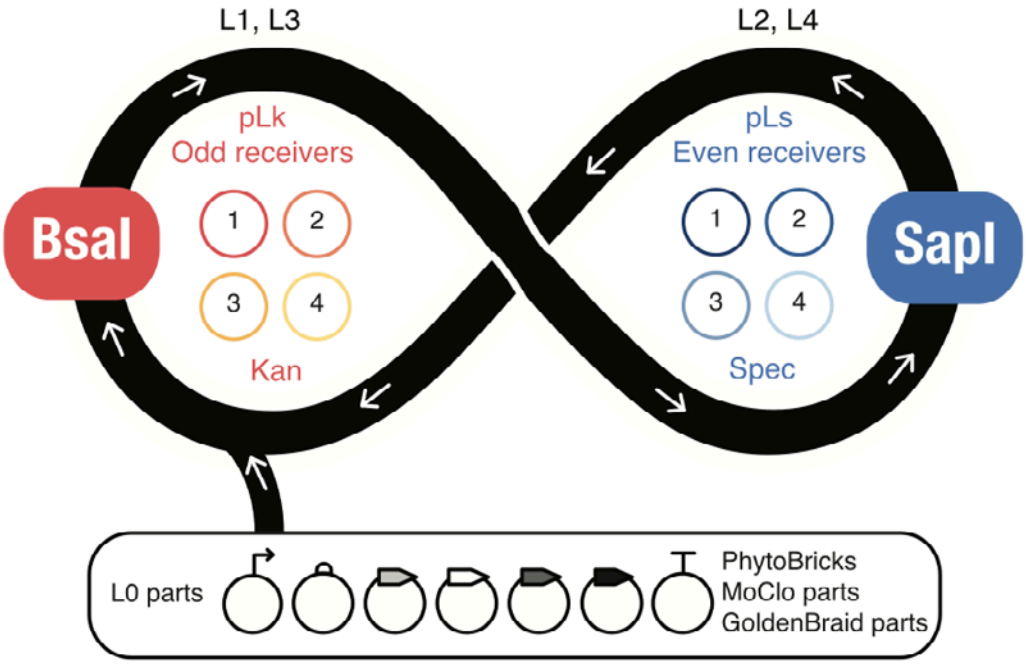
We have adopted Benchling (www.benchling.com) as a free research platform with tools for note-taking, molecular biology, and sample tracking.

The web-based platform provides (i) an electronic lab notebook with drag & drop import for images of any file type, protocols, time-stamping, and PDF backups, and (ii) a design and analysis suite for synthetic biology experiments including a feature library and auto annotation, automated primer design, Type IIS cloning wizard, plasmid visualisation, lineage tracking, BLAST and sequence alignment. (iii) Benchling also provides a biological registry to track Level 0 DNA parts, plasmid constructions, samples, and microbial and plant isolates.

Benchling provides different templates for standardised entry of metadata with options for bulk import and export. This is useful for database curation and compilation of DNA synthesis orders. It is a platform for collaboration that allows sharing of a project with a small number of users, an organisation or the entire community.

Principal contacts: Susana Sauret-Gueto & Nicola Patron

Workflow for standardised DNA assembly



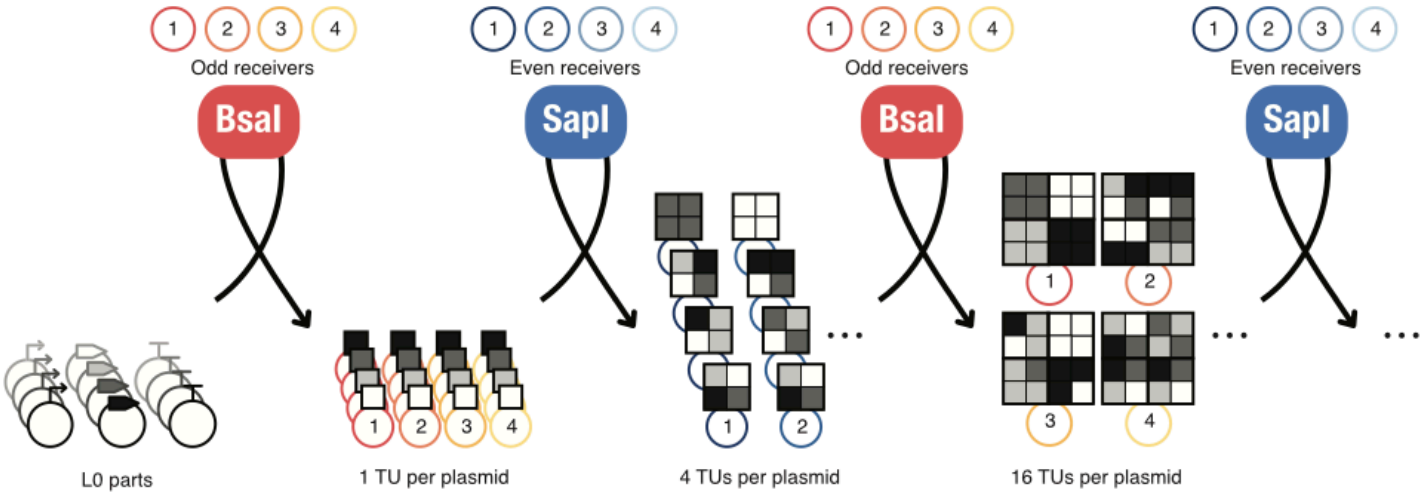
Loop Assembly for simple DNA construction

CAMBRIDGE, PUC CHILE

As part of a collaboration between the University of Cambridge and the Universidad Católica de Chile, Pollak and Federici have devised a new method for gene assembly based on two Type IIS restriction endonuceases, BsaI and SapI. Loop Assembly allows rapid and efficient production of large DNA constructs, is compatible with widely used Level zero (L0) DNA parts such as Phytobricks, and can be easily automated.

Loop Assembly requires the alternating use of two Type IIS enzymes, Bsa1 (6-base-pair recognition sequence, 4 base overhang) and Sap1 (7 base-pair recognition sequence, 3 base overhang), and two sets of complementary plasmid vectors that allow efficient and ordered construction of 1, 4, 16, 64 gene fragments.

Principal contacts: Jim Haseloff & Fernan Federici



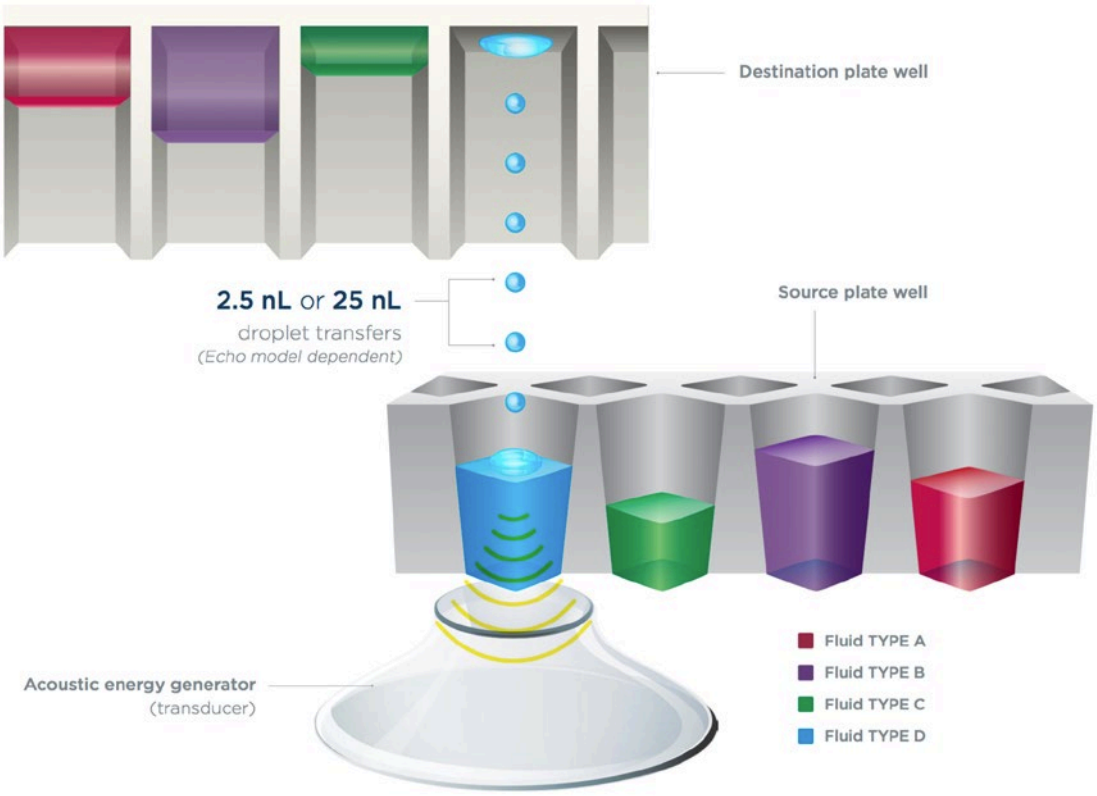
Automated Loop assembly and validation

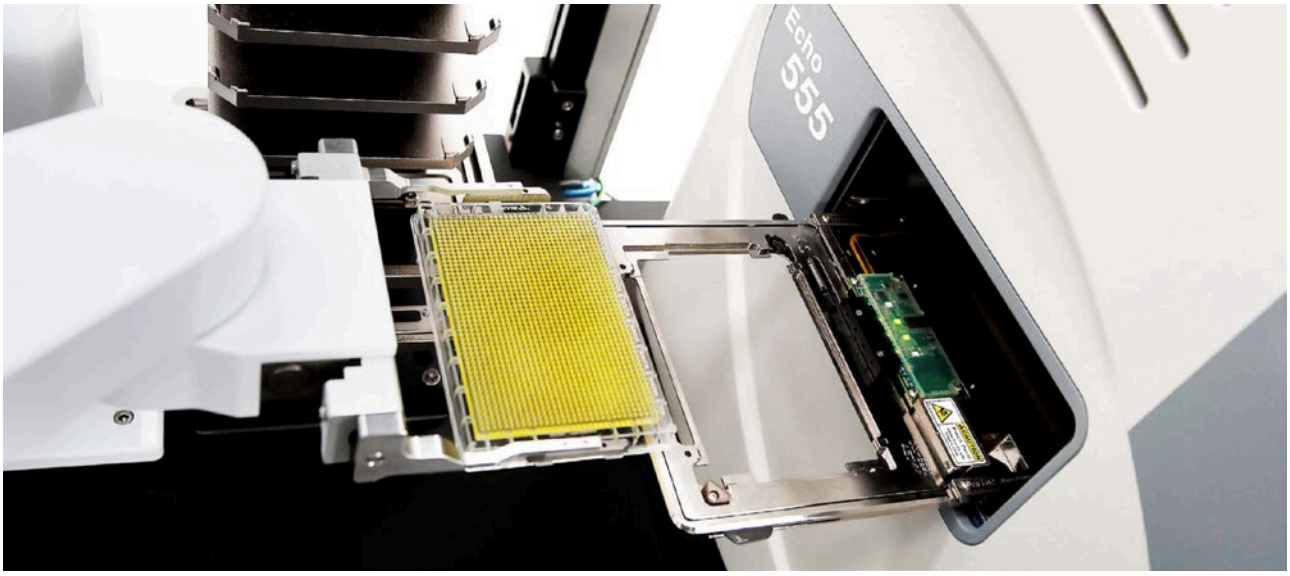
CAMBRIDGE, PUC CHILE, EARLHAM INSTITUTE

Like other "Golden-gate"-based protocols, Loop Assembly does not require purification of individual DNA fragments, side products are recut during the ligation reaction to drive efficient formation of end-products. Loop Assembly is well suited to automation. OpenPlant researchers at the Earlham Institute and Cambridge are developing methods using acoustic-focusing non-contact liquid handling robots, which increases speed and scale of assembly, while reducing consumable costs and allowing reactions to be performed in nanolitre volumes.

Principal contacts: Susana Sauret-Gueto & Nicola Patron

Workflow for standardised DNA assembly



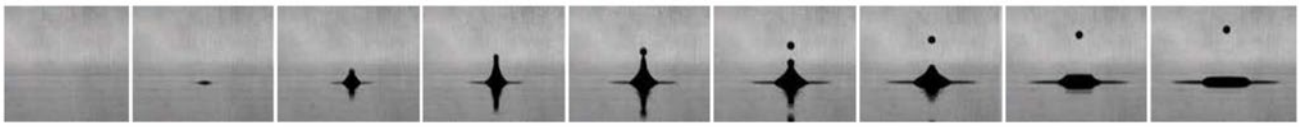


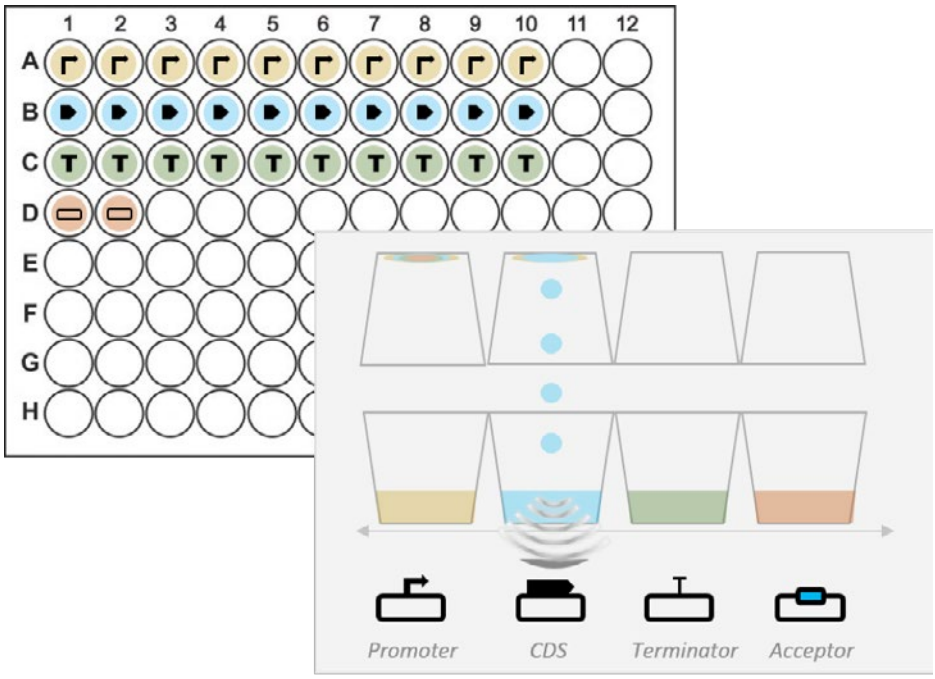
Robotic assembly of DNA reactions

Both the OpenPlant Cambridge Lab and the Earlham Foundry have purchased Labcyte 550 Echo instruments for programmable nanoscale liquid handling. The devices use an acoustic focusing probe to transfer 2.5 nL droplets from source plates to chosen destination wells. The droplets are transferred at up to 200 Hz, allowing the assembly of small scale reactions with precision and flexibility..

New reaction assemblies can be easily reprogrammed, and no tips are consumed during use. The easy programmability and use of the Labcyte Echo is well suited for use in a multi-user environment, compared to dedicated pipetting robots and fixed pathways for automated sample handling.

(Images: Labcyte)





Nanoscale automated DNA assembly and verification

EARLHAM INSTITUTE

We have automated and miniaturized Type IIS–mediated assembly of plasmid constructs. We use a modular setup that harnesses an automated freezer working in a 96-well-plate format, and a suite of liquid handling robots to array source plates, assemble larger constructs using acoustic energy from standard DNA parts and perform the downstream microbiological workflow.

Multiple assemblies can be verified in parallel using miniaturized Nextera XT libraries on an Illumina MiSeq Platform or using a novel 'SMRTGate' protocol co-developed with the Liverpool GeneMill (D'Amore et al (2017) BioTechniques 63:1 13–20).

Principal contacts: Nicola Patron and Anthony Hall (Earlham)

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OpenMTA for free exchange of DNA parts

OPENPLANT & BIOBRICKS FOUNDATION

Current IP practices and restrictive licensing threaten to restrict innovation as the scale of DNA systems increases. We believe that the field needs to explore new “two-tier” intellectual property models that will protect investment in applications, while promote sharing of DNA components and freedom-to-operate for small companies in commercial applications of Synthetic Biology.

We are collaborating with the Biobricks Foundation on an Open Materials Transfer Agreement (OpenMTA). This is a simple, standardized legal tool that enables individuals and organizations to share their materials and associated data on an open basis.

The primary purpose of the OpenMTA is to eliminate or reduce transaction costs associated with access, use, modification, and redistribution of materials and associated data. This in turn will help minimize waste and redundancy in the scientific research process and promote access to materials and associated data for researchers in less privileged institutions and world regions. (http://www.openmta.org)

Principal contact: Linda Kahl

Global DNA distribution

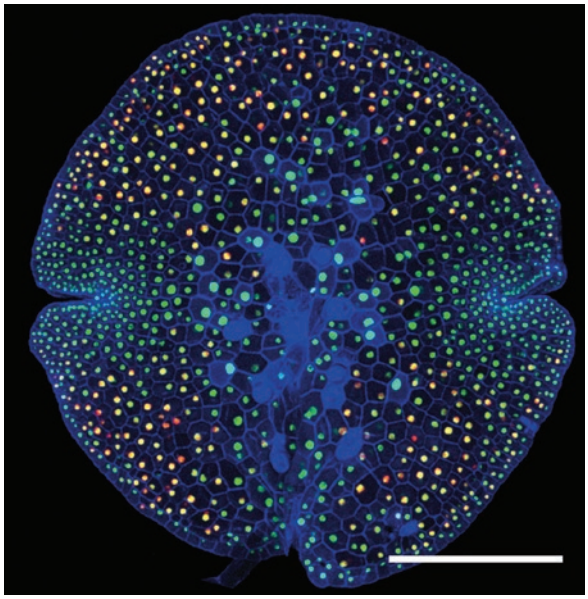
CAMBRIDGE, EARLHAM INSTITUTE & ADDGENE

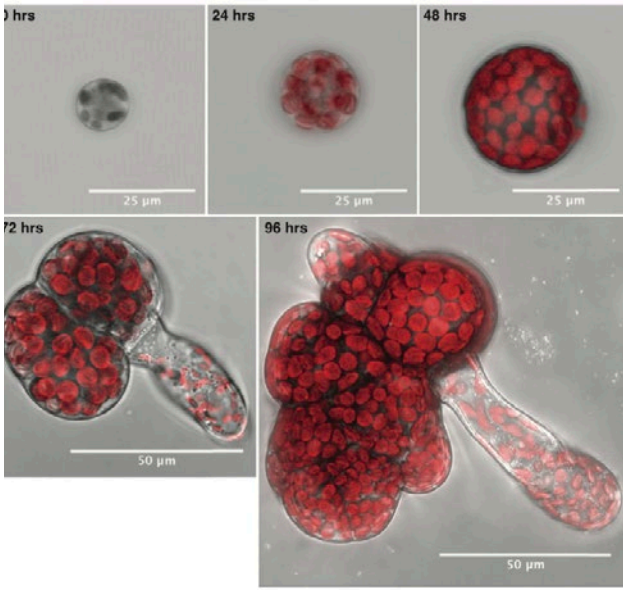
In order to use the the OpenMTA as a framework for the free distribution for DNA parts and circuits. Many existing DNA plasmid vectors are maintained under commercial licenses, and are not suitable for open distribution. We have constructed a series of IP-free plasmid backbones for Level 0 parts and pOdd and pEven vectors for Loop assembly.

We are submitting OpenPlant DNAs to the not-for-profit company Addgene, to facilitate global access from academia and industry. Further, Addgene employs a standard materials transfer agreement for exchange between institutions worldwide. We are working with Addgene to introduce routine use of the OpenMTA internationally in 2018.

In addition to vector sets for rapid assembly, we have synthesised a large library of DNA parts for circuit construction in Marchantia, including core promoters, protein fusion tags, terminators, reporter genes and other genetic effectors. These will be shared under the terms of the OpenMTA.

Principal contacts: Susana Sauret-Gueto & Nicola Patron





Testing synthetic promoters in Marchantia

CAMBRIDGE

Around 400 synthetic core promoters have been designed. Key genes, mainly regulatory genes and key effector genes have been identified in Marchantia. Sequences upstream of each gene, including the presumptive leader sequence, and running to the ATG start codon have been extracted. Candidate proximal promoter domains have been domesticated, cured of BsaI and SapI sites, flanked by convergent BsaI sites, and sent for DNA synthesis and cloned into a universal Level 0 plasmid vector. This pUAP1 vector was constructed by Nicola Patron, and is custom built for sharing DNA parts used in many types of Type IIS assembly procedures. Bernardo Pollak has constructed plant transformation vectors with multi-spectral fluorescent protein outputs, for ratiometric characterisation of gene expression. This allows direct observation of patterns of gene expression in transformed Marchantia tissues, as shown above for a young gemma.

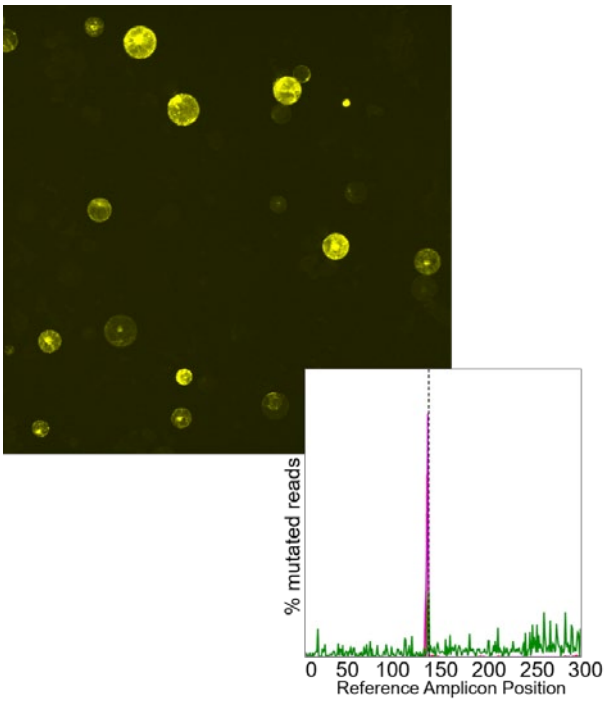
Principal contacts: Susana Sauret-Gueto

Gene expression in germinating sporelings

CAMBRIDGE

Marchantia spores can be harvested and germinated in synchronised fashion. Germination is accompanied by rapid expansion, differentiation of chloroplasts, the first cell divisions, formation of a pronounced apical-basal axis and continued growth and specialisation. Samples can be collected across this period, RNAs extracted, and analysed by high-throughput RNA sequencing to obtain a map of shifting patterns of transcription during these initial phases of plant development. The transcriptome data has been used to investigate genes involved in early chloroplast differentiation and division.

Principal contact: Jim Haseloff



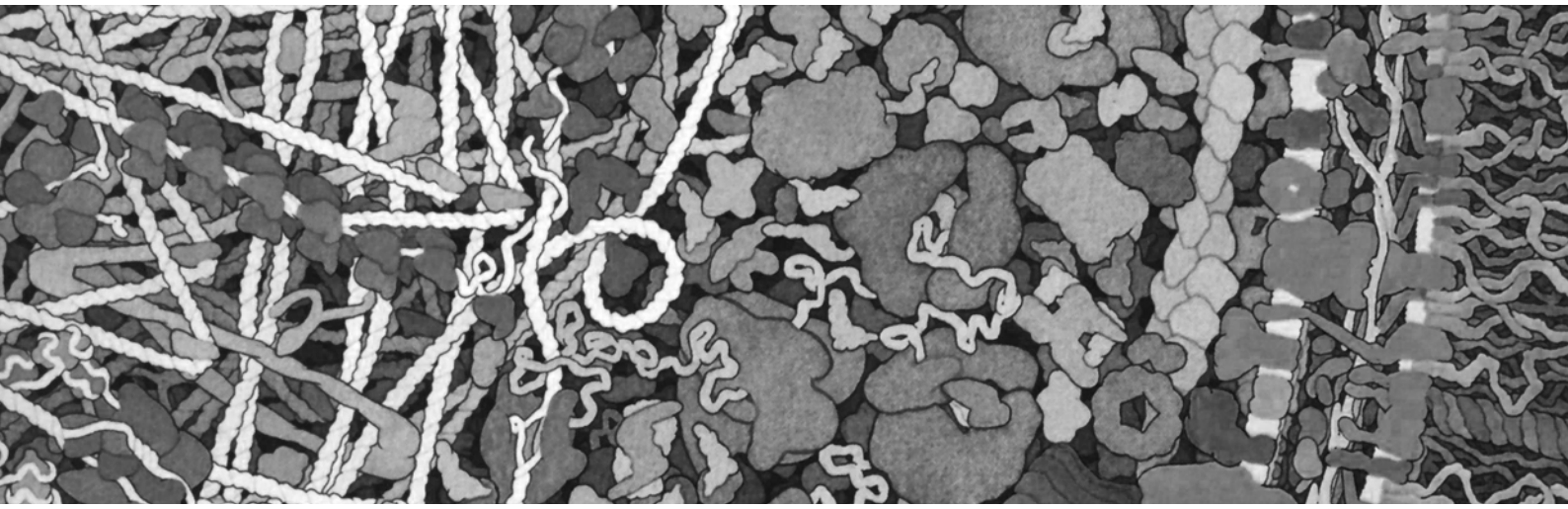
Expanded toolkit for plant genome engineering

EARLHAM

Molecular tools adapted from the Clustered Regularly-Interspaced Short Palindromic Repeats (CRISPR) loci that confer adaptive immunity in bacteria and archaea have been applied for genome engineering in eukaryotes. RNA-guided Cas (CRISPR Associated) proteins have been used to induce targeted mutagenesis at endogenous loci in numerous plant species. However, the efficiency of editing varies between species and between targets, mutations are often observed at non-target loci and use of wild-type Cas9 limits engineering to target loci containing a canonical NGG motif.

Oleg Raitskin has developed an expanded toolbox of molecular tools for RNA-guided Cas-mediated plant genome engineering to improve specificity and to increase the number of potential target sites available in plant genomes. To compare and quantify the efficiency and specificity of multiple Cas9 variants, Oleg coupled automated DNA assembly to a transient workflow using protoplasts and Illumina MiSeq targeted resequencing.

Principal contacts: Oleg Raitskin & Nicola Patron



Workpackage C:
New mechanisms for regulation of gene expression

RNA-based mechanisms for gene regulation complement conventional transcriptional regulation, and can be highly flexible and modular. These mechanisms are common in nature, and are only now being harnessed for synthetic systems.

This workpackage contains the following projects:
Riboswitches in new chassis (C3) and Riboregulator circuits (C4) (Alison Smith, Cambridge). The aim of this work is to develop new methods of transgene delivery into the green alga Chlamydomonas reinhardtii and in technology to control gene expression. The project leaders participate in an international project for the construction of a Chlamydomonas MoClo kit of Golden Gate domesticated DNA parts, which will foster the development of synthetic biology in algae.

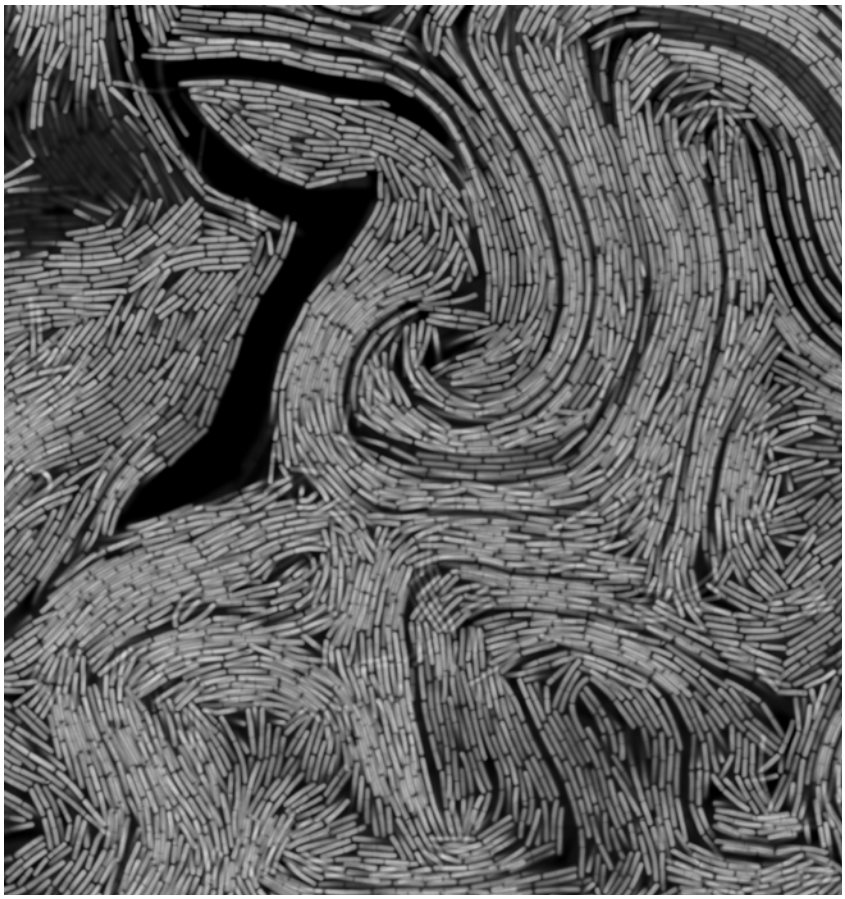
In addition, workpackage C benefits work with Cyanobacteria circuits (B3) (James Locke). The projects share common aims in quantitative methods to measure gene circuit output. It also benefits from present and past projects in the Baulcombe lab, which provide tools, mutant strains and methods for RNA silencing.

Investigators
David Baulcombe; Alison Smith (Cambridge)

Staff Employed
Francisco Navarro (PDRA; Baulcombe lab). Started May 2015
Gonzalo Mendoza-Ochoa (PDRA; Smith lab). Started Nov 2017

Partners
An OpenPlant Fund grant has established new collaborations between OpenPlant PDRA Francisco Navarro and John Innes Centre bioinformatics specialist, Marielle Vigouroux, to develop a codon optimisation tool for Chlamydomonas.
Tim O'Leary, Department of Engineering, University of Cambridge

C



Online resources:

Smith lab
(www.plantsci.cam.ac.uk/research/alisonsmith)

Baulcombe lab
(www.plantsci.cam.ac.uk/research/davidbaulcombe)

Milestones:

C1: RNA silencing modules for regulation of genes in a land plant and an alga.

Deliverable C1.1: Identification of endogenous siRNA and miRNA loci from a land plant and an alga that could serve as the backbone for construction of RNA silencing modules (month 6, Baulcombe).

Deliverable C1.2: Assembly of test modules incorporating the backbones and demonstration that they can be used to silence gene expression in a land plant and an alga (month 18, Baulcombe).

Deliverable C1.3: Analysis of variant test modules in different tissue/growth states to characterize effective silencing systems and targeting rules (month 24, Baulcombe).

C2: Validation of RNA silencing modules for regulation of genes in a land plant and an alga
Deliverable: Testing of RNA silencing modules for validation of artificial silencing systems with feedback-based regulation. (month 30, Baulcombe).

C3: Standardised riboswitch parts for transgene regulation in different chassis.
Deliverable: Public release of DNA parts encoding riboswitches, characterised for use in plants and algae (month 48, Smith, Osbourn).

C4: Riboregulators for plastid transgene regulation.
Deliverable: Public release of DNA parts encoding TPR/PPR proteins characterised as components for regulation of plastid gene expression (month 60, Smith).

Progress to date:

Objective C1: RNA silencing modules for regulation of genes in a land plant and an alga.

We have identified endogenous miRNA precursors for synthetic gene circuit constructs. miR1157 and miR1162 backbones have been selected as carriers of amiRNAs for gene circuits. They have been previously characterized and successfully engineered to target new sequences. We have reduced the complexity of the endogenous miRNA precursor miR1157 and expressed it from a non-coding region of a construct. We have tested that this modified miRNA precursor is still able to efficiently repress a target sequence.

We have chosen two different Chlamydomonas strains as hosts of gene circuit constructs, the cell wall-less mutant CC-1883 and the wild type CC-1690. While the transformation protocol of CC-1883 is well established, we have optimised an electroporation protocol for the transformation of the wild type strain. Using this protocol, we have been able to obtain a sufficient number of transformants for our experiments.

Strong silencing of transgenes in Chlamydomonas made us test several strategies to optimize gene expression. In addition, the limited availability of molecular sequences useful for gene expression in Chlamydomonas implied the construction of more than 500 plasmids over the last two years. The results and methodology developed here can be easily extrapolated to land plants.

RNA silencing modules based on miRNA-precursor sequences from the green alga *Chlamydomonas reinhardtii* were generated and optimized. A modular strategy for fast and reliable cloning was implemented, based on Golden Gate. A series of reporter systems were constructed in the alga to demonstrate that modules were able to silence gene expression in vivo. These reporter systems allowed us to characterize the effective silencing systems, targeting rules and mode of action of artificial miRNAs in Chlamydomonas.

Objective C2: Validation of RNA silencing modules for regulation of genes in a land plant and an alga.

We have developed a Chlamydomonas parts kit for Golden Gate cloning. We have increased the number of available parts of the Chlamydomonas MoClo Kit, allowing fast and efficient construction of DNA molecules. Construction is based on modular assembly of DNA parts following the OpenPlant common syntax. A similar modularity strategy has also been applied to the construction of miRNA precursors. miRNA precursors have been cloned into several sites of the DNA constructs, and the impact in the expression of the cassette and in miRNA maturation have also been assessed. We have used an endogenous gene (*MAA7*) to confirm the functionality of miRNAs. Repression of *MAA7* confers resistance to the metabolic drug 5-fluoroindole offering an easy and fast reporter sytem.

Fluorescence proteins are being used as reporters of gene expression and silencing, allowing us to study the miRNA function both at population and single cell level. Nine different fluorescent proteins, which were codon optimized for expression in Chlamydomonas, have been tested to find suitable reporters. We have selected two fluorescent proteins with non-overlapping spectra that are expressed to detectable levels. Methods of fluorescence measurement have been established using OpenPlant fluorescence plate readers in Haseloff lab. Work in collaboration with James Locke is establishing single cell measurements.

Transgene expression is frequently suppressed in Chlamydomonas. We have tested several methods to overcome suppression in order to obtain detectable amounts of a fluorescence reporter. Selection using the antibiotic zeocin rendered transformants with high expression levels of the transgene in the wild type background. In addition, we are also using a mutant strain that confers high transgene expression without interfering with the miRNA pathway. We expect that the results obtained with our reporter systems will help to better understand pathways of miRNA-mediated control in the alga..

We used resources and knowledge obtained from previous deliveries to construct a simplified version of an incoherent feed-forward loop, which is the most common regulatory motif in natural gene networks. We have characterized the capacity of this circuit to buffer transcriptional noise.

We are setting up simple circuits in Chlamydomonas in which both miRNA and target molecules are produced from synthetic DNA constructs. These circuits carry two reporter systems: one that reports for the miRNA expression, and a second that reports for the miRNA-dependent silencing. We are currently testing the functionality of this circuit. This circuit will be used to characterize the kinetics and key parameters of the basic unit of the miRNA-dependent gene silencing, including targeting rules of miRNAs in Chlamydomonas. The use of fluorescent reporters is revealing cell-to-cell variability in the activity of the circuit described above. Understanding this variability will be useful to confer more robustness to our synthetic gene circuits.

With these milestones we have developed a platform for characterisation of miRNAs as tools to control gene expression. The characterization of the mode of action and regulatory properties of miRNAs is prerequisite for the design of synthetic circuits. The platform to characterize miRNAs in the green alga Chlamydomonas consists of (i) a reporter system for miRNA production, and (ii) a reporter system for miRNA-dependent silencing of target a mRNA. Both reporter systems are based on fluorescent proteins, which allow quick, *in vivo*, and single cell evaluation of silencing events. For the construction of the reporter systems we evaluated several fluorescent proteins, constructed a set of Golden Gate domesticated DNA parts for gene expression in *Chlamydomonas reinhardtii*, employed strategies to increase gene expression, and tested methods of gene expression measurement. miRNA precursors from endogenous miRNAs in the alga were selected to be used as scaffold of synthetic miRNA.

This platform has been used to search for synthetic miRNAs and miRNA recognition sites that can deliver strong repression of an mRNA. It has been also valuable to scan the mRNA molecule for sites where miRNA-mediated strong repression can be exerted.

By using this platform, we have established that the level of repression of an mRNA by a miRNA depends on various factors, including:

- miRNA abundance: the higher the miRNA concentration, the stronger the repression. We observe that one single recognition site on the mRNA can bring strong repression.
- sequence complementarity: perfect complementarity between a miRNA and an mRNA delivers the strongest repression, while sequence mismatches in the cleavage site of the miRNA modifies the repression curve. We are now characterizing the role of sequence complementarity in the mode of action of the miRNA on its target.

With this preliminary characterization, we are now starting to construct miRNA-based gene synthetic circuits in the alga *Chlamydomonas reinhardtii*. In particular, we are evaluating the properties of a miRNA-mediated incoherent feedforward loop, which is the most common regulatory motif in natural gene networks, and it is thought to confer robustness to gene expression variability. We are addressing the modelling of our circuits in collaboration with Tim O'Leary from the Department of Engineering in the University of Cambridge.

Objective C3: Standardised riboswitch parts for transgene regulation in different chassis.

To be able to engineer microalgae in a modular and predictable way, we have contributed to the development of a Golden Gate-based Modular Cloning (MoClo) toolkit for the green microalga *Chlamydomonas reinhardtii*. This toolkit will be composed of around 130 functionally validated and standardised DNA parts. Once completed, it will enable rapid engineering of Chlamydomonas for both fundamental research and green biotechnology. As an extension of the Chlamydomonas MoClo toolkit, we are developing new molecular tools to be able to conditionally control the expression of genes of interest. To achieve this goal, our aims are (i) to identify riboswitches from diverse organisms that have already been characterised and shown to regulate gene expression in their native hosts (ii) follow a synthetic biology approach to further characterise the different elements of these riboswitches (iii) use this new information to generate new riboswitches (iv) test the responsiveness of these riboswitches for the control of transgene expression in different photosynthetic eukaryotic organisms (including microalgae and plants).

Objective C4: Riboregulators for plastid transgene regulation.

Regulation of chloroplast gene expression via TPR/PPR proteins encoded by the nucleus has been established by Aleix Gorchs Rovira. NAC2 is a TPR protein required for expression of psbD, a PSII subunit. Introduction into a nac2 mutant of a construct encoding NAC2 under control of a vitamin B12-repressible promoter (P_{METE}) restores photosynthesis, but this can be down-regulated by increasing concentrations of vitamin B12 in the medium. The binding site of NAC2 is the 5'UTR of psbD, and if this is fused to another gene, this too will be regulated by this genetic circuit. Similarly, thiamine can be used to tune down photosynthesis in strains where MRL1 (a PPR protein required for efficient translation of rbcL) is regulated by a thiamine-repressible riboswitch ($THI4_{RS}$). Crosses have resulted in generation of strains containing both P_{METE}-NAC2 and $THI4_{RS}$-MRL1, and also the endogenous 5'UTRs for psbD and rbcL have been replaced with nonresponsive elements. These strains can now be used to introduce metabolic enzyme genes into the chloroplast and have their expression regulated by vitamins.

Aleix Gorchs Rovira and Stefan Grossfurthner (Smith-UCam lab) have produced several MoClo parts for introduction of sequences into the chloroplast genome of Chlamydomonas. These include promoters, ribosome-binding sites, terminators and homologous regions for insertion around the genome. These will facilitate engineering of the chloroplast genome to introduce several transgenes at different sites. In addition, protocols have been established to allow chloroplast transformation by electroporation, increasing the frequency of transformants.

Publications

Moses T, Mehrshahi P, Smith AG, Goossens A (2017) **Synthetic biology approaches for the production of plant metabolites in unicellular organisms.** *J Exp Bot, 68: 4057-4074.*

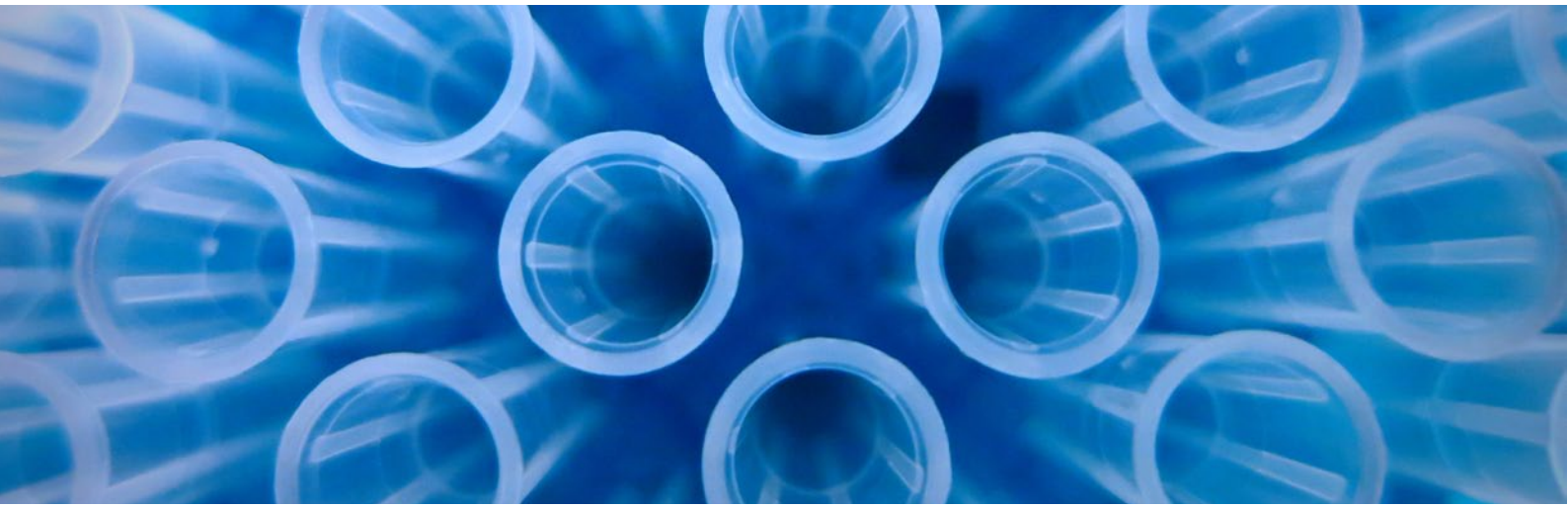
Nguyen GTDT, Scaife MA, Helliwell KE, Smith AG (2016) **Role of riboswitches in gene regulation and their potential for algal biotechnology** *J Phycol 52: 320-328*

Scaife MA, Smith AG (2016) **Towards developing algal synthetic biology**. *Biochem Soc Trans 44: 716-722*

Scaife MA, Nguyen GTDT, Rico J, Lambert D, Helliwell KE, Smith AG (2015) **Establishing Chlamydomonas reinhardtii as an industrial biotechnology host.** *Plant J, 82: 532-546.*

Navarro FJ and Baulcombe D. **miRNA-mediated regulation of synthetic gene circuits in the green alga *Chlamydomonas reinhardtii.*** *Manuscript in preparation*

Crozet P, Navarro FJ, Baulcombe D, Sauret-Gueto S, Mehrshahi P, Gorchs-Rovira A, Sayer A, Gangl D, Geisler K, Smith A and other co-authors to be confirmed from the groups of Stéphane Lemaire, Olaf Kruse, Poul Erik Jensen, Michael Schroda, Felix Willmund and José-Luis Crespo. **A Versatile MoClo kit for synthetic biology in the unicellular alga *Chlamydomonas reinhardtii*.** *Manuscript in preparation.*



Workpackage D: Genome Engineering

Hierarchical DNA assembly methods are a necessary part of genome construction and modification. Large-scale assemblies require specialized cloning vehicles like artificial chromosomes for construction and transfer of genetic circuits to target organisms. Recent developments are producing efficient tools for modifying chromosomal sequences in situ.

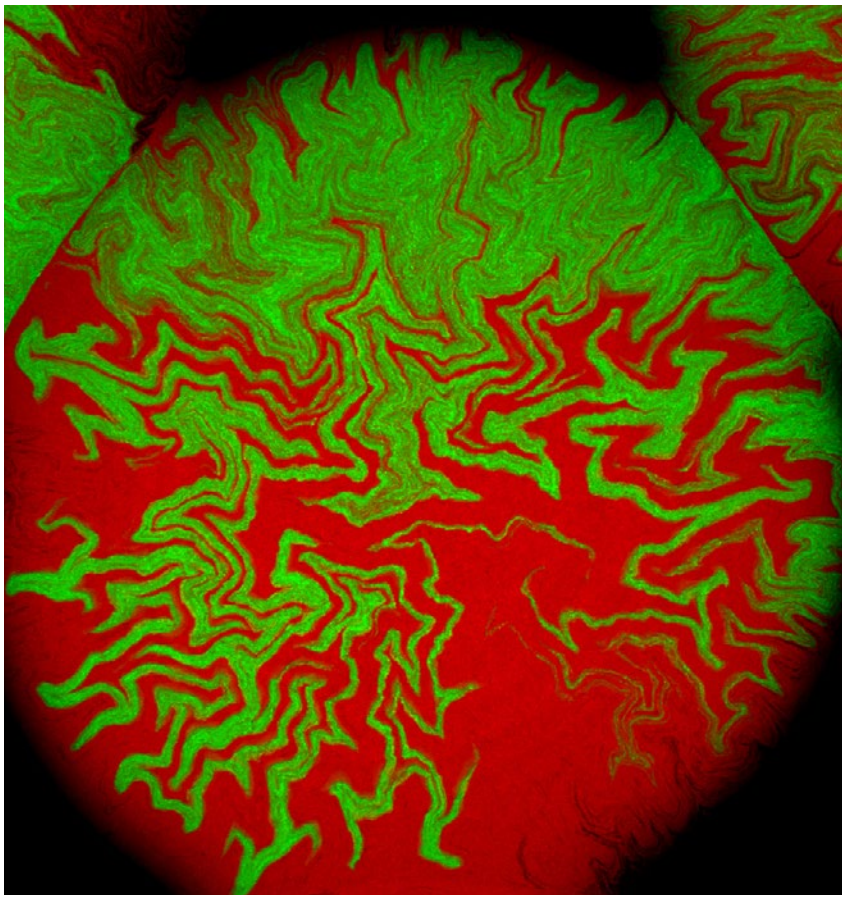
The genome editing tools and large-scale gene assembly technologies developed in workpackage D will be of direct benefit to all the trait-engineering work-packages (F, G, H, I & J). Specifically, vectors for chloroplast manipulation and methods to achieve homoplasty are being developed for use in workpackage F and molecular tools for targeted genome editing are being developed for use in workpackages G, H and I. Increased accessibility to engineering tools for the M. polymorpha chloroplast will have direct implications on workpackages: F4, F5 and C4, as they all involve chloroplast engineering. The chloroplast optimised fluorescent reporter library will be of direct use to several work packages, specifically F1-5, C3 and C4. Additional reporters will increase our ability to probe transcription/translation in plants. Additionally, the standardised assembly technologies from workpackage B have been applied to the technologies developed in workpackage D.

Investigators
Nicola Patron; Jim Haseloff; Jim Ajioka; Giles Oldroyd; James Locke; Christopher Howe; Alison Smith; Sebastian Schornack; Julian Hibberd

Staff Employed
Oleg Raitskin (PDRA; Patron lab). Start Jan 2015. Re-employed at EI Sep 2016
Douglas Griffith (PDRA, Locke lab). Start Jul 2015, end Nov 2016
David Willey (PDRA, Ajioka lab). Start Sep 2015, end Mar 2016
Orr Yarkoni (PDRA, Ajioka lab). Start May 2016
Philip Carella (PDRA, Schornack lab). Start Sep 2016
Stephen Rowden (PDRA, Howe lab). Started Sep 2017

Partners
Collaborations to accelerate the application of genome engineering technologies have been established:
Harwood lab, JIC (Patron)
Smith Lab, JIC (Workpackage G)
Raines lab, University of Essex (Patron)
Mazzo lab, University of Campinas, Brazil (Patron)
Haseloff lab, University of Cambridge (Workpackage A)

D



Milestones:

D1: Construct and characterise yeast artificial chromosome vectors as plant shuttle systems.
Deliverable: Public release of YAC or BAC based vectors for plant genome engineering (month 36, Ajioka, Patron).

D2: Plastid genome vectors for chloroplast transformation.
Deliverable: Public release of vectors for efficient plastid transformation (month 48, Haseloff, Ajioka, Smith).

D3: System for facilitating homoplasty after chloroplast transformation.
Deliverable: Public release* of tools and vectors for reverse host-restriction in plastids (month 60, Haseloff).

D4: Establish vectors for ds-break mediated gene deletion, mutation and addition.
Deliverable: Public release of CRISPR/Cas9 or TALEN based tools to delete, mutate and deliver exogenous DNA to specific genomic loci in several model and crop plant species. (month 60, Schornack, Patron, Jones, Oldroyd, Hibberd, Haseloff).

Online resources:

Patron lab
(www.earlham.ac.uk/patron-group)

Smith lab
(www.plantsci.cam.ac.uk/research/alisonsmith)

Ajioka lab
(www.path.cam.ac.uk/directory/james-ajioka)

Progress to date:

D1: Construct and characterise yeast artificial chromosome vectors as plant shuttle systems.

We have constructed an vector for a plant shuttle system in *B. subtilis* via genomic integration. This will allow for easy distribution and storage of *M. polymorpha* genome fragments via spores containing plant genomic DNA that can be stored at room temperature indefinitely and germinated simply via LB inoculation. This will allow for easy genome extraction and/or *in vivo* manipulation of the plant genome. Integration plasmids with appropriate homologous ends have been designed and constructed for this purpose.

D2: Plastid genome vectors for chloroplast transformation.

A. Marchantia chloroplasts

Christian Boehm (Haseloff lab) established plastid transformation in Marchantia. He has created the first fluorescent markers for liverwort plastid transformation. A panel of codon optimised fluorescent reporters spanning most of the visual spectrum has been designed and synthesised. Five variants of the following fluorescent reporters have been designed: iRFP670, mCardinal, mPlum, mCerulean, mNeptune, mRaspberry, mTurquoise, mWasabi, eBFP, Sirius and TagCFP, the top candidates for synthesis were chosen on the basis of GC content and likelihood of passing synthesis QC. mRaspberry, mNeptune, mCerulean, iRFP670 and eBFP have successfully been cloned into a chloroplast transformation vector (pCS CL0*B) and work on the remaining reporters is ongoing.

The 120KB Marchantia chloroplast genome is a highly attractive target for large DNA manipulation. The size of the chloroplast genome is beyond the range of conventional plasmid cloning strategies, but remains relatively small, easier to handle *in vitro* and and of great interest for metabolic engineering.

We have demonstrated chloroplast transformation and successful development of fluorescent protein markers for Marchantia chloroplasts.

However, historical errors in the identity of the Marchantia chloroplast genome has meant that these vectors may be suboptimal due to sequence mismatches. Although the chloroplast genome was one of the first sequenced plastid genomes (Ohyama *et al*., J Mol Biol. 203:281-98, 1988), it is now clear that the biological sample was derived from cell cultures of *Marchantia paleacea* not *polymorpha*). We have re-sequenced the chloroplast genome from Cambridge isolates of *M. polymorpha* and re-annotated and curated the validated genome sequence. Missing ORFs have been identified, partial annotations have been completed and putative promoter sites identified throughout the entire chloroplast genome. A new generation of transformation vectors with corrected sequences has been constructed, and is being tested for improved efficiency.

In addition, the chloroplast genome has been partitioned into putative transcription units as functional “chunks” for refactoring. This conceptual reorganisation follows the model of MarpoDB and opens up the plastid genome for better phylogenetic comparisons and functional description.

Manual curation of the plastid genome annotation has clarified the presence and predicted function for a number of poorly annotated genes such as: MapoCp048, MapoCp058, ORF167 and a few where the nearest homolog is still >55%, (MapoCp087, MapoCp088, MapoCP005, MapoCp023). We have also identified restriction endonuclease sites for SbfI, ApaLI, AdeI, Tth111I, SexAI and CspCI, which have been identified as enzymes with no cut sites in areas of the genome which would be difficult to engineer, such as tRNAs and rRNAs – and therefore useful for synthetic genome manipulations. A multiple cloning site style approach is being explored for the genome design, where these cut sites can subsequently be used to easily access the genome for further modification.

B. Chlamydomonas chloroplasts

In Smith (UCam) lab we have generated a range of MoClo compatible parts for chloroplast transformation of *Chlamydomonas reinhardtii*. We are collaborating with groups in Paris, Copenhagen, and Bielefeld and Kaiserslautern in Germany to generate a Chlamy MoClo kit, which will be accompanied by a paper. Once this has been accepted the kit will be available – probably sometime in 2018.

The commonality between chloroplast genome regulatory sequences in different higher plants means that parts are probably completely interchangeable. However there are significant differences to the genome in *C. reinhardtii* so our parts are unlikely to be usable in other systems. In any case the homologous regions (HRs) needed to insert the sequences into the genome need to be completely specific.

Aleix Gorchs Rovira and Stefan Grossfurthner (Smith-UCam lab) have produced several MoClo parts for introduction of sequences into the chloroplast genome of *C. reinhardtii*. These include promoters, ribosome-binding sites, terminators and homologous regions for insertion around the genome. These will facilitate engineering of the chloroplast genome to introduce several transgenes at different sites. In addition, protocols have been established to allow chloroplast transformation by electroporation, increasing the frequency of transformants

In a parallel project, regulation of chloroplast gene expression via TPR/PPR proteins encoded by the nucleus has been established by Aleix Gorchs Rovira. NAC2 is a TPR protein required for expression of psbD, a PSII subunit. Introduction into a nac2 mutant of a construct encoding NAC2 under control of a vitamin B12-repressible promoter (P_{METE}) restore photosynthesis, but this can be down-regulated by increasing concentrations of vitamin B12 in the medium. The binding site of NAC2 is the 5’UTR of psbD, and if this is fused to another gene, this too will be regulated by this genetic circuit. Similarly,

thiamine can be used to tune down photosynthesis in strains where MRL1 (a PPR protein required for efficient translation of rbcL) is regulated by a thiamine-repressible riboswitch ($THI4_{RS}$). Crosses have resulted in generation of strains containing both P_{METE}-NAC2 and $THI4_{RS}$-MRL1, and also the endogenous 5'UTRs for psbD and rbcL have been replaced with nonresponsive elements. These strains can now be used to introduce metabolic enzyme genes into the chloroplast and have their expression regulated by vitamins.

D3: System for facilitating homoplasmy after chloroplast transformation.

Eftychis Frangedakis (Haseloff Lab) has been appointed to lead a team to develop more efficient methods of gene delivery into Marchantia chloroplasts. Cas9 has been codon-modified for efficient use in Marchantia and other plants. Marchantia plants tolerate presence of the gene, and transgenic lines can be maintained, where simple delivery of a suitable guide sequence will trigger genome modification events, after Cas9-mediated cleavage of the genome. This system was established by Bernardo Pollak and he, Owen Male and Eftychis Frangedakis have used the system to target the homologues of genes known to be important for chloroplast division in higher plants. Gene knockouts produced aberrant, oversized chloroplasts in the targeted lines. Many cells only one or a few grossly enlarged chloroplasts. The plants appear phenotypically unaffected, at least when grown under laboratory conditions. This is similar to analogous phenotypes seen in Arabidopsis. We believe that these lines may be useful for plastid transformation experiments - effectively increasing the target size, and facilitating gene conversion. We are targeting a wider set of genes to determine if the chloroplast numbers per cell can be further reduced, and testing the lines for improved organelle transformation and homoplasty.

D4: Establish vectors for ds-break mediated gene deletion, mutation and addition.

Oleg Raitskin (Patron lab) he has demonstrated RNA-guided Cas9-mediated targeted mutagenesis and gene deletion in model species (Nicotiana, Arabidopsis). This has also been established in the Schornack lab, where an internal access website for genome editing at UCam has been set up. Sebastian Schornack is collaborating with the Henderson lab, advising on TALEN design. Raitskin has developed a digital-droplet based PCR assay to quantify the efficiency of double-stranded break creation, allowing us to compare different components and design the most efficient toolkit. In addition, has developed a suite of Cas9 variants and an associated toolkit for targeted mutagenesis and gene deletion in multiple plant species. Currently these are being compared for specificity and efficiency using next-generation sequencing technologies and digital-droplet based PCR. Assembly of plasmid vectors for targeted mutagenesis is being automated at the DNA foundry at the Earlham Institute (Patron lab). A protoplast assay for rapid assessment of constructs has been established for tobacco, Arabidopsis and barley. Recombinant Cas9 protein has been produced and purified and trials are in progress for the delivery of the protein-RNA complex to plant cells are in progress. The Patron lab has demonstrated RNA-guided Cas9-mediated targeted mutagenesis and gene deletion has been demonstrated in multiple species including *Nicotiana benthamiana*, Arabidopsis, tomato (collaboration with Banfield lab), potato (for carbohydrate engineering, PDRA Aytug Tuncel; Smith Lab (JIC); workpackage G), barley (collaboration with Harwood, Uauy & Wulff Labs, JIC; Lawrenson et al, 2015), *Brassica oleracea* (collaboration with Harwood lab; Lawrenson et al, 2015). Plants (*Nicotiana tabacum*, *Nicotiana benthamiana*, Arabidopsis and barley) with disrupted selection cassettes have been created to enable efficient recovery of targeted insertion events. Preliminary attempts to repair this cassette by simultaneous delivery of nucleases and repair template are ongoing (Oleg Raitskin, in collaboration with Raines Lab, UEssex). Work on investigating Cas proteins from untested CRISPR systems with novel functionality is ongoing (Oleg Raitskin, Patron lab). The development of Cas9 and TALE-based ligand-inducible orthogonal transcription factors is in progress (MSc student, Patron lab)

Publications

Rowden S, Bombelli P and Howe C. (In Press) **Design and study of bio-electrochemical system for biotechnological applications and metabolic investigation** *in 'Photosynthesis: Methods and Protocols' ed Covshoff S. Springer.*

Volpi e Silva N and Patron N (2017) **CRISPR-based Tools for Plant Genome Engineering.** *Emerging Topics in Life Sciences doi: 10.1042/ETLS20170011*

Salzman AE (2017) **Controlling Relative Gene Expression Using Orthologous Regulatory Elements: Exploring Future Prospects of Tuneable Control for the Manufacture of Natural Products.** *Masters thesis.*

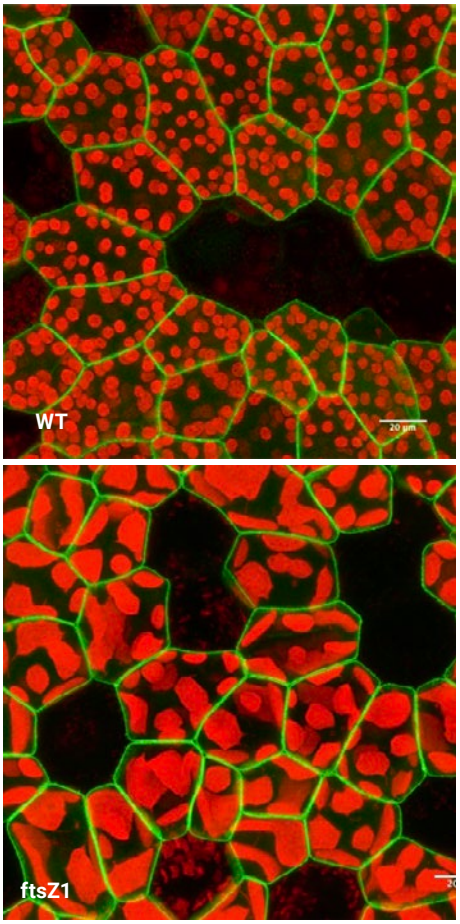
Raitskin O, Patron NJ, (2016). **Multigene Engineering with RNA guided Cas9 Nuclease.** *Current Opinion in Biotechnology 37; 6975*

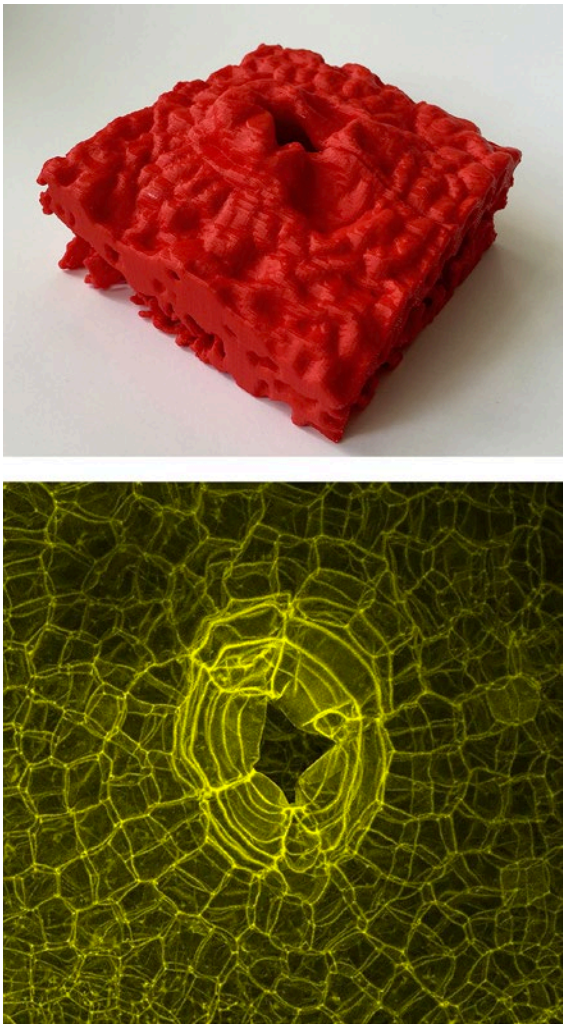
Patron, NJ (2016) **DNA assembly for plant biology.** *Current Protocols in Plant Biology 1:604-616*

Lawrenson T, Shorinola O, Stacey N, Liu C, Østergaard L, Patron NJ, Uauy C, Harwood, W (2015). **Induction of targeted, heritable mutations in barley and *Brassica oleracea* using RNA guided Cas9 nuclease.** *Genome Biology 16; 258*

Raitskin O, Schoudma C, Patron N. **Comparing efficiency and specificity of targeted mutagenesis in plant cells.** *In preparation.*

Yarkoni O, Ajioka J. **Manual annotation and curation of the *M. polymorpha* chloroplast genome.** *In preparation.*





Giant chloroplasts in Marchantia

CAMBRIDGE

Cas9 can be codon-modified for efficient use in Marchantia and other plants. Marchantia plants tolerate presence of the gene, and transgenic lines can be maintained, where simple delivery of a suitable guide sequence will tigger genome modification events, after Cas9-mediated cleavage of the genome. This system has been established by Bernardo Pollak. Owen Male used the sytem to target the homologues of genes known to be important for chloroplast division in higher plants. Gene knockouts produced abberant, oversized chloroplasts in the targeted lines - see the images above taken with Susana Sauret-Gueto. Eftychis Fragedakis is now targeting a wider set of genes to determine if the chloroplast numbers per cell can be further reduced, as found in Arabidopsis mutants. These manipulations may be useful for improved chloroplast transformation and homoplasty techniques.

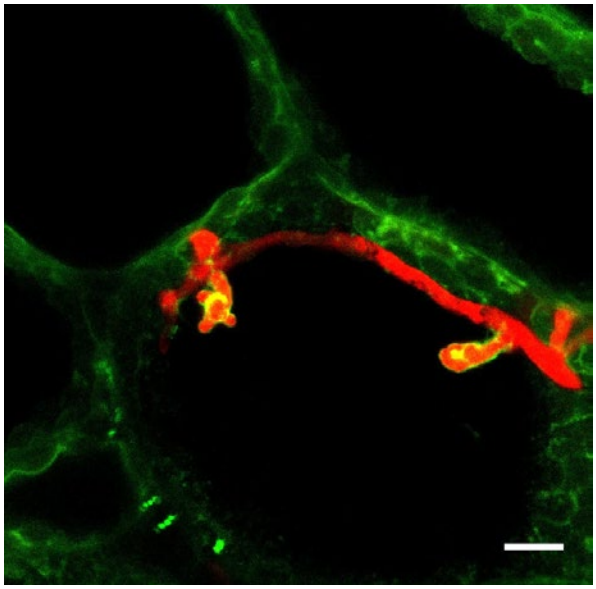
Principal contact: Eftychis Fragedakis

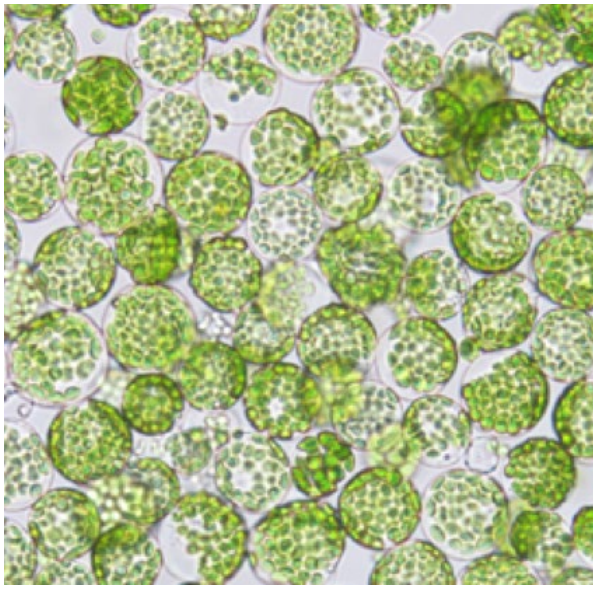
Reconstruction of Marchantia air chambers

CAMBRIDGE

As Marchantia grows, cell division at growing points, or meristems, produces tissues that undergo self-organisation via additional cell divisions and differentiation events, to form air chambers. These chambers are comprised of cellular "floors", "walls", "roof" and air pore. The air chambers are packed with specialised cell filaments, that consist of highly photosynthetically active cells. The air chambers form uninterrupted arrays on the top surface of the plant, and are likely a relic of an early attempt to adapt to gas exchange and photosynthesis in a terrestrial environment.
Marta Tomaselli has been applying optical clearing and image reconstruction techniques to analyse these cell complexes, including 3D printing of cellular features

Principal contact: Marta Tomaselli





Host-pathogen interactions in liverworts

CAMBRIDGE

Numerous studies describe interactions between symbiotic microbes and early land plants like Marchantia, yet our understanding of how pathogens manipulate these plants is poorly understood. To address this, we have recently established a robust pathosystem between the filamentous oomycete pathogen *Phytophthora palmivora* and the model liverwort *Marchantia polymorpha*. We discovered that *P. palmivora* colonizes air chambers of the dorsal photosynthetic layer of liverworts to establish disease. Moreover, our work has revealed that *P. palmivora* forms intracellular structures within *M. polymorpha* cells. The plant recognises these structures and deploys host cellular trafficking machinery proteins. This work suggests that the formation of microbial structures in plants is evolutionary conserved and successfully exploited by pathogens. Our work lays the foundation for the identification of Marchantia pathogen-responsive promoter elements and the identification of non-vascular plant specific pathogen mechanisms.

Image: Intracellular structures formed by Phytophthora (red) inside Marchantia cells and labelled with a Marchantia protein (green/yellow) Size bar: 10 micrometer. Picture by P. Carella

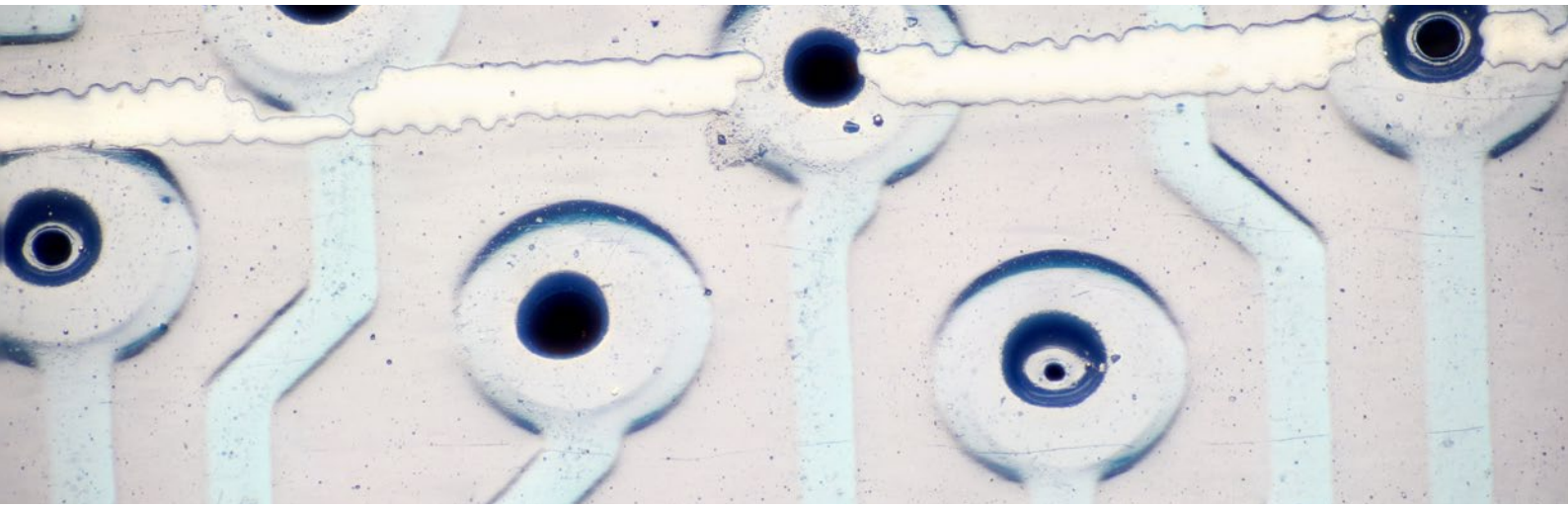
Principal Contacts: Philip Carella & Sebastian Schornack

DNA-free genome engineering

JOHN INNES CENTRE & EARLHAM

Direct delivery of programmable nucleases such as Cas9 in a complex with the guide RNA, known as the ribonuclease (RNP) complex, avoids the introduction of DNA into the cell. Oleg Raitskin and Aytug Tuncel have developed protocols for the production of the ribonuclease complex and delivery to protoplasts of tobacco and potato, demonstrating DNA-free targeted mutagenesis. Regeneration of potatoes with mutations in the target genes is underway.

Principal contacts: Oleg Raitskin, Aytug Tuncel, Nicola Patron & Alison M. Smith



Workpackage E: Digital Tools

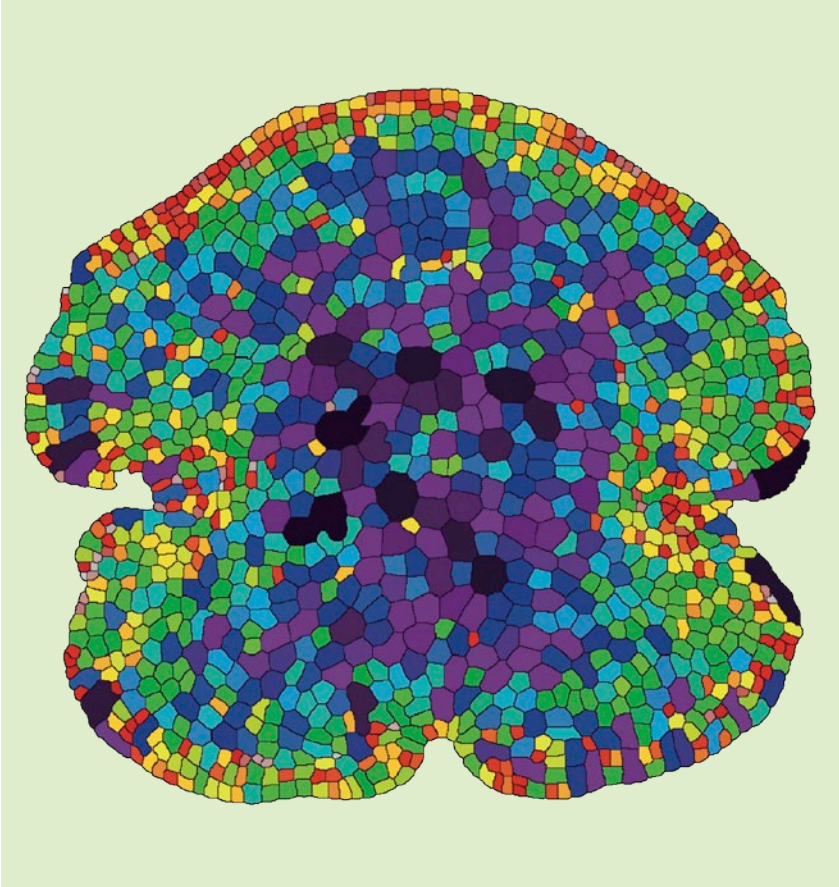
Software tools play an increasingly important role in Synthetic Biology experiments, as we automate experiments, and the systems we construct increase in scale. We need computational models in order to accurately predict the behaviour of biological systems, which are governed by multiscale parallel and feedback regulated genetic, physical and chemical interactions.

The Digital Tools workpackage aims to provide software to automate DNA assembly and the quantification of gene expression in plant in addition to providing models for gene expression and cell growth. This supports Work Packages A, B, D and others producing vectors, tools and parts.

Investigators
James Locke; Jim Haseloff; Jim Ajioka; Nicola Patron; Giles Oldroyd

Staff Employed
Bruno Martins (PDRA, Locke lab). Started Jan 2017

Partners
Mihails Delmans; Bernardo Pollak (HaseloffLab)
Nathan Hillson, JBEI
Tim Rudge, PUC, Chile
Hackster.io
Addgene
Benchling



E

Online resources:

Repository of open source code for the Haseloff lab:

- **Cellmodeller**
- **LoopDB**
- **MarpoDB**
- **PartsDB**
- **Platypus**

(github.com/HaseloffLab)

Milestones:

E1: Software for automated DNA assembly.
Deliverable: Implementation of software for a DNA assembly pipeline, in collaboration with Nathan Hillson, JBEI (month 12, Ajioka, Patron, Haseloff, Oldroyd).

E2: Software for automated quantification of gene expression in planta.
Deliverable: Public release* of open source software routines for automated processing of gene expression data in microbes and plants (month 24, Haseloff).

E3: Software models for gene circuits.
Deliverable: Web-based access to developments in modelling of gene circuits in the OpenPlant community, and web access to parameters of DNA parts via JBEI-ICE API (month 36, Haseloff, Locke).

E4: Software models for cell growth.
Deliverable: Public release* of open source software for multi-scale modelling of cellular growth in microbes and plants (month 60, Haseloff, Locke).

Progress to date:

Objective E1: Software for automated DNA assembly.

Agreement on a standard genetic syntax for plant DNA parts (Patron et al., 2015) has provided a coordinated approach to Milestones in all foundational workpackages, as a basis for building an automated DNA assembly process and the establishment of a central database/registry for plant parts. This underpins Work Packages A, B, C, D and E.

Software for a DNA assembly pipeline was first implemented as part of the Device Editor function accessible from the JBEI-ICE software (https://j5.jbei.org/j5manual/pages/23.html). We contracted the establishment of a one-click OSX installation package for JBEI-ICE. We have shared the Xcode development tools with the authors, led by Nathan Hillson, JBEI. This could be extended by use of other software, including NEB Golden Gate (https://goldengate.neb.com/editor), with commercially available solutions in Geneious (http://www.geneious.com).

However, the utility and scalability of the Type IIS assembly technique across the OpenPlant community has meant that we have looked at customised solutions that have the potential for better integrated access to genome resources and automated assembly.

Building on early work, we have adopted a two step approach to managing standardised DNA parts. These involve a customised database solution, MarpoDB, and Benchling, a free web-based solutions that facilitates part sharing and management. Benchling has proved popular as it is robust and maintenance free, and has IIS assembly tools. In particular, the ROC group (Researchers for OpenPlant, Cambridge) have integrated Benchling into a shared Phytobrick-based workflow.

Objective E2: Software for automated quantification of gene expression in planta.

For work with the model plant *Marchantia polymorpha*, we have produced MarpoDB, which is an open source database for MarpoDB describes that presents the Marchantia genome from an engineer's perspective, rather than a geneticist's. The database handles the Marchantia genome as a collection of parts. This is highly useful for automatically mining new parts, and managing part description, and part characterisation. We think that this break from standard genome database architecture is essential for tackling the refactoring of synthetic plant genomes. MarpoDB also provides a useful container for gene expression data, and integration of cellular features via Plant Ontology terms. (http://marpodb.io)

MarpoDB has been designed to facilitate the definition and extraction of synthetic DNA elements to be synthesised as standardised DNA parts. For example, we have identified 400 core promoter candidates, and extracted these from the Marchantia genome. The extracted sequences have been domesticated, removing Bsa1 and Sap1 recognition sequences if necessary, and chemically synthesised. The refactored parts have been cloned into pUAP1, a specially prepared vector designed for public distribution under the OpenMTA (vector details published in Patron et al., 2015).

Key genes, mainly regulatory genes and key effector genes have been identified in Marchantia. The Haseloff Lab has constructed and tested pLoop plant transformation vectors with multi-spectral fluorescent protein outputs, for ratiometric characterisation of gene expression. This allows direct observation of patterns of gene expression in transformed Marchantia tissues, and use of ratiometric imaging for quantitative measurement of gene expression in planta. The Haseloff lab has developed an image processing pipeline that uses matplotlib, scipy, skimage, tifffile, numpy and can be installed using pip (python package manager) or other package manages such as easy_install. The software is freely available at https://github.com/bpollakw/thesis.

MarpoDB has the facility to collate characterisation data for standardised DNA parts. We are using Marchantia as a test case for the assembly of Level zero parts and characterisation data that can be mined for the construction and modelling of synthetic gene circuits.

In addition, the Haseloff lab has developed 3-parameter measurement techniques for quantifying gene expression in cell suspensions in such as way that extrinsic noise is minimised and a reliable estimate of the intrinsic properties of gene promoters can be made (Rudge etal., 2016; Grant et al., 2016). This relies on software models for gene expression, cell growth, and the use of a coexpressed marker to reduce variation. A computational framework has been established to allow automated analysis of microplate reader data, and this has been made available on Github (https://github.com/HaseloffLab/Platypus).

Objective E3: Software models for gene circuits.

Bruno Martins (Locke lab) is using synthetic circuits to rewire the cyanobacterial circadian clock. He has used constructs to probe the circadian clock and its outputs at the single cell level in *S. elongatus*. He has built models of the coupling of the cyanobacterial clock, that are publicly available at the Molecular Systems Biology website and are written in SBML to enable easy sharing of the code. He is now extending this work, making the clock produce different period outputs based on his models.

Mihails Delmans and Jeanet Mante (Haseloff lab) are developing software-based classification schemes for whole-organism descriptions of the dynamics of gene expression at the cellular scale in Marchantia gemma.

Objective E4: Software models for cell growth.

CellModeller is a software package that has been developed in the Haseloff Lab for modelling of plant and microbial cellular systems. It combines genetic, chemical and physical systems for multiscale modelling of cellular growth. It has unique features in the precision and flexibility of the integrated

cellular modelling environment and use of GPU-based software acceleration. The early developers of the software (Rudge, Dupuy, Mackenzie, Kan, Steiner) have moved to new positions across the world, and we have constructed a hub for continued collaboration and community building around the platform. Software code, binaries and documentation for CellModeller are freely available online via a dedicated website (www.cellmodeller.org) with a Google support forum, github repository (http://haselofflab.github.io/CellModeller/) and youtube videos (https://www.youtube.com/results?search_query=CellModeller). The latest features include cell-cell adhesion and cell shape, as well as algorithms for whole colony-scale segmentation from confocal microscopy datasets of growing microbes.

Publications

CR. Boehm, PK. Grant and J Haseloff, (2018) **Programmed hierarchical patterning of bacterial populations**. *Nature Comms. accepted.*

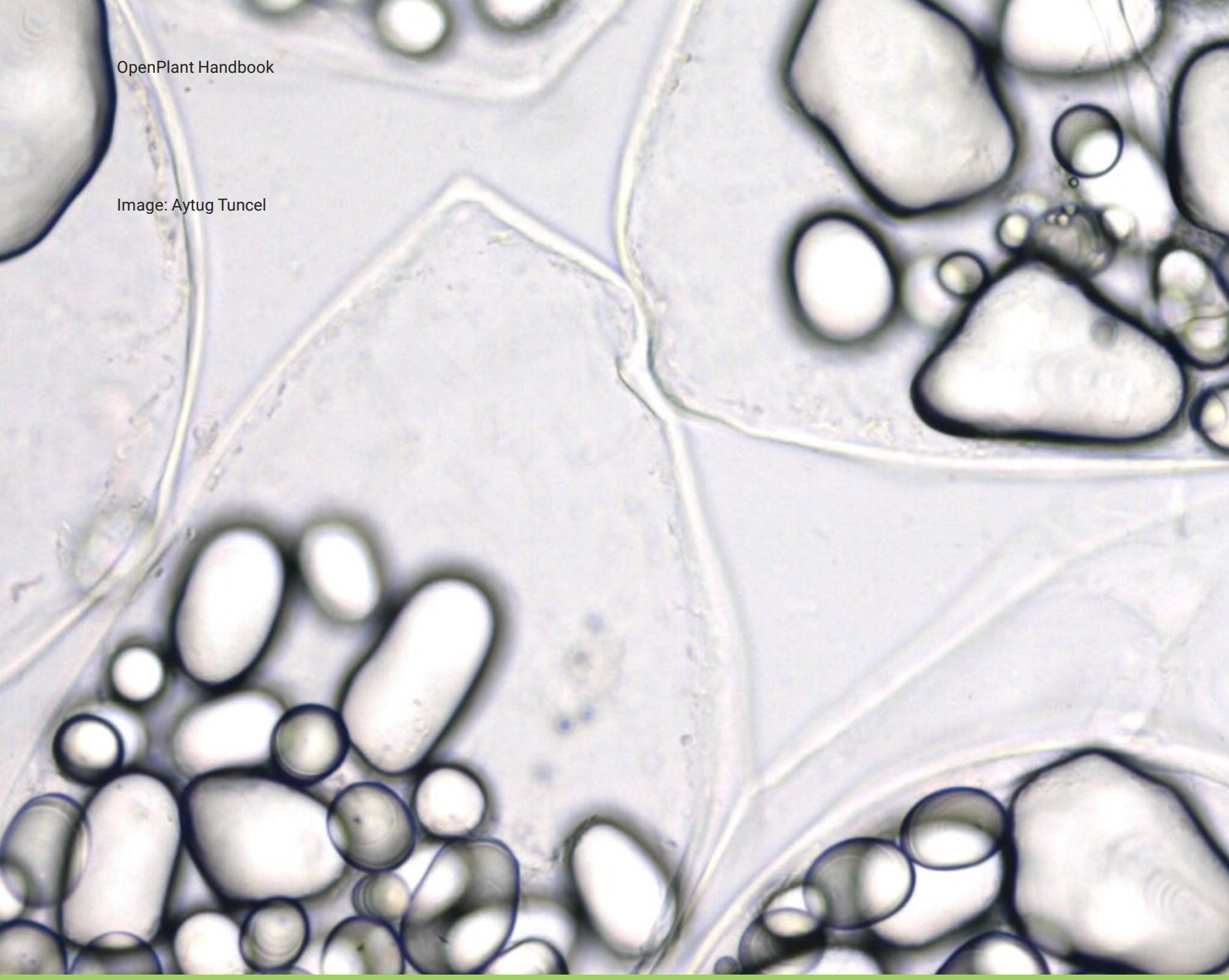
Nuñez IN, Matute TF, Del Valle ID, Kan A, Choksi A, Endy D, Haseloff J, Rudge TJ, Federici F (2017). **Artificial Symmetry-Breaking for Morphogenetic Engineering Bacterial Colonies.** *ACS Synth Biol. 6(2):256-265. doi: 10.1021/acssynbio.6b00149.*

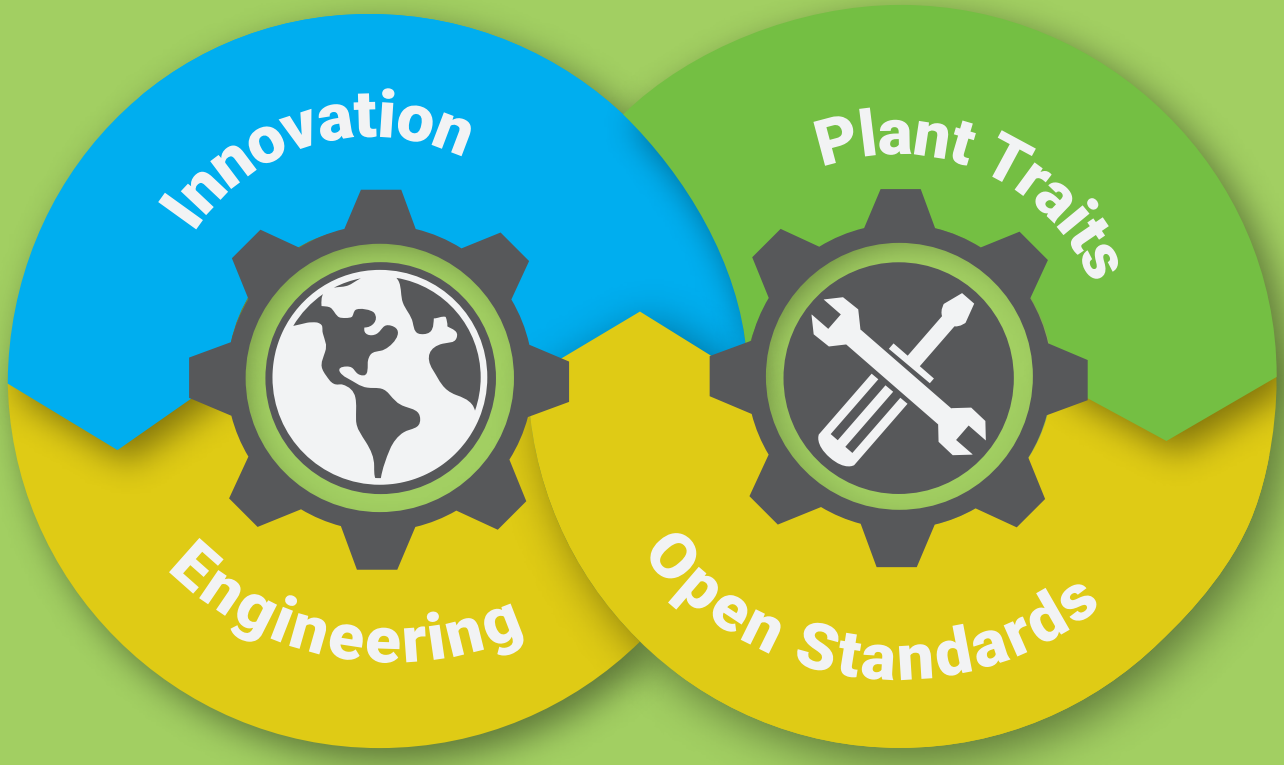
Martins et al. (2016). **Frequency doubling in the cyanobacterial circadian clock.** *Molecular Systems Biology 12(12):896. doi: 10.15252/msb.20167087.*

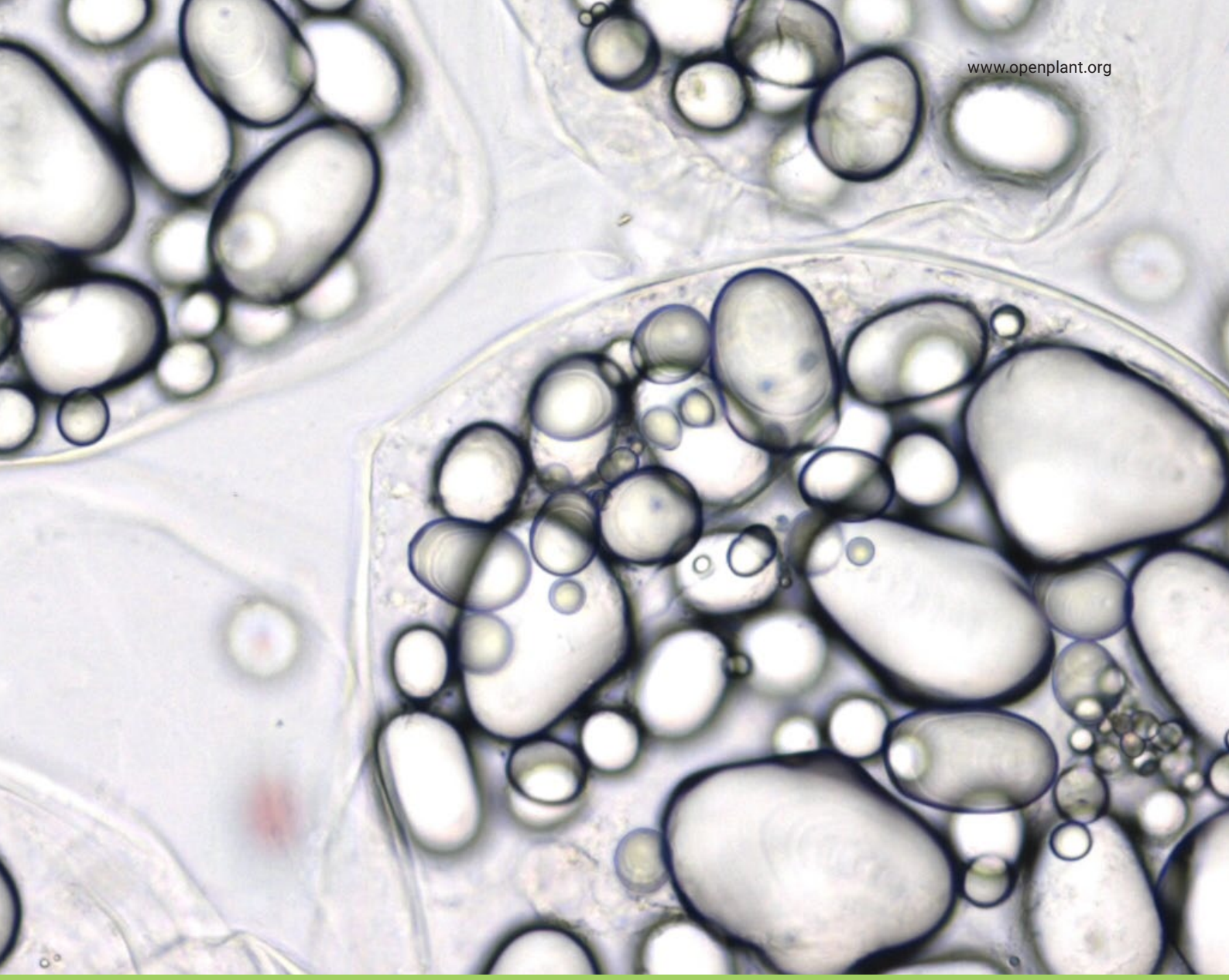
Rudge T, Brown J, Federici F, Dalchau N, Phillips A, Ajioka J, & Haseloff J (2016). **Characterization of intrinsic properties of promoters.** *ACS Synthetic Biology 5:8998.*

Grant PK, Dalchau N, Brown JR, Federici F, Rudge TJ, Yordanov B, Patange O, Phillips A, Haseloff J (2016). **Orthogonal intercellular signaling for programmed spatial behavior.** *Molecular Systems Biology 12:849861*

Image: Aytug Tuncel



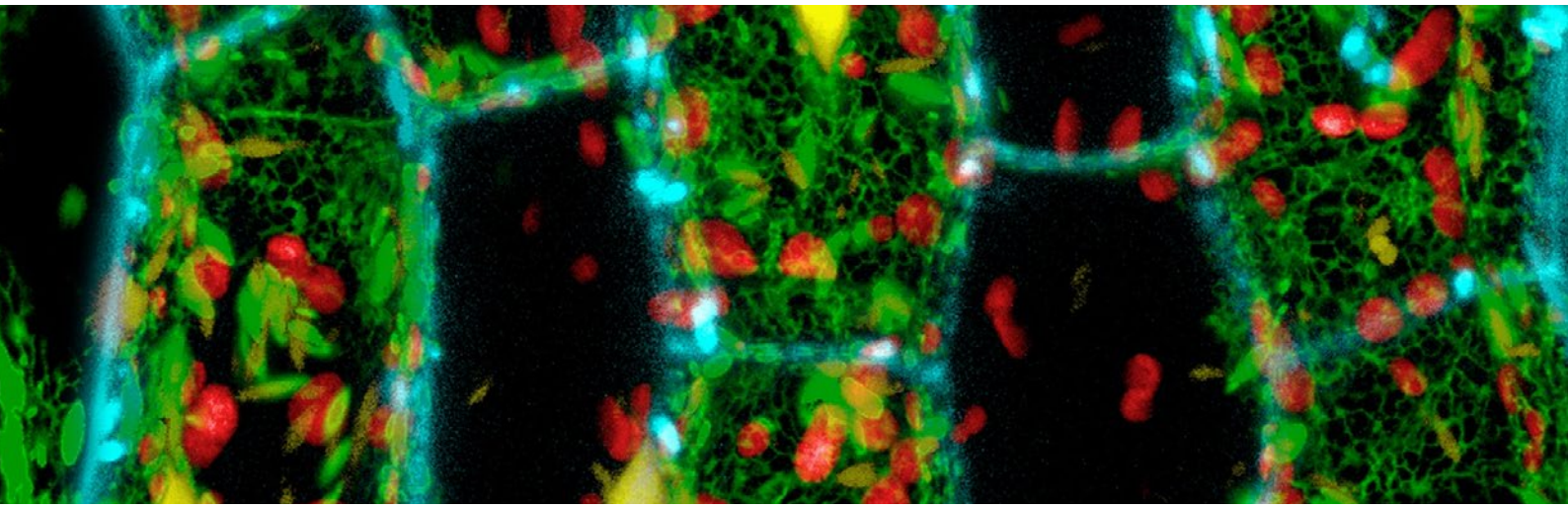




Engineering plant traits

In a second group of workpackages, new standards, tools and work practices are being implemented to engineer new traits in plants.

Workpackage F: Altered photosynthesis and leaf structure.
Workpackage G: Changes in plant carbohydrate content.
Workpackage H: Engineered pathways for the metabolic engineering of natural products.
Workpackage I: New forms of symbiosis and nitrogen fixation for crop plants.
Workpackage J: Methods for high level production of biomolecules by transient expression.



Workpackage F:
Modules for engineering photosynthesis and leaf metabolism

Plant leaves are biofactories that can accumulate valuable products in a number of discrete compartments both within and between cells. Furthermore, they also fine tune synthetic pathways in response to environmental signals. While significant progress has been made in defining cell specific gene expression in roots, this has not been achieved in leaves. This is a bottleneck in engineering this easily harvested organ, and there is no central repository of genetic modules to facilitate this. We aim to provide a library of elements that can be used to drive expression of both nuclear and plastid encoded genes in specific compartments of specific cells of leaves, and in addition to control that expression over the day-night cycle. These modules will be registered and made available in the OpenPlant repository.

Workpackage F aims to employ standardised DNA parts for the assembly of a collection of tools useful for engineering photosynthesis in plants. There will be strong interactions with the standards being established in Workpackages B and D, along with application of the parts in Workpackages A, G, H and J.

Investigators
Julian Hibberd; Alex Webb; Jim Haseloff; Alison Smith (JIC)

Staff Employed
Ivan Reyna-Llorens (PDRA; Hibberd lab) Started Oct 2015.
Lukas Mueller (PDRA; Webb lab) Started March 2017.

Partners
Jim Ajioka; Nicola Patron; Christian Boehm

F

Milestones:

F1: Protein scaffolds for cell specific and targeted intracellular expression in leaves.
Deliverable: Artificial protein scaffolds from bacterial systems that can be assembled in planta. Public release* of DNA parts for scaffolds and cognate ligands (month 24, Hibberd).

F2: DNA motifs that generate cell specific expression in leaves.
Deliverable: Leaf specific promoter motifs will be identified by sequencing of RNAs from isolated cell types and functional testing, and released publically* (month 36, Hibberd).

F3: Transcription factors and target cis-elements for engineering co-ordinated expression of synthetic pathways in leaves.
Deliverable: Leaf specific transcription factors and characterised cis elements will be released publically* (month 36, Hibberd).

F4: Inducible and cell-specific expression of genes in the chloroplast genome
Deliverable: Plastid targeted systems for regulation of plastid gene expression will be characterised and released publically* (month 60, Webb, Haseloff).

F5: Circadian control of synthetic promoters and gene control in chloroplasts
Deliverable: Public release* of synthetic promoters for expression at defined phases in the day-night cycle in plants (month 60, Webb, Smith/JIC).

Online resources:

Hibberd lab
(hibberdlab.com)

Smith lab
(www.jic.ac.uk/directory/alison-smith)

Webb lab
(www.plantsci.cam.ac.uk/research/alexwebb)

Progress to date:

This workpackage started in October 2015, as the groundwork for the establishment of the common syntax for plant DNA parts was consolidated. The first part of the work in the Hibberd laboratory was focused on the identification of leaf-specific promoter motifs and development of artificial protein scaffolds for metabolic engineering.

Related to this work, Christian Boehm in the Haseloff lab has established plastid transformation in Marchantia, and developed refactored cyan fluorescent protein markers for plastid expression. The use of fluorescent protein markers in Marchantia chloroplasts has been highly problematic for a number of years. There have been no reports of their successful use for chloroplast transformation. Christian has successfully engineered the cyan fluorescent protein gene for use in chloroplast transformation experiments in Marchantia. He is now working on binary systems for spatio-temporal control of gene expression in plastids.

This workpackage will combine efforts on nuclear and plastid genome based efforts to engineer photosynthesis, and will link with work on plastid genome engineering in workpackage D.

Objective F1: Protein scaffolds for cell specific and targeted intracellular expression in leaves.

The first milestone for the Hibberd laboratory is to develop artificial protein scaffolds from bacteria and assemble these *in planta* for metabolic engineering. The Hibberd lab has designed, synthesized and verified parts for the GBD, SH3, PDZ domains and their cognate ligands, all of which derive from metazoans. These DNA parts follow the Phytobrick standard (Patron et al., 2015) In addition, they have produced modules for the cohesin and its dockerin from bacterial systems that use the cellulosome complex. These parts were chosen based on previous work in bacterial systems where they have been used to increased flux through metabolic pathways. Each module has been placed into *Arabidopsis thaliana*, and shown to interact via BiFC coupled with confocal laser scanning microscopy. They are ready for public release.

Objective F2: DNA motifs that generate cell specific expression in leaves.

The second milestone is to identify DNA motifs that generate cell specific expression in leaves. Stable transgenic lines of *Arabidopsis thaliana* have been produced, which contain epitope-tagged nuclei and ribosomes driven by cell-specific promoters. Focus has been on two promoters that drive specific expression in bundle sheath cells of leaves. By combining functional testing *via* production of truncations with computational analysis, we have identified one positive regulator in *cis* that is necessary and sufficient to drive cell specific expression in leaves, and one negative regulator that represses expression in mesophyll and veinal cells. Both are ready for public release.

Objective F3: Transcription factors and target cis-elements for engineering co-ordinated expression of synthetic pathways in leaves.

The Hibberd lab has compiled a list of transcription factors that are preferentially expressed in bundle sheath cells of *A. thaliana*, and identified three transcription factors of particular interest Of these, one interacts directly with the positive regulatory DNA element identified in F2 above. Thus, these parts could be used for coordinate expression of genes in designated cells. These parts are ready for public release.

Objective F4: Inducible and cell-specific expression of genes in the chloroplast genome

Lukas Mueller is characterising synthetic promoters and reporters for analysis and manipulation sugar metabolism and circadian oscillations in *Marchantia polymorpha*.

Objective F5: Circadian control of synthetic promoters and gene control in chloroplasts

The genetic architecture of the circadian system in Marchantia has been analysed by Lukas Muller (Webb/Haseloff labs). The Marchantia genome lacks homologs to CCA1 and LHY and contains only one homolog to the PRR5/7/9 family in Arabidopsis. This has informed the choice of genes for synthesis of DNA parts.

Promoter regions (3kb upstream of 5'UTR) of putative circadian clock genes and putative clock output genes were identified in the Marchantia genome, domesticated and equipped with the respective cloning tags (following the common syntax) for Loop assembly. The promoters of the following genes were generated: MpCAB2, MpRVE, MpPRR, MpCCR2. In addition, the coding sequence of the luciferase PLUS enzyme was made compatible with the loop assembly system and equipped with the respective cloning tags. Functionality of the luciferase PLUS enzyme in Marchantia was confirmed *in vivo* with a constitutively expressed promoter.

Transgenic lines expressing luciferase driven by the CAB2 promoter were generated in order to run high-throughput circadian assays in Marchantia. Different successful transformants are being screened for the transgenic trait and a protocol is developed to compare Marchantia to Arabidopsis using instruments available in the Webb lab. High resolution observation of dynamic patterns of gene expression is complicated by the perdurance of stable fluorescent protein reporters. Muller is testing a strategy based on the fusion of synthetic degrons to fluorescent proteins to cause destabilisation of the reporter according to the N-terminal rule. This is expected to shorten the half-life of fluorescent reporters. Half-lives of the GFP signals will be measured following heat shock induction, to identify the most appropriate marker for observing circadian responses.

Publications

Reyna-Llorens I, Burgess S.J., Reeves G., Singh P., Stevenson S.R., Williams B.P., Stanley S. and Hibberd J.M. (2018) **Ancient duons may underpin spatial patterning of gene expression in C4 leaves.** Proceedings of the National Academy of Sciences, USA doi/10.1073/pnas.1720576115.

Yu, Z., Boehm, C.R., Hibberd, J.M., Abell, C., Haseloff, J., Burgess, S.J., Reyna-Llorens, I., (2017). **Droplet-based microfluidic analysis and screening of single plant cells**. BioRxiv pre-print: doi: https://doi.org/10.1101/199992

Burgess, S.J., Reyna-Llorens, I., Jaeger, K., Hibberd, J.M. (2017). **A transcription factor binding atlas for photosynthesis in cereals identifies a key role for coding sequence in the regulation of gene expression.** *BioRxiv pre-print doi: https://doi.org/10.1101/165787*

Reyna-Llorens I, Hibberd JM (2017). **Recruitment of pre-existing networks during the evolution of C4 photosynthesis.** *Philos Trans R Soc Lond B Biol Sci. 372(1730). pii: 20160386. doi: 10.1098/rstb.2016.0386.*

Kümpers BM, Burgess SJ, Reyna-Llorens I, Smith-Unna R, Boursnell C, Hibberd JM (2017). **Shared characteristics underpinning C4 leaf maturation derived from analysis of multiple C3 and C4 species of Flaveria.** J Exp Bot. 68(2):177-189. doi: 10.1093/jxb/erw488.

Hibberd JM, Furbank RT (2016). **Wheat genomics: Seeds of C4 photosynthesis.** *Nat Plants. 2(11):16172. doi: 10.1038/nplants.2016.172.*

Hibberd JM, Furbank RT (2016). **In retrospect: Fifty years of C4 photosynthesis.** *Nature. 538(7624):177-179. doi: 10.1038/538177b.*

Aubry S, Aresheva O, Reyna-Llorens I, Smith-Unna RD, Hibberd JM, Genty B (2016). **A Specific Transcriptome Signature for Guard Cells from the C4 Plant Gynandropsis gynandra.** Plant Physiol. 170(3):1345-57. doi: 10.1104/pp.15.01203.

Williams, B.P., Burgess, S.J., Reyna-Llorens, I., Knerova, J., Aubry, S., Stanley, S., and Hibberd, J.M. (2016). **An Untranslated cis-Element Regulates the Accumulation of Multiple C4 Enzymes in Gynandropsis gynandra Mesophyll Cells.** *Plant Cell, 28(2), p454-65.*

Workpackage G: Carbohydrate engineering

Plants provide unrivalled opportunities for provision of sugars and polysaccharides for biorefining, biofuels, animal feed, food and other industrial uses. The main goal of this workpackage is to improve the quality and increase the yield of target polymers, and to alter their structure for higher value applications. The targets will be plant cell wall polymers that important to these applications: xylan, mannan, and novel digestible glucans. The objectives will be achieved by building a registry of polysaccharide synthesis pathway genes and transcription factors that can be co-ordinately expressed using tested promoters from this and other workpackages.

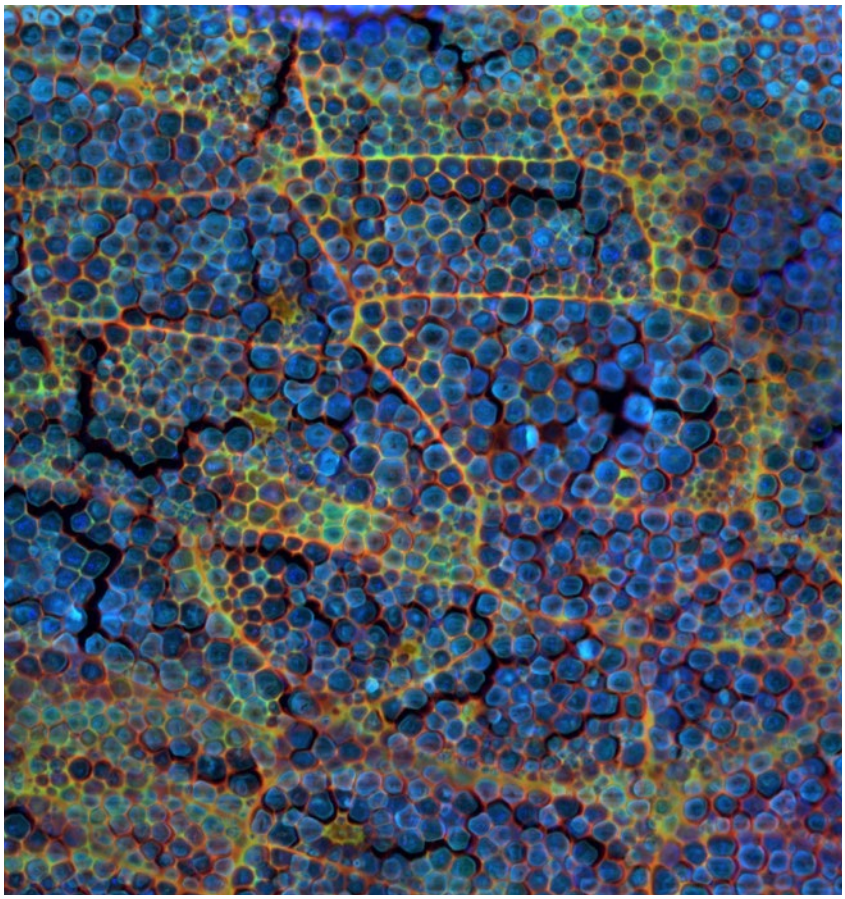
This workpackage will use DNA assembly technologies and genome editing technologies developed in workpackages B and D.

Investigators
Paul Dupree; Rob Field; Alison Smith (JIC); Nicola Patron

Staff Employed
Aytug Tuncel (PDRA; Smith lab at JIC) Started Jan 2015
Henry Temple (PDRA; Dupree lab) Started Feb 2017
Louis Wilson (OpenPlant DTP student in Dupree lab) Started Apr 2017

Partners
Alison Smith (JIC) obtained Norwich Research Park Innovation funds to establish a transformation method for potatoes in the JIC BRACT transformation group to support a CRISPR/Cas9-mediated carbohydrate engineering project. A collaboration with the Quadram Institute, Norwich, is providing access to state-of-the-art starch analytical techniques.

G



Milestones:

G1: A resource of inducible expression systems in fibre cells.
Deliverable: Public release* of fibre cell specific promoters (month 54, Dupree).

G2: A resource of genes for engineering ectopic mannan synthesis.
Deliverable: Public release* of glycosyltransferase gene tools for ectopic synthesis of mannans (month 60, Dupree).

G3: A resource of genes for engineering xylan synthesis.
Deliverable: Public release* of glycosyltransferase genes that direct modification of xylans in planta (month 60, Dupree).

G4: A tool-kit of algal glucan-active enzymes.
Deliverable: Public release* of carbohydrate-active enzymes mined from red algae genomes (month 24, Field).

G5: Cytosol-targeted expression of glucan-active enzymes.
Deliverable: Characterisation of new enzymes for glucan synthesis and modification in plants (month 36, Smith/JIC, Field).

G6: Engineered plants producing cytosolic glucans.
Deliverable: Transgenic plant lines as models for biosynthesis of novel glycans (month 36, Smith/JIC, Field).

Online resources:

Dupree lab
(www.bioc.cam.ac.uk/dupree)

Smith lab
(www.jic.ac.uk/directory/alison-smith/)

Field lab
(www.jic.ac.uk/directory/rob-field/)

Progress to date:

Objective G1: A resource of inducible expression systems in fibre cells.

Work started in 2017 with the appointment of PDRA Henry Temple and OpenPlant PhD student Louis Wilson to the Dupree lab. They are using the secondary cell wall-specific *Arabidopsis thaliana* IRX3 promoter for expression of genes of interest. Henry started preparing several constructs for expressing specific glycosyltransferases (GTs) activities using tissue specific promoters.

Objective G2: A resource of genes for engineering ectopic mannan synthesis.

Work in the Dupree lab on galactomannan, synthesized by CSLA2 and GMGT1, has progressed well, so the lab are extending this objective to the modification of mannan structure.

Objective G3: A resource of genes for engineering xylan synthesis.

Phylogenetic analysis using several candidates of different species, show conifer putative Xylan Arabinosyltransferases cluster in three different groups within GT-61 clade B (Anders et al., 2011). This analysis led us to choose two candidates of each specific conifer group and we have cloned them into GoldenGate compatible vectors based on use of the OpenPlant common syntax. We have generated constructs for conifer GT61 expression under specific promoters for secondary cell wall tissues. We have now transformed several *Arabidopsis* backgrounds (which possess specific modifications in Xylan biosynthesis) with some of the constructs generated. Using transgenic lines, we will be able to evaluate the activity of these GTs *in vivo* and evaluate the consequences of these modifications on the properties of the biomass and plant cell walls.

Conifer arabinosyl transferases, driven by the *A. thaliana* IRX3 promoter (Objective 1), have been shown to be active in secondary cell walls in transgenic *A. thaliana* plants (Lyczakowski et al., 2017). Novel arabinogalactan methyltransferases have also been identified (Temple et al., in revision). The Dupree lab are using the HyperTrans system in *Nicotiana benthamiana* to obtain protein for biochemical analysis of glycosytransferase activities.

Objective G4: A tool-kit of algal glucan-active enzymes.

A complete informatics analysis has been conducted on two transcriptome data-sets generated by the Field lab for the photosynthetic protozoan *Euglena gracilis*, cultured under autotrophic and heterotrophic conditions (O'Neill et al., 2015). This identified an unexpectedly large repertoire of carbohydrate-active enzymes, including many involved in storage beta-glucan metabolism and a range of what appear to be hemi-cellulose-synthesising enzymes, although Euglena is not known to produce such glycans. All data is available via the JIC web site: http://jicbio.nbi.ac.uk/euglena/

Further analyses of algae, such as Emiliania and Prymnesium, is ongoing together with an Innovate UK funded project, to assess their repertoire of polysaccharide and natural product glycosylation capabilities to feed into synthetic biology and industrial biotechnology studies.

We have employed advanced bioinformatics and structure homology prediction approaches to identify candidate algal beta-1,3-glucan phosphorylases. Heterologous expression studies are underway. An homologous bacterial phosphorylase has been crystallised and X-ray diffraction data will be acquired shortly.

The Field lab is exploring artificial, *in vitro* metabolic cycles, driving production of glucose-based oligosaccharides from cheap and readily available sucrose by using sucrose phosphorylase and glucan phosphorylases. We have now achieved this goal for oligosaccharides based on amylose (a-1,4-linked), cellulose (b-1,4-linked) and latterly b-1,3-linked glucan. In our search for the necessary but elusive b-1,3-glucan phosphorylase, we have identified a new family of carbohydrate-active enzymes from Euglena, which is currently being classified by our collaborator Bernard Henrissat. These enzymes, which are only distantly related to the obvious GH94 b-1,4-glucan phosphorylases, are proving to be versatile tools for the generation of immune stimulatory b-1,3-glucans (up to dp ca 25) for evaluation in fish feed in a BBSRC/Newton Fund project with India and Bangladesh.

Objective G5: Cytosol-targeted expression of glucan-active enzymes.

The Field lab is exploring artificial *in vitro* metabolic cycles driving production of glucose-based oligosaccharides from cheap and readily available sucrose by using sucrose phosphorylase and glucan phosphorylases. They have achieved this goal for oligosaccharides based on amylose (a-1,4-linked), cellulose (b-1,4-linked) and latterly b-1,3-linked glucan. In the search for the necessary but elusive b-1,3-glucan phosphorylase, they have identified a new family of carbohydrate-active enzymes from *Euglena* (Rejzek et al., 2017; Kuhaudomlarp et al., 2018; Panpetch et al., in revision); other new enzyme families from algae and bacteria are currently being evaluated.

Objective G6: Engineered plants producing cytosolic glucans.

PDRA Aytug Tuncel (Smith lab) is applying and testing the genome editing tools and technologies developed in the Patron lab (Workpackage D) to generate novel, commercially or nutritionally valuable glucans in model plant and crop species. The primary objective is the creation of potatoes that contain digestion-resistant starches with two major nutritional benefits: reduced calorie intake from consumption of chips, crisps and other potato-based snack foods and increased supply of complex carbohydrates to the microbiota of the lower gut that

reduces risk of several diseases including colorectal cancer and type II diabetes. Constructs encoding RNA-guided Cas9 to target starch branching enzymes in the potato genome have been assembled and delivered to potatoes by the BRACT group.

We have screened several potato plantlets and identified mutants which have successful editings in different isoforms of the starch branching enzymes. These mutant plants, which still retain the wild type form of the genes of interest, are currently being grown to maturity in greenhouse to be re-examined for increased gene editing. In addition, we constructed new vectors which are expected to improve editing efficiency and will soon be used for transformation to create second generation of mutants. As an alternative approach, we are also implementing and optimizing a protocol to isolate protoplasts from potato which can be subsequently transformed with the new constructs and regenerated into plants with a high chance obtaining starch branching enzyme null mutants.

A first round of transformation of stem explants produced plants partially edited for genes encoding one or both isoforms of starch-branching enzyme (SBE) A large fraction of starch granules from tubers of plants with partial editing of both SBE genes has major structural defects around the hilum, consistent with abnormal polymer structure. Various starch and metabolic analyses of the tubers are under way in collaboration with Quadram Institute (formerly Institute of Food Research) researcher Fred Warren, whose lab is equipped for a range of starch analytical techniques. A second round of transformation using improved constructs has produced more frequent, more extensive editing of SBE genes: tubers will be harvested for analysis in the autumn. Aytug Tuncel has also developed methods to transform protoplasts isolated from potato leaves. Initial results are very promising: SBE genes are completely edited in some regenerated calli and some calli apparently lack any DNA from the construct.

Additional funding was secured to establish a transformation method for potatoes in the BRACT transformation group at the John Innes Centre.

Publications

Lyczakowski JJ, Wicher KB, Terrett OM, Faria-Blanc N, Yu X, Brown D, Krogh KBRM, Dupree P, Busse-Wicher M. (2017). **Removal of glucuronic acid from xylan is a strategy to improve the conversion of plant biomass to sugars for bioenergy.** *Biotechnol Biofuels;10:224. doi: 10.1186/s13068-017-0902-1.*

O'Neill EC, Kuhaudomlarp S, Rejzek M, Fangel JU, Alagesan K, Kolarich D, Willats WGT, Field RA (2017). **Exploring the glycans of *Euglena gracilis*.** *Biology* 6(4), 45

Rejzek M, Hill L, Hems ES, Kuhaudomlarp S, Wagstaff BA, Field RA (2017). **Sugar nucleotide profiling.** *Methods in Enzymology – Chemical glycobiology* 597, 209-239

O'Neill EC, Saalbach G, Field RA, (2016). **Gene Discovery for Synthetic Biology: Exploring the Novel Natural Product Biosynthetic Capacity of Eukaryotic Microalgae.** *Methods in Enzymology,* 2016, 576, 99-120

O'Neill et al., (2015). **The transcriptome of Euglena gracilis reveals unexpected metabolic capabilities for carbohydrate and natural product biochemistry.** *Mol. BioSyst. DOI: 10.1039/C5MB00319A.*

Temple H, Mortimer J, Tryfona T, Yu X, López-Hernández F, Sorieul M, Anders N, Dupree P* **Identification of arabinogalactan methyltransferases in the DUF579 family.** *In revision.*

Yu L, Pereira CS, Lyczakowski JL, Kotake T, Yu X, Li A, Mфgelsvang S, Skaf MS, Dupree P. **The patterned structure of galactoglucomannan synthesized by CSLA2 and GMGT1 suggests it may bind to cellulose**. *Manuscript in preparation.*

B. A. Wagstaff, M. Rejzek, S. Kuhaudomlarp, L. Hill, I. Mascia, S. A. Nepogodiev, R. A. Field. **NDP-β-L-rhamnose biosynthesis across the algal taxonomic groups: van evolutionary perspective.** *New Phytologist, submitted*.

S. Kuhaudomlarp, N. J. Patron, B. Henrissat, M. Rejzek, G. Saalbach, R. A. Field. **Identification of *Euglena gracilis* β-1,3-glucan phosphorylase and establishment of a new glycosyl hydrolase family GH** *J. Biol. Chem., doi: 10.1074/jbc.RA117.000936.*

P. Panpetch, R. A. Field, T. Limpaseni. **Co-expression of isoamylase genes from cassava *Manihot esculenta* Crantz 'KU50' tubers confirms the requirement for heteromeric complex formation for enzyme activity.** *Plant Mol. Biol., in revision.*

Workpackage H:
Tools for Engineering Plant Natural Products

Plants produce a rich and diverse array of natural products. These compounds have important ecological functions, providing protection against pests, diseases, ultraviolet-B damage and other environmental stresses. They are also exploited as pharmaceutical drugs, agrochemicals, within the food and drink industry, and for a wide variety of other industrial biotechnology applications. Although plants are potentially a tremendous source of diverse and valuable natural products, identifying the pathways for the synthesis of these compounds is more complicated than in microbes because the genomes are larger and more complex. However advances in sequencing technology coupled with the recent discovery that the genes for natural products pathways are in many cases organised in operon-like clusters within plant genomes; now makes it possible to access the genes and enzymes of specialised metabolism in plants far more readily and so to harness and exploit metabolic diversity using synthetic biology approaches.

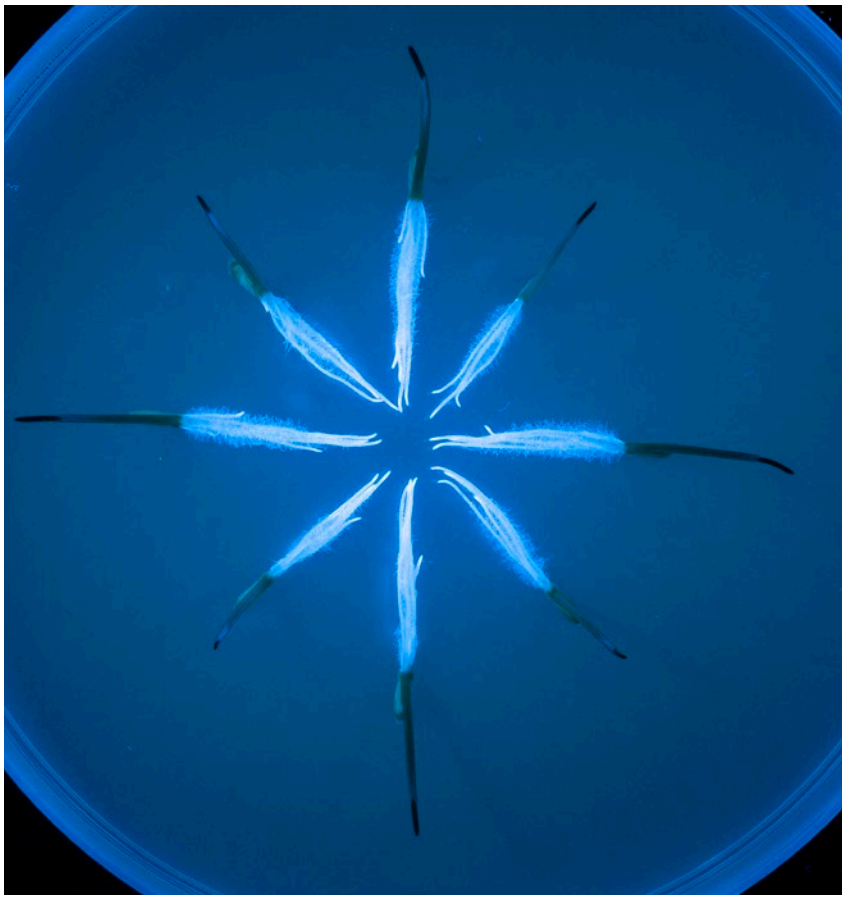
The HyperTrans plant expression system (Workpackage J) is being heavily used by the Martin and Osbourn labs. This platform supports the testing and investigation of metabolic pathways and the creation of new compounds. In turn, these projects inform and enable further optimisation of this powerful tool.

Investigators
Cathie Martin; Anne Osbourn; Paul O'Maille; Sarah O'Connor

Staff Employed
Yang Zhang (PDRA; Martin lab at JIC). January 2015 – January 2016
Don Nguyen (PDRA; O'Maille lab at JIC). February 2015 – February 2016
Hans-Wilhelm Nützmann (PDRA; Osbourn lab at JIC). Sept 2014 - Sept 2017
Michael Stephenson (PDRA; Osbourn lab at JIC). Started February 2015
Benjamin Lichman (PDRA; O'Connor lab at JIC). Started February 2016
Noam Chayut (PDRA; Martin lab at JIC). August 2016 - January 2018
Zhenhua Liu (PDRA; Osbourn lab at JIC). Started November 2017

Partners
Alain Goossens, VIB, Ghent
Norfolk Plant Sciences
Croda (BBSRC High Value Chemicals from Plants NIBB Proof of Concept project)
Marnix Medema (University of Wageningen)
Dr. DaeKyun Ro, University of Calgary, Canada
An OpenPlant Fund grant has established new collaborations between the Osbourn and Haseloff labs for producing triterpenes in Marchantia.
Collaboration with Susan Duncan, EI, as part of OpenPlant Fund project 'Advancing the ability to image single RNA molecules at the cellular level'

H



Online resources:

Osbourn lab
(www.jic.ac.uk/directory/anne-osbourn/)

Martin lab
(www.jic.ac.uk/directory/cathie-martin/)

O'Connor lab
(www.jic.ac.uk/directory/sarah-oconnor/)

Milestones:

H1: A database and resource of parts for enzyme building blocks for natural product synthesis.
Deliverable: Genome mining data and public release* of plant DNA parts for synthesis and modification of natural product synthesis (month 60, Osbourn, Martin, O'Connor).

H2: Optimised enzymes for terpene production.
Deliverable: Public release* of DNA parts encoding improved enzymes for terpene synthesis (month 36, O'Maille, Osbourn).

H3: Transcription factors for control of natural product production.
Deliverable: Public release* of DNA parts encoding transcription regulators for terpene and alkaloid synthesis (month 60, Osbourn, Martin).

H4: Synthetic metabolons for improved phenylpropanoid production
Deliverable: Characterisation of model synthetic metabolons for phenylpropanoid biosynthesis (month 60, Martin).

H5: Synthetic metabolic clusters for deployment into crop plants
Deliverable: Use of the synthetic metabolon toolkit for rapid assembly and testing in Marchantia and Arabidopsis (month 36, Osbourn).

Progress to date:

Objective H1: A database and resource of parts for enzyme building blocks for natural product synthesis.

The number of biosynthetic gene clusters that have been reported for plant natural product pathways continues to grow (Nützmann et al., 2016; Owen et al. 2017). The Osbourn lab is collaborating with the lab of Marnix Medema (University of Wageningen) to develop and optimise computational methods for pathway discovery. This has led to the release of plantiSMASH, a customised algorithm for mining for biosynthetic gene clusters in plant genomes (Kautsar et al. 2017).

In addition, the O'Connor lab at JIC has recently produced the plant-derived iridoid alkaloid strictosidine in yeast (Brown et al. 2015). PDRA Benjamin Lichman has discovered additional enzymes in this pathway to generate more "building blocks" for this work. He has generated a proteome database for trichomes of iridoid producing plants and is now searching this database for new candidate pathway enzymes.

The Osbourn lab has developed improved agro-infiltration methodology for production of triterpenes using the HyperTrans transient plant expression system (Reed et al., 2017). This has enabled purification of gram-scale quantities of triterpene in just a few weeks, without any need for re-engineering of the host. They have also shown that this platform can be used for quick and easy combinatorial biosynthesis without the need for generation of multi-gene constructs, simply by mixing *Agrobacterium* strains harbouring different expression constructs prior to infiltration, and have used this approach to generate and purify a suite of bespoke triterpene analogs and demonstrate differences in anti-proliferative and anti-inflammatory activity in bioassays, providing proof of concept of this system for accessing and evaluating medicinally important bioactives. Together with new genome mining algorithms for plant pathway discovery and advances in plant synthetic biology, this advance provides new routes to synthesize and access previously inaccessible natural products and analogs and has the potential to reinvigorate drug discovery pipelines.

PDRA Benjamin Lichman in the O'Connor group has progressed with identifying new building blocks in the biosynthesis of iridoids. Specifically, Lichman has identified a number of biosynthetic genes that derivatize the iridoid scaffold and that modulate the stereochemistry of the iridoid ring system. An example of a gene duplication and neofunctionalization of a short chain alcohol dehydrogenase (SDR) has been discovered. The original function of the SDR was hypothesized to be to oxidize the substrate nepetalactol to nepetalactone. This homologue of this SDR has an intriguing non-redox role in controlling the stereochemical course of the ring cyclization. In sum, these new genes can now be used to generate a wide variety of iridoid analogs, which have potentially important agrichemical activity.

PDRA Noam Chayut is working on a project for Plant Sourced L-Dopa production for Parkinson's treatment. The current cost of L-DOPA makes it unavailable for deprived populations worldwide. In addition, there is a growing demand for 'natural' or plant sourced pharmaceutical substances in the first world. L-DOPA, a product of tyrosine hydroxylation, is an intermediate metabolite in biosynthesis of violet and yellow betalain pigments, in Beta vulgaris (beetroot). Natural steady state levels of L-DOPA are very low, usually undetectable. The goal of this project is to block the turnover of L-DOPA in beetroot to allow its accumulation to levels that could enable low-tech accessible production in a plant system. Hairy beet roots induced by *Agrobacterium rhizogenes* could be identified easily in soil-grown plants due to their lack of gravitropism. Using an emerging technology for genome editing (CRISPR/Cas9-mediated gene silencing), the DODA in red and in yellow beet was permanently knocked down. The transformed, mutated hairy roots showed lower downstream betalain pigmentation in comparison to empty vector control hairy roots in both tested genotypes. L-DOPA accumulation was verified by high-pressure liquid chromatography and accumulated in µg quantities. These results proved that the concept for agriculturally produced L-DOPA for pharmaceutical uses is very promising.

Projects in the Osbourn, Martin and O'Connor labs continue to identify and functionally characterise a range of DNA parts for natural product synthesis. The development of a deeper understanding of regulation of biosynthesis pathways, bioinformatics-based methods for mining plant genomes for biosynthetic gene clusters, novel practical tools and improved synthetic biology pipelines are rapidly accelerating the discovery, characterisation and practical utilization of new pathways and chemistries. Several collaborative projects with industry are advancing this research in a commercial context.

Anne Osbourn has recently secured a BBSRC Super Follow-on Fund grant to identify genes for the synthesis of the triterpene glycoside QS-21 from soapbark (*Quillaja saponaria*) and reconstitute the pathway in *N. benthamiana* using transient plant expression technology. QS-21 is an adjuvant used in anti-malaria and shingles vaccines. Engineering the QS-21 pathway in *N. benthamiana* will serve as a flagship proof of concept project to demonstrate the power of the transient plant expression platform for accessing high-value products from plants.

Objective H2: Optimised enzymes for terpene production.

PDRA Don Nguyen (O'Maille lab) has identified, characterised and mutated a set of cytochrome P450 enzymes from the Asteraceae family. Enzymes characterised from *Barnadesia spinosa* have been engineered into yeast to generate oxygenated sesquiterpenes. Work on optimising enzymes for triterpene production is currently underway in the Osbourn lab.

Objective H3: Transcription factors for control of natural product production.

Hans-Wilhelm Nützmann (Osbourn lab) showed that plant biosynthetic gene clusters are strongly marked by Polycomb-mediated histone 3 leucine 27 trimethylation when in their off state, and by the histone 2 variant H2A.Z when in their on state (Nützmann and Osbourn, 2015; Yu et al, 2016), and that these features can be exploited to identify new biosynthetic gene clusters. Recent work has focused on generation of a capture HiC map and FISH analysis to investigate the three-dimensional positioning of biosynthetic gene clusters in the nucleus in *A. thaliana.* These methods have been successfully established and validated. Data analysis is being carried out in collaboration with colleagues at the Barbraham Institute. Nützmann has also established single cell RNA FISH methodology with Dr Susan Duncan, Earlham Institute.

Hans Nützmann (Osbourn lab) has used yeast one hybrid analysis to identify several transcription factors that bind to promoters of genes within a root-expressed triterpene metabolic gene cluster (the thalianol cluster) which we have characterised from *Arabidopsis thaliana*. These transcription factors were evaluated for their transactivation capacities using a tobacco protoplast transient expression system (with Alain Goossens, VIB, Ghent). The observed activity was dependent on co-incubation with a second transcription factor. The dual regulatory activity may represent a novel mechanism in the control of clustered metabolic pathway genes. A candidate transcription factor for a biosynthetic gene cluster from oat (the avenacin cluster) has recently been identified and functional analysis is underway.

The Martin lab has developed vectors for transient induction of gene expression in tomato fruit. These new tools and resources were used to test candidate transcription factors controlling lycopene, alpha tocopherol and ascorbate production in tomato. Yang Zhang used these tools to test the activity of a new pathway for flavone synthesis in Scutellaria baicalensis. This resulted in co-authorship on a paper published in Science Advances in April 2016. These vectors are based on the HyperTrans vectors, but have been modified to improve fruit expression using the E8 promoter as well as vectors for expression driven by the 35S promoter. A reliable protocol for inoculation of fruit for optimised gene expression has been established. All vectors are compatible with the OpenPlant common syntax. RNA-seq data for *S. lycopersicum* x *S. pennellii* tomato introgression lines has been screened for trans-eQTLs affecting expression of genes encoding enzymes of monoterpenoid biosynthesis. Candidate transcription factors are being identified from those intervals showing strong eQTLs. A new set of introgression lines (*S. lycopersicum* x *S. lycopersicoides*) have been grown and fruit gathered for new RNA-seq analysis for characterisation of additional eQTLs.

Similar strategies could be employed to identify trans-eQTLs controlling glycoalkaloid biosynthesis in tomato.

The Martin lab have robust evidence supporting two positive regulators of lycopene biosynthesis and a repressor of alpha tocopherol biosynthesis in tomato. They have deleted the gene encoding one of the activators of lycopene biosynthesis using CRISPR/Cas9 and are testing its role by phenotypic characterisation of the fruit.

Objective H4: Synthetic metabolons for improved phenylpropanoid production

Yang Zhang (Martin lab) has focussed on characterising the targets of AtMYB12 and SlMYB12 in tomato since these genes seem to offer a more effective means of enhancing phenylpropanoid metabolism than constructing synthetic metabolons. Consequently, emphasis has been shifted to improving our transcription factor tools and further funding was sought to improve the usefulness of AtMYB12 in engineering metabolism in tomato. Here the idea is to reduce the responsiveness of flavonoid biosynthesis to AtMYB12 by mutagenizing the promoter of Chalcone Synthase 1 which contains the AtMYB12 binding motif. This would allow ectopic expression of AtMYB12 in fruit to induce tyrosine and tryptophan biosynthesis, but to reduce the subsequent draw on these amino acid pools by flavonoid biosynthesis, without eliminating flavonoid biosynthesis completely. Mutagenesis is proposed using CRISPR/Cas9 genome editing in a collaborative project with SME Persephone Bio.

We have achieved levels of resveratrol in tomatoes of 6 mg g^{-1} DW and levels of genistin of 80 mg g^{-1} using this strategy. Characterisation of the effects of AtMYB12 on carbon flux in tomato suggests that use of AtMYB12 could enhance natural product accumulation from a range of hydrophobic and aromatic amino acids.

Jie Li (Martin lab) was awarded an OpenPlant project with Greg Reeves from Julian Hibberd's group in Cambridge to test the production of capsaicin in tomato. Jie has introduced VpVAN, pAMT, KASI, KASIIIb, BCAT, BCKDH, ACS1 and CS genes into *N. benthamiana* using the HyperTrans system to determine whether the proteins are active and also whether, in combination, they will make capsaicin.

Progress to date:

Objective H5: Synthetic metabolic clusters for deployment into crop plants

The Osbourn lab has shown that the promoters for two of the genes from the oat avenacin cluster retain their characteristic expression patterns (in the epidermal cells of root meristems) when introduced into diverse plant species as promoter-reporter constructs (Kemen *et al*. PNAS, 111:8679, 2014). Building on this, analyses have been expanded to test a total of nine promoters from this cluster, confirming that they behave in the same way. These promoters therefore represent an important resource for driving the expression of heterologous gene-sets in the root tips of plants. Three promoters from the avenacin cluster were used to successfully drive the expression of a three-gene pathway for a plant defence compound (dhurrin) from sorghum in *Arabidopsis thaliana* roots. This opens up opportunities for engineering expression of other multi-gene traits (e.g. nitrogen fixation genes) in the roots and also for manipulating the root microbiome through expression of different types of metabolites.

Three independent operon-like synthetic constructs were developed for transformation into *M. polymorpha* chloroplasts for *de novo* synthesis of mono-, sesqui- and triterpenes. Because of the two issues listed above, a new direction is being trialled and evaluated, using nuclear transformation of Marchantia with chloroplast targeting of the proteins.

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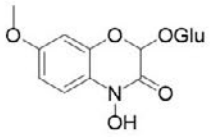
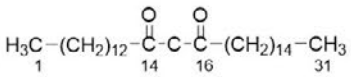
Zea mays



DIMBOA-Glu

Pest and disease resistance

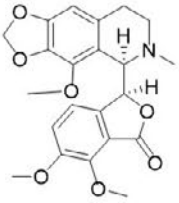
Hordeum vulgaris



β-Diketones

Protective leaf waxes

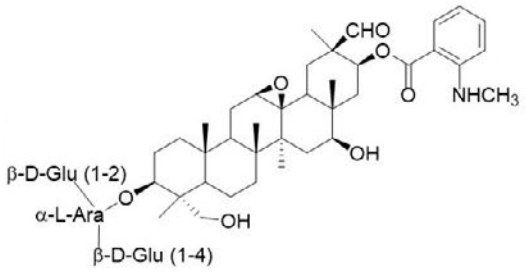
Papaver somniferum



Noscapine

Medicinal compound

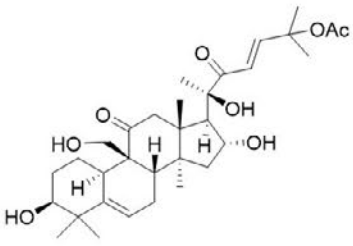
Avena strigosa



Avenacin A-1

Disease resistance

Cucumis sativus



Cucurbitacin C

Bitterness

Adapted from Nützman et al., 2016

Genome mining with plantiSMASH

JOHN INNES CENTRE-WAGENINGEN UNIVERSITY

The diversity of plant natural products is a source of great potential for industrial, agricultural and medical application. There is much to be gained from harnessing this diversity and engineering biosynthesis of high value chemicals into alternative chassis for rapid and sustainable production, discovery of novel chemicals, and synthesis of new-to-nature molecules. Advances in genome sequencing and the discovery of plant biosynthetic gene clusters have opened the doorway for systematic mining of plant genomes for new enzymes and pathways (Nützmann et al., 2016).
A collaboration between the Osbourn and Medema labs (Wageningen) has led to the development and optimisation of a customised algorithm for mining plant genomes for biosynthetic gene clusters, called plantiSMASH. plantiSMASH is a versatile online analysis platform to automate the discovery of gene clusters and biosynthesis pathways in plants (Kautsar et al. 2017). The plantiSMASH web server, precalculated results and source code are openly available at http://plantismash.secondarymetabolites.org

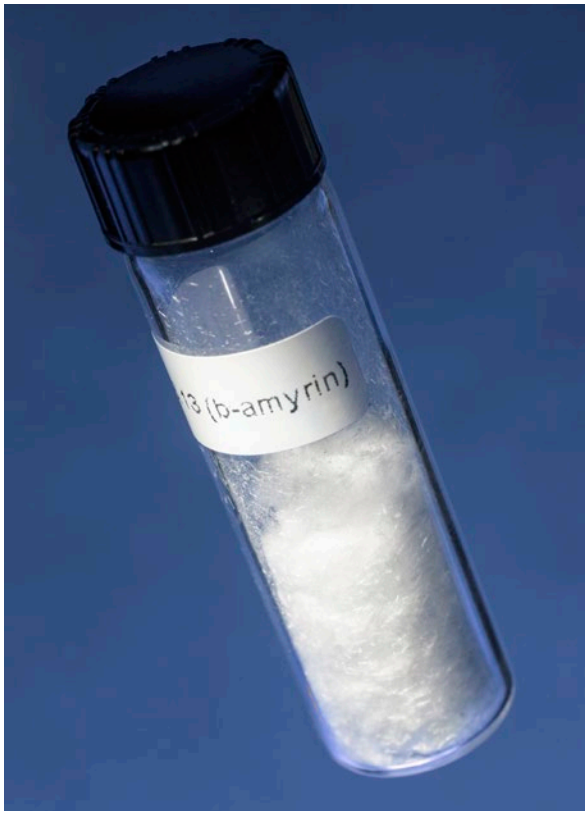
Principal contact: Anne Osbourn

Plants as Bio-factories

JOHN INNES CENTRE

The Hypertrans® system, developed by Professor George Lomonossoff and Dr Frank Sainsbury at the John Innes Centre, has established a unique position for the UK for rapid transient expression of proteins in plants. The technology is extremely powerful and was used under licence by the Canadian company Medicago to produce 10m effective doses of H1N1 (swine flu) VLP Vaccine in just 30 days, meeting the US Defense Advanced Research Projects Agency test requirements for control of emerging diseases.
Traditional methods for vaccine production using chicken embryos take 6-9 months. In contrast, plant-based production is much faster and can be rapidly up-scaled. Hypertrans® technology has also been used for expression of biosynthetic pathways, production of antibodies, antigens, and enzymes, and to solve the structure of particles to near atomic resolution when no parent virus particle is available. The system has huge potential for nanotechnology and nanomedicine applications with virus-like particles delivering cargo to cells, diagnostics and biosensor design. The Hypertrans® system provides a rapid testing and production platform and a valuable teaching and training tool.

Principal contact: George Lomonossoff



Production pipeline for small molecules

JOHN INNES CENTRE

Plants have long been recognised as a rich source of biologically active small organic molecules, and many commonly prescribed drugs are natural products or directly derived analogues. The triterpenes represent one of the most diverse families of plant natural products. However, the lack of easy access to these compounds via synthetic chemistry has hindered their exploration as potential leads for drug development. Through the utilisation of transient expression, the Osbourn lab have developed a plant based platform for the preparative production of triterpenes. The utility of this system has been demonstrated through the facile gram-scale production of the archetypal pentacyclic triterpene β-amyrin, and via the generation of novel analogues. This has afforded the opportunity to probe the structure activity relationships of biologically active β-amyrin derivatives (Reed et al. 2017). Advances in bioinformatics and the growing wealth of plant genomic data is likely to rapidly broaden the scope of potential enzymes which could be exploited through this system to access even greater chemical diversity. Such advances have the potential to reinvigorate drug discovery pipelines.

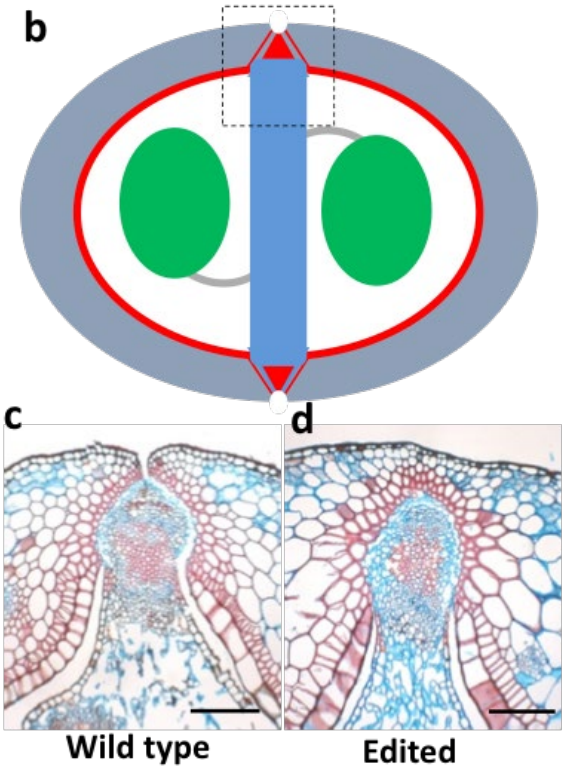
Principal contact: Anne Osbourn

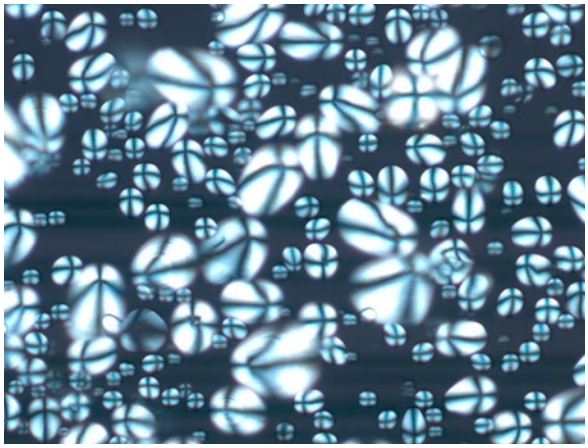
Scale-up for application at Leaf Systems®

NORWICH RESEARCH PARK

In January 2017, a purpose-built facility for plant production opened on the Norwich Research Park. Leaf Systems® International Ltd was designed for the scale-up of proteins metabolite and complex natural product production for research, clinical trials and bio-medical applications using the Hypertrans® expression platform. The new facility contains state of the art containment facilities to produce and engineer its feedstock plants, and incorporates industry standard downstream processing to ensure production quality and bio-security. The new facility is currently being used to scale up production of a number of test molecules, including Bluetongue virus-like particles, a virus-free polio vaccine, and a diagnostic for Zika virus.

Website: www.leafexpressionsystems.co.uk





Targeted gene knock-out in crops

JOHN INNES CENTRE & EARLHAM INSTITUTE

Genome editing using CRISPR /Cas9 allows us to introduce small mutations in specific target genes to knock-out their function. The mutant plants produced are extremely valuable for determining the function of the target genes. Such gene-edited plants may also have useful characteristics that could be incorporated into improved crops. We have used this technology successfully in wheat, barley, Brassica oleracea, potato and tomato. Genome editing in crops using CRISPR / Cas9 is now being offered as a resource to the research community. This activity is funded by BBSRC through the Bioinformatics and Biological Resources (BBR) fund.

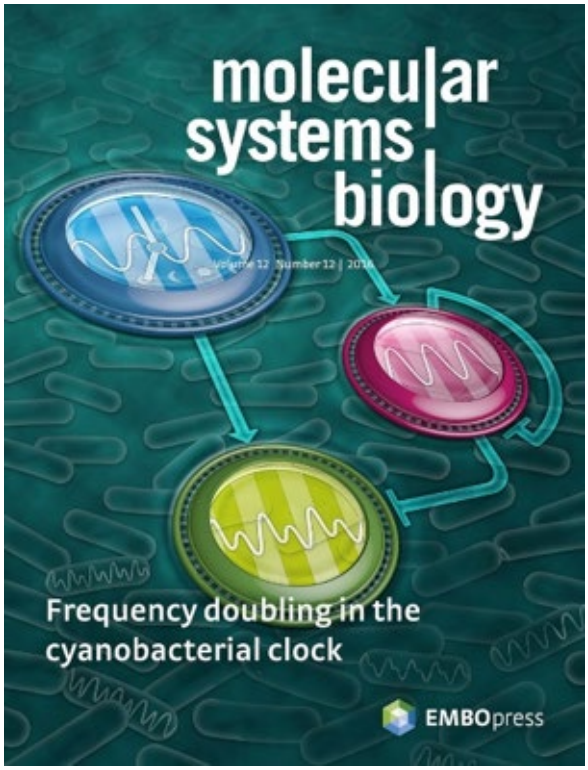
Principal contact: Wendy Harwood

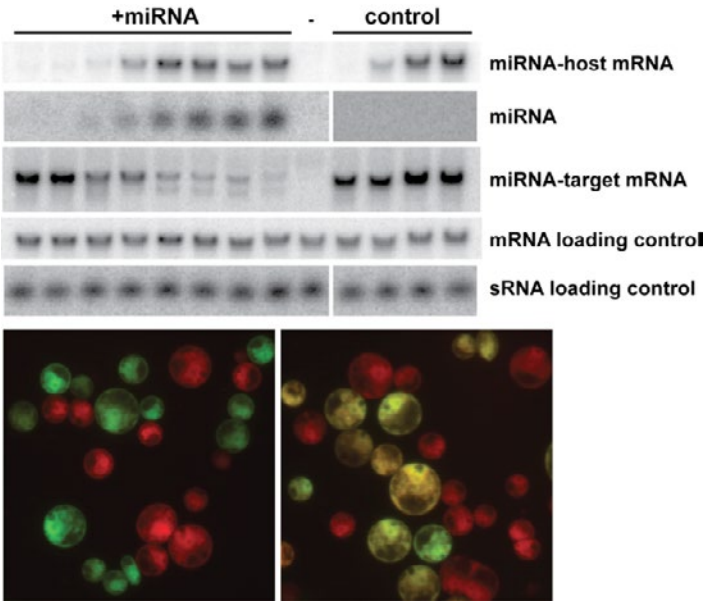
Potatoes with altered starch properties

JOHN INNES CENTRE & JAMES HUTTON INSTITUTE

We are growing genome-edited potato plants expected to have altered starch polymer structures and hence improved nutritional quality. The novel starch granules in the tubers will be resistant to digestion, so when used as food the tubers will provide more dietary fibre and less elevation of blood glucose than normal potatoes. The plants were regenerated from protoplasts transformed with CRISPR-Cas9 constructs that have mutated the starch branching enzyme genes without integration of foreign DNA.

Principal contact: Alison Smith (JIC)





Re-wiring frequency in cyanobacterial circuits

CAMBRIDGE

Rational design of oscillators is a goal of synthetic biology, but natural systems are already endowed with reliable oscillators in the form of circadian (24 hour) clocks. Understanding how to harness clocks to generate specific (non-circadian) frequencies, and how to systematically integrate clocks with other pathways will give us powerful tools, enabling the assembly of complex and dynamic synthetic circuits.
In the first stage of the project, we used single-cell time-lapse microscopy and mathematical modelling to study the coupling of the circadian clock to a circuit that controls expression of the key photosynthesis gene psbAI in the cyanobacterium S. elongatus. We observed frequency doubling in the expression of psbAI, i.e., it peaks twice a day with a period of 12 hours rather than 24 hours. We also observed two peaks in single-cell growth rates, suggesting frequency doubling can affect the global state of the cell. Using an iteration of theory and experiment, we determined the network design principles underlying the dynamics of frequency doubling (Martins et al. MSB, 2016). We are now perturbing this network to generate different frequencies, as predicted by our mathematical models.

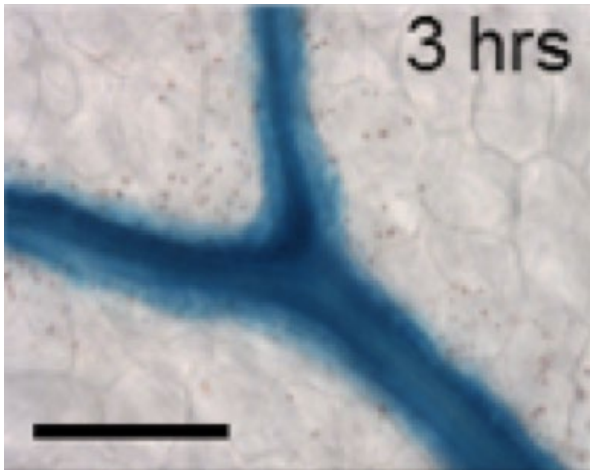
Principal contact: James Locke

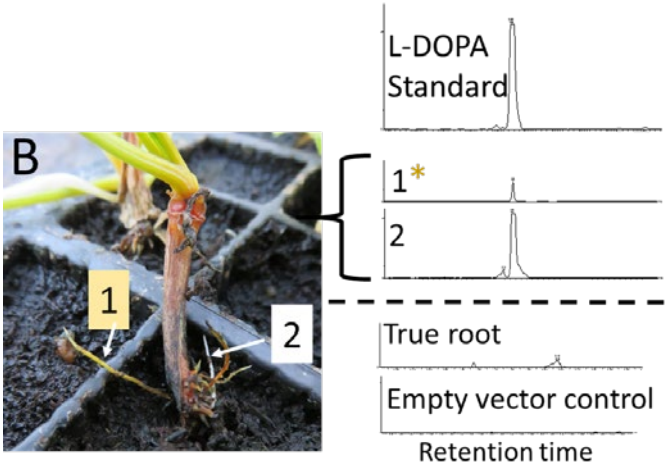
Modulating gene expression in green algae

CAMBRIDGE

Since their discovery, miRNAs have been revealed as a promising tool for the efficient and specific modulation of gene expression, with applications ranging from human health to biotechnology. Our synthetic biology approach to characterise miRNA-mediated gene silencing in the green alga Chlamydomonas has rendered a better understanding of their action, and consequently, more control over their output. Gene silencing is followed by reporters and can be studied at single cell and population level. In addition, the standardization of DNA parts for gene expression has made possible the exchange of tools within the algal community. This work has established the basis for further engineering of gene expression in plants using miRNAs.

Principal contact: Francisco J Navarro





A synthetic module for expression in specific leaf cells

CAMBRIDGE

A unifying aspect of multicellularity is the spatial patterning of gene expression associated with different cell-types. Here we report the first DNA part that can be used to direct gene expression to specific cells of leaves. By combining transcription factor analysis with truncation and oligomerisation analysis, we identified a short tuneable fragment that can be used to control gene expression in bundle sheath cells of leaves.

Principal contact: Julian Hibberd

Using beetroot for local production of L-DOPA

JOHN INNES CENTRE, SESVANDERHAAVE, & FONDAZIONE EDMUND MACH DI SAN MICHELE ALL'ADIGE

We have determined that beetroot can be developed as an effective production system for L-DOPA by gene editing to mutagenise the gene encoding DODA in beetroot in hairy roots. To translate these foundational experiments we have generated a mutagenized M2 population of YrYr/rr/blbl beet (yellow) by EMS mutagenesis. DODA mutants will be screened by looking for white seedlings/roots in the M2.

Principal contact: Cathie Martin

Workpackage I: Nitrogen Fixation

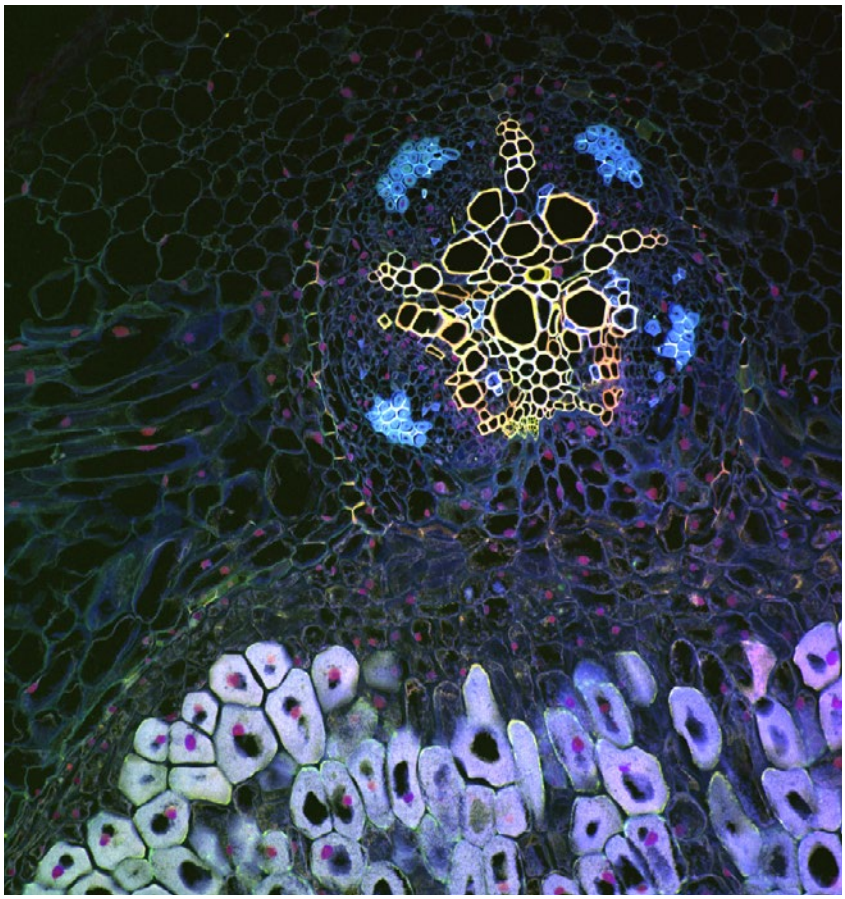
We have initiated an engineering strategy to transfer the recognition of rhizobial bacteria from legumes to cereals, as the first step towards engineering N-fixing cereal crops. This is a strategically important challenge and this Gates and BBSRC-funded programme represents one of the most ambitious engineering strategies in plant signalling. Marchantia provides a fantastic platform for testing synthetic biology approaches in engineering symbiosis signalling that is directly linked to a strategic programme in cereals.

Workpackage I feeds into Workpackage A, assisting in the establishment of Marchantia as a simple plant chassis for synthetic biology through the development and testing of methods and tools, and Workpackage B by producing parts that can be included in the parts collection. Workpackage I tests and uses genome editing tools produced in Workpackage D.

Investigators
Giles Oldroyd; Jim Haseloff; Sebastian Schornack; Nicola Patron

Staff Employed
Pierre-Marc Delaux (PDRA; Oldroyd lab), Started Sep 2014 - Ended Aug 2015
Philip Carella (PDRA, Schornack lab). Started Sep 2016

Partners
Gates-funded ENSA project (Engineering Nitrogen Symbiosis for Africa)
Three OpenPlant Fund grants have been funded for new collaborations between the Oldroyd group and groups in Cambridge, to explore the evolution of symbiosis signalling using Marchantia paleacea as a model, to develop modules for studying LysM receptor-like kinases and to develop novel cell reporters for high resolution imaging.



Milestones:

I1: Establishment of Marchantia as a model system for signalling in symbiosis.
Deliverable: Description of laboratory co-cultivation and marker techniques for symbiotic interactions between Marchantia spp. and Glomermycota fungi (month 12, Oldroyd, Haseloff, Schornack).

I2: Assembly of genetic components required for engineering Nod factor signalling.
Deliverable: A toolkit of transcription factors and signalling components for engineering synthetic responses to Nod factors (month 24, Patron, Oldroyd).

I3: Optimisation of gene circuits for synthetic Nod factor signalling in Marchantia.
Deliverable: Transgenic lines for optimising the function and activity of an ectopic Nod signalling pathway in (month 36, Oldroyd).

Online resources:

Oldroyd lab
(www.slcu.cam.ac.uk/directory/giles-oldroyd)

ENSA - Engineering Nitrogen Symbiosis for Africa
(www.ensa.ac.uk/)

Progress to date:

Objective I1: Establishment of Marchantia as a model system for signalling in symbiosis.

Robust methods have been established for co-cultivation of Marchantia spp. with Glomermycota fungi and visualisation of the fungus colonisation. In addition, a high-throughput transformation system has been developed for *Marchantia paleacea* and marker systems have been developed for secretion system pathway and tonoplast labelling.

The Schornack lab have established liverwort cultivation on vermiculite. Furthermore, we have established reproducible colonisation of several liverwort species (*Marchantia spp*., *Lunularia cruciata*) with Glomeromycota fungi (*Funnelliformis mossae*, *Rhizophagus irregularis*) in this vermiculite system and detection of the fungus using staining and high resolution confocal fluorescence microscopy. Entry of hyphae in all cases occurred through substrate oriented rhizoids. Distribution of hyphal colonisation within liverwort thalli differed markedly between *Marchantia spp*. and *Lunularia spp*. but was always restricted to the storage cell layers. Constructs have been developed for secretion system pathway and tonoplast labelling and used to confirmed their functionality in *Marchantia polymorpha*.

A *Marchantia paleacea* isolate was selected as a chassis, because of its ability to form arbuscular mycorrhizal symbiosis. A rapid transformation protocol has been developed and successfully moved onto a 96-well format, allowing high throughput transformation of *M. paleacea*. The genome of *M. paleacea* was sequenced using paired end libraries and illumina sequencing, providing a genome of sufficient quality for our studies in this project. Protocols for genome editing using CRISPR-Cas9 in *M. paleacea* have been established, and *NOP1* gene knockouts have been created to test the efficiency of CAS9 endonucleases in *M. paleacea*. Mutation of the *NOP1* gene creates an easily scorable and non-lethal phenotype.

Following a phylogenetic analysis, the three main TFs (IPD3, NSP1 and NSP2) in *M. paleacea* were used in complementation assays in Medicago *nsp2* mutants. Expression of *MpaNSP1* in a Medicago *nsp1* mutant rescued its symbiotic defect. By contrast, *MpaNSP2* did not complement the Medicago *nsp2* mutant. To confirm this result, trans-activation of the Medicago *pENOD11* promoter, known to be targeted by these TFs, was conducted in *Nicotiana benthamiana*. Expression of *MtNSP1* and *MtNSP2* strongly activated this promoter whereas expression of *MpaNSP1* and *MpaNSP2* resulted in a weak signal. Combining *MtNSP2* with *MpaNSP1* yielded the same result than the combination of Medicago genes. By contrast, *MpaNSP2* with *MtNSP1* was only weakly active. MpaNSP1 seems fully functional in a nodulation context whereas MpaNSP2 is not.

Objective I2: Assembly of genetic components required for engineering Nod factor signalling.

Because we needed to develop a new liverwort platform that was able to associate with AM fungi, we could not rely on the existing liverwort genome sequences. We have sequenced the genome of *M. paleacea* using paired end libraries and illumina sequencing. This has provided a genome of sufficient quality for our studies in this project. For the 250Mb *M. paleacea* genome we have 27,530 scaffolds and an N50 of 87kb. Clearly with so many scaffolds the genome is still quite fragmented and we are currently exploring using PacBio sequencing as a means to integrate scaffolds. However, our analysis of the existing genome reveals many complete gene models with good levels of promoter sequences attached. Thus for many genes the existing genome provides sufficient resolution.

Phylogenetic analysis revealed the presence of the three main TFs (IPD3, NSP1 and NSP2) in *M. paleacea*. The corresponding coding sequences were synthesized and cloned under the control of a 35S promoter using the Golden Gate system to allow constitutive expression in Medicago roots. Similar constructs with the orthologs from Medicago (Mt) were also generated. The experiments with *M. paleacea IPD3* (*MpaIPD3*) are in progress. Expression of *MpaNSP1* in a Medicago *nsp1* mutant rescued its symbiotic defect. By contrast, *MpaNSP2* did not complement the Medicago *nsp2* mutant. MpaNSP1 seems fully functional in a nodulation context whereas MpaNSP2 is not. To confirm this result, trans-activation of a Medicago promoter known to be targeted by these TFs, *pENOD11*, has been conducted in *Nicotiana benthamiana*. Expression of *MtNSP1* and *MtNSP2* strongly activated this promoter whereas expression of *MpaNSP1* and *MpaNSP2* resulted in a weak signal. Combining *MtNSP2* with *MpaNSP1* yielded the same result than the combination of Medicago genes. By contrast, *MpaNSP2* with *MtNSP1* was only weakly active. These assays in heterologous system confirm that *MpaNSP2* is not functional in a nodulation context. Future engineering efforts in Marchantia will be thus focussed on NSP2.

Since the beginning of the OpenPlant project within the Oldroyd lab, Marchantia as a model system has been further integrated into the Bill and Melinda Gates Foundation Engineering Nitrogen Symbiosis for Africa project, which was recently awarded Phase 2 funding.

Objective I3: Optimisation of gene circuits for synthetic Nod factor signalling in Marchantia.

In parallel to the assays in heterologous systems, promoter:GUS fusion of Medicago promoters known to be targeted by the symbiotic TFs (*pENOD11*, *pNIN*) have been transformed in *Marchantia paleacea*. The transformed thalli are now regenerating. Activation of these promoters will be tested in *Marchantia paleacea* in response to symbiotic signals and to Glomeromycota fungi. We anticipate that *pENOD11* will not be activated because of MpaNSP2. A set of constructs, containing modified version of MpaNSP2 or MtNSP2 have been designed and will be tested in *M. paleacea* transformed *with pENOD11:GUS*. This will be the first step toward engineering nodulation signalling in *M. paleacea*. With the availability of a high-throughput transformation system, other constructs

(combination of TFs, auto-active kinase, auto-active TFs, promoter:reporter) are now going to be tested.

We have also been developing CAS9 knockouts in *M. paleacea*. For this we have designed a number of different constructs to test the efficiency of CAS9 endonucleases in *M. paleacea*. We are using the *NOP1* gene as our target, as used by the Haseloff lab. Successful knock-outs cause loss of air chambers on thalli of *M. polymorpha*, and create an easily scorable and non-lethal phenotype. We have generated a number of constructs that test different promoters to drive Cas9; codon optimisation of Cas9; different U6 promoters and different sgRNA backbones. All constructs have been transformed into *M. paleacea* and efficiency of CAS9-directed knockouts will be assessed. Using this information we will develop symbiotically relevant CAS9-directed mutants.

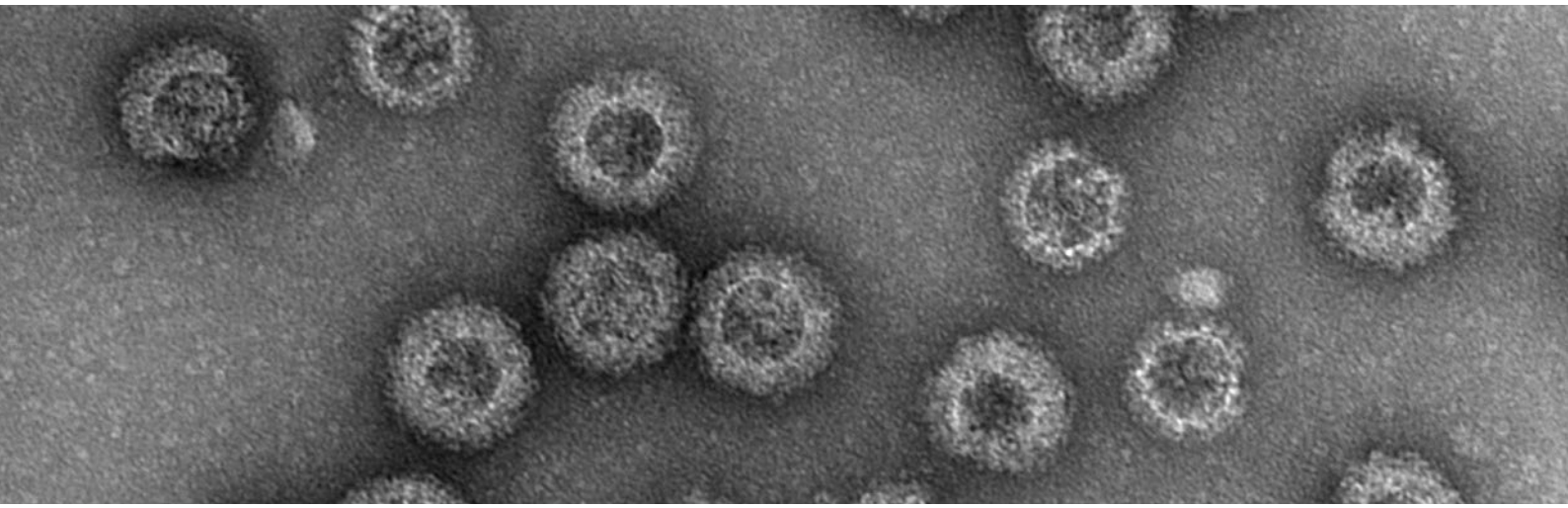
Publications

Carella, P., Gogleva, A., Tomaselli, M., Alfs, C., and Schornack, S. (2017) ***Phytophthora palmivora* establishes tissue-specific intracellular infection structures in the earliest divergent land plant lineage.** *bioRxiv 188912; doi: https://doi.org/10.1101/188912 (Under Review, PNAS)*

Carella P, Schornack S (2017). **Manipulation of Bryophyte Hosts by Pathogenic and Symbiotic Microbes.** *Plant Cell Physiol. doi: 10.1093/pcp/pcx182.*

Delaux PM, Radhakrishnan G, and Oldroyd G (2015). **Tracing the evolutionary path to nitrogen-fixing crops.** *Curr. Op. Plant Biol. DOI:10.1016/j.pbi.2015.06.003*

Delaux PM, Radhakrishnan G, Jayaraman D, Cheema J, Malbreil M, Volkening J, Sekimoto H, Nishiyama T, Melkonian M, Pokorny L, Rothfels C, Sederoff H, Stevenson D, Surek B, Zhang Y, Sussman M, Dunand C, Morris R, Roux C, Wong G, Oldroyd G, Ane JM (2015). **Algal ancestor of land plants was preadapted for symbiosis.** *Proc. Natl. Acad. Sci USA 112: 1339013395*



Workpackage J:
Virus-based systems for bioproduction

The CPMV-HT technology, and its associated pEAQ vectors (Sainsbury & Lomonossoff, 2008; Sainsbury et al., 2009), developed by George Lomonossoff (JIC) has established a unique position for the UK for rapid transient expression of proteins in plants through Agrobacterium-mediated infiltration of Nicotiana benthamiana leaves. The CPMV-HT is a highly flexible system and will be developed for a range of applications in the field of plant synthetic biology.

Workpackage J provides a plant expression technology for use by others within the OpenPlant consortium and throughout the world. The technology is already well integrated and being used in Workpackage H. Work to extend the range of hosts for protein expression is carried out through interaction with Workpackage A.

Investigators
George Lomonossoff; Anne Osbourn

Staff Employed
Eva Thuenemann (PDRA; Lomonossoff Lab). Started Nov 2014; on maternity leave Aug 2016 - Aug 2017.
Miriam Walden (PDRA; Lomonossoff Lab). Feb - Nov 2016.

Partners
LeafSystems® International Limited

J

Online resources:

Lomonossoff lab
(www.jic.ac.uk/directory/george-lomonossoff/)

Leaf Expression Systems
(www.leafexpressionsystems.co.uk)

Milestones:

J1: A series of expression vectors with defined translational characteristics.
Deliverable: Distribution of new viral expression cassettes with fine-tuned levels of translation efficiency (month 24, Lomonossoff, Osbourn).

J2: Modification of the CPMV-HT system to permit expression in alternative hosts.
Deliverable: Distribution of new viral expression cassettes with extended host ranges (month 48, Lomonossoff).

J3: Methods for the delivery of expression vectors to a variety of hosts.
Deliverable: Distribution of new bacterial strains for the intracellular delivery of the viral vectors to a range of new plants hosts (month 60, Lomonossoff).

Progress to date:

The HyperTrans technology developed by the Lomonossoff lab at JIC has proved to be extremely powerful for transient expression of structural proteins in plants and has been used for production of antibodies and antigens, vaccines, empty virus-like particles for nanotechnology applications and human gastric lipase enzyme for use in a model gut system. Workpackage J was originally scheduled to start in year two. However, as projects from several workpackages are heavily using the technology, the start date for the workpackage was brought forward.

Objective J1: A series of expression vectors with defined translational characteristics.

A series of vectors has been made to enable fine-tuning of protein expression levels by making changes in the 5'- and 3'-UTRs (Meshcheriakova et al., 2015). In addition, versions of the CPMV-HT sequences were made compatible with the OpenPlant common plant syntax (Patron et al., 2015) by the Patron and Osbourn groups. These modified vectors were made available to the consortium.

A new synthetic version of the 5' UTR used in HT system has been developed and shown to be twice as effective as the original HT sequence. This work also revealed that the 3' UTR derived from CPMV RNA-2 is probably optimal and difficult to improve upon. The new synthetic version of the HT system will be distributed under an open MTA via OpenPlant.

In collaboration with the Centre for Bioengineering at the Russian Academy of Sciences (CB-RAS), we have developed a new vector system (pEFF) which combines the high translational benefits of the CPMV-HT system with the replication ability of potato virus X (PVX). This system will be extremely useful in cases where virus spread throughout a host is desirable.

The ability to produce defined levels of proteins has been exploited to produce synthetic stabilised virus-like particles (sVLPs) of poliovirus as part of a WHO-funded collaboration involving JIC, University of Leeds, University of Oxford and the National Institute of Biological Standards and Control (NIBSC). The efficient production of sVLPs requires the expression of different amounts the precursor of the structural proteins (P1) and the protease (3CD) necessary for its processing. The resulting plant-produced sVLPs are able to protect model animals against challenge with poliovirus. In a further demonstration of the power of synthetic biology, we have made use of transient expression to produce TMV-based nano-rods of defined length that have the potential to metallised to give nanowires.

Versions of the CPMV-HT system that are compatible with the OpenPlant common syntax have been developed by the Patron and Osbourn groups.

Objective J2: Modification of the CPMV-HT system to permit expression in alternative hosts.

Work is underway to investigate and broaden the range of hosts amenable to protein expression using HyperTrans. The CPMV-HT system has recently been tested in Marchantia (in collaboration with Jim Haseloff's group, Workpackage A) and has been used successfully in the BY2 cell pack system (developed by Fraunhofer Institute, Aachen, Germany) by the Lomonossoff group. The BY2 system in particular has potential for high-throughput screening of CPMV-HT expression constructs. A training workshop will be run at JIC in July 2016 for anyone interested in the system.

The HyperTrans plant expression system has proved to be highly amenable for expression of plant natural product biosynthetic pathways. The Osbourn lab has generated suites of structural variants of triterpenes using this platform in sufficient quantity for structural analysis by NMR and for assays for various applications (including surfactant properties, with industry; also anti-inflammatory/anti-cancer properties).

A training workshop on the BY2 cell pack system ("cookies"; developed by Fraunhofer Institut, Aachen, Germany) was run by the Lomonossoff group in July 2016. This allowed for anyone interested in the system to become familiar with the system.

Staff involved in this workpackage are advising on the commissioning of the translational facility (Leaf Expression Systems®; LES) designed for scaling up production using the HyperTrans system. The LES building was opened on 23/01/17 and is currently being used to scale up production of a number of test molecules.

One of the main applications of the CPMV-HT technology has been the production of synthetic virus-like particles (VLPs). We have now extended this application to situations where no structure of the parent virus particle is available. This has enabled the structure of particles of potato leafroll virus (PLRV) to be solved to near atomic resolution. The ability to produce synthetic VLPs and then solve their structures merely from knowledge of the genomic sequence has huge implications for synthetic biology.

Industry partnership and Commercialisation

George Lomonossoff and Eva Thuenemann were involved in scientific committees advising plans for development of the LES translational facility until the opening of the facility in Jan. 2017. George Lomonossoff continues to act as a consultant to the facility. The Lomonossoff laboratory hosted a visit from Marcus Fehr from BASF, Germany, to evaluate the possibility of transiently producing fungal protein in plants.

The HyperTrans technology is currently still being used under licence by the Canadian company Medicago for production of specific vaccines, particularly against influenza. There have been meetings between Medicago and LES in Norwich regarding progressing various collaborations.

We supply pEAQ vector kits containing the HyperTrans technology to laboratories worldwide under MTAs.

Publications

Brillault. L., Jutras, P. V., Dashti N., Thuenemann E. C., Morgan G., Lomonossoff G.P., Landsberg M. J., Sainsbury F. (2017). **Engineering Recombinant Virus-like Nanoparticles from Plants for Cellular Delivery.** *ACS Nano. doi: 10.1021/acsnano.6b07747.*

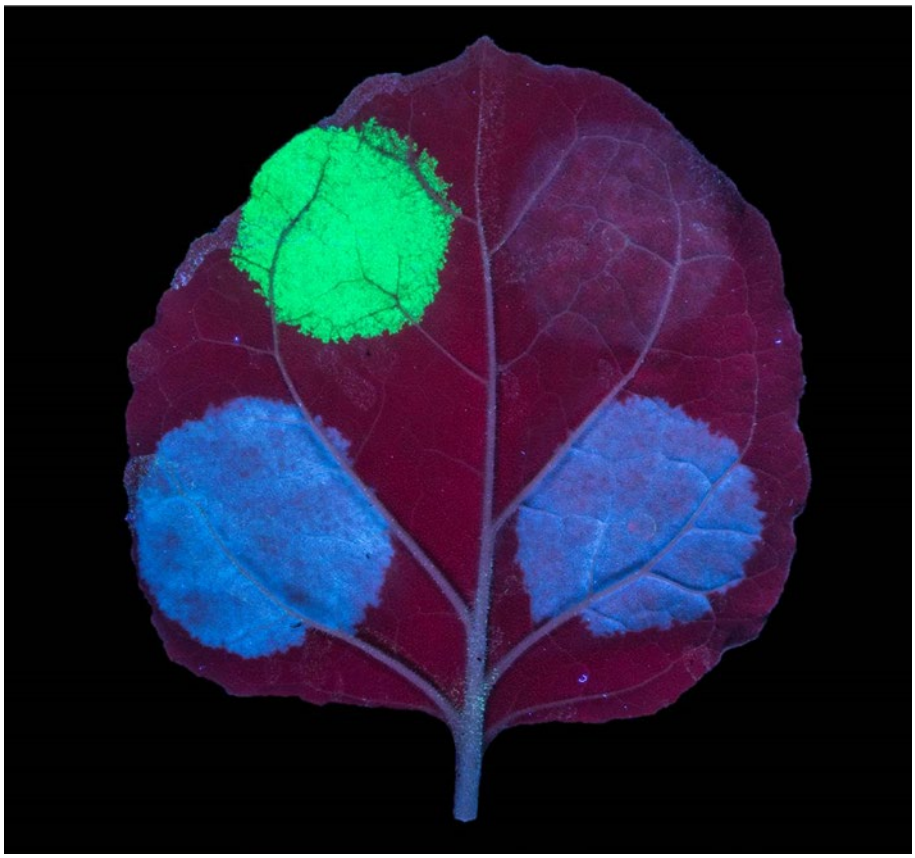
Saxena P., Thuenemann E. C., Sainsbury F., Lomonossoff G. P. (2016). **Virus-Derived Vectors for the Expression of Multiple Proteins Plants.** *"Methods and Protocols" Springer New York Heidelberg Dordrecht London 1385:39-54.*

Lomonossoff G. P. and D'Aoust, M.A. (2016). **Plant-produced biopharmaceuticals: A case of technical developments driving clinical deployment.** *Science 353, 1237-1240.*

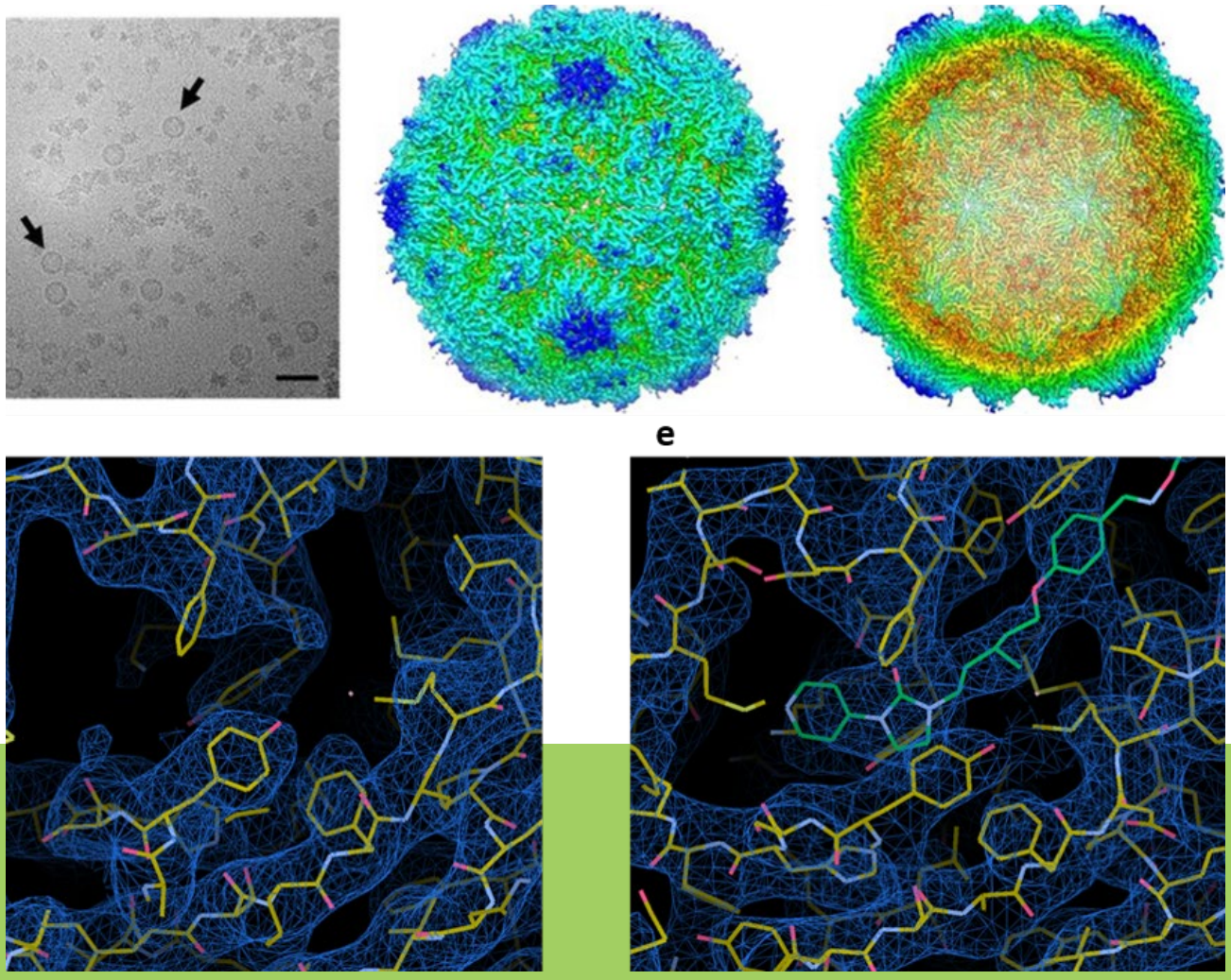
Meshcheriakova et al., (2015). **Fine-tuning levels of heterologous gene expression in plants by orthogonal variation of the untranslated regions of a nonreplicating transient expression system.** *Plant Biotechnology Journal. DOI: 10.1111/pbi.12175.*

Peyret H., Lomonossoff G. P. (2015). **When plant virology met Agrobacterium: the rise of the deconstructed clones.** *Plant Biotechnology Journal 13, 11211135.*

Sainsbury F, and Lomonossoff GP, (2014)**. Transient expressions of synthetic biology in plants.** *Current Opinion in Plant Biology. DOI: 10.1016/j.pbi.2014.02.003.*



Nicotiana benthamiana as a host organism to produce componants of avenacin, a fluorescent antimicrobial produced by oat roots, which protects the roots from soil pathogens. Image: Aymeric Leveau, Osbourn lab.

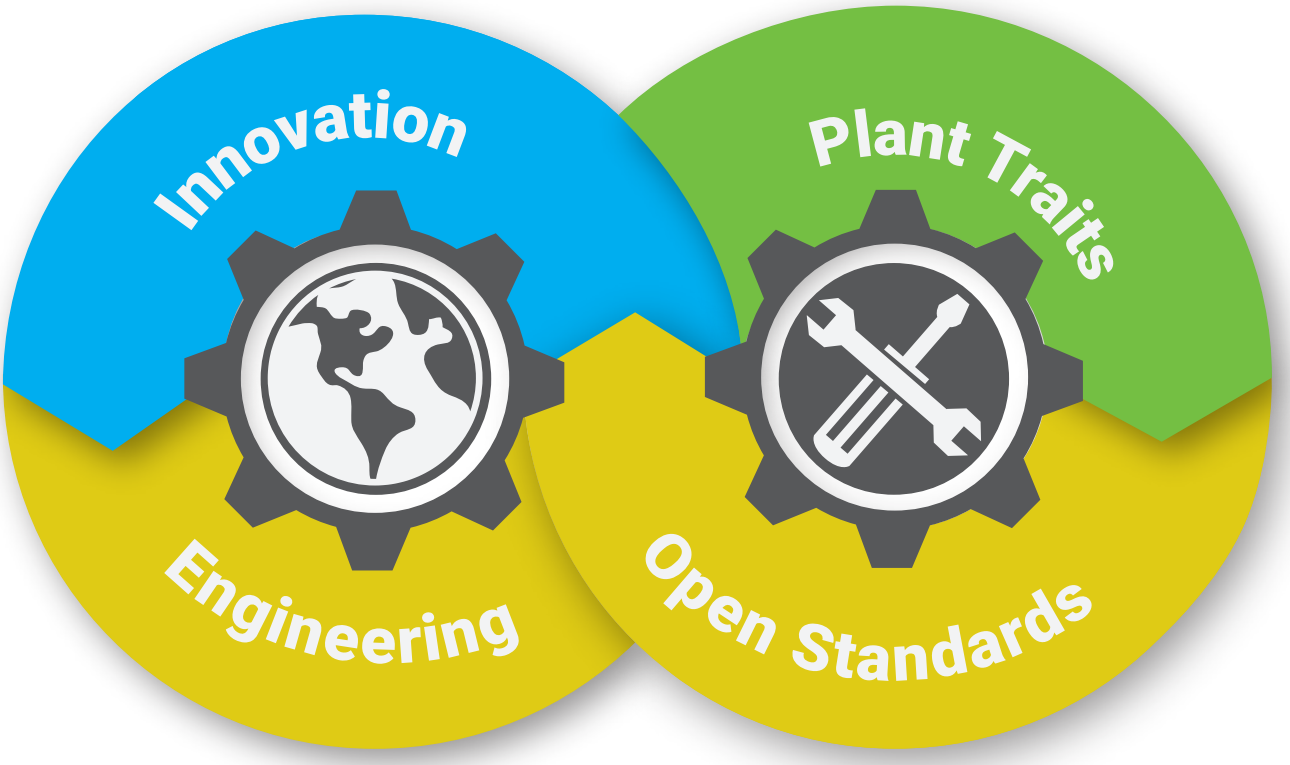


Production of synthetic poliovirus particles in plants

JOHN INNES CENTRE, LEEDS, OXFORD, NIBSC

The Hypertrans® system, developed by Professor George Lomonossoff and Dr Frank Sainsbury at the John Innes Centre, has been extensively used for the rapid transient expression of proteins in plants. As part of a World Health Organisation (WHO) – funded collaboration, we have used this system to produce synthetic non-infectious poliovirus particles for use in the WHO worldwide polio eradication campaign. The synthetic particles consist only of the protein shell and lack the virus nucleic acid; however, to ensure they are stable enough to act as an effective vaccine, it was necessary to first identify mutations in the protein which stabilised the particles. The stabilised proteins were able to form particles in plants that have a structure equivalent to that of natural poliovirus. These particles were able to protect mice against poliovirus and thus have the potential to act as an effective vaccine.

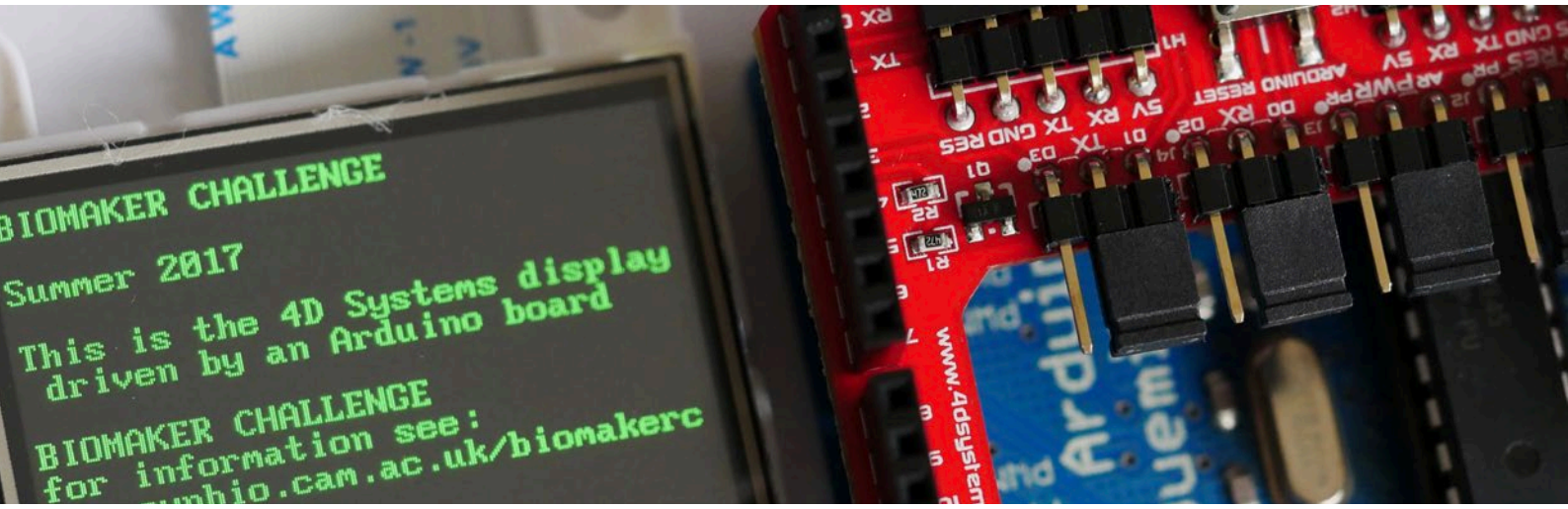
Principal contact: George Lomonossoff



Promoting innovation through sharing

In a third group of workpackages, OpenPlant is promoting responsible research and innovation through interdisciplinarity and frameworks for equitable sharing and global exchange.

Workpackage K: Mini-funds to seed novel interdisciplinary exchange and innovation.
Workpackage L: Outreach activities, training and tools for open exchange of DNA parts and other reagents in biotechnology.
Workpackage M: Project management and communication



Workpackage K:
OpenPlant Fund for interdisciplinary research and exchange

A micro-fund has been established to support seed projects on a competitive basis, to provide small grants to promote a forum for innovation and scientific exchange in the UK. Workers from participating institutions will be invited to complete a short application form and to present their pitches at an open forum. A team of judges will award funds directly. This will create a forum and storefront for ideas in plant synthetic biology. A pilot scheme has been run successfully as part of the Cambridge Nanoforum.

The OpenPlant Fund provides support for projects relevant to all work packages and fosters interdisciplinary exchange within and between the teams working on the different packages at each OpenPlant institution. The open hardware development and training component also especially supports OpenPlant pathways to international exchange.

Investigators
Jim Haseloff, Anne Osbourn

Staff Employed
Colette Matthewman (Project Manager). Started October 2014.
Jenny Molloy (Project Coordinator). Started February 2015.

Partners
Oliver Hadeler, Programme Manager of CamBridgeSens and the Sensor CDT, Department of Chemical Engineering and Biotechnology
Ionscope Ltd (CEO has contributed in-kind technical assistance, participated in judging panel)
Cambridge Consultants (Representative has contributed in-kind technical assistance to teams and participated in judging panel)
Emre Ozer, Principal Research Engineer, ARM, Cambridge
Stefanie Reichelt, Head of Light Microscopy at Cancer Research UK, Cambridge
Alexandre Kabla, Department of Engineering, University of Cambridge
Dan MacLean, The Sainsbury Laboratory, Norwich
Microsoft Research (Representative has contributed in-kind technical assistance to teams and participated in judging panel)
New England Biolabs (offered in-kind support to Biomaker Challenge teams)

K

Milestones:

K1: Annual funding round to support small-scale innovative research projects.
Deliverable: Distribution of awards and public documentation of project results (annually, months 12-60, Haseloff, Osbourn).

K2: Annual support for open source hardware development and training.
Deliverable: Co-sponsorship of student training, and development and documentation of open source hardware and bioinstrumentation (annually, months 12-60, Haseloff, Osbourn).

Online resources:

OpenPlant Fund details
(www.openplant.org/fund/)

Biomaker Challenge
(www.biomaker.org)

Biomaker platform on hackster.io
(www.hackster.io/biomaker/projects)

Code on Github
(github.com/BioMakers)

Progress to date:

Objective K1: Annual funding round to support small-scale innovative research projects.

The OpenPlant Fund was established to support seed projects on a competitive basis through the annual distribution of up to twenty £5000 grants following a lightweight application process and public pitching event. The aim of the fund is to promote the development of plant Synthetic Biology as an interdisciplinary field and to facilitate exchange between The University of Cambridge, the John Innes Centre and The Sainsbury Laboratory for the development of open technologies and responsible innovation in the context of Synthetic Biology. Within in this work package we have aimed to promote open source hardware for science through supporting technical development and also the necessary training required to deliver and implement such hardware in synthetic biology laboratories.

To date, over 60 OpenPlant Fund projects have been funded (sixteen in 2015; fourteen in July 2016; ten in Dec 2016; eleven in Jul 2017, ten? in Dec 2017). Twenty grants were awarded in the latest rounds, following submissions and a series of competitive pitches in July and December 2017. The range of topics of the projects remains broad, covering DNA part development and testing, research method development, cell-free biology, open lab hardware, schools outreach, international capacity building, IP policy, software and more. Project teams are primarily led by graduate and postdoctoral researchers, with a couple of undergraduate-led projects also granted.

In 2015, the OpenPlant Fund attracted sixteen proposals, all of which were successful. The topics ranged from DNA part development to open lab hardware, schools outreach, international capacity building, IP policy, software and more. All teams are multidisciplinary and span Cambridge and Norwich. The teams were supported prior to making their applications by mixer events with lightning talks, pitch training and networking opportunities combined with substantial effort on the part of the management team in making connections and linking collaborators.

Fourteen proposals were funded in the 2016 OpenPlant Fund calls. The topics ranged from DNA part development to open lab hardware, schools outreach, international capacity building, IP policy, software and more. All teams are multidisciplinary and span Cambridge and Norwich. The teams were supported prior to making their applications by mixer events with lightning talks, pitch training and networking opportunities combined with substantial effort on the part of the management team in making connections and linking collaborators.

The management team made a decision to run future rounds as themed opportunities in order to more effectively seed interactions around the key tools, technologies and outreach initiatives required for plant synthetic biology. Ideas for focus areas were collected at the All-Hands Meeting in May 2016 and include: Imaging, Microfluidics, Functional materials and Education and public engagement. These formed the basis for one-day mixer events featuring time for presentations from a diverse range of potential collaborators, followed by brainstorming time with the intention that teams coalesce around ideas and develop them into proposals for submission several weeks afterwards.

In 2017, we have been actively encouraging applications related to use of cell-free extracts from bacteria, plants, yeast or other organisms to transcribe and translate engineered DNA. Cell-free synthetic biology is gaining popularity for prototyping genetic circuits and metabolic pathways and has many applications from production of biologics to paper-based diagnostic tests and biosensors. Cell-free systems also offer opportunities for low-cost curriculum development, negating the issues of GM licensing in teaching environments. To date we have funded five projects related to cell-free synthetic biology.

To date, the OpenPlant Fund has supported 61 interdisciplinary and cross-institute projects (sixteen in Jul 2015; fourteen in Jul 2016; ten in Dec 2016; eleven in Jul 2017; eleven in Dec 2017). These projects range from DNA part development and testing, research method development, cell-free biology, open lab hardware, schools outreach, international capacity building, to IP policy, software and more. Half of all applicants are post-docs and over a third are PhD students. The projects are multidisciplinary, often building new collaborations between Cambridge and Norwich, and with external partners including companies, institutes and universities from the UK and abroad. A number of the OpenPlant Fund projects have already openly shared their tangible outputs in the form of new technologies and publications.

Objective K2: Annual support for open source hardware development and training.

OpenLabTools in the University of Cambridge Engineering Department has been sponsored to offer open biohardware related summer projects to engineers and these placements are currently underway. Further training and development is taking place via the iGEM teams in both Cambridge and Norwich. Both are facilitated by OpenPlant-supported labs and OpenPlant has provided £10k in sponsorship to the Cambridge-JIC team to enter the biohardware track.

Outside of the specific deliverables for this work package, a Cambridge-Norwich team of graduate students and postdocs are organising an open labware competition for 2016 that will provide 3D printers to winning labs, thus increasing capacity for rapid prototyping and distributed manufacture of open hardware within OpenPlant. Discussions are underway with several individuals and organisations regarding synthetic biology training and links with countries in the global South, where it is felt that open hardware and tool development could provide a key point of collaboration and a pathway to international exchange.

(i) Cambridge-JIC iGEM 2015 team
The 2015 team project was entitled ‘OpenScope’ - an open source microscope.

The team undertook a two week crash course in Plant Sciences featuring lectures and activities with faculty from Cambridge and Norwich, including OpenPlant PIs Dr. Jim Ajioka (Pathology & Arsenic Biosensor, University of Cambridge), Dr. Jim Haseloff (Plant Sciences & OpenPlant, University of Cambridge), Dr. George Lomonossoff (JIC) and Dr Nicola Patron (TSL Norwich). Inspired by a 3D printed microscope designed by Dr Richard Bowman (Department of Physics, University of Cambridge), they chose to build an ultra low-cost 3D-printed motorised web-streaming autofocus microscope for synthetic biologists,. This allows researchers to tailor the microscope to their needs - allowing imaging on any lab-bench, in the incubator, fume-hood or the field using remote access and battery power, or use many OpenScopes for parallel rapid preliminary screening. The microscope offers fluorescence imaging for schools and laboratories with small budgets, based on low-cost (£200 in its most expensive set-up) and easily sourced components.

Functionalities developed include Brightfield (max. 0.4 µm optical resolution, 1 µm movement resolution in xy-plane), Fluorescence (qualitative, GFP imaging) and Darkfield. The stage of the microscope can be translated using 3 stepper motors, which in turn can be controlled via the Arduino using WebShell. OpenScope is internet accessible from anywhere in the world via WebShell and MicroMaps, which also allows unambiguous and accurate distance measurements anywhere within its field of view, easy high quality image and time-lapse capture at any time. An ImageJ plugin was developed to combine the power of ImageJ's annotation tools with the simplicity of OpenScope.

OpenScope was awarded a gold medal at the iGEM Giant Jamboree in Boston, and was nominated by the judging team for four awards: Best Hardware Project, Best Software Tool, Undergrad, Best Applied Design, Undergrad and Best Poster, Undergrad.

A working set-up of OpenScope and the software was demonstrated to huge interest from other teams and supervisors. A number of team supervisors mentioned that they were considering using OpenScope as an alternative to commercial microscopes in their teaching laboratories, both to reduce costs and as setting up the microscope would be an educational experience in itself.

(ii) The 2016 Cambridge-JIC iGEM team, supported by OpenPlant and Cambridge Consultants, built a toolbox for chloroplast transformation in the microalgae Chlamydomonas reinhardtii (Chlamy) by targeting all the transformation steps for improvement. They built a library of tested parts optimised for Chlamydomonas chloroplasts, or those of related species, built a low-cost gene gun and multifunctional incubator for their cells, and modelled the behaviour of transformed systems. Finally, they designed a wet lab tool which could help achieve essential homoplasmy (transformation of all copies of chloroplast DNA) in one generation instead of 2-3 months of selection (http://2016.igem.org/Team:Cambridge-JIC). The 2016 Cambridge-JIC iGEM team, InstaCHLAM, were awarded a gold medal at the iGEM Giant Jamboree in Boston, and were awarded a gold medal , and won the Best Plant Synthetic Biology prize.

(iii) In 2017, we launched a new programme, called the Biomaker Challenge. This programme is co-funded by OpenPlant and the Isaac Newton Trust, and coordinated by OpenPlant, the Synthetic Biology SRI and CamBridgeSens. The Biomaker Challenge is a four-month programme challenging interdisciplinary teams to build low-cost sensors and instruments for biology. Successful applicants receive a Biomaker Toolkit and a discretionary budget for additional sensors, components, consumables and 3D-printing worth up to £1000. The challenge has been established to encourage frugal, open source and DIY approaches to biological experiments. There was a huge amount of interest in the Challenge, and we funded 41 projects in this first year. All teams are expected to exhibit their device at a Biomaker Fayre on Saturday 21 Oct 2017 as part of Cambridge Open Technology week.

Outputs from the OpenPlant Fund and Biomaker Challenge projects are now being published to the website www.biomaker.org. This website brings together information and outcomes of projects from the OpenPlant Fund, Biomaker Challenge and the previous Cambridge Synthetic Biology Fund. We have also established a Biomaker web channel at https://www.hackster.io/biomaker/projects for simpler and more accessible documentation of projects.

Publications

Juhas M, Ajioka JW, (2017). **T7 RNA polymerase-driven inducible cell lysis for DNA transfer from Escherichia coli to Bacillus subtilis.** *Microb Biotechnol.; 10(6):1797-1808. doi: 10.1111/1751-7915.12843.*

Nuñez I, Matute T, Herrera R, Keymer J, Marzullo T, Rudge T, Federici F (2017). **Low cost and open source multi-fluorescence imaging system for teaching and research in biology and bioengineering.** *PLOS One* https://doi.org/10.1371/journal.pone.0187163

Sotta, N., Duncan, S., et al. (2017). **Rapid transporter regulation prevents substrate flow traffic jams in boron transport.** *eLife; 6:e27038. DOI: 10.7554/eLife.27038*

Yu, Z., Boehm, C.R., Hibberd, J.M., Abell, C., Haseloff, J., Burgess, S.J., Reyna-Llorens, I., (2017). **Droplet-based microfluidic analysis and screening of single plant cells.** *BioRxiv pre-print: doi: https://doi.org/10.1101/199992*

Juhas M, Ajioka JW. (2016). **Lambda Red recombinase-mediated integration of the high molecular weight DNA into the Escherichia coli chromosome.** *Microb Cell Fact. 5;15(1):172.*

Juhas M, Ajioka JW. (2016). **Integrative bacterial artificial chromosomes for DNA integration into the Bacillus subtilis chromosome.** *J Microbiol Methods 125:1-7. doi: 10.1016/j.mimet.2016.03.017.*

Duncan, S et al. (2016). **A method for detecting single mRNA molecules in Arabidopsis thaliana.** *Plant Methods 12:13. DOI: 10.1186/s13007-016-0114-x*

Liddicoat, J. and Liddell, K. (2016). **Open Innovation with Large Bioresources: Goals, Challenges and Proposals.** *https://papers.ssrn.com/sol3/papers.cfm?abstract_id=2888871*

Synthetic Biology meets the real world

OpenPlant funds interdisciplinary team-based projects that explore the intersection of electronics, 3D printing, sensor technology, low cost DIY instrumentation and biology - and policy workshops and outreach events. These projects aim to build open technologies and promote development of research skills and collaborations. They tap into existing open standards and a rich ecosystem of resources for microcontrollers, first established to simplify programming and physical computing for designers, artists and scientists. These resources provide a simple environment for biologists to learn programming and hardware skills, and develop real-world laboratory tools. Further, the OpenPlant projects provide a direct route for physical scientists and engineers to get hands-on experience with biological systems, and we are developing low-cost open reagents and protocols for easier access to cell-free DNA programmable systems.

Enabling the innovators

History
Since 2014, we have funded small interdisciplinary projects and catalysed new collaborations between several hundred students, researchers and academics across Cambridge, Norwich and beyond. A listing of recent OpenPlant projects is provided here. A more comprehensive collection of information can be found at www.biomaker.org. The projects have generated a large number of electronic prototypes, software, 3D printed devices and biological elements. We hope that these resources prove useful and can be built upon by others, especially to initiate new low-cost approaches to quantitative biology and engineering for teaching and research.

SynBio Strategic Research Initiative Fund
The University of Cambridge SynBio Fund supported eighteen innovative, open and interdisciplinary projects relevant to Synthetic Biology over 2015-16. The aim of the fund was to promote the development of Synthetic Biology as an interdisciplinary field at the University of Cambridge. (www.synbio.cam.ac.uk)

OpenPlant Fund mini-projects
OpenPlant Fund aims to promote the development of plant Synthetic Biology as an interdisciplinary field and to facilitate exchange between The University of Cambridge, the John Innes Centre and the Earlham Institute for the development of innovative and open projects relevant to plant Synthetic Biology, and responsible innovation and outreach in this context. The projects receive £4000 over six months, with an additional £1000 for outreach or follow-on work after reporting on their progress. Funds are managed through a cost centre managed by a faculty sponsor, to help manage integration of the project with existing research loads. All outputs of the projects are open and shared.
(www.openplant.org/fund)

Biomaker Challenge micro-projects
Starting in Summer 2017, the Biomaker Challenge is a four-month programme challenging interdisciplinary teams to build low-cost sensors and instruments for biology. From colorimeters to microfluidics and beyond, we're looking for frugal, open source and DIY approaches to biological experiments. Participants receive a £250 Biomaker Starter Kit and a discretionary budget for additional sensors, components, consumables and 3D-printing worth up to an additional £750.

Up to 50 grants are awarded annually and all teams exhibit their device at a public Open Technology event and Biomaker Fayre in October.

The Biomaker Challenge leverages additional support from the University of Cambridge Research Policy Committee through the Synthetic Biology Strategic Research Initiative and CamBridgeSens Strategic Research Network. We are actively promoting wide participation both within Cambridge and Norwich, and with external partners - including international collaborations with individuals, companies and institutions. In particular, the new Biomaker Challenge has been designed to be easily portable between institutions and open to industrial collaboration. (www.biomaker.org)

Cell-free gene expression
We are encouraging applications related to use of cell-free extracts to transcribe and translate engineered DNA. Cell-free synthetic biology is gaining popularity for prototyping genetic circuits and metabolic pathways and has many applications from production of biologics to paper-based diagnostic tests and biosensors.

Innovative projects
OpenPlant funding has proved to be a highly effective way of providing key support for independent small projects and promoting valuable new collaborations among young researchers, along with the development and documentation of open source biology, hardware and bioinstrumentation. In a short period of time, we have seen some notable outcomes. For example, our funds have provided seed money for the evolutionary development of 3D printed microscopes across several projects: "Open source 3D-printed microscope", Richard Bowman, Stefanie Reichelt, Hugh Matthews & Jeremy Baumberg, £5K; "High Performance Mechanisms for Low Cost Science", Richard Bowman, Stefanie Reichelt, Hugh Matthews, £5K; "OpenScope", Cambridge-JIC iGEM2015 team, £10K; and "OpenScope", SynBio Student Society, £5K. These early projects promoted experimentation with a novel, clever and open design. This has subsequently mutated into a family of 3D printed microscopes, optical devices and accessories - and found global use in community labs, schools, social enterprises and research labs.

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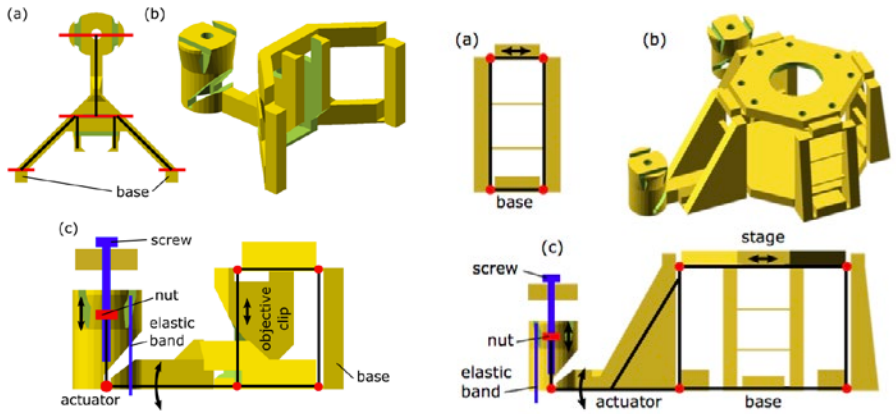

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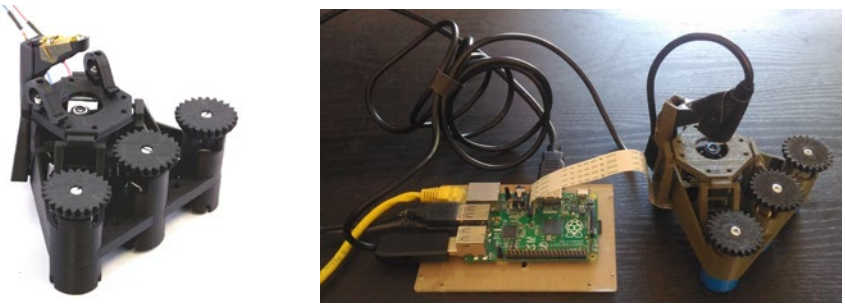

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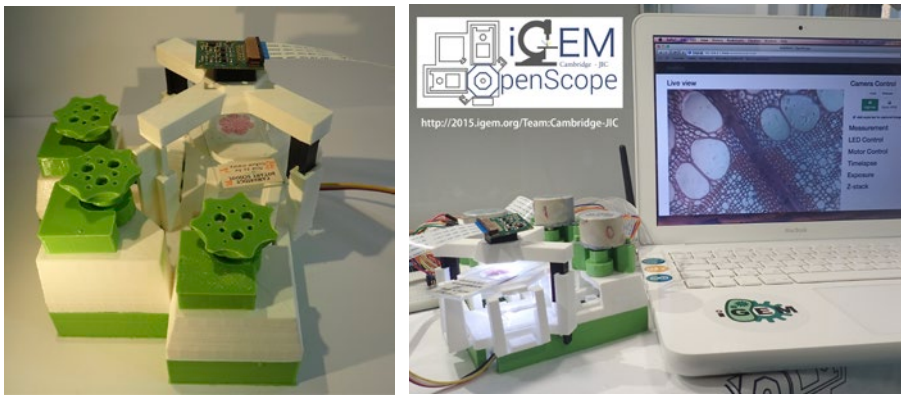

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The current version can be found at https://github.com/rwb27/openflexure_microscope.

An example: evolution and spread of open technology

OpenFlexure 3D printed microscope

1. Richard Bowman and colleagues in Cambridge develop a design for a monolithic, 3D printed microscope stage, based on a novel plastic flexure translation stage. This design and its implementation is later published as: *A one-piece 3D printed flexure translation stage for open-source microscopy* JP Sharkey, DCW.Foo, A Kabla, JJ Baumberg, and RW Bowman. **Review of Scientific Instruments** 87, 025104 (2016).

2. The stage design is complemented by low cost Raspberry Pi board and Pi camera with inverted lens to build low cost inverted video microscopes, in the UK and beyond, eg. Public Labs in the US (https://publiclab.org/notes/mathew/04-17-2016/making-an-openflexure-microscope).

3. The Cambridge-JIC iGEM2015 team builds an upright version of the microscope in consulation with Richard. They add motorised focus and translation, and develop a software package for remote image collection and data processing (http://2015.igem.org/Team:Cambridge-JIC)

4. Richard Bowman, Alex Patto and colleagues develop an award-winning social enterprise, WaterScope, around use of the low-cost microscope for rapid automated screening of mini-colonies from water-bourne bacterial contamination. The microscope technology is made freely available. Microscopes are printed in Africa (http://www.waterscope.org).

5. Richard Bowman continues to develop the microscope optics, producing versions that incorporate low-cost, high-performance objectives and cheap tube lens.

Versions of the OpenFlexure microscope are being built and modified worldwide. The stage design is modular and has been used for fibre optic alignment, and integrated into other microcope designs..

OpenPlant Fund case studies: Outreach, Policy and RRI

Strengthening synthetic biology capacity in Africa

Richard Smith-Unna (UCam), Dr Vicky Schneider (Earlham Institute), Dr Jelena Aleksic (TReND), Richard Pilling (Intel), Dr Chinyere Okoro (Sanger Institute), Dr Ibukun Akinrinade (University of Bingham, Nigeria), Dr Vicky Schneider (Earlham Institute)

Two OpenPlant Funds were awarded to support the activities of TReND Africa in developing synthetic biology capacity through their ongoing training activities in Kenya and Nigeria.

One grant supported a bioinformatics training workshop that ran from 30th November to 5th December 2015 for 37 students from nine African countries. The course was held at ICIPE in Nairobi, Kenya and involved six days of theory and practical work, starting from the principles of Unix and programming, through to advanced scientific programming and visualisation. Towards the end of the week students worked on specific analysis methods in various areas of genetics and genomics, with a special focus session on synthetic biology delivered by Richard Smith-Unna. An ongoing student-led study group, coordinated online, will help the students keep the momentum from the course going and the course was also repeated with a new cohort in 2016. The course materials were made freely available online.

Dr Jelena Aleksic explained why such courses can have such an enormous impact in Africa “In an environment where all scientists above undergraduate level are expected to lecture regularly, the impact of advanced training courses quickly goes beyond the original participants. All our students hold Masters qualifications or above and work at African research institutions. We estimate that each of our course attendees will have the chance to pass on some of those skills to an additional 200 colleagues within the first year alone, and many more on an ongoing basis from there.”

The second grant supported setting up an open synthetic biology lab in Abuja, Nigeria. The team were able to develop a synthetic biology lab in Bingham University, Abuja, Nigeria by collecting over 550 kg of equipment donations from Institutes in Switzerland and the UK and shipping to Nigeria in May 2016. This included molecular biology equipment such as a PCR machine, centrifuges and consumables which enabled a course to be run in January 2017 providing a robust introduction to molecular biology and gene editing techniques including cloning, CRISPR, DNA, RNA and protein methods. The course also included a Science Policy Lecture supported by the European Molecular Biology Organisation.

Exploring the boundary of science, art and design

Co-lab: Dr Paolo Bombelli (Department of Biochemistry, University of Cambridge), Dr Paloma Portela Torres (UCL), Lena Asai (Goldsmiths, London), Juan Manuel García Arcos (CRI, Paris), Ke Fang (CRI, Paris) and 3D models of viruses: Vanessa Bueno (Earlham Institute, Roger Castells Graells (JIC), Elisabeth Gill (Engineering, UCam), Charlie Owen (JIC)

Co-Lab (meaning “collaboration” in Japanese) is a hands-on workshop, facilitated by designers and researchers, aiming to foster conversation and interdisciplinarity in biology. The workshops brings artists, designers and scientists to meet and initiate conversation to explore the possibilities of collaboration in biological design.

This 5th edition of Co-Lab workshop was themed around plant science and synthetic biology and was hosted in Cambridge and Norwich, including Makespace Cambridge, the Department of Plant Sciences and the John Innes Centre. It was facilitated by Open Science School, a non-profit organization based in the Center for Research and Interdisciplinarity of Paris and was held in collaboration with Doing It Together Science (DITOs), an EU citizen science project.

The Co-Lab aimed to spur discussion of plant synthetic biology from an ethnographic point of view and consisted of three ideation workshops and a ‘Big Making Days’ prototyping workshop where teams worked on interdisciplinary projects around synthetic biology and engineering life. The programme included pigment extraction, making electricity with plants (hosted by Dr Paolo Bombelli), Ethnography activity, and series of participatory lectures. The workshops collectively attracted over 50 attendees from a wide variety of backgrounds.

One project conceived at the event was successful in applying for its own OpenPlant Fund to develop accessible 3D models of molecules for schools. The VRICKS team created kits of 3D models of molecules for schools and outreach activities to facilitate the understanding of viral structures, polymers and synthetic biology projects. The kits included complete structures and also pieces to be assembled as 3D puzzles and will be a tool for teachers and researchers to teach about their subject in an interactive manner.

3D printed models of viruses have been distributed among scientists and teachers from UK, Germany, Spain, Jordan and Kenya and the project team is receiving requests from scientists and teachers to produce more models that include, for example, viruses, proteins, nanoparticles, self-assembly models and bacteria. In June 2017, Roger Castells Graells received an UEA Engagement Student Award for outstanding contribution to Public & Community Engagement, including this OpenPlant Fund project and has used the models to illustrate talks and posters at five scientific conferences.

https://www.biomaker.org/projects/co-lab-openplant-interdisciplinary-workshops

OpenPlant Fund case studies: Outreach, Policy and RR

Synthetic biology for schools

Dr Jenni Rant (The SAW Trust), Dr Tim Rudge (Universidad Catolica, Chile), Tim Marzullo (Backyard Brains, Inc), Juan Keymer (Universidad Catolica, Chile), Nadia Radzman (John Innes Centre), Dr Colette Matthewman (John Innes Centre), Samantha Fox (John Innes Centre), Lawrence Pearce (John Innes Centre), Dr Nicola Patron (Earlham Institute), Dr Fernán Federici (University of Cambridge/ Universidad Catolica, Chile), Lalitha Sundaram (Department of Pathology, University of Cambridge), Dr Steven Burgess (Department of Plant Sciences, University of Cambridge), Dr Ben Miller (School of Biological Sciences, University of East Anglia)

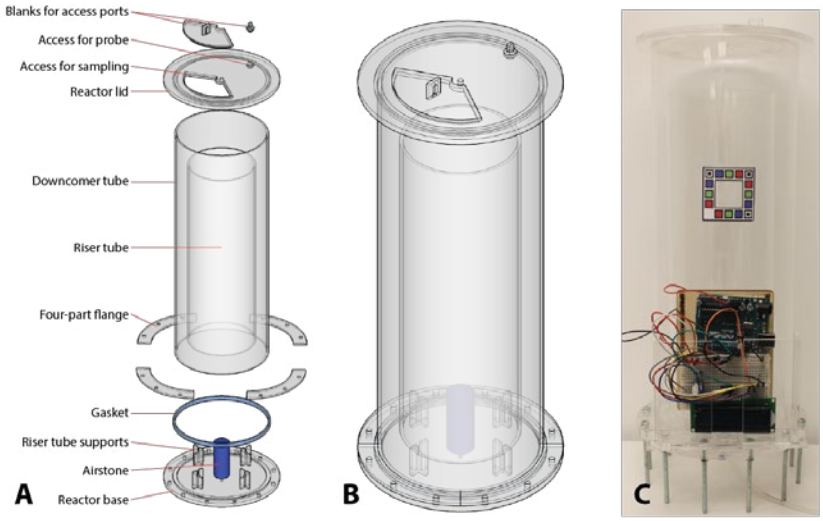
The synthetic biology community in Norwich and Cambridge are working on several ideas for developing educational materials, tools and practicals to bring multidisciplinary science and synthetic biology into schools. To increase their overall impact, we propose to create a complete package of activities, supporting information and hardware that can be successfully used in schools to introduce synthetic biology with a focus on plant chassis, and to provide learning opportunities across a wide range of disciplines. Our intention within the scope of this project was to target the resources for local schools, but we are looking for national and international opportunities for dissemination.

The Big Algal Open Experiment

Dr Paolo Bombelli (Biochemistry, University of Cambridge), Dr Brenda Parker (Biochemical Engineering, UCL), Dr James Lawrence (Biochemical Engineering, UCL), Marc Jones (PhD student in Computational and Systems Biology, John Innes Centre)

Algae are amazing: they recycle over half of the carbon dioxide we exhale, and form the basis of many food chains, yet we still understand very little about how they grow. In future, we may wish to cultivate algae for food, fuel, or to clean up wastewater so we need to understand more about their biology! The Big Open Algae Experiment team aim to help us enhance our algal knowledge by performing the biggest parallel algae experiment in history. They are inviting universities and citizen scientists to participate in an open-source data collection experiment on outdoor microalgal growth.

Up and down the UK, they'll be running experiments using a bioreactor they designed and asking people to submit their recordings of how well the algae are growing. Following and recording the algal growth will be easy and fun. This is thanks to a smart-phone app: the Alg-app. The Alg-app will enable everyone having access to a smartphone to get involved. During the OpenPlant Fund project, low-cost bioreactors, the website and app were constructed (http://bigalgae.com/about). In this last two years we have been running the "Big Algae Experiment" and have interacted with school groups at Latitude Festival, the New Scientist Festival, Rugby School and through events in Cambridge. Big Algae was showcased at the CRI, Paris and at the Open Source Tech conference in Santiago, Chile. As part of UCL Engineering's programme of CPD activities for teachers, we have also organised sessions with STEM teachers to demonstrate the bioreactors and train them in how to run the experiments. The concept is being developed into an 'Algaegotchi' pet with the Iaac Advanced Architecture Group in Barcelona.



Report Summaries

Responsible Innovation and Open innovation with Large BioResources: Goals, Challenges and Proposals
Dr Kathy Liddell (Centre for Law, Medicine and Life Sciences, Faculty of Law, University of Cambridge), Dr John Liddicoat (Centre for Law, Medicine and Life Sciences, Faculty of Law, University of Cambridge), Dr Rob Doubleday (Centre for Science and Policy), Dr Nicola Patron (Earlham Institute).
On 28 January 2016, the Centre for Law, Medicine and Life Sciences together with the Centre for Science and Policy, and OpenPlant hosted a workshop on responsible and open innovation with large bio-resources. Discussions were stimulating and highlighted the different approaches taken by the two fields to policies of openness. The outcomes have since been published as an Open Access report on SSRN (https://ssrn.com/abstract=2888871).

Workshop on Genetic resources in the age of the Nagoya Protocol and gene/genome synthesis (ongoing)
Professor Jim Haseloff (University of Cambridge), Dr Dominic Berry (University of Edinburgh), Dr Deborah Scott (University of Edinburgh)
The ongoing improvement of gene and whole genome sequencing and synthesis technologies presents possibilities of new practices, and demands discussion and debate in light of the long history of global bioresource management. This workshop in November 2016 acted as a venue for collecting information on current developments, sharing views, highlighting potential areas of concern, and establishing grounds upon which to build better understanding of the interactions between and implications of the Nagoya Protocol and gene synthesis for collection, circulation, and use of genetic resources. A report is in preparation.

Ongoing Projects

Developing teaching resources for rapid, open and combinatorial genetic circuit fabrication in cell-free systems
Fernan Federici (Plant Sciences, UCam and PUC, Chile), Nicola Patron (Earlham Institute), Bernardo Polak (Plant Sciences, UCam)

Developing Cell-Free Genetic Circuits and their Electronic Counterparts as Educational Tools for SynBio Students
Cambridge University Synthetic Biology Society

Plug and play synthetic biology education resource
Dr Katia Smith-Litière (Biomakespace), Dr Payam Mehrshahi (Plant Science, UCam), Patrick Hickland (Plant Science, UCam), Tony Naggs (Biomakespace), Marek Balint (Biomakespace), Roger Mason (Biomakespace)

Focus Stacking for Teaching and Publication in Plant Sciences
Jennifer Deegan (Plant Sciences, UCam), Richard Mortier (Computer Science and Technology, UCam, Tim Deegan (Computing industry), Christopher Whitewoods (JIC), Matthew Couchman (JIC), Aleksandr Gavrin (SL, UCam)

Open Source Resources for Teaching Synthetic Biology in Low-Resource Settings
Sabrina Gonzalez-Jorge (Plant sciences, UCam), Hans Pfalzgraf (UEA and JIC), Alexis Moschopoulos (University of Leeds), Aseda Addai-Deseh (Kumasi Hive), Anna Lowe (Kumasi Hive)

Accessible 3D Models of Molecules
Vanessa Bueno (Earlham Institute, Roger Castells Graells (JIC), Elisabeth Gill (Engineering, UCam), Charlie Owen (JIC)

OpenPlant Fund case studies: Hardware

Whiskeroscope: rodent whisker inspired sensor for use in analysis of plant tissue structure

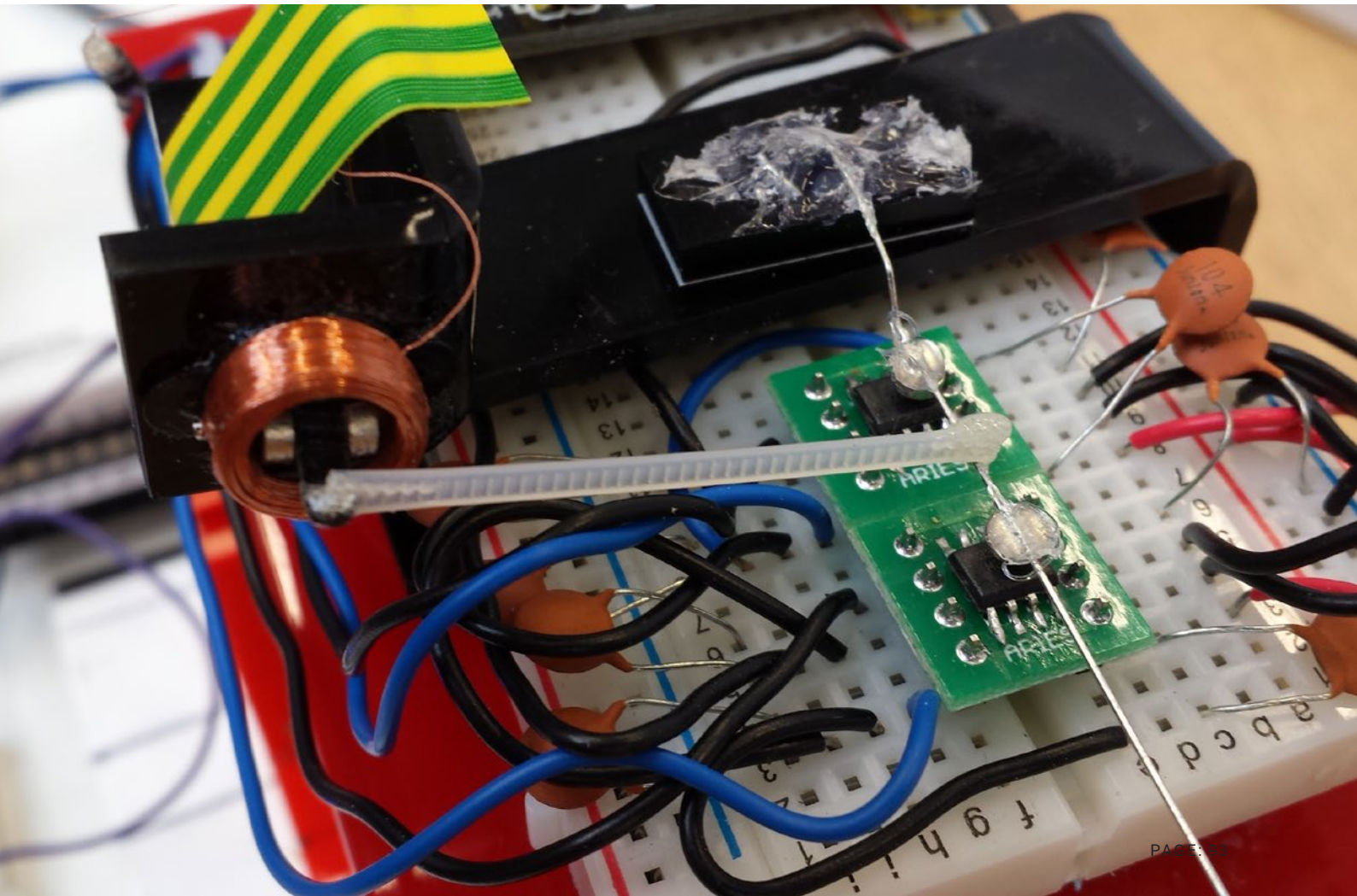
Jan Lyczakowski (Department of Biochemistry, University of Cambridge), Abhimanyu Singh (Independent, previously Department of Engineering, University of Cambridge). Christie Nel (Independent, previously Stellenbosch University)

Understanding mechanical properties of plant biomass is crucial for multiple industries, including building construction and production of lignocellulosic biofuels. Current methods to analyse mechanical properties of biomass are slow and provide little accuracy. The aim of the project was to develop a prototype of a novel type of mechanical sensor which addresses challenges outlined above and required a range of skills. Team member Jan Lyczakowski reported “It has been great working with an engineer and an informatician to design, construct and optimise a device relevant to my research.”

The OpenPlant Fund has allowed me and my collaborators to engage in an exciting, interdisciplinary project.. In addition to the financial support, the OpenPlant has provided much guidance on how to structure and develop our project.

The device is inspired by rodent whiskers and relies on two inputs, obtained using thin steel rod, to quantify stiffness. During each measurement the primary, macromotion, dataset is obtained by analysing the extent to which the whisker bend during the contact with the material. Additional information is obtained by overlaying the macromotion data with the impact of the whisker contacting the material on its micro-oscillation. The instrument successfully discriminated between materials with unlike mechanical properties (steel and foam) and differently aged stem samples from willow.

Whiskeroscope was also applied to study *Arabidopsis thaliana* stems with altered composition of secondary cell walls. The project and the background information on plant cell walls were demonstrated to the wider public as Jan Lyczakowski explains: “Thanks to participating in the OpenPlant Fund we were also given an opportunity to showcase our project during the Cambridge Science Festival. It was really great to demonstrate our prototype to the public and discuss how by analysing and modifying plant biomass we can generate sustainable and renewable biomaterials.”



OpenPlant Fund case studies: Hardware

An open source autonomous imaging station for high schools, universities, and emerging DIY scientific communities

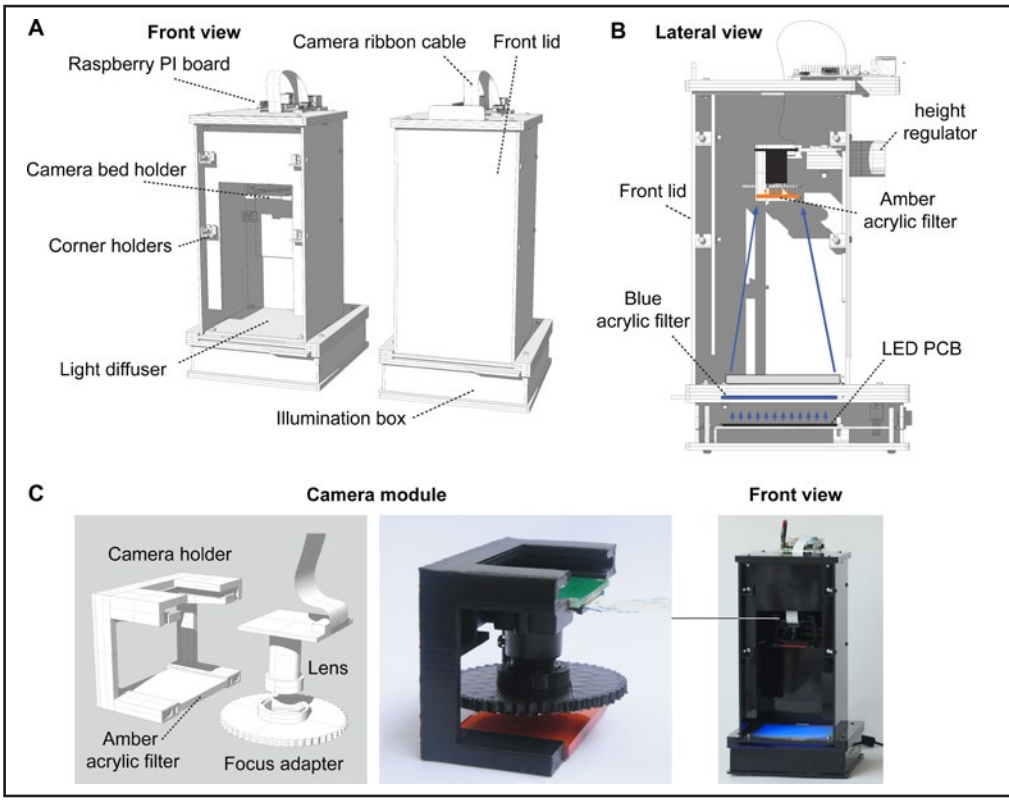
Fernán Federici (University of Cambridge/Universidad Catolica, Chile), Neil Pearson (Earlham Institute), Tim Rudge (Department of Engineering, Universidad Catolica, Chile), Tim Marzullo (Backyard Brains, Inc), Juan Keymer, (Universidad Catolica, Chile)

The advent of easy-to-use open source microcontrollers, off-the-shelf electronics and customizable manufacturing technologies has facilitated the development of inexpensive scientific devices and laboratory equipment. The team developed and published (Nuñez et al., 2017) an imaging system that integrates low-cost and open-source hardware, software and genetic resources. The multi-fluorescence imaging system consists of readily available 470 nm LEDs, a Raspberry Pi camera and a set of filters made with low cost acrylics. This device allows imaging in scales ranging from single colonies to entire plates.

The team also developed a set of genetic components (e.g. promoters, coding sequences, terminators) and vectors following the standard framework of Golden Gate, which allowed the fabrication of genetic constructs in a combinatorial, low cost and robust manner. In order to provide simultaneous imaging of multiple wavelength signals, they screened a series of long stokes shift fluorescent proteins that could be combined with cyan/green fluorescent proteins for 3-channel fluorescent imaging. Open source Python code was developed to operate the hardware to run time-lapse experiments with automated control of illumination and camera and a Python module to analyze data and extract meaningful biological information. To demonstrate the potential application of this integral system, the team tested its performance on a diverse range of imaging assays often used in disciplines such as microbial ecology, microbiology and synthetic biology.

Isaac Nuñez appreciated the opportunity to work on the project with the support of OpenPlant: “OpenPlant funds were important because we are generating a real impact in research and teaching through interdisciplinarity. This project not only introduced us to new modes of work based on good practices, documentation and open source licensing but also allowed us to learn from different fields such as open hardware, design, FOSS and advanced DNA fab methods.”

In order to highlight the benefits of employing an open framework, the team formed an industry partnership with the Open Source company Backyard Brains (TM), which has significant experience in creating and distributing open educational and research technology for neuroscience in Latin America and worldwide (backyardbrains.com, backyardbrains.cl). In collaboration, the team assessed the potential use of their imaging statuon in a high school environment, per author Tamara Matute “ We have been able to use these resources in workshops in high schools, community spaces and cultural centres; and implement advanced practicals to teach in vitro synbio, DNA fab and microbiology. The open source and low cost nature of the resources has allowed citizens to better understand the principles behind gene expression analysis and modelling”



Together, their results demonstrate the successful integration of open source hardware, software, genetic resources and customizable manufacturing to obtain a powerful, low cost and robust system for education, scientific research and bioengineering. The paper was selected as Editor’s Pick for the PLOS Open Source Toolkit Channel in December 2017.

Nuñez, I., Matute, T., Herrera, R., Keymer, J., Marzullo, T., Rudge, T., & Federici, F. (2017). Low cost and open source multi-fluorescence imaging system for teaching and research in biology and bioengineering. *PLOS One*, *12*(11), e0187163.

Plant-ProChip 2.0: High throughput transformation of plant protoplasts

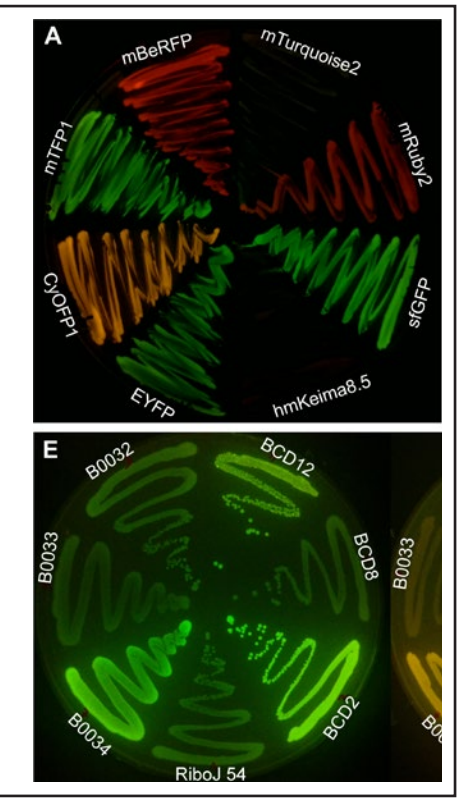
Ivan Reyna-Llorens (Plant Sciences, UCam), Steven Burgess (Plant Sciences, UCam), Ziyi Yu (Chemistry, UCam), Gregory Reeves (Plant Sciences, UCam), Christian R. Boehm (Plant Sciences, UCam)

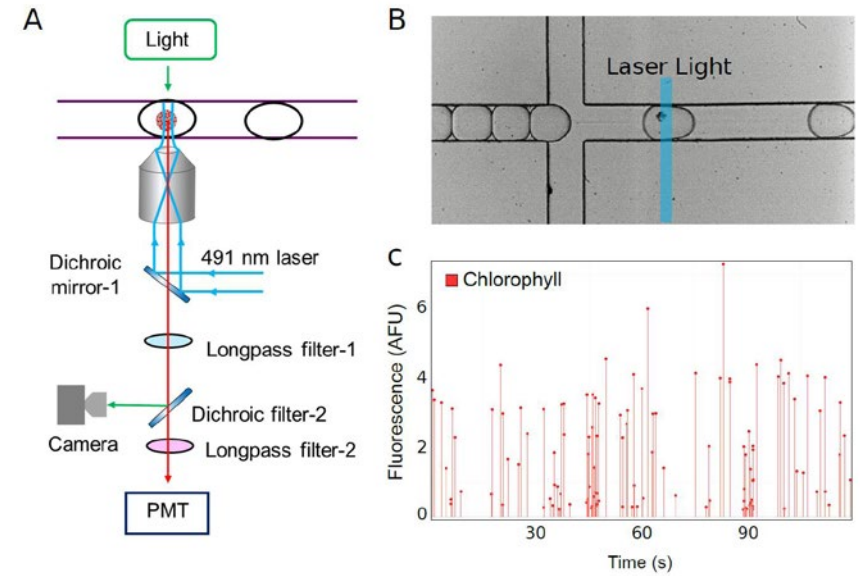
A current limitation for plant synthetic biology involves high-throughput screening of genetic parts in plants. Current approaches require testing circuits in individual plants, through transient or stable transgenics. Applying these techniques to entire libraries is not feasible at a laboratory scale. Droplet-based microfluidics has been used to facilitate high throughput analysis of individual prokaryote and mammalian cells. However, there is a scarcity of similar workflows applicable to rapid phenotyping of plant systems.

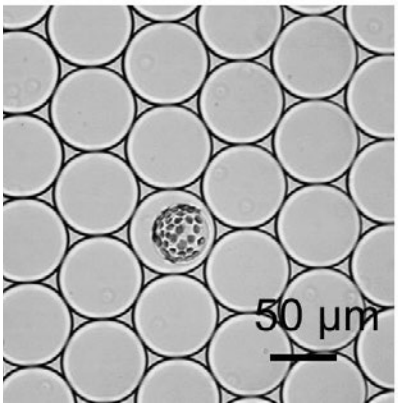
In the first stage of this project, the team aimed to develop a high-throughput screen for the analysis of promoter sequences in plant protoplasts. As a result, they successfully isolated, encapsulated and analysed protoplasts from the model species, *Marchantia polymorpha* and *Arabidopsis thaliana* using a PDMS microfluidic device. The team then received a second OpenPlant Fund to to develop microfluidics for both transient and stable protoplast transformation protocols at a high-throughput scale.

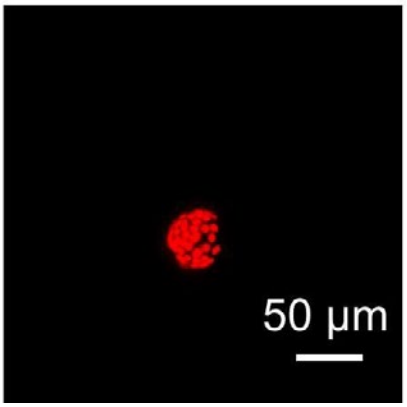
They have now published a preprint (Yu et al., 2017) reporting on-chip encapsulation and analysis of protoplasts isolated from the emergent plant model *Marchantia polymorpha* at processing rates of >100,000 protoplasts per hour. They used their microfluidic system to quantify the stochastic properties of a heat-inducible promoter across a population of transgenic protoplasts to demonstrate that it has the potential to assess gene expression activity in response to environmental conditions. They further demonstrated on-chip sorting of droplets containing YFP-expressing protoplasts from wild type cells using dielectrophoresis force. This work opens the door to droplet-based microfluidic analysis of plant cells for applications ranging from high-throughput characterisation of DNA parts to single-cell genomics.

Yu, Z., Boehm, C. R., Hibberd, J. M., Abell, C., Haseloff, J., Burgess, S. J., & Reyna-Llorens, I. (2017). Droplet-based microfluidic analysis and screening of single plant cells. *bioRxiv*, 199992.









Report Summaries

Open Labware for plant electrophysiology
Dr Carlos A. Lugo (EBI, previously The Sainsbury Laboratory), Dr Marco Aita (Sainsbury Laboratory, University of Cambridge), Christian R. Boehm (Department of Plant Sciences, University of Cambridge), Guru Vighnesh Radhakrishnan (John Innes Centre), Dr Marielle Vigouroux, (John Innes Centre)
In order to investigate electrical responses to mechanical and other external stimuli, this project consisted of replicating an open source Arduino shield which receives, amplifies and transmits "ECG"s from plant tissues into a computer or other circuits. The resultant board's schematics and other experimental tools such as manipulators and signal transducers were published on a dedicated project page including files for producing boards and 3D printed parts.The team received a second Open Plant Fund grant for further development of monitoring and data gathering capabilities of the shields and automation of the micromanipulators. A number of kits were given away to schools and several workshops were run with schools and members of the public, including a popular Science Makers event.

Building a low-cost desktop plant experiment box
Dr Marco Aita (Sainsbury Laboratory, University of Cambridge), Dr Marielle Vigouroux (John Innes Centre), Dr Carlos Lugo (EBI)
Doing experiments in plant biology is a difficult task because experimental conditions are difficult to control and growth chambers can be very expensive and optimised for plant growth rather than running experiments. This project developed small independent "experimental boxes" which are optimised for in-vivo recording of single plants under different environmental conditions and cost <£1000 each.

Wireless, portable, low cost, open source hardware for monitoring plant electrophysiology
Dr Pakpoom Subsoontorn (Plant Science, University of Cambridge), Sakonwan Kuhaudomlarp (JIC), Dr Kyle Lopin (Naresuan University, Thailand), Dr Settha Tangkawanit (Naresuan University, Thailand)
A prototype of a low-cost tool was developed for measuring plant electrical signal coupled with radio modules for long-distance data collection, costing < £40. It can sense and transmit signals from Venus flytrap responding to tactile inputs and distinguish the action potential from other disturbances. This has applications in detecting stress responses. The team received a second grant to improve upon the prototypes but ended early due to relocation of team members.

Establishing 3D Printed Microfluidics for Molecular Biology Workflows (ongoing)
Steven Burgess (Department of Plant Sciences, University of Cambridge), Tom Meany (Department of Plant Sciences, University of Cambridge), Richard Bowman (Department of Physics, University of Cambridge), Oleg Raitskin (Earlham Institute), Neil Pearson, (Earlham Institute)
With synthesis of DNA becoming cheaper, and plasmid construction automated, the testing of biological parts is becoming a bottleneck in the design-build- test cycle. Analysis of single cells offers a procedure for rapid screening of parts and this has been facilitated by advances in microfluidics. The downside of these approaches is that they tend to rely on expensive, specialist equipment, meaning they are out of reach to most molecular biology laboratories. This project developed a 3D printed fluorescent microscope, 3D printed syringe pump and a droplet generator prototype using open source designs.

Light sheet microscopy of cell sheet folding in *Volvox*
Stephanie Hoehn (DAMPT, University of Cambridge), Pierre Haas (DAMPT, University of Cambridge), Karen Lee (JIC)
Dr. Stephanie Höhn and Dr. Pierre Haas (DAMTP, Cambridge) are studying embryonic cell sheet folding events in Volvox and in collaboration with Dr. Karen Lee (John Innes Centre, Norwich) they are now studying the development of the feeding bladders of the aquatic carnivorous plant Utricularia (Bladderwort) using light sheet fluorescence microscopy (LSFM) to study developmental processes *in vivo*. This OpenPlant Fund project enabled them to improve their hardware and the resulting image quality, doubling the thickness of a sample for which they can acquire useful fluorescence data. This significantly increases the variety of future applications including studies on the morphogenesis of entire embryos in the multicellular micro-alga *Volvox* and the development of feeding structures of the aquatic carnivorous plant Bladderwort.

Development of a Low-Cost Micro-Environment Device for Root-Nutrient Interaction (ongoing)
Tyler McCleery (JIC), Ziyi Yu (Chemistry, UCam), Zhijun Meng (Chemistry, UCam), Veronica Grieneisen (JIC)
Standard lab conditions for plant growth typically involve homogeneous nutrient conditions, but actual field conditions are rarely homogeneous. This project developed a low-cost microfluidic device that can finely control rapid changes in the micro-environment surrounding the root structure. To date, the team have grown roots in working prototypes built using soft lithography and established a preliminary protocol to fabricate low-cost versions of these devices. In the coming months they will test root growth in the low-cost devices and study root growth in a dynamic or heterogeneous nutrient environment.

The Green Mother Machine Reloaded
Christian Schwall (Biochemistry, UCam), Philipp Braeuninger-Weimer (Engineering, UCam) , Bruno Martins (Sainsbury Laboratory, UCam), Arijit Das (Sainsbury Laboratory, UCam), Chao Ye (Sainsbury Laboratory, UCam), Toby Livesey (Biochemistry, UCam), Antony Hall (UEA)
In this project the team wanted to build a microfluidic device which allows the observation of *Synechococcus elongatus* PCC 7942, a well-studied cyanobacterium, at the single cell level. They based their design on a well-established device called the mother machine and tailored it to the specific needs of *Synechococcus elongatus*. One of the biggest challenges in adapting the mother machine was keeping the cells alive and loading the cells into the growth channels. They successfully optimized the loading and survival of *Synechococcus elongatus* in the green mother machine by improving the loading protocol and the experimental setup. In addition, they tested various prototypes for the robust media switching between different media.

Ongoing Projects

Universal precise large area colony scanning stage with measurement and selection tool integration (ongoing)
Tobias Wenzel (Department of Physics, University of Cambridge), Luka Mustafa (Institute IRNAS Race), Ji Zhou(Earlham Institute), Nick Pullen (John Innes Centre), Neil Pearson (Earlham Institute)

Bench-top Controlled Environment Growth Chamber for Speed-Breeding and Crop Transformation
Oscar E. Gonzalez-Navarro (Quadrum Institute and JIC), Ricardo H. Ramirez-Gonzalez (Crop Genetics, JIC), Sreya Ghosh (Crop Genetics, JIC), Marcela Mendoza-Suarez (Plant Science, Uni. of Oxford), Luis Hernan (Architecture, Newcastle Uni.), Carolina Ramirez-Figueroa (Architecture,Newcastle Uni.)

Open-Cell: An Open-Source 3D Printable System for High-Throughput Cell-Free Screening
Clayton Rabideau (Chemical Engineering and Biotechnology, UCam), Stefan Grossfurthner (Plant Sciences, UCam)

Development of open source camera trap powered by plant microbial fuel cell (pMFC)
Paolo Bombelli (Biochemistry, UCam), Rachael Kemp (Zoological Society of London), Alasdair Davies (Zoological Society of London)

OpenPlant Fund case studies: Biology

Development of new codon optimisation tools and development of a synthetic gene expression system in the green alga *Chlamydomonas reinhardtii*

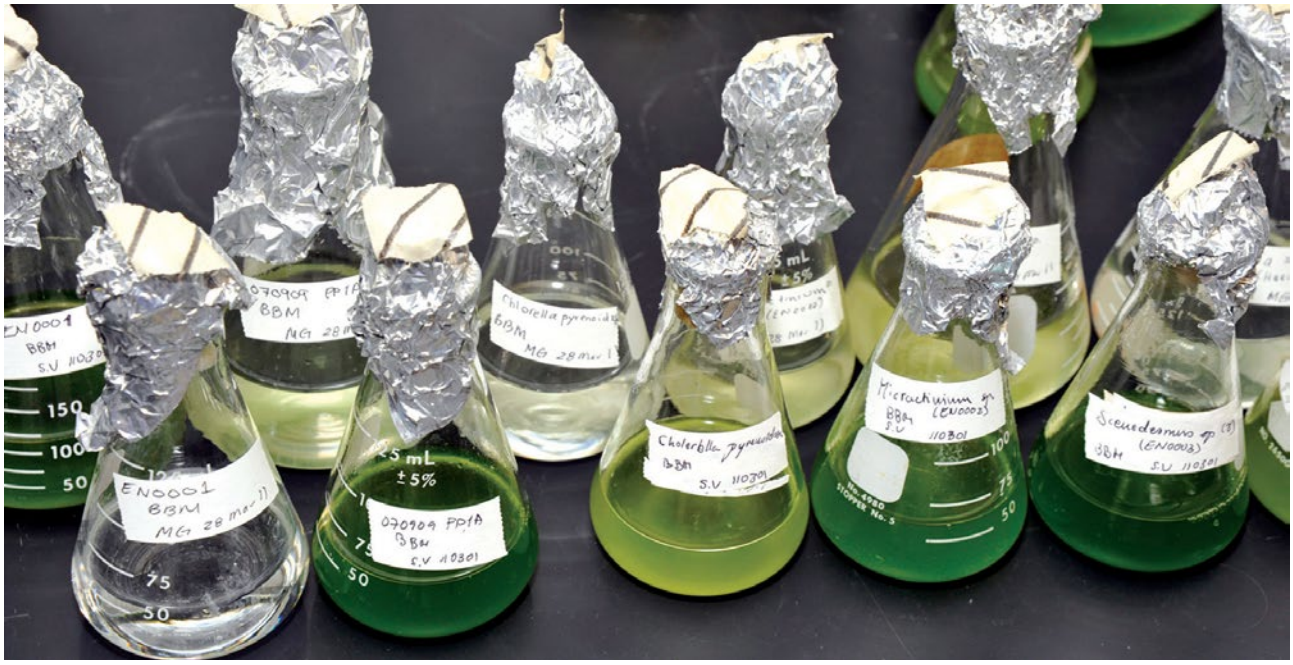
Francisco Navarro (Department of Plant Sciences, University of Cambridge), Marielle Vigouroux (John Innes Centre)

Most organisms share the same genetic code, based on three nucleotide codons that encode for one amino acid. However, synonymous codons (which specify a single amino acid) are not used at equal frequency by different species. Dr Francisco Navarro and Dr Marielle Vigouroux were interested in assessing the impact of codon usage in protein production in the green alga *Chlamydomonas reinhardtii* which has an unusually high GC content in its coding sequences. *Chlamydomonas* sequences have 68% GC content, while *Arabidopsis* is only 44% and human is 52%. The high GC content strongly biases codon selection towards GC-rich codons, making codon optimization a necessary step for the expression of genes from other species in the alga and hence an important consideration for any synthetic biology project.

The team performed sequence analysis of publicly available datasets to identify codon usage of genes transcribed under different conditions, at different expression levels, and along the length of coding sequences. The conclusion of this analysis was that codon selection in *C. reinhardtii* is very robust, characterized by a strong preference towards GC-rich codons, for most amino acids with only few exceptions. In addition, the team found that codon preference was not uniform along coding sequences. The results pointed out several considerations to take into account for the codon optimization of transgenes. In order to test gene variants with different levels of codon optimization, Francisco and Marielle developed an experimental platform for measuring the production of a reporter protein.

The platform was designed to test the expression of different gene variants of a fluorescent protein that differed in the choice of synonymous codons. *Chlamydomonas* transcription regulatory sequences (promoters, terminators and UTRs) were used to drive the expression of the reporter gene. The team were able to implement a versatile cloning strategy and tested the expression of several fluorescent reporters in different strain backgrounds. The DNA parts generated followed -specific MoClo DNA parts following the common syntax for gene assembly in plant synthetic biology co-authored by many members of OpenPlant. Some of these parts have been contributed to a *Chlamydomonas*-specific MoClo kit, created by a consortium of European labs, including from the Univeristy of Cambridge, which will be an open resource for the *Chlamydomonas* community.

Marielle and Francisco enjoyed and appreciated the opportunity to set up their collaboration through the OpenPlant Fund “There is much interest in algal biotechnology, and the abundant publicly available data can be used to aid its development . The OpenPlant fund award allowed us to explore codon usage in green alga. In addition, it was an excellent opportunity to create a link between the John Innes Centre and the University of Cambridge. We enjoyed pitching our idea in front of the panel, and it is exciting to see that our results become into an open resource to the scientific community “



Hot Tomato: Complementation of the Capsaicin Biosynthetic Pathway to Engineer Spicy Tomatoes

Greg Reeves (Department of Plant Sciences, University of Cambridge), Chris Boursnell (Department of Plant Sciences, University of Cambridge), Jie Li (John Innes Centre)

Chili pepper (Capsicum spp.) is the most cultivated spice crop in the world with an annual value over $20B (USD). The spicy flavour of chili peppers is due to the accumulation of capsaicinoids and interestingly, the orthologous genes for the whole capsaicin pathway are present in the tomato genome but are not expressed at the same stages in fruit development as in chili pepper. This project aimed to utilise synthetic biology approaches to overexpress enzymes from chili pepper to complement endogenous tomato genes—forming a functioning capsaicin pathway to yield spicy tomatoes. This experiment offers a tool to building synthetic pathways in plants through complementation of existing components and furthers understanding the evolution of secondary metabolites in plants.

The team identified missing genes in tomato for the capsaicin biosynthetic pathway by comparative bioinformatic analyses. They selected and cloned eight genes in the pathway into single and multi-gene transformation constructs which have initially been tested in tobacco (Nicotiana benthamiana) by infiltrating with Agrobacterium tumefaciens to give transient expression. The lines have been screened for capsaicin accumulation via spectroscopy and several lines absorbed the wavelength for capsaicin (280 nm), suggesting capsaicin accumulation, but analysis in on-going. The next stage of the project will be stable tomato transformation with all eight chili pepper genes to produce tomatoes with heritable spiciness.

The work has been presented at the OpenPlant Forum, NIAB and the Cambridge Science Festival as part of a ‘Synthetic Biology and the Senses’ exhibit.

OpenPlant Fund case studies: Biology

Advancing the ability to image single RNA molecules at the cellular level

Susan Duncan (John Innes Centre), Susana Sauret-Guet (Department of Plant Sciences, University of Cambridge), Christian Boehm (Department of Plant Sciences, University of Cambridge)

Plant biology currently lags behind other fields in the study of cell-to-cell variation and subcellular localisation of mRNA. Dr Susan Duncan (John Innes Centre) helped to establish the first single molecule fluorescent in situ hybridisation (smFISH) method for plants, allowing each RNA molecule to be visualised as a single fluorescent dot in *Arabidopsis thaliana* root meristem tissue. This technique revealed subcellular localisation of coding and non-coding RNA and provided data to enable the estimation of the frequency of transcriptional firing events.

The high level of back ground autofluorescence emitted by many green plant tissues currently limits smFISH analysis to a single tissue type but with the support of the OpenPlant Fund, the team were able to optimise the existing technique and adapt it for use in other Arabidopsis tissues and to enable RNA imaging in the liverwort Marchantia polymorpha. Improvements allowed researchers to image three, rather than two, RNA targets simultaneously and led to amplified signals which were compatible with confocal imaging. RNA could also be visualized in more differentiated root cells, e.g. root hairs. OpenPlant funding also enabled Susan to collaborate on a project about boron transport and generate data for a publication (Sotta et al., 2017).

Dr Susan Duncan reported “Our work has provided a strong foundation for improved RNA imaging that will open up numerous avenues for Arabidopsis and Marchantia research in the future.” She was able to promote the technique at a range of conferences and through publications including a commentary piece on the technique (Duncan & Rosa, 2017). Susan was also made an expert collaborator by LGC, in recognition of her ongoing efforts to promote plant smFISH and this led to further conference presentations and planning a funded workshop.

Duncan, S., & Rosa, S. (2017). Gaining insight into plant gene transcription using smFISH. Transcription, 1-5.

Sotta, N., Duncan, S., et al. (2017). Rapid transporter regulation prevents substrate flow traffic jams in boron transport. eLife; 6:e27038. DOI: 10.7554/eLife.27038

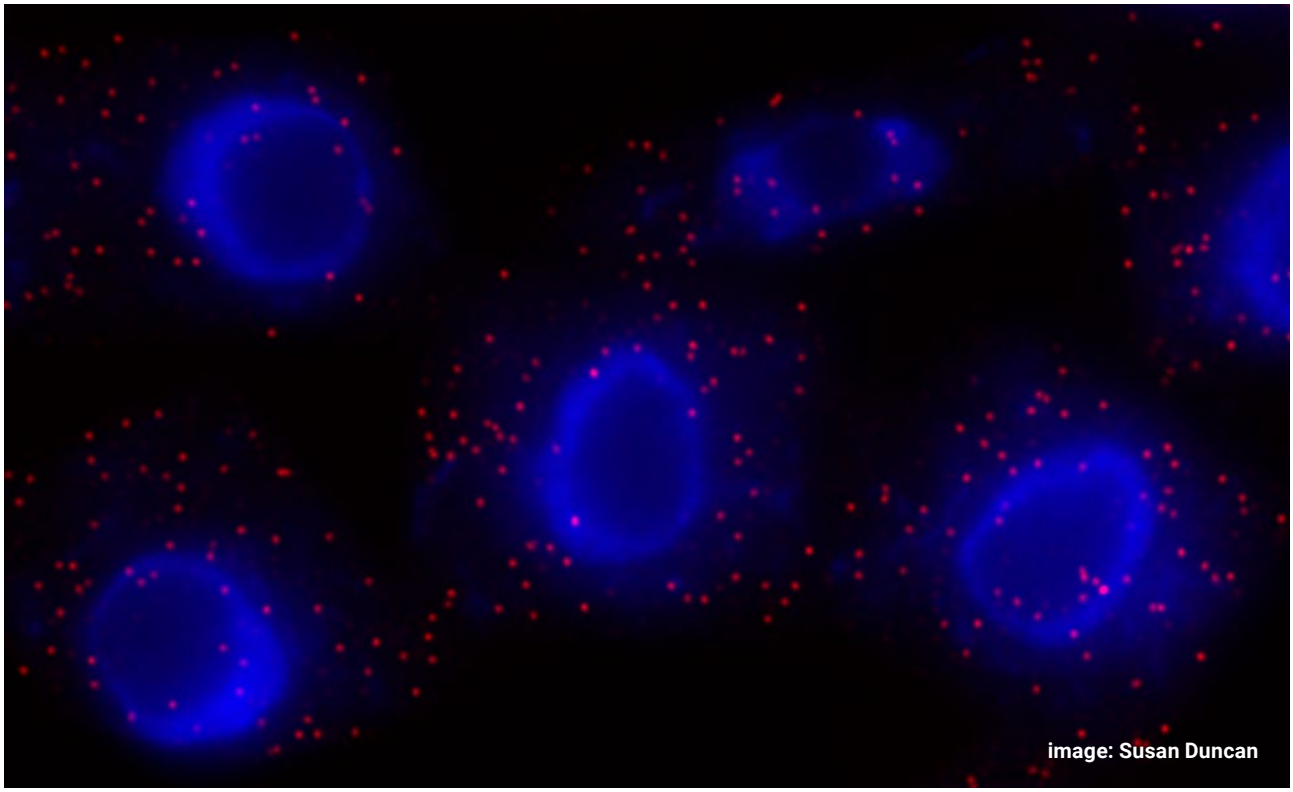


image: Susan Duncan

Report Summaries

Developing novel selection markers for plant transformation to advance live-imaging techniques
Dr Fernán Federici (University of Cambridge/Universidad Catolica, Chile), Dr Katharina Schiessl (John Innes Centre), Leonie Luginbuehl (John Innes Centre), Guru Rhadakrishnan (John Innes Centre)
A total of 25 DNA parts were synthesised, including tissue specific promoters and coding sequences of fluorophores and chromophores. Level 1 and level 2 GOLDEN GATE plasmids were generated and transformed into Medicago Hairy roots. Subsequently, selection markers were tested to see if they were detectable under the stereomicroscope and images were taken using confocal microscopy. It was found that the nuclear-envelope localised fluorophore dtomato, expressed under the Lotus UBIQUITIN promoter, was detectable under the stereomicroscope and could therefore provide a novel selection marker for live imaging. Furthermore, it was found that the BEARSKIN promoter was not detectable in the lateral root cap but expressed at the base of the induced hairy root callus. No significant colour change was observed in the roots transformed with the chromoproteins.

The use of synthetic biology tools to define the roles of LysM receptor-like kinases in legumes and cereals
Feng Feng (John Innes Centre), Ronelle Roth (Department of Plant Sciences, University of Cambridge)
This team synthesised a number of golden gate modules including gene promoters, coding sequences and terminators and made the final constructs required to characterise LysM receptor-like kinases in legumes and cereals. They then expressed the constructs in *Nicotiana benthamiana* to check protein expression and are now focusing on transforming the constructs in Medicago and rice to detect defence and symbiosis phenotypes.

Quick analytical system for plastid genome modifications
Mario Juhas (Department of Pathology, University of Cambridge)
This project set out to provide the synthetic biology community with a quick Pulsed-Field Gel Electrophoresis (PFGE)-based analytical system for plastid genome modifications. The project led to a number of educational resources, including protocols for the sample plugs preparation for PFGE of plastid and BAC DNA and for PFGE analysis of plastid and BAC DNA. The protocols and results have been published in Open Access journals.

Juhas, M., & Ajioka, J. W. (2016). Integrative bacterial artificial chromosomes for DNA integration into the Bacillus subtilis chromosome. *Journal of Microbiological Methods*, *125*, 1-7.

Juhas, M., Wong, C., & Ajioka, J. W. (2016). Combining Genes from Multiple Phages for Improved Cell Lysis and DNA Transfer from Escherichia coli to Bacillus subtilis. *PLOS One*, *11*(10), e0165778.

Juhas, M., & Ajioka, J. W. (2016). Lambda Red recombinase-mediated integration of the high molecular weight DNA into the Escherichia coli chromosome. *Microbial cell factories*, *15*(1), 172.

Channeling targeted DNA double strand breaks into alternative repair pathways
Dr Ian Henderson, Dr Natasha Yelina, Patrick Diaz (Department, of Plant Sciences, University of Cambridge), Dr Sebastian Schornack (The Sainsbury Laboratory, University of Cambridge), Meiogenix (Paris)
The team expressed TAL DNA binding domains fused to the FokI nuclease under meiotic promoters (e.g. DMC1, SPO11) in Arabidopsis. The aim of the work is to target DNA double strand breaks to specific sites in the genome, in order to bias initiation of meiotic recombination. Preliminary data showed that while these nucleases are expressed in meiotic-stage floral buds they do not support wild type levels of crossover recombination when the endogenous nuclease (SPO11-1) is mutated. To investigate this further, they are performing whole genome DNA sequencing and mutation discovery as well as extended phenotypic analysis.

Engineering Marchantia polymorpha chloroplasts for the production of high-value specialised terpenes
Aymeric Leveau (John Innes Centre), Tessa Moses (John Innes Centre), Christian R. Boehm (Department of Plant Sciences, University of Cambridge)
The aim of this project was to develop three independent operon-like synthetic constructs to achieve *de novo* synthesis of mono-, sesqui- and triterpenes in *M. polymorpha* chloroplasts. GoldenGate modules of coding sequences to be expressed in *M. polymorpha* were synthesized. However, two major issues were encountered during the project, including problems with transforming *M. polymorpha* chloroplasts with large constructs, and an assembly defect of the 2A peptide system used for generating the clusters. To circumvent these obstacles, constructs allowing nuclear transformation of M. polymorpha and subsequent chloroplast targeting of the proteins were designed and this is currently being evaluated.

Implementation of a synthetic transcriptional AND gate in the chloroplast of Chlamydomonas reinhardtii
Christian Boehm (Department of Plant Sciences, University of Cambridge),Payam Mehrshahi (Department of Plant Sciences, University of Cambridge),Hannah Laeverenz-Schlogelhofer (Department of Physics, University of Cambridge)
The chloroplast is among the most attractive substrates for biological engineering but there are few suitable systems for controlling the expression of transgenes from the chloroplast genome. This team proposed to develop a synthetic transcriptional AND gate implemented in the chloroplast of *Chlamydomonas reinhardtii*, based on a modified T7 bacteriophage RNA polymerase (T7RNAP). They designed optimized yellow and cyan fluorescent reporters and a T7RNAP gene which they domesticated to be compatible with Golden Gate assembly. The team then designed nuclear transformation vectors encoding intact and split T7RNAP variants under control of constitutive expression signals or riboswitches. They attempted introduction of the optimized fluorescent reporters into the C. reinhardtii chloroplast genome, and are waiting to confirm establishment of homoplasmy prior to demonstration of AND gate functionality.

A synthetic biology approach to investigating arbuscular mycorrhizal symbiosis in Marchantia paleacea
William Summers (Department of Plant Sciences, University of Cambridge), Uta Paszkowski (Department of Plant Sciences,

University of Cambridge), Giles Oldroyd (John Innes Centre), Andrew Breakspear (John Innes Centre), Guru Radhakrishnan (John Innes Centre)
D14-LIKE (D14L) has recently been identified as being vital for the establishment of arbuscular mycorrhizal (AM) symbiosis in rice (*Oryza sativa*). Mutation of this gene results in a complete breakdown in communication between the plant and fungus (Gutjahr et al 2015). The liverwort lineage includes members that engage in AM symbioses; whilst others do not, offering a good experimental system for the question 'Does the essential function of D14L in AM symbiosis exhibit widespread conservation?'. The OpenPlant Fund project developed eight D14L constructs which will be made openly available to others wishing to use them. Transformation protocols for M. paleacea were also developed to enable future knockout experiments.

DNA-mediated fusion of spheroplasts with synthetic liposomes (ongoing)
Lorenzo Di Michele, (Physics, UCam), Martin Howard (JIC), Pietro Cicuta (Physics, UCam)
The project aims to control the fusion of spheroplasts or mammalian cells with artificial liposomes using amphiphilic DNA nanostructures. Liposomes are interesting because they can be used to efficiently deliver large amounts of cargoes into the live cells, needed for instance for genetic editing. Various candidate DNA nanostructures were designed, assembled and characterised and their ability to induce fusion of liposomes of different size with other liposomes was successfully demonstrated. In parallel, a protocol for the preparation of giant spheroplasts from filamentous E. coli was implemented and the possibility of functionalising the spheroplasts with the DNA nanostructures demonstrated. The team is are currently attempting the fusion of spheroplasts with liposomes, but preliminary results are inconclusive.

Ongoing Projects

A cell-free sensor platform for the quantification of arsenic concentrations in drinking water
Genevieve Hughes (Earth Science, UCam), Elise Siouve (Biotechnology, UCam), Carolina Orozco (Biotechnology, UCam), Sina Schack (Biochemistry, UCam), Lisa Hecker (Biophysics, UCam), Alexandru Grigoroiu (Biomedical Engineering, UCam), Sammy Mahdi (Electrical Engineering, UCam, James Vereycken (Organic Chemistry, UCam), Francesco Tonolini (Physics, UCam), David-Benjamin Grys (Electrical Engineering, UCam), Tess Skyrme (Aerospace Engineering, UCam), Ralf Mouthann (Physics, UCam)

Cell-free diagnostics for the surveillance of livestock viruses
Laura Mitchell (Chemistry, UChem), Raghd Rostom (Wellcome trust Sanger Institute and UCam), Emily Groves (Medicine, UCam), Andre Zylstra (Babraham Institute and UCam), Punika Ratchachittapong (summer intern, UCam)

Development of Manufacturing Capability for Rare Sugar Nucleotides
Tom Simmons (Glycoscience, UCam), Jan Lyczakowski (Biochemistry, UCam), Henry Temple (Biochemistry, UCam)

Engineering of Chlamydomonas reinhardtii to produce betalain pigments and the use of riboswitches to direct metabolic flux
Alfonso Timoneda (Plant Sciences, UCam), Dr Payam Mehrshahi (Plant Sciences, UCam)

CGSENS: Visualization of CG methylation using a fluorescence protein biosensor
Dr Marino Exposito-Rodriguez (Biological Sciences, UEssex), Dr Sara Lopez-Gomollon (Plant Sciences, UCam)

GardenSeq: Chasing the invisible diversity of microbial life forms in vegetable garden beds with a portable DNA-sequencer
Maximilian Stammnitz (Veterinary Medicine, UCam), Meltem Gürel (Computational Biology, UCam), Philipp Braeuninger-Weimer (Electrical Engineer, UCam), Daniel Elías Martin-Herranz (Bioinformatics, Wellcome Trust Genome Campus, UCam), Daniel Kunz (Wellcome Trust Sanger Institute, UCam), Christian Schwall (Sainsbury laboratory, UCam)

Single cell pollen meiosis screening in wheat
Ashleigh Lister (Earlham Institute), Dr Iain Macaulay, (Earlham Institute), Dr Matt Clark (Earlham institute), Prof Graham Moore (Crop Genetics, JIC), Prof Peter Shaw (Cell and developmental Biology, JIC), Dr Azahara Martin (Crop Genetics, JIC), Dr Lola Santome (Crop genetics, JIC)

Harvesting the genetic value of interspecific wheat introgressions
Tobias Barber (NIAB and UCam), Dr Alison Bentley (NIAB), Dr Keith Gardner (NIAB) Dr Chris Wright (Earlham Institute), Dr Jaroslav Dolezel (Centre of Plant Structural and Functional genomics, Czech Republic)

Identifying nutrient-status dependent elements regulating the wheat transcriptional response to neighbours
Stéphanie Swarbreck (Plant Sciences, UCam), David Swarbreck (Earlham Institute)

Actin visualization: to disclose mechanisms of host cell reorganisation during interactions with microbes
Aleksandr Gavrin (SL, UCam), Wendy Harwood (JIC)

Comparative analysis of cell free and in planta protein synthesis systems
Susan Duncan (EI), Laura-Jayne Gardiner (EI), Quentin Dudley (EI), Philippa Borrill (JIC), Pallavi Singh (Plant Sciences, UCam)

Cell-free proteins synthesis as a resource for generating plant proteins
Quentin Dudley (EI), Susan Duncan (EI), Nicolas Larus-Stone (Department of Computer Science, UCam)

Towards an efficient transformation system for legumes
Abhimanyu Sarkar (JIC), Julia Russell (JIC)

Design of synthetic plant and mammal gene regulatory networks using nonparametric Bayesian approaches
Marc Jones (Computational and Systems Biology, JIC), Iulia Gherman (Biology, UYork), Anastasiya Sybirna (Wellcome/CRUK Gurdon Institute, UCam)

Establishing a Procedure for Rapid Identification of Genetic Parts for Use in Algal Biotechnology
Kher Xing Chan (Cindy) (Department of Plant Sciences,

University of Cambridge), Steven Burgess (Department of Plant Sciences, University of Cambridge), Marielle Vigouroux (John Innes Centre)

Translating Nitrogen Use Efficiency from models to crops (ongoing)
Mariana Fazenda (Plant Sciences, UCam), Matthew Milner (NIAB), Mario Caccamo (NIAB), Dan Swan (Earlham Institute)

OpenPlant Fund case studies: Software

Documentation Tool for Open Plant Technologies

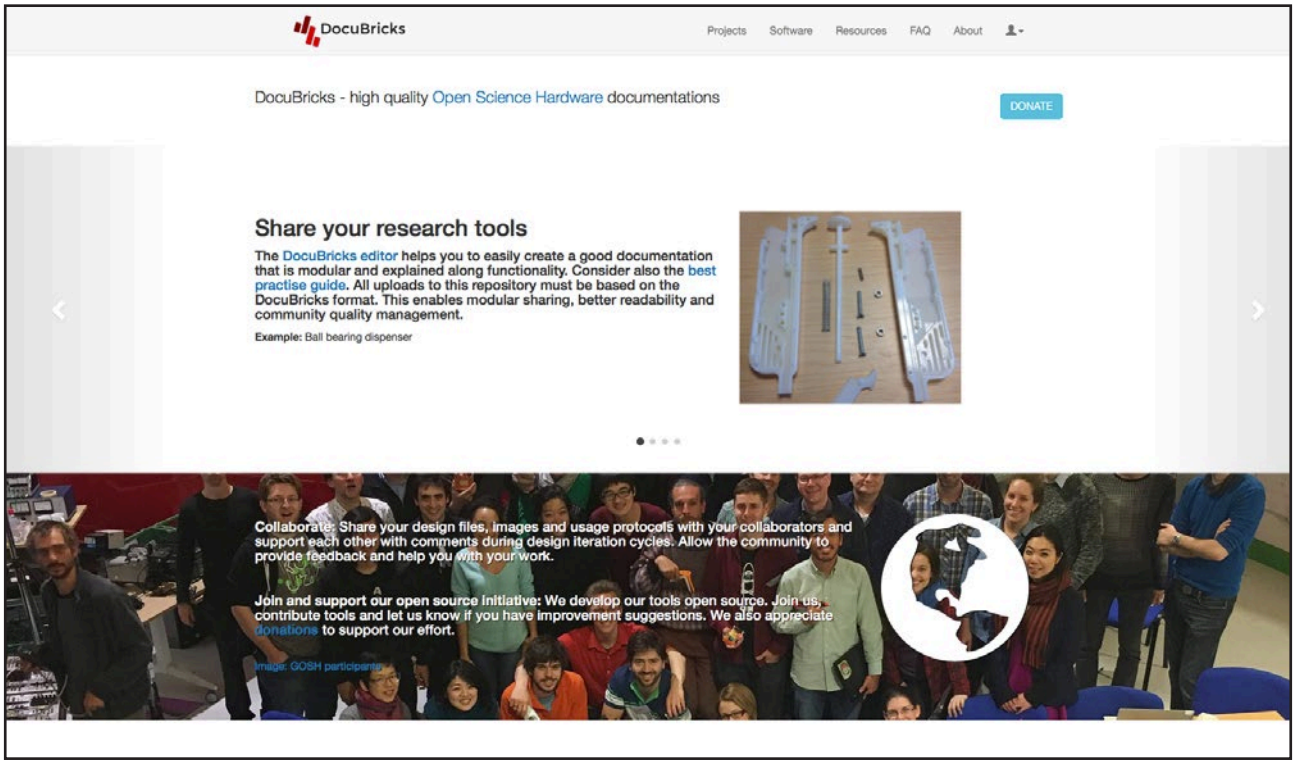
Tobias Wenzel (Department of Physics, University of Cambridge), Johan Henriksson (EMBL-EBI), Carlos Lugo (EMBL-EBI), Luka Mustafa (Shuttleworth Foundation Fellow, IRNAS)

There is a growing trend to produce open hardware for science due to its potential benefits for reproducibility of scientific results. Making knowledge production more accessible for everyone also has personal and group-level benefits such as avoiding losses of knowledge when team members leave, saving time as well as resources and increasing the quality of scientific outcomes. OpenPlant Fund and Biomaker Challenge have produced several hardware designs which are openly shared. However, many of the designs for scientific instrumentation are complex and the DocuBricks team felt that available tools were insufficient to enable useful sharing of open source hardware projects that integrate different types of components.

They built an open source hardware documentation software and an online repository called DocuBricks (DocuBricks.com) that allows projects to be documented in a modular fashion. The aim is to make it easier for users to assess how the project solves a problem, whether the information is complete and if calibration strategies and protocols are provided. They also wanted to provide a database for scientists who would like to cite the documentation and files in a publication as well as demonstrate the community impact of each module of their work.

This software tool is itself open source and saves documentations in an accessible XML format. Thirteen projects are currently live on the site and the team are continuing to develop DocuBricks to serve as a high quality repository for Open Science Hardware. The database is citeable and is now a recommended repository for hardware published in the new Open Access 'Journal of Open Hardware' published by Ubiquity Press.

DocuBricks has been presented at numerous meetings including OpenCon Cambridge, MozFest, Gathering for Open Science Hardware and featured on PLOS Blogs, in Nature News, BioTechniques, the Mozilla Open Leaders programme and a Global Young Academy report on open hardware. Tobias Wenzel reported that "The OpenPlant Fund award was a unique opportunity for us to seriously implement and test our idea (DocuBricks). Beyond the flexible funding it also connected us to an amazing community of users, advocates and activities that really made a difference!"



Report Summaries

Open Pi-Image: A low cost-open source plant growth imaging and analysis platform

Professor Alex Webb (Department of Plant Sciences, University of Cambridge), Dr Dan MacLean (The Sainsbury Laboratory)
The Open Pi-Image team designed and constructed a near infrared image capture system based on a Raspberry Pi computer, PiNoir camera and custom 3D printed parts, with designs shared openly online. This runs an extensible and modular open source software suite we developed called Open Pi Image that controls automated image capture and spawns image analysis. The Pi software can be accessed on any external system (e.g. a laptop) via a web server running on the Pi and the system can be embedded in inaccessible places. Open Pi Image is designed to incorporate new user provided scripts for analysis and can be easily extended and customised.

Facilitating synthetic biology literature mining and searching for the plant community
Dr Robert Davey (Earlham Institute), Dr Ksenia Krasileva (Earlham Institute/TSL), Dr Nicola Patron (Earlham Institute), Richard Smith-Unna (UCam), Dr Peter Murray-Rust (UCam)
A two-day workshop was held in March 2016 centred on novel methods for discovering information about plants from the existing literature ("Content Mining"). Most people were running within an hour and a typical example was "find all you can about diseases of oats" using Europe PubMed Central (with over 1 million Open Access papers). This retrieves about 500 papers, which were further filtered for chemicals, diseases, species, etc. and displayed within a minute or two, significantly increasing the speed of knowledge-driven scientific discovery. Participants contributed code to the project and helped construct scrapers and dictionaries to extract more information from papers related to plant synthetic biology. The group will run a second workshop and are seeking external grant funding for further collaboration.

Ongoing Projects

Time Series Analysis of Environmental Variables to enable GxE studies: A Machine Learning and Open Hardware Approach to Plant Research Experimentation
Daniel Reynolds (EI), Aaron Bostrom (EI), Joshua Ball (EI)

R for Proteomics' training proposal
Jan Sklenar (TSL, Norwich), Laurent Gatto (Computational Proteomics Unit, UCam), Marielle Vigouroux (JIC), Govind Chandra (JIC)

BIOMAKER.ORG

ELECTRONICS : 3D PRINT : SENSORS : INSTRUMENTATION : BIOLOGY

A new mini-fund for innovation and skills training

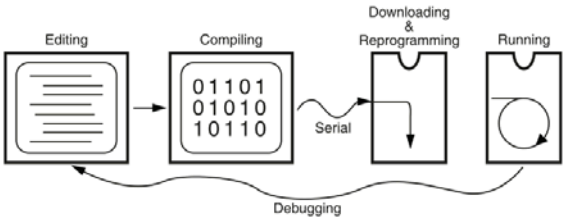
The Biomaker Challenge is an interdisciplinary team-based opportunity to explore the intersection of electronics, 3D printing, sensor technology, low cost DIY instrumentation and biology. The Biomaker Challenge aims to promote collaboration between disciplines, tapping into commodity electronics and open technologies for instrumentation to build research skills and collaborations.

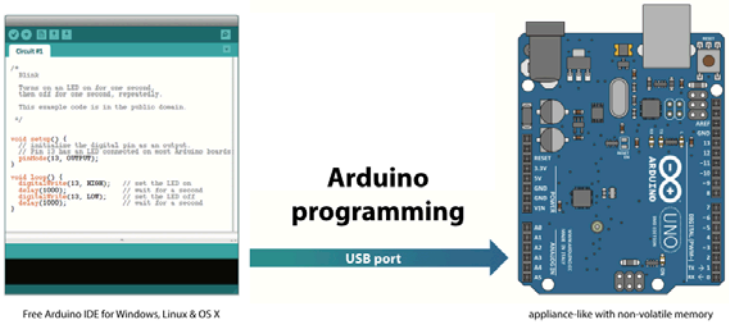
We have chosen Arduino-based hardware (www.arduino.cc) as our starting point. The Arduino community has established open standards and rich ecosystem of resources for simple microcontrollers, first established to simplify programming and physical computing for designers and artists. Arduino circuit boards can be plugged into the USB port of any laptop, and a simple cross-platform programming environment used to program the board. A program is simply loaded to non-volatile memory on the Arduino board, which will execute this program loop whenever the board is powered on - behaving as a dedicated appliance or instrument. Arduino boards include many input/output ports, and are intended to interface with sensors and actuators.

The Arduino system provides a simple environment for learning programming and hardware skills, and developing real-world laboratory tools for biologists. Further, the Biomarker Challenge provides a direct route for other scientists and engineers to get hands-on experience with biological systems.

The effort is sponsored by BBSRC/EPSRC through OpenPlant Synthetic Biology Research Centre (www.openplant.org) and the University of Cambridge Research Policy Committee through the Synthetic Biology Strategic Research Initiative (www.synbio.cam.ac.uk) and CamBridgeSens, the Sensors Strategic Research Network (www.sensors.cam.ac.uk).

The Biomaker Challenge Starter Kits contain teaching materials to allow anyone with no previous experience to learn programming and interfacing to the Arduino microcontroller board





Biomaker Starter Kit

Each team in the Biomaker Challenge receives a Starter Kit that will allow even inexperienced individuals to develop skills, and provide a platform for exploring more challenging applications. The kit includes:

ARDX Prototyping Kit. The ARDX Starter Kit for Arduino is a great learning resource with components to build 13 different projects. The kit provides a manual with instructions arranged as a series of lessons. These provide a simple way of learning how to wire electronic circuits and programming the Arduino microcontroller. For example, the kit comes complete with a set of paper circuit templates that you lay over the breadboard and push the components through - to remove the worry of wiring the project incorrectly. No experience necessary.

Grove Modular Sensor/Actuator Kit. Grove is a modular electronics platform for Arduino-based quick prototyping that does not involve soldering. Simply plug the Grove modules into the Grove shield and leverage the example code provided for each Grove module. Grove is a modular, ready-to-use tool set. Much like Lego, it takes a building block approach to assembling electronics. The Grove Starter Kit contains 10 of the most popular Grove modules and an Arduino shield with Grove connectors.

Sidekick Basic Component Starter Kit. This contains basic components to build 7 different projects, and include an additional small circuit breadboard and more hook-up wire. The kit is provided by SeeedStudios (http://wiki.seeed.cc/Sidekick_Basic_Kit_for_Arduino_V2/).

Giant Prototyping Board for Arduino. The Gtronics Proto Shield Plus allows you to plug in Arduino boards, and to integrate these with custom shields and components on a large plug board - minimising tangled hook-up wires. On-board push buttons and a LCD are provided to facilitate debugging of program flow and to interrogate hardware during testing.

Programmable Touchscreen. The Biomaker Starter Kit will contain a 4D Systems 3.2" gen4 touch-responsive programmable display from 4D Systems (with memory card, Arduino interface and programmer), with information about programming environments. An Arduino library for direct serial communication with the display is available - along with more sophisticated Workshop4 development tools, including ViSi-Genie, a graphical programming tool that allows simple access to a wide range of display widgets like gauges, switches, sliders, readouts, etc., for creating customised interfaces for Arduino-based instruments. The programmable displays can be easily adapted for Raspberry Pi board computers. These programmable touchscreens allow the simple prototyping of sophisticated user interfaces, to match the flexible and programmable control of hardware by microcontroller-based instruments.

The teams are also provided with additional support of up to £750 over the summer, for additional components and materials, including access to a 3D printing service with both fused deposition modelling (FDM) and stereolithography (SLA) printing services, and teams will be expected to share their projects on Github. The Biomaker Challenge culminates in a public Open Technology exhibition. All teams will be expected to demonstrate their creations at this public event. Prizes will be awarded for especially creative and/or enabling projects.

For more information, go to
https://www.biomaker.org

Workpackage L:
OpenPlant Forum: responsible innovation

This workpackage involves activities of the annual OpenPlant Forum, the annual working group and workshops and other public engagement activities with the SAW Trust. The OpenPlant Forum will provide a platform for exploring the potential applications of reprogrammed biological systems, and a framework for exploring the wider implications of the potentially disruptive new technologies. The SAW Trust provides training in project design to scientists working in collaboration with professional artists and writers who come together as teams to deliver projects themed on the scientists' research topics.

This workpackage spans all other workpackages in OpenPlant. The annual Forum meeting encourages attendance from all OpenPlant participants and OpenPlant Fund recipients, and all workpackages are represented. Each year, a working group is established for in depth investigation of a topic relevant to the Forum theme. The SAW Trust provides training in project design to scientists working in collaboration with professional artists and writers who come together as teams to deliver projects themed on the scientists' research topics. SAW workshops are coordinated by Dr Jenni Rant and opportunities exist for all workpackages and OpenPlant Fund projects to interact.

Investigators
David Baulcombe
Dale Sanders
Jim Haseloff
Anne Osbourn

Staff Employed
Colette Matthewman (Project Manager, Norwich)
Jenny Molloy (Project Coordinator, Cambridge)

Partners
Jenni Rant - The SAW Trust
Linda Kahl – BioBricks Foundation

L

Online resources:

OpenPlant Forum
(www.openplant.org/forum/)

OpenPlant RI news
(www.openplant.org/outreach/)

OpenPlant reports
(www.openplant.org/reports/)

Milestones:

L1.1: Annual symposia on a series of themes related to plant synthetic biology
Deliverable: Devise and convene annual meetings (annually months 12-60, Haseloff, Osbourn).

L1.2: Recruitment of annual working groups
Deliverable: Appoint working groups around the symposia themes, with membership rotating to suit (annually, months 12-60, Haseloff, Osbourn, Baulcombe, Sanders).

L1.3: Annual published report
Deliverable: Document the themed output of the working group and symposium speakers (annually, months 12-60, Haseloff, Osbourn).

L2: SAW workshops
Deliverable: Co-sponsor SAW Trust workshops (annually, months 12-60, Osbourn).

Progress to date:

Responsible Research and Innovation (RRI) activities are integrated into the OpenPlant SBRC through a number of cross-cutting activities. Central to this are efforts to create mechanisms for the exchange of resources and information by developing enabling tools for sharing such as standards (the OpenPlant common syntax; Patron *et al*., 2015) and IP solutions (OpenPlant IP Working Group; Open MTA), resources such as DNA parts collections (see workpackage reports) and shared protocols (OpenPlant protocols are shared on http://protocols.io/), and building an open community for plant synthetic biology (e.g. through OpenPlant Forum; OpenPlant Fund workshops to strengthen synthetic biology capacity in Africa). The OpenPlant Forum is an important vehicle for bringing together a multidisciplinary community to discuss important questions in Responsible Research and Innovation. Smaller meetings such as the OpenPlant All-Hands meeting, ROC meetings, and interdisciplinary workshop (e.g. Co-lab OpenPlant workshops) provide further opportunities for discussions on issues related to RRI. To support these activities and enable our PDRAs to contribute more extensively, we delivered a workshop on RRI, ethics and argumentation, and openness attended by all OpenPlant-funded PDRAs and some associates.

Jenny Molloy coordinates quarterly meetings of the Virtual Institute of Research and Innovation (VIRI) in Cambridge, also attended by Colette Matthewman as a representative from Norwich. These meetings bring together members of the science departments with members of the Centre for the Study of Existential Risk (CSER) and the Centre for Science and Policy (CSaP) in Cambridge to discuss matters related to RRI and to discuss opportunities for collaboration. Resulting from these collaborations, OpenPlant researchers from all three institutes have become involved in a Bioengineering Horizon Scanning Exercise organised by CSER.

The OpenPlant Fund grant proposals have proved an excellent resource for the development of more targeted RRI activities, enabling the following workshops: 1. Responsible Innovation and Open Innovation with Large BioResources, 2. Genetic resources in the age of the Nagoya Protocol and gene/genome synthesis and 3. Co-lab OpenPlant - interdisciplinary workshops of science, art and design.

This workpackage involves activities of the annual OpenPlant Forum, the annual working group and workshops and other public engagement activities with the SAW Trust. The OpenPlant Forum provides a platform for exploring the potential applications of reprogrammed biological systems, and a framework for exploring the wider implications of the potentially disruptive new technologies. Each year, in association with the Forum, a working group has been established for in depth investigation of a topic relevant to the Forum theme. The SAW Trust provides training in project design to scientists working in collaboration with professional artists and writers who come together as teams to deliver projects themed on the scientists' research topics.

Objective L1.1: Annual symposia on a series of themes related to plant synthetic biology

The 2015 inaugural Forum focussed on access, openness and enabling technologies and attracted a strongly interdisciplinary, intersectorial group of ~90 participants. Sessions addressed themes such as frameworks for open innovation, open DNA parts and assembly, foundational systems, plant based bioproduction and open technologies. Twenty four experts were recruited to the first year's working group on the topic of IP solutions for OpenPlant and the wider SynBio community. A meeting of the IP Working Group took place on 30 July 2015. The purpose of the meeting was to bring together academic researchers, technical experts, and legal practitioners to explore the challenges and solutions for creating and sustaining an international platform of open technologies for plant synthetic biology. The aim of this working group is the creation of standard legal tools as well as recommendations for policies and practices that will enable the adoption of open platforms for biotechnologies at the institutional level. A report on the meeting has been drafted and is currently open for comments from the working group, after which it will be made available online. Linda Kahl from the BioBricks Foundation used feedback from the IP working group to shape the OpenMTA that she is drafting for use by OpenPlant.

The 2016 OpenPlant Forum focussed on the theme of reprogramming agriculture. There are huge opportunities for delivering social, environmental and economic benefits through efforts to reprogramme plants and agriculture, but there are both technical and social bottlenecks that affect progress in this field. The 2016 OpenPlant Forum brought together over one hundred people from various disciplines to hear some of the recent advances in crop and feedstock engineering, discover tools to support innovation in this field, and to discuss and reflect on ethical, legal, social, and economic considerations. Sessions included reprogramming feedstocks, innovation in crops, ELSA, creating tools and developing methods for plant synthetic biology, and enabling innovation through openness and exchange. The Forum was coupled to an industry showcase and networking event to develop new interactions between academic researchers and companies working in the synthetic biology space.

The 2017 OpenPlant Forum canvassed fast and frugal techniques for engineering biology, with applications in research, education and international development. The Forum was coupled with the OpenPlant Fund pitches, an industry forum focussing on building a sustainable global bioeconomy, a post-doc organised networking event and the first meeting of a new working group to establish open curriculum resources, using cell-free systems.

Objective L1.2: Recruitment of annual working groups

Twenty four experts were recruited to the first working group on the topic of IP solutions for OpenPlant and the wider SynBio community. The group was convened at the OpenPlant Forum

in July 2015 and continued exchanging ideas and information at monthly teleconferences until December 2016. The purpose of these meetings was to refine the design goals of the Open Materials Transfer Agreement (OpenMTA) - a collaboration between the BioBricks Foundation and OpenPlant to enable open exchange of plasmids and other biological materials. A report has been completed and distributed as part of a 'soft launch' of the OpenMTA in the final quarter of 2016. Smaller groups were convened throughout 2016 to discuss specific challenges in successfully introducing a mechanism for open transfer within institutions, which requires both strategic partnerships and strategic timing. Progress towards a launch is well underway but a meeting of the larger working group has not been considered timely until the point at which they could add most value to ensuring the OpenMTA is implemented and meets its aims. Co-organisers are in active discussion about the next steps in this process.

Following the work of these groups, we have established a web presence for the OpenMTA in partnership with the BioBricks Foundation at https://www.openmta.org/. This includes video tutorials to describe the OpenMTA in a concise and accessible manner. OpenPlant researchers contributed to the production of the video. The IP working group contributed to a list of FAQs, hosted on the website, to provide more specific and targeted information and to pre-empt questions from researchers and institutions. Linda Kahl has developed a set of short video case studies, targeted at technology transfer offices, dealing with specific uses and impacts of the OpenMTA. The IP working group report has been published on the OpenPlant website.

In 2017, a new working group was established that focused on curriculum development and teaching resources for fast and frugal biotechnology. The first meeting of the working group took place in July 2017, immediately after the OpenPlant Forum. A range of key players were invited, including researchers at the cutting edge of the technology, educationalists and outreach experts. The working group focused on the educational opportunities offered by cell-free systems and freeze-dried components to provide a platform for teaching synthetic biology at low-cost and without use of GMOs. The first meeting lay the groundwork for assembly of open and modular curriculum components that could be combined into different teaching frameworks across multiple disciplines, drawing on existing work from organisations such as BioBuilder, the National Centre for Biotechnology Education, Science and Plants in Schools and Raspberry Pi.

In addition to the formal working groups, OpenPlant has been involved in a range of activities in relation to Access and Benefit Sharing through the Nagoya Protocol:

- In November 2016, OpenPlant co-funded and participated in a workshop with the Engineering Life project at Edinburgh University. The workshop was on "Genetic resources in the age of the Nagoya Protocol and gene/genome synthesis". This workshop is being written up into a paper.
- We ran the Global Gardens SAW workshop as a co-learning experience with the general public, exploring access and benefit sharing. More details are below. There are plans in place to analyse and write up the outcomes of this activity, and to build upon it.
- Nicola Patron participate in working group on Plant Genetic Resources and Sustainable Development Goals: Needs, Rights and Opportunities (Rockerfeller Foundation, Bellagio Center, Nov 2016). A report has been submitted to the Plant Genetic Resources in Food and Agriculture Treaty. A publication is in review and a follow-up workshop will take place in September 2017.
- In January 2016, Colette Matthewman presented on Synthetic Biology and the Nagoya Protocol at a HVCfP NIBB workshop "Sharing nature's genetic resources – implementing the Nagoya Protocol on Access and Benefit Sharing" in London.

Objective L1.3: Annual published report

Reports have been published and distributed in print form, and are freely available as PDF documents at https://www.openplant.org

Objective L2: SAW workshops

Three OpenPlant-SAW workshops were run in the first year of OpenPlant:

SAW at Ludham primary school (16.03.15). A full day workshop with 32 kids (age 8-11 year-old), covering DNA, mendelian genetics, synthetic biology and Marchantia as a model plant.

Cambridge Botanic Gardens Festival of Plants (16.05.15). Nature's biofactories and Marchantia activities. Several OpenPlant PDRAs were involved, and engaged with the general public at this event which attracts thousands of people each year.

SAW at the HVCfP Synthetic Biology Workshop (13.07.15). An evening workshop run as part of SynBio workshop. Feedback on the event was overwhelmingly positive.
OpenPlant joined efforts with ERASynBio to organise the summer school for early career researchers "An Introduction to Synthetic Biology in Plant Systems" in Norwich (14-20.09.15). A meeting report of this event has been accepted for publication in the next edition of New Phytologist (Carmichael et al., 2015). A synthetic biology day was arranged as part of this year's Year 10 Science Camp in Norwich (30.06.15), involving activities on understanding and creating gene circuits, engineering nitrogen symbiosis in plants and a debate addressing responsible research and innovation.

In the second year we strengthened the collaboration between OpenPlant and The SAW Trust and increased the scale and impact of outreach and public engagement activities. OpenPlant exhibits were run at the Youth STEMM Awards mid-year conference (Jan 2016), the Cambridge Science Festival (Mar 2016; with OpenPlant Fund Whiskeroscope project), and Latitude Festival (July 2016). For these exhibits, we have designed and developed a modular set of activities and displays that we can mix and match for different events. This enables us to easily adapt to different size events, and shift the focus of the exhibit for different events. These activities will be exhibited at the Norwich Science Festival in October.

A key achievement was securing a place to exhibit in the Kids Area at Latitude Festival in July 2016. Latitude is a mixed arts

Progress to date:

festival that attracts over 10,000 visitors a year, who enjoy the rich mix of thought-provoking performances and interactive workshops. Combined efforts of OpenPlant, the SAW Trust, and OpenPlant Fund grant award winners, The Big Algal Open Experiment, led to the creation of stand entitled "The Power of Plants". This was an exhibit that led visitors on a journey looking at traditional uses of plants, how plant selective breeding has produced the food crops that we recognise today, tracking the evolution of our relationship with plants through science to introduce the synthetic biology approach, and some of the modern uses of plants and algae that bioengineering enables. The exhibit attracted much attention and was received with great positivity. The first day of the festival, Schools Day, saw the exhibit fully booked with 1 hour workshops for school and public groups repeated throughout the day. We ran an open, drop-in exhibit for the second and third days of the festival.

OpenPlant Fund grant award winners, Carlos Lugo and Marielle Vigouroux, worked with The SAW Trust to develop and deliver a 1-day workshop for Year 6 pupils at Stapleford Community Primary School (South Cambridgeshire). More details can be found on the OpenPlant blog: http://openplant.org/blog/2016/02/openplant-science-art-and-writing-workshop-a-success/

In March 2016, Jenni Rant ran a workshop to introduce OpenPlant PDRAs and associates to The SAW Trust methods of exploring science through art and poetry, and to give them a chance to experience these themselves. Jenni has also run a train the trainer workshop with the UK Centre for Mammalian Synthetic Biology, Edinburgh.

In the third year there has been an increase in impact of the OpenPlant and The SAW Trust partnership through the following activities:

SAW developed workshop to enable dissemination and share best practice with other research centres. Jenni Rant delivered two of these workshops for SynthSys and the UK Centre for Mammalian Synthetic Biology (University of Edinburgh), and a similar collaboration has been established with Warwick Integrative Synthetic Biology Centre, to deliver workshops in the coming year.

Two SAW Trust primary school workshops were run on the theme of plants as green factories for producing vaccines and high value natural products. The latter was filmed to show SAW in action: http://sawtrust.org/news/saw-in-action/

Two primary school workshops were organised by OpenPlant intern, Emma McKechnie-Welsh, under guidance of Jenni Rant, covering biodiversity, plant evolution and genetics. Blog: https://www.openplant.org/blog/plant-science-saw-projects-at-tunstead-primary-school

A weekend interactive exhibit at Norwich Science Festival (Oct 2016), incorporating OpenPlant Fund project "Accessible 3D models of molecules", including a public effort to make the largest virus like particle possible out of paper "protein pieces" decorated by visitors. We got to 100 pieces! Blog: https://www.openplant.org/blog/2017/3/23/openplant-stand-at-the-first-norwich-science-festival

Colette Matthewman and Jenni Rant secured an outreach grant from the Biochemical Society to create a robot that explains the processes of transcription and translation in a fun and interactive way. They worked together with artist Molly Barrett and JIC researchers, Nadia Radzman and Ioannis Tamvakis, to design DNA Dave the robot who was a huge hit at Cambridge Science Festival, at the OpenPlant exhibit "Synthetic Biology and the Senses" (Mar 2017). Adults and children alike found this tool for explaining the key scientific principles behind transcription and translation both informative, accessible and fun. Blog: https://www.openplant.org/blog/2017/3/23/cambridge-science-festival-stand-20-improved-design

In a collaborative project between OpenPlant, the SAW Trust, Social Scientist Dr Nick Lee (Social Scientist, Warwick Integrative Synthetic Biology Centre) and the Writers Centre Norwich, we ran a unique co-learning workshop called The Global Garden, based on the SAW method. Using practical science, art and poetry, as well as discussions of case studies, the Global Garden explored cultural attitudes to societal problems, biotechnological solutions and issues arising from the use of plants as a source of natural products. This workshop explored biodiversity, traditional and modern uses of plants, access and benefit sharing and feelings on natural vs synthetic products and the use of these descriptors in different contexts. The exploration of these issues, helping researchers to think about the social, as well as scientific, impact that biodiversity might have on people. Likewise, participant feedback indicated that the activities carried out as part of the Global Garden encouraged group members to think about important issues that they had not considered before. The group's engagement with the day showed how a versatile workshop such as the Global Garden can help people to understand complex scientific research that has so far had limited reach beyond academic circles and can help researchers to understand the values, concerns and optimisms that publics hold in relation to the science. Katie Beckett, Access and Benefit Sharing Project Manager at Regulatory Delivery, participated in the workshop and said "To have the time and space to think creatively about the use of genetic resources and the key issues that face society, was an entirely refreshing experience. Creative language and art as communication tools is not something that has come up in my day job, but this workshop taught me the role they can play and of impact they can impart."

OpenPlant and SAW teamed up this year to deliver a science tent for exploration of "Marvellous Medicines" in Kidztown at Boomtown festival (Aug 2017), which attracts over 50,000 people each year. This was the first time that the kids' area offered a science tent. The interactive exhibition included a natural products periodic table filled with different plants for extracting colours, scents, and citric acid. Kids could choose a

selection of plants to make a potion from, which would change colour, fizz and give off a scent as the experiment progressed. Throughout, the children learned about a plant's ability to make different compounds that define their features such as colour, scent and taste. They extracted the colour pigment themselves and used other natural extracts to complete their potions, observing how we can use things that plants make for our own products. The older children also learned about pH and colour indicators, a classic chemistry practical they will no doubt carry out in secondary school. A further use for plants was discovered in the art stand: the plant materials could be used as 3D elements to decorate the potion bottles.
Blog: http://sawtrust.org/news/boomtown-festival-august-2017/

The OpenPlant partnership with the SAW Trust delivers large and important aspects of our ethical, social and policy programme. To date, we have delivered six OpenPlant-themed workshops in primary schools, designed and delivered by research scientists in collaboration with SAW, influencing the educational approach to these topics. We engaged with the public by delivering inspiring interactive exhibits at the Cambridge and Norwich Science Festivals (2017 & 2017) with exhibits such as "Synthetic Biology and the Senses", and "Synthetic Biology Solutions". We also delivered science activities to engage Children in the Kids areas at Latitude Festival (mixed arts festival with around 10,000 visitors annually) with "The Power of Plants" and at Boomtown festival (music festival with around 50,000 visitors annually) with "George's Marvellous Medicines". This was the first time that the Boomtown kids' area has offered a science tent.

In 2017, Dr Colette Matthewman and Dr Jenni Rant (SAW Trust) secured funding to create a machine to explain the processes of transcription and translation in a fun and interactive way. Working together with artist Molly Barrett and JIC researchers they designed DNA Dave the robot who was a huge hit at Cambridge and Norwich Science Festivals. Adults and children alike found this tool both informative, accessible and fun, and teachers claimed that they saw "lightbulb" moments where there pupils understood these complex scientific processes that were proving difficult for them to grasp in a classroom environment. We have had several requests from teachers for DNA Dave to be taken into their schools.

The SAW Trust delivered a workshop for OpenPlant researchers as part of a focus day on responsible innovation, ethics, openness and society. The workshop introduced researchers to the SAW methodology and gave them a chance to experience this first hand, through creation of artwork and poetry based on their own OpenPlant science, enabling our researchers to reflect upon and explore their own science. Further to this, the SAW Trust has developed a workshop that trains scientists, artists, writers and teachers to use the SAW methodology. This workshop has been run at the UK Centre for Mammalian Synthetic Biology Research (University of Edinburgh) and in China. In 2018, a conference established by the SAW Trust in collaboration with Norfolk County Council will share best practice in science learning between schools locally and internationally.

Publications

Halewood M, Chiurugwi T, Sackville Hamilton R, Kurtz B, Marden E, Welch E, Michiels F, Mozafari J, Sabran M, Patron N, Kersey P, Bastow R, Dorius S, Dias S, McCouch S, Powell W.(2018) **Plant genetic resources for food and agriculture: opportunities and challenges emerging from the science and information technology revolution.** doi: 10.1111/nph.14993.

Wintle BC, Boehm CR, Rhodes C, Molloy JC, Millett P, Adam L, Breitling R, Carlson R, Casagrande R, Dando M, Doubleday R, Drexler E, Edwards B, Ellis T, Evans NG, Hammond R, Haseloff J, Kahl L, Kuiken T, Lichman BR, Matthewman CA, Napier JA, ÓhÉigeartaigh SS, Patron NJ, Perello E, Shapira P, Tait J, Takano E, Sutherland WJ. (2017). **A transatlantic perspective on 20 emerging issues in biological engineering.** *Elife; 6. pii: e30247. doi: 10.7554/eLife.30247.*

Kahl L, Molloy J, Patron NJ, Matthewman C, Haseloff J, Grewal D, Johnson R, Endy D. **Expanding options for material transfer via the OpenMTA.** *In review*

Jenny Molloy and Linda Kahl (2016). OpenPlant Intellectual Property Working Group Meeting Report.

Halewood M, Chiurugwi T, Sackville-Hamilton R, Kurtz B, Marden E, Welch E, Michiels F, Mozafari J, Sabran M, Patron NJ, Mooney P, Kersey P, Bastow R, Dorius S, Dias S, McCouch S, and Powell. **Plant genetic resources for Food and Agriculture: opportunities and challenges emerging from the science and information technology revolution.** *In revision*

Parry G, Patron NJ, Bastow R, Matthewman C (2016). **Meeting review: GARNet/OpenPlant CRISPR-Cas Workshop.** *Methods in Plant Biology 12:16*

Carmichael et al. (2015). **An introduction to synthetic biology in plant systems: ERASynBio/ OpenPlant summer school for early career researchers, September 2014.** *New Phytol . DOI: 10.1111/nph.13433.*

OpenPlant Intellectual Property Working Group Meeting Report. 2016. http://www.openplant.org

The SAW Trust

Engaging Society

The Science Art and Writing (SAW) Trust (registered charity no. 1113386) specialises in helping researchers improve and exercise key professional development skills through participation in enriching engagement experiences with communities. Working across the disciplines with professional artists and writers, and using intriguing scientific images, SAW workshops invite people to engage with scientists and explore science through practical activities, poetry and the visual arts. For scientists, this forms an important part of engaging with society, encouraging reflection on the motivations for and applications of our research. Working in a cross-disciplinary team fosters interesting discussions and builds new networks. We encourage scientists to use their own images where possible and have also set up a new open access image library where images can be downloaded for free. To see more please visit: http://images.norwichresearchpark.ac.uk.

OpenPlant's work with the SAW Trust is enabling scientists to take a multidisciplinary approach to introducing synthetic biology to the public and is providing a platform to exchange ideas. OpenPlant scientists have delivered SAW workshops based on their work in several primary schools (Ludham, Stapleford and Tunstead), created interactive stands for public events (Boomtown Festival 2017, Latitude Festival 2016, Cambridge Science Festival 2016/2017, Festival of plants, Norwich Science Festival 2016/2017), been awarded extension funding to develop resources (Biochemical Society Grant, Synthetic Biology for schools project) and also experienced their own research through a responsible innovation-themed SAW session as part of an OpenPlant workshop entitled Ethics, Openness, Outreach and the Media. The collaborative Global Garden workshop series provide a forum for people from varied backgrounds to learn about the high value chemicals produced by plants, explore the issues surrounding the commercial development and use of these products, and share their opinions and ideas.


FENCE

Outputs of SAW workshops

Poetry and art form important parts of our SAW projects. The following examples were produced during an OpenPlant SAW project in a primary school in Norfolk and a Global Garden workshop with adults.

Untitled

Generated from skeletal threads.
A network of molecules
Co-existing.
Fuel that makes it go.
It is what they make of it.
Deconstructed and building.
Understood in limited terms,
Taken apart.
Examined and freely exhumed.
Its functions unfold
Ambiguously.
Record the results fairly.
Fight the exploitation,
Individual and corporation.
It is different but the same,
Claimed.

Untitled

Mother made a gift.
She left it to be discovered.
For years it was enjoyed,
In blissful ignorance.
The gift had no value.
At this stage,
There was no proof of purchase.
It was not gift wrapped.
No label to say who it was for.
No instructions or list of ingredients.
She left it to be discovered.

DNA by Maisie, age 9

Life Instructor,
Body Builder,
Colour Constructor,
Shape Sculptor,
Plant Grower,
Trait Tailor,
Constant Creator,
Similarity Shaper,
Difference Decider,
I Am
The DNA.

Sharing SAW with other Synthetic Biology Research Centres

The flexibility of the SAW approach, along with the value to participants and the impact created make it an excellent tool for the synthetic biology community more widely. To share best practise, we developed a SAW workshop to enable dissemination of the initiative to other Synthetic Biology Research Centres and in April 2016 and 2017, the University of Edinburgh hosted SAW workshops for scientists from SynthSys and the UK Centre for Mammalian Synthetic Biology together with artists, writers and teachers.

Participants worked together on practical science, poetry and art activities that have been tried and tested in school SAW projects to see how their research themes can work in this format. Teams of scientists, artists and writers were then formed to develop new SAW projects that have since been delivered in Edinburgh schools. These were the first SAW projects to take place in Scotland.
These excellent projects were captured through an array of twitter posts and articles on the web. The scientists gained valuable experience, both by building confidence to widen their own approach to public engagement and through establishing a broader network of professionals from multiple disciplines, with which they can continue to deliver innovative outreach projects.

Practices for responsible innovation

Biomakespace planning

Interdisciplinary workshops

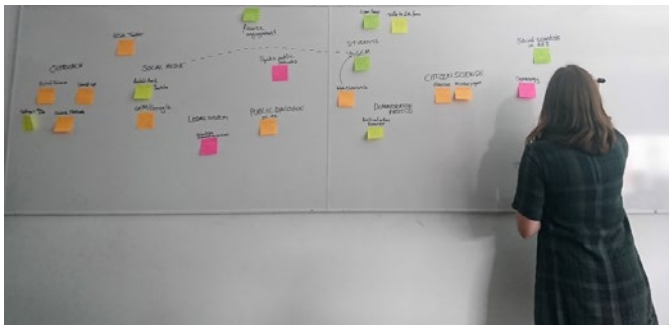
The cultivation of crops and pastures is driven by global population pressure, and responsible for unsustainable impacts on natural environments. An overarching aim of the OpenPlant project is to provide a map of feasible technical approaches for improving bioproduction and agriculture – considering possible economic models, opportunities and social implications. This includes consideration of the adoption of different forms of IP ownership, open source technologies, new business models in biotechnology, scientific codes of practice, responsibility for design and implementation, bioengineering accreditation, third world exchange, design for sustainability, decentralisation, UK policy development, evaluation of environmental impact (at the point of conception and design, rather than implementation), guidelines for best practice in new biological systems and real-world agronomy.

Responsible Research and Innovation (RRI) activities are integrated into the OpenPlant SBRC through a number of cross-cutting activities. Central to this are efforts to create mechanisms for the exchange of resources and information by developing enabling tools for sharing such as standards and IP solutions, DNA part collections, shared protocols, and an open community for plant synthetic biology; along with OpenPlant Fund workshops to strengthen synthetic biology capacity in Latin America and Africa.

The OpenPlant Forum is an important vehicle for bringing together a multidisciplinary community to discuss important questions in Responsible Research and Innovation. Smaller meetings such as the OpenPlant All-Hands meeting, ROC meetings, and interdisciplinary workshops provide opportunities to explore issues related to responsible innovation. To support these activities and enable our PDRAs to contribute more extensively, we deliver workshops on RRI, ethics and argumentation, and openness attended by OpenPlant-funded PDRAs and many associates. OpenPlant participates in quarterly meetings of the Virtual Institute of Research and Innovation (VIRI) in Cambridge. These meetings bring together members of the science departments with members of the Centre for the Study of Existential Risk (CSER) and the Centre for Science and Policy (CSaP) to discuss matters related to RRI and to discuss opportunities for collaboration. Resulting from these collaborations, OpenPlant researchers from all three institutes have become involved in a Bioengineering Horizon Scanning Exercise organised by CSER.

Public exhibitions

Engagement Highlights



Public Perceptions of Synthetic Biology

JOHN INNES CENTRE, UNIVERSITY OF EAST ANGLIA

OpenPlant joined forces with synthetic biologists and social scientists from the University of East Anglia to organise a scoping workshop on public engagement in synthetic biology, looking at: (i) the diversity of synthetic biology engagement processes; (ii) public views and concerns identified in these processes; (iii) the opportunities moving forward. The workshop, held in July 2017, was attended by synthetic biologists and social scientists from the UK Synthetic Biology Research Centres and the Eastern Academic Research Consortium (Eastern ARC) as well as a representative from the NGO, Society Inside. The workshop highlighted the vast range of synthetic biology engagement activities, and thus the models available for all to use. Additionally, the workshop collected suggestions on how the community can build upon the work that has already taken place.

Principal contact: Colette Matthewman

DNA Dave, the robot!

JOHN INNES CENTRE, SAW TRUST

In December 2016, a group of enthusiastic scientists met with artist Molly Barrett to begin work on a robot that would explain the processes of transcription and translation to a lay audience. The world was introduced to DNA Dave, the robot, at the 2017 Cambridge Science Festival. Regardless of age, the public were really excited to discover what the robot could do, and the process of transcription and translation of DNA to proteins was well explained by operating Dave's buttons, cogs and switches. One parent commented "I love how accurately you simplify the process for the young ones". DNA Dave was also very well received at the 2017 Norwich Science Festival, where he was visited by schools throughout learning week. One teacher commented that it was amazing to see the "lightbulb moment" as her pupils came to understand how proteins are created using instructions from DNA, a process that is challenging to teach in a classroom setting. There has been a lot of interest from schools in borrowing DNA Dave to complement classroom teaching. The creation of the robot was funded through an Outreach Grant from the Biochemical Society.

Principal contact: Jenni Rant

Global Garden Workshop

NORWICH

A Global Garden workshop was run as a collaboration between OpenPlant researchers at the John Innes Centre, the Science Art Writing (SAW) Trust, Social Scientist Dr Nick Lee (Warwick Integrative Synthetic Biology Centre) and the Writers Centre Norwich. The workshop was advertised to the public, and explored biodiversity, traditional and modern uses of plants, access and benefit sharing and feelings on natural vs synthetic products. Participants were immersed in the theme through practical science, art, poetry and a set of case studies that raised a variety of questions leading to discussions of issues around the use of plants as sources of drugs and other high value products. This co-learning experience highlighted to researchers the values, concerns and optimisms of publics in relation to the use of plants as a source of natural products.

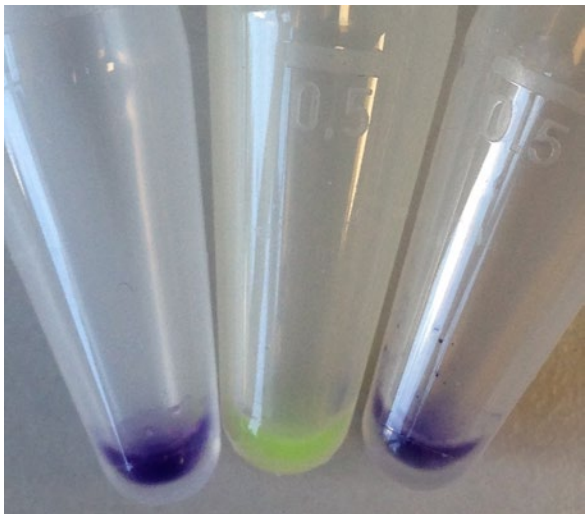
Principal contact: Jenni Rant

Norwich Science Makers Network

NORWICH

OpenPlant has supported the establishment of a Norwich Biomakers meetup group to bring together a cross disciplinary network of people to learn from each other, share ideas and skills and shape interdisciplinary and collaborative project plans. The network provides an umbrella under which a variety of activities can be captured, and can feed into programmes such as the OpenPlant Fund and Biomaker Challenge. Norwich Biomakers was established in September 2017, and has to date run four monthly meetups and gathered together over 100 members. Activities have led to two new interdisciplinary collaborations between biologists and technologists as well as the initiation of the first Biomakers project.
website: www.meetup.com/Norwich-Science-Makers/

Principal contact: Colette Matthewman



Building the Biomakespace in Cambridge

CAMBRIDGE

A Biomakespace in Cambridge is being built by a group of researchers, scientists, engineers, technologists and curious minds - to create an innovation space for biology and biological engineering. This effort is being driven by OpenPlant personnel and supported by OpenPlant initiatives. The Biomakespace is situated within the historic old MRC Laboratory of Molecular Biology building.

It intends to make bioengineering and manufacturing technologies accessible to a wide spectrum of innovators and enthusiasts to develop projects and ideas in an informal setting, with space for experimental biology and fabrication tools focused on scientific applications. The space aims to build a cross-disciplinary and cross-sector community for synthetic biology in the city, with a focus on open technology and innovation. It will also provide activities such as training and skills sharing sessions, networking events and foster links with innovation and seed funding schemes and local bioincubator spaces and accelerator programmes. (https://biomake.space)

Principal contact: Jenny Molloy

Cell-free biology workshops

CAMBRIDGE-NORWICH

Recent technical advances in the preparation of microbial cell-free extracts have given rise to a new class of highly efficient systems for gene expression that are cheap to deploy and have huge potential benefit for the provision of a wide variety of diagnostics, sensors, vaccines and research materials. Further, the extracts can be stored desiccated, stable for over a year, and reactivated at the point of use by hydration. The cell free extracts can be programmed by the addition of DNA to allow rapid and simple prototyping of gene circuits for diagnostics or bioproduction.

In vitro biology provides a number of key advantages for the design, assembly and testing of DNA encoded circuits for diagnostics and environmental sensing. Cell-free extracts avoid the complications, delays and regulatory uncertainty associated with uncontained of GMOs, while providing opportunities for high level, low cost training and capacity building.

The emerging technology enables engineering of DNA circuits without the need for genetic modification and in a low cost manner that makes it accessible for researchers in low resource settings. OpenPlant is sponsoring efforts to develop new educational and training materials for use in the UK and developing countries.

Principal contact: Jim Haseloff

Responsible innovation for global agriculture and conservation

Past experiences with GM technologies have shown that they cannot be developed in isolation from social, ethical and environmental considerations, and OpenPlant supports work on the wider implications of the technology at local and global scales, including discussions on the potential impact of Synthetic Biology on environmental conservation and sustainable human practices. These bring together a wide range of engineers, scientists and policy developers to explore the implications for adopting new technologies and different models for sustainable agriculture, bioproduction and land use.

The OpenPlant initiative has been funded with three main aims:

To create a hub for interdisciplinary exchange between Cambridge and Norwich, between the fundamental and applied sciences, that will underpin advances in UK agriculture and bioproduction.

To establish systems for the open exchange of new plant tools and DNA components that will promote commercial innovation and international scientific exchange.

To explore the wider implications of the technology at local and global scales. This will bring together a wide range of engineers, scientists and policy developers to explore new technologies and possible models for sustainable agriculture, bioproduction and land use.

Africa

Great challenges and potential for sustainable bio-based solutions, but poor infrastructure, and clear need for capacity building.

Latin America

Strong agricultural base, and scientific community keen for better and more accessible educational resources.

Europe

Well developed technical base, but widespread concerns about genetic modification limit investment in field use of GMOs.

Australasia

Important farming and mining sectors with strong technical and educational base - recent investments in synthetic biology.

North America

Large scale investments, increasingly driven by private sector and DARPA. Retreat from multilateral agreements by USA.

Asia

Heavy and coordinated investment in food security and new biotechnologies by Chinese government and industry.

OpenPlant supports the standardisation and global sharing of open technologies for plant synthetic biology. We aim to catalyse responsible innovation for sustainable agriculture and conservation. OpenPlant provides new tools, and a point of exchange for young scientists and entrepreneurs in the UK and worldwide. It is pioneering a programme of open curriculum development through the use of low-cost, freeze-dried, programmable cell-free systems.

Synthetic Biology in Africa

The OpenPlant obtained funding for a 'Practical Synthetic Biology' workshop in Africa - to exploit recent technical advances in biology that have given rise to cell-free and transient expression systems that are cheap to deploy and have large potential benefit for diagnostics, sensors, vaccines and research materials. The workshop was help in collaboration with the University of Pretoria, which has initiated the construction of the Future Africa campus, intended to provide a hub for Africa's leading scientists and scholars.

The workshop found:

1. The field of Synthetic Biology is introducing low-cost, breakthrough technologies for a wide range of practical challenges including diagnostics, environmental conservation, microbial bioproduction, crop improvement and human health. These are of critical importance to the future well-being and economic development of sustainable societies across Africa.

2. Synthetic biology offers new tools and approaches:
   - Standardised, modular DNA parts and rapid assembly of genetic circuits for reprogramming biological systems.
   - Cell free expression systems that do not require containment, and can be freeze-dried and stored at ambient temperatures to eliminate the need for refrigeration.
   - Transient gene expression in contained hosts, and transgene-free genome editing to avoid the costs, resources and regulatory hurdles associated with the deployment of genetically modified organisms.
   - Legal frameworks, repositories and open technologies for the open exchange of genetic materials.
3. These new technologies are relatively low-cost, but their adoption in Africa is limited by deficits in technical training, poor access to new research materials, inadequate laboratory facilities, and lack of strategic partnerships with other African and international research institutions.

4. The UK and Africa share a common goal with the need to develop improved synthetic biology training in schools, universities, community labs and industry.

5. International efforts to develop open standards and protocols for DNA parts and tools will provide a major impetus for technology transfer to Africa.

6. We recommend that (i) biotechnology is fertile area for UK-Africa exchange, and that (ii) capacity-building based on open technologies and exchange be a major component of any funding initiative.

7. Synthetic biology can provide better solutions for: (i) rapid-response production of vaccines and biologics, (ii) point-of-use diagnostics and field biosensors, (iii) agricultural crop improvement using non-transgenic (genome editing) tools, and (iv) harnessing local biodiversity to build a sustainable bioeconomy.

8. In each of these applications, the development of practical solutions and social impact requires:
   - Standardised curricula for training and biotechnology education in resource-poor communities and institutes.
   - Building local expertise through exchange and shared knowledge.
   - Establishing in-country facilities for generation and exchange of open-source tools and materials.

We have organised follow-up workshops and meetings, focusing on knowledge transfer for cell-free biology.

2014-2019

OpenPlant Fund

The OpenPlant Fund supports workshops in Africa and Cambridge to support teaching efforts, capacity building and development of open resources for technology transfer.

2016

Cell-free workshops

OpenPlant identifies the potential of cutting edge developments in the field to contribute to education and innovation in low resource environments., using non-GMO reagents that do not require a cold-chain. The same technology can be applied to paper-based assays and field tests. Workshops in cell-free biology are held in Cambridge

2017

Bakubung workshop

OpenPlant and the Earlham Foundry coordinate a workshop in South Africa to explore the limits and opportunities for new technologies to affect development of a modern bioeconomy in Africa. We conclude that OpenPlant can contribute positively through promoting open practices and exchange, and facilitating access to new technologies that offer radical improvements for learning and research in the new biology.

2017-2019

Biomaker Challenge

OpenPlant initiates a new model for project-based learning, the Biomaker Challenge. This offers relatively low-cost, broad participation, entry-level access to new fields to promote interdisciplinarity and a common set of tools and skills for training. Further, the model is easily transportable between institutions and countries. The Biomaker Challenge produces new prototypes for bioinstrumentation, and we wish to add cell-free systems to facilitate addition of DNA-based programming to the Challenge.

2018-future

Open Curriculum

OpenPlant is planning future workshops and meetings to recruit a coalition of international experts in cell-free biology and curriculum development, and to produce open source frameworks and materials for teaching in low resource environments. These include working practices for *in vitro* DNA assembly, distribution of non-GM freeze-dried reagents and low-cost instrumentation for quantitative analysis.

Engineering for the bioeconomy in Africa

Sustainable technologies and the bioeconomy in Africa

New biological engineering approaches offer the prospect of breakthrough approaches to the reprogramming of living systems, and the rapid development of new sustainable production systems in the face of increasing global demand for sustainable and resource-efficient solutions to challenges of food production, materials, energy, health, climate change and environmental sustainability. In the process of transitioning from a fossil carbon economy, we are seeing the rapid growth of the bio-based economy in developed countries. Identified in the 2016 OECD Science, Technology and Innovation Outlook as one of the 10 key technology trends of the future, Synthetic Biology is a new field defined by the application of engineering principles to living systems for useful applications in health, agriculture, industry and energy. Internationally, there are large and ongoing investments in the field, which generally require substantial investment in laboratory infrastructure and the deployment of stably transformed, approved genetically modified organisms (GMOs) into the environment. Except in a minority of countries like South Africa where GMO biosafety and regulatory frameworks are well established and specific GMO applications with emphasis on crop improvement may be well received (http://www.biosafety.org.za/), both of these issues can be highly problematic in the context of developing countries and can be circumvented through the application of recent low-cost, cell-free and transient synthetic biology systems.

Benefits of the new biological technologies

Cell-free and transient expression systems are easy to implement, relatively free of biosafety evaluation requirements and cheap to deploy. Underpinning this field is the open-source distribution of modular and standardised synthetic DNA components, which facilitates international exchange, knowledge transfer and innovation. These new technologies avoid complications, delays and regulatory uncertainties associated with uncontained use of GMOs, eliminate requirements for cold chain setups for transport and storage, and provide new options for high level collaboration on education, training and interdisciplinary research between UK and African scientists in low-resource environments. The promotion of open, low-cost, low-resource technology platforms allows for the in-country development of solutions for local problems, while building capabilities in an emerging technology that will be valuable for education, research, innovation and economic development. These novel non-GM approaches offer new prospects for (i) low cost diagnostics and environmental sensors, (ii) programmable cell-free expression systems, (iii) vaccine production for rapid responses to emerging viral threats, (iv) biomining and bioproduction, (v) new breeding techniques in plants based on genome editing using CRISPR/Cas9, (vii) new opportunities for training and education in UK and Africa, and (viii) an opportunity to engage societies concerned about GM technology.

Need for capacity building in Africa

The development of a thriving biological products-based economy (bioeconomy) in African countries is constrained by a general lack of funding, skills and infrastructure at multiple levels, from secondary school, undergraduate and postgraduate training to basic and applied research, pilot-scale testing and commercialisation. Concurrently, limited public and political understanding of biotechnology and its socio-economic benefits and risks also stultify the uptake of biotechnology in society and industry. A number of world-class African centres with training and research capacity exist, generally in better-resourced countries, or where major foundation funding has been invested. However, these model centres are typically weakly connected to research institutions in neighbouring countries and the region as a whole. These challenges could be overcome in part through access to the latest low-cost, open-source, widely shareable and scalable synthetic biology technologies that can be applied to accelerate basic training and capacity building in biotechnology, while stimulating research addressing unique challenges of importance to the African continent. Fast, flexible and scalable Synthetic Biology technologies will be important components of an agile response to emerging challenges such as infectious diseases, and biotic and abiotic stresses impacting on food security in African countries, while supporting the growth of national bioeconomies.

Risks of inaction or exclusion

The timing of implementing emerging biotechnologies will be critical for sustainable development of the African bioeconomy. The risks of inaction, exclusion or failure to adopt these technologies are profound. Long term negative impacts include:

(i) losing a generation of talented young Africans who could otherwise contribute to building the African bioeconomy, (ii) African nations and scientists falling behind the international scientific community, (iii) significant mortality and morbidity due to the inability of African countries to rapidly respond to emerging diseases (e.g. Ebola) and food security threats (e.g. novel crop pathogens). Rapid response to such emergencies will greatly depend on facility infrastructure and scale-up capabilities to achieve sufficient vaccine or therapeutic supply in a short time-frame, in addition to developing diagnostics for rapidly determining infection and disease spread.

Open technologies

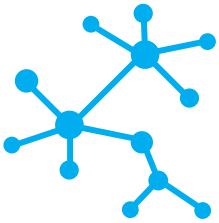
Open technologies allow for an unrestricted legal right to use, reuse and redistribute materials for all purposes, including commercial applications. Beyond legal aspects, open approaches provide mechanisms for organizing knowledge production that favour (a) universal versus restricted access e.g. availability of specific molecular tools unencumbered by intellectual property, (b) universal versus restricted participation e.g. greater involvement of beneficiaries in shaping projects using those tools, and (c) collaborative versus centralized production e.g. multiple partners working together for a common goal (Smith et al., 2008). For example, open designs for making lab equipment are intended to be documented in such a way that others can make the equipment locally, allowing social and professional communities to grow around the shared resource.

The workshop participants supported openness as an underpinning concept for GCRF calls, where all applicants should be required to justify their approach to sharing or protecting their work and how their strategy maximises ODA-relevant impact. Global challenges benefit more from unrestricted deployment of scientific knowledge and tools.

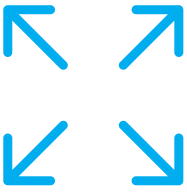
Openness has a range of potential impacts (see table opposite). Importantly, while the group were unanimous in the need to consider open approaches in synthetic biology for the African bioeconomy, they agreed that this is likely to form part of a two-tier strategy. Openly licensed or public domain tools will accelerate the pace of innovation, but at some point, investment and enterprise may require protection of specific applications - in order to best add value, create economic benefits and sustain activities. There are some situations where openness may impede deployment or run contrary to the needs of the beneficiaries (e.g. through precluding necessary investment in a technology or disadvantaging indigenous populations).

There are many examples of successful companies with intellectual property based on open technologies and a wide variety of business models that are not based on intellectual property as the key mechanism for capturing value, but instead rely on differentiation by quality, manufacturing, distribution channels and marketing, among other mechanisms. These approaches are well-suited for use in emerging bioeconomies.

Freedom-to-operate for entrepreneurs and companies without onerous and expensive licensing requirements, enabling value creation and small-scale, local enterprise.

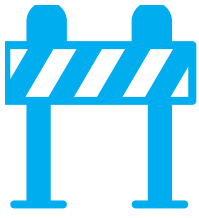


Decentralised collaboration for pre-competitive innovation



Scalable projects, particularly in small and under-resourced scientific communities.

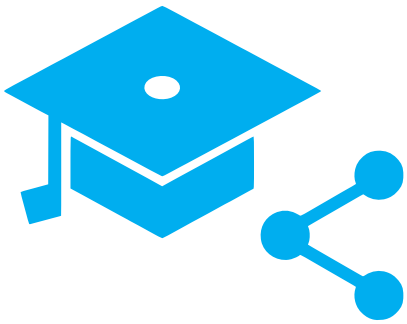
Acceleration of research through sharing and reduce time to translation and deployment.



De-risk and reduce duplication of effort and inadvertent lock-in.

Bakubung Report

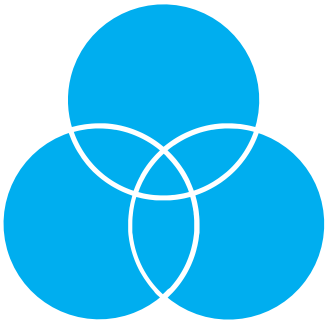
Synthetic biology and open-source applied biology tools that are pragmatic, safe and cost-effective have the potential to stimulate bioeconomic growth and address African challenges in healthcare, agriculture, education and the environment. OpenPlant is recruiting leading international experts to explore the latest developments in synthetic biology, bioengineering and DIY biology, their potential as training tools for students and future innovators, and practical opportunities for deployment in Africa.
This effort has been started with a symposium and workshop that was held in Pretoria and Bakubung, South Africa in February 2017, supported by the BBSRC and Global Challenges Research Fund (GCRF), a £1.5bn UK fund dedicated to the support of challenge-driven research in developing countries. The workshop resulted in the publication of a report that canvassed the difficulties and opportunities for promoting innovation in the emerging bioeconomy in Africa. In response, OpenPlant is coordinating an international programme for development of open materials for biological education in low resource environments. (https://www.openplant.org/global-challenges/)



Education and Training

Modern biotechnology is exemplified by the growing field of Synthetic Biology, where formal engineering principles and practices are being incorporated into biology. Generally, the costs of biological fabrication and testing are low compared to other high technology industries, and living products, such as crops, pharmaceuticals and bioproduction systems, can be self-propagating. A number of parallels can be drawn between writing computer software and writing DNA code, where participation can be relatively cheap, the output can have a high value, and progress can be self-sustaining. However these are both knowledge-based activities, and success is directly linked to (i) availability of adequate education and training resources, (ii) opportunities to access these, and (iii) the ability to transfer skills and innovations from an educational system to market.

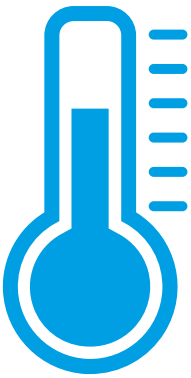
The Synthetic Biology field is providing new resources and approaches that offer prospects for dramatic improvements to teaching. For example, standardisation and modularisation of DNA engineering allows “de-skilling” and acceleration of complicated assembly processes, and new in vitro systems offer remote bioproduction and simple testing of DNA circuits without cold-chains, expensive laboratory equipment and containment facilities. The National Centre for Biotechnology Education (NCBE) in the UK has pioneered the co-development of pedagogy and accessible, cheap curriculum materials. Many African educational institutions suffer from underfunding and large student numbers, and would benefit from access to low-cost, state-of-the-art teaching materials. Just as the biological components of the kits are becoming more modular and easier to use, there is an opportunity to develop modular curriculum elements that could be easier to implement in different educational and training environments.



Interdisciplinarity

Interdisciplinarity is pervasive in Synthetic Biology applications and should be strongly encouraged. An understanding of the social, political, economic and cultural contexts of project sites, along with their legal and regulatory environments, should be demonstrated at the outset. Responses to contextual opportunities and challenges identified should be integrated into the project design through interdisciplinary collaboration throughout the project lifecycle. Particular attention should be paid to the perspectives of end users, from problem identification to implementation. Examples might include:

- Collaboration with the social sciences to understand the perspectives of end users and other stakeholders before, during and after the intervention.
- Collaboration with disciplines making downstream use of the new technologies, such as the medical or agricultural sciences.
- Using new and traditional media and visual and performing arts to generate informed public dialogue about the opportunities and risks of the new technologies and to facilitate enhanced understanding.
- Identification of policy, regulatory and legal gaps and bottlenecks; and interdisciplinary collaborations to devise mechanisms to address these.
- Collaboration with other biological and physical sciences and engineering to address technological challenges.
- Collaboration with industrial designers who use a user-centred design approach to develop context-appropriate tools and devices.



Eliminate the cold-chain

While synthetic biology approaches could be applied to solve problems in diverse areas such as diagnostics and bioproduction, the timescales for deregulation and release of bioengineered organisms are uncertain, particularly in the many African countries where regulatory processes are not yet in place or GMO technology is contested (Adenle et al., 2011). Additionally, while there is a strong demand for low-cost materials and resources for teaching applied sciences such as biotechnology, the lack of underlying infrastructure in the low-resource environments of many African countries means that maintaining a typical molecular biology laboratory is fraught with challenges. For practical training in synthetic biology in African schools and universities to succeed, and to stimulate the establishment of bio-maker spaces to facilitate informal education and innovation, there is a requirement for non-GM, rapid, cheap and safe materials.

Cell-free expression systems can be used for the development and optimization of synthetic gene networks. They have already been leveraged for the rapid screening of gene constructs and the application of the paper-based platforms for programmable in vitro diagnostics of human pathogens (Pardee et al., 2016),

and can be coupled to existing microfluidics expertise at the Council for Scientific and Industrial Research (CSIR) in South Africa.

This technology has the potential to overcome the bio-containment issues inherent with the use of live cells as biological chassis since living cells are not required. As a consequence, deploying tools outside of the laboratory environment (e.g. point-of-use biosensors for diagnostic applications) is more likely with cell-free systems with their vastly reduced regulatory burden and cell-free applications are therefore more likely than cell-based systems to achieve social impact in the short to medium term.

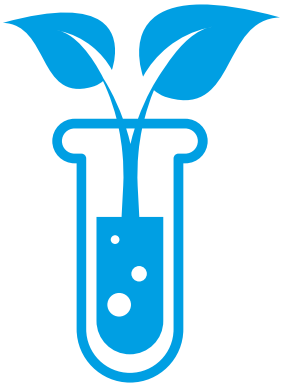
The avoidance of living cells also makes cell-free systems particularly well adapted to education in low-resource environment (Garamella et al., 2016), where cell extracts and reagents can be stabilized for storage at ambient temperatures, negating the need for cryostorage and reliable electricity supply that are required for living cells. They are likely to be an effective and affordable system for implementation in African training and education programs fashioned on competition models such as iGEM (www.igem.org).

Capacity building

Capacity building is a key requirement for developing partnerships in African research institutions and developing the African bioeconomy through Synthetic biology. Academic research capacity is generally underdeveloped in low- and middle-income countries (LMICs). Broad expansion of academic capacity to established and limi ted-resource research institutions is important to achieve impact (Van der Stocken et al., 2016). Access to standard biological reagents and laboratory equipment required for synthetic biology research are limited due to relatively high costs (e.g. international import costs, unfavourable currency exchange rates and price markups by local distributors). This drives up operational costs and is slowing the evolution of the continent's bioeconomy. Furthermore, the training capacity to implement basic molecular biology techniques (e.g. DNA manipulation and cloning) and use new synthetic biology tools (e.g. high-throughput DNA construct assembly, cell free systems, plant transient expression systems) to investigate biological problems is generally lacking (Vicente-Crespo et al., 2016).

There are currently no established African facilities that develop and manufacture materials and reagents required for synthetic biology such as basic molecular biology reagents and enzymes. Similarly, there are no facilities that make or supply open-source laboratory hardware (e.g. 3D-printed gel tanks, pipettes, microscopes) or biological parts and cell-free expression systems available to regional education and research institutions. The establishment of national or regional suppliers of low-cost, reliable, basic molecular biology consumables and equipment, together with strategic investment into facility upgrades, would significantly enhance the continent's capacity in Synthetic Biology at all levels, from educational labs to high-throughput, centralised bioeconomy research facilities.

The adoption of cutting edge Synthetic Biology tools and standard approaches would significantly enhance research capabilities; because much smaller reagent sets are required and the reagents are often non-proprietary. Synthetic Biology approaches are often cheaper than traditional molecular biology (e.g. Golden Gate Cloning and Gibson Assembly DNA assembly methods, compared to Gateway®). Furthermore, by combining the supply of inexpensive, locally manufactured reagents and open-source DNA parts with workshops to coach trainers, best practice can be shared effectively across African universities and research institutes. Co-ordinating the supply of reagents and open-source parts with UK-led training workshops for current and potential customers will accelerate knowledge transfer and technology uptake at facilities where Synthetic Biology research is relevant and needed. These could take place in the UK. However, greater reach would be achieved if UK scientists provide local workshops co-ordinated with the distribution of open components and tools. A combination of locally made and distributed tools, technologies and training would provide a powerful mechanism to build and sustain the African bioeconomy through practical application of Synthetic Biology.



Biodiversity

The Convention for BioDiversity (CBD) and the recent supplementary agreement known as the Nagoya Protocol on Access and Benefit Sharing, recognise the sovereign rights of nations to derive benefits from their biodiversity. The CBD has already recognised eight biodiversity hotspots in Africa. Three are in South Africa, in whole or in part, and the South African plan for developing a sustainable bioeconomy emphasizes harnessing the country's unique biodiversity to create jobs, industry and revenue, aiming to create a skilled local workforce and facilitate translation into sustainable businesses.
The advanced technologies and expertise available in the UK for metabolite analysis, genomics, data mining and pathway

(re)construction using metabolic engineering offer an excellent training opportunity for African scientists to become skilled in these areas, so complementing their interests in particular indigenous flora and their traditional uses. This would build skills that could support authentication of the quality of traditional medicines and open up opportunities and lead to new areas of innovation. As all such work is required to be compliant with the CBD/Nagoya Protocol and national laws such as the South African Biodiversity Act (2004, 2006), a social science stream should be integrated into any such initiatives. In particular, translational research of African native species raises concerns of, for example, the inequitable exploitation of indigenous knowledge, or the possibility of irresponsible bioprospecting of sensitive fauna and flora that could lead to their decimation for financial gain.

While knowledge exchange to allow local populations to harness their biodiversity may be an achievable short-term goal in South Africa, which has scientific infrastructure as well as relevant laws and treaties (see http://www.wipo.int/wipolex/en/profile.jsp?code=ZA), it would be hard to replicate in African nations without similar regulatory frameworks. However, initiatives like Future Africa could be leveraged to establish a conduit for inclusive knowledge exchange with the wider African scientific community and core activities such as training and capacity building would enable fair and equitable access to the necessary knowledge, skills and expertise in Synthetic Biology to allow scientists across Africa to harness the unique biodiversity of their local environs to create sustainable bioeconomies that have the potential to solve local problems in health and industry and that achieve global impact.

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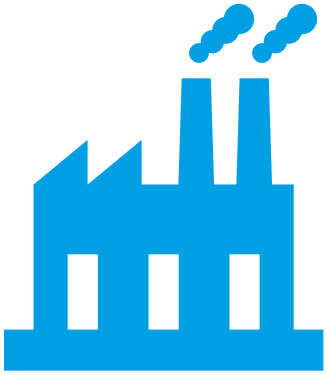


Future directions

Biological engineering through synthetic biology, and in particular new, rapid and non-GM tools, has the potential to develop the bioeconomies of several African countries and address major challenges facing the continent. In identifying and prioritising key applications of current synthetic biology technologies - local expertise, training tools, capacity building and facility development are core focus areas that will achieve practical solutions with social impact.

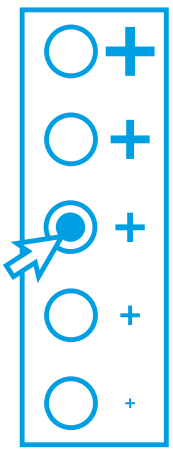
Knowledge exchange between UK and Africa-based institutions, local production and distribution centres for open-source tools and materials, as well as cost-effective educational tools for synthetic biology training and interdisciplinary innovation in resource challenged regions or institutions were regarded as key opportunities, among others.

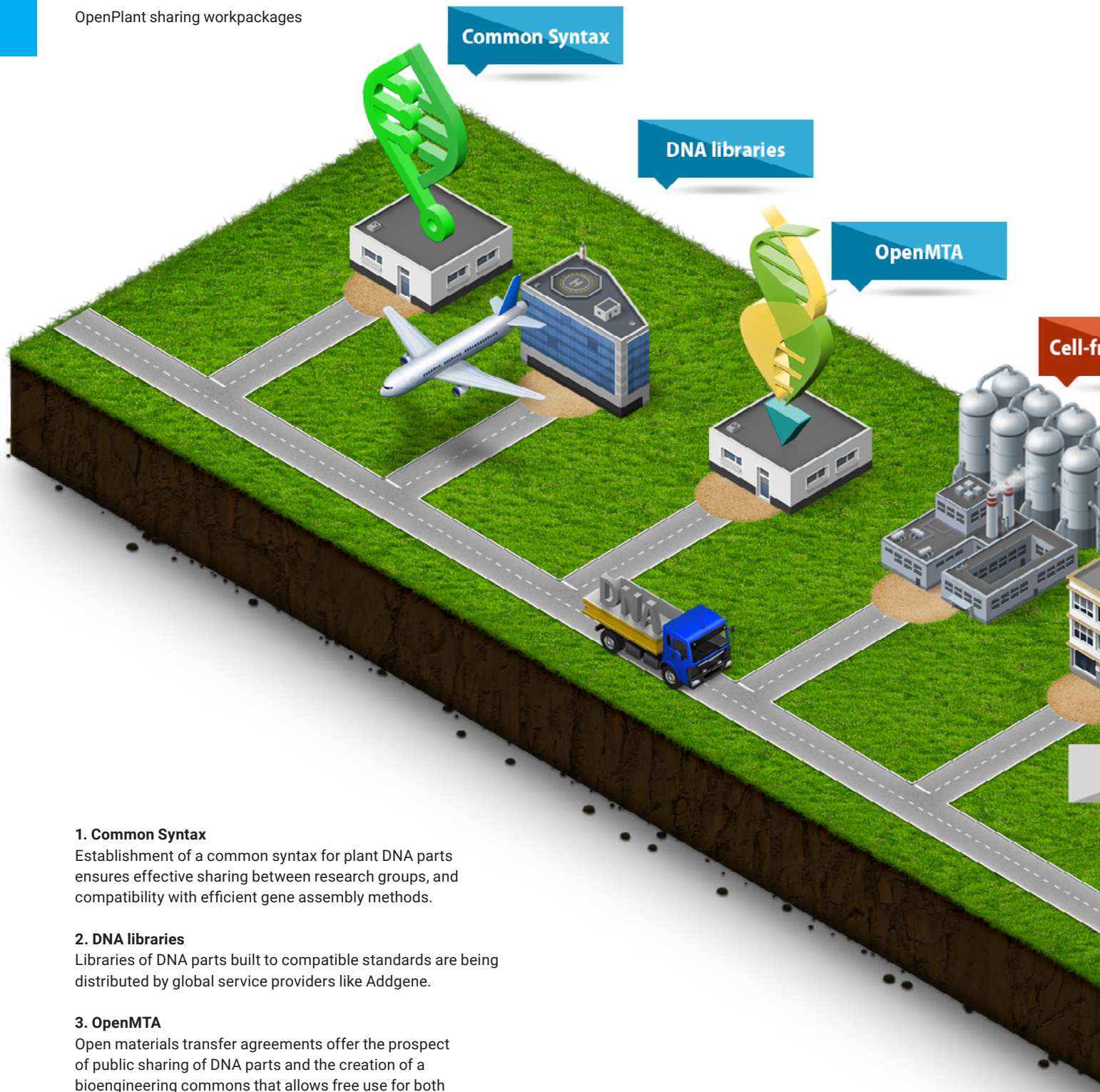
The participants favour leveraging GCRF-funded projects to increase knowledge and open materials transfer and develop local capacity for cell-free and transient bioproduction systems with co-funded infrastructure at leading African and UK institutions.

It is intended that new strategic and synergistic partnerships between such “anchor” institutions across the African continent will facilitate the transfer of skills, synthetic biology materials and expertise to less-equipped regions to achieve the maximum impact.

The full version of the Bakubung report can be downloaded from www.openplant.org

After the Bakubung meeting, a follow-up workshop was held in Cambridge, UK in order to explore the development of curriculum materials based on (i) the distribution of freeze-dried reagents for (ii) DNA programming of cell-free extracts using (iii) low-cost instrumentation and (iv) open source teaching resources. This resulted in the formation of an international group of scientists, educators, designers and engineers to implement this idea.





1. Common Syntax
Establishment of a common syntax for plant DNA parts ensures effective sharing between research groups, and compatibility with efficient gene assembly methods.

2. DNA libraries
Libraries of DNA parts built to compatible standards are being distributed by global service providers like Addgene.

3. OpenMTA
Open materials transfer agreements offer the prospect of public sharing of DNA parts and the creation of a bioengineering commons that allows free use for both research and commercial use.

4. Cell-free extracts
Non-GMO, freeze-dried extracts can be developed at low cost for *in vitro* gene expression. The lyophilised extracts can be shipped, stored and used without need for cold-chain, special containment procedures or expensive equipment.

5. Open Curricula
Cell-free extracts can be programmed with DNAs in order to test particular hypotheses, and paired with matching open sourcer educational materials.

6. Education & Outreach
Educational materials based on freeze-dried cell-free extracts and low cost microbioreactors are well suited for flexible delivery of teaching material in school, university and industrial training and outreach events.

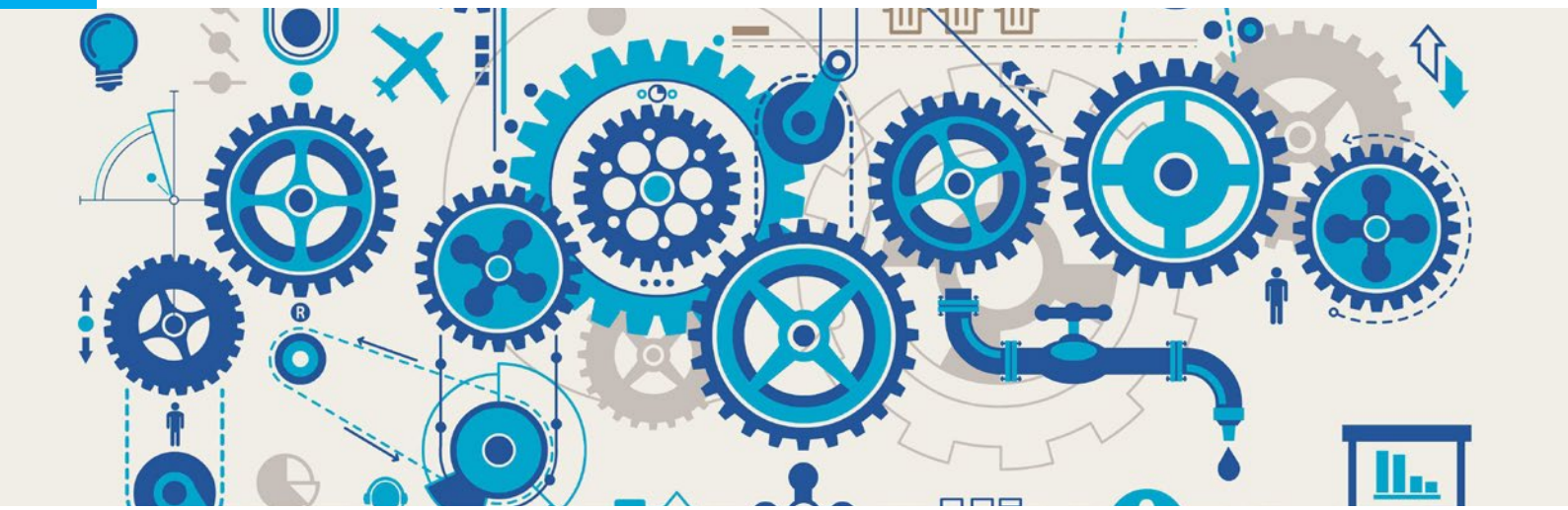
7. Health
Cell-free extracts can be used for training, and to develop simple diagnostics and field assays for health and veterinary applications.

8. Environment
Cell-free extracts can form the basis of cheap paper-based sensors for ubiquitous environmental surveillance.

9. Bioeconomy
Cell-free systems and open curriculum materials have the potential to revolutionise new teaching methods, and to help train the innovators and engineers required to take full advantage of the growing global bioeconomy

Roadmap for implementation

Programmable cell-free systems
The development of standards for DNA part curation, exchange and assembly has opened new opportunities for education and global knowledge transfer in synthetic biology. In particular, the new DNA technologies are being coupled to use of the latest generation of cell-free gene expression systems, which are relatively cheap, simple to employ and capable of high levels of activity. The use of cell-free extracts obviates the need to work with genetically modified organisms, with the attendant requirements for containment, culture and disposal. Further, cell-free extracts can be freeze-dried, and distributed at room termperature. They allow DNA-programmed experiments to be run as simple biochemical assays in low-resource settings, and provide quick and quantitative results. This provides a new route to improved education, training for industry and real-world field uses for engineered DNA circuits. OpenPlant is providing support for an international group of scientists, educators, designers and engineers to drive this forward.



Workpackage M:
Governance, Management & Communication

This workpackage is responsible for the overall management and coordination of the project. This involved key participants and coordinators from within the projects and external advisors. The Coordination and Management groups play the major role in monitoring progress, and contingency planning. This workpackage is responsible for running project management meetings, and ensuring coordination of activities between the Cambridge and Norwich sites. It is also responsible for coordinating the OpenPlant Forum and associated pump-priming and outreach activities.

Management team
David Baulcombe; Dale Sanders; Jim Haseloff; Anne Osbourn

Staff Employed
Colette Matthewman (Project Manager, Norwich)
Jenny Molloy (Project Coordinator, Cambridge)

M

Milestones:

M1: Monthly meetings of the Coordination Group
Deliverable: Monthly reports to Management Group (months 1-60, Haseloff, Osbourn).

M2: Quarterly meetings of the Management Group
Deliverable: Quarterly progress review and report (quarterly, months 3-60, Haseloff, Osbourn, Baulcombe, Sanders).

M3: Annual meetings of the Advisory Board
Deliverable: Annual report of overall progress (annually, months 12-60, Haseloff, Osbourn, Baulcombe, Sanders).

M4: Organisation of annual All-Hands meetings
Deliverable: Presentations and web based documentation of workpackage efforts (annually, months 12-60, Haseloff, Osbourn).

M5: Management of the OpenPlant Fund
Deliverable: Annual summary of funding allocation and outcomes for existing projects (annually, months 12-60, Haseloff, Osbourn, Baulcombe, Sanders).

M6: Management of the OpenPlant Forum
Deliverable: Selection of annual theme for Forum, suggestions for invited speakers and review of costs (annually, months 12-60, Haseloff, Osbourn, Baulcombe, Sanders).

M7: Coordination of SAW activities
Deliverable: Report of co-funded SAW Trust activities, and indentification of opportunities for participation by OpenPlant scientists (annually, months 12-60, Osbourn).

M8: Coordination of OpenLabTool activities
Deliverable: Report of co-funded OpenLabTools activities (annually, months 12-60, Haseloff).

Resources

Working documents held on Google drive

Archives held in Basecamp

Progress to date:

Objective M1-2: Monthly meetings of the Coordination Group & Quarterly meetings of the Management Group

Coordination of activities between the Cambridge and Norwich sites has worked effectively with weekly meetings between the Norwich-based Project Manager and Cambridge-based Coordinator to review progress and day to day needs. Monthly coordination group meetings and quarterly management group meetings are also held, with progress reports produced for the meeting. The minutes from the management meetings are shared with the Science Advisory Board via Basecamp. Science Advisory Board Meetings are held annually, held at the end of each OpenPlant Forum, which are scheduled to take place in the last week of each July. An annual report of progress for the OpenPlant project is produced each year, and these have been made available on the www.openplant.org website. The OpenPlant annual reports are submitted to the scientific advisory board for review. The SAB also provides a formal response for us and the funding agencies, and in turn, feedback is provided by BBSRC and EPSRC for consideration by the OpenPlant management group.

Objective M4: Organisation of annual All-Hands meetings

OpenPlant held a 1-day All-Hands meeting in May 2016, which was well attended by both PIs and PDRAs from the OpenPlant research centre. The main focus of the meeting was on tools and technologies being developed / used in OpenPlant labs and mechanisms for sharing information around these. The meeting provided a chance for PDRAs to update the network on their progress and for discussions around cross-cutting topics. The following five topics were points of discussion:

1. Tools and technologies (biological, hardware, software, automation etc.)
2. Open Innovation and exchange of resources
3. Opportunities for research and application
4. Training and community building
5. Commercialisation, engagement and impact

Several action points came out of the meeting, including new scientific collaborations, suggested topics for themed OpenPlant Fund calls, the establishment of a Slack account as an online communication forum for OpenPlant, and a PDRA led forum for discussion around methods and tools for sharing information.

A meetup group called “Researchers related to OpenPlant in Cambridge” (ROC) has been established in Cambridge. This group are holding monthly meetups discussing topics including sharing of information and resources. Information from these meetings is fed into a Slack discussion thread to share with researchers that are not able to attend the meetings. A PDRA-led meeting took place at the John Innes Centre prior to the OpenPlant Forum to bring together Norwich and Cambridge researchers and expand the ongoing discussions. The outcomes of this meeting were fed into a panel discussion at the Forum on enabling innovation through openness and exchange. Actions that have already been taken as a result of these discussions are the establishment of an OpenPlant project to share protocols on protocols.io: https://www.protocols.io/groups/openplant-project, and the establishment of an OpenPlant Benchling account for testing out DNA parts information sharing prior to public release.

Objective M5: Management of the OpenPlant Fund

The OpenPlant Fund rounds have been managed by the coordination group and awarded over 60 projects of £4000 each, with an additional £1000 that follows adequate performance and a the report (see Workpackage K). No major management issues have arisen and the call and judging mechanism were deemed appropriate following regular debriefings by the coordination group. Substantial coordination time has been invested in making connections between potential applicants, holding networking and events and a training event to prepare for the competition pitches. Generally, high quality applications have been received, and there has been a high rate of success for applications.

After the first OpenPlant Fund rounds, the OpenPlant management team made a decision to run future rounds as themed opportunities in order to more effectively seed interactions around the key tools, technologies and outreach initiatives required for plant synthetic biology. Ideas for focus areas were collected at the All-Hands Meeting in May 2016. For more details on the projects see Workpackage K.

The OpenPlant Fund has been joined by the Biomaker Challenge, which is also being managed and coordinated by Colette Matthewman and Jenny Molloy. This has resulted in two funding calls per year and effort is put into building new inter-institutional and interdisciplinary collaborations through mixer events, linking people up through email and sharing project ideas in newsletters. Management of the Biomaker Challenge has led to the organisation and implementation of a series of workshops, including use of Github for shared documentation, and development of shared software and hardware skills.

Objective M6: Management of the OpenPlant Forum

The 2015 OpenPlant Forum took place at the University of Cambridge 28-29 July with around 90 attendees. These represented the majority of the consortium and many external participants from other projects, institutions, organisations and companies. Intellectual property and enabling technology was chosen as the theme. Invited speakers included Jane Calvert (University of Edinburgh) on forms of openness, Linda Kahl (BioBricks Foundation) on open material transfer agreements, David Rejeski (Wilson Centre) on regulation and synthetic biology, Chas Bountra (University of Oxford) on open science translating to innovation from the perspective of drug discovery and more.

The 2016 OpenPlant Forum took place at the John Innes

Conference Centre, 25-27 July with over 100 attendees. There was a good representation from the consortium and national and international external participants from other projects, institutions, organisations and companies, including several post-docs from the Warwick SBRC. The focus was on reprogramming agriculture, and invited speakers included Allan Green (CSIRO), Johnathan Napier (Rothamsted), Matthew White (AB Sugar), Spencer Adler (Bioeconomy Capital), and Tom Knight (Ginkgo Bioworks). Discussion panels were highly multidisciplinary, and included Monique Simmonds (Kew Gardens), Wieke Betten (Ethicist, VU Amsterdam), Dominic Berry (Science Historian, University of Edinburgh), Tobias Wenzel (Department of Physics, University of Cambridge), and Peter Murray-Rust (ContentMine). There was also an opportunity for the OpenPlant PDRAs to present their research, either orally or as a poster. This gave the SAB a chance to see the latest research progress.

The 2017 OpenPlant Forum was held at Downing College, Cambridge and canvassed fast and frugal techniques for engineering biology, with applications in research, education and international development. It provided a showcase for new technical developments in lyophilised cell-free systems and curriculum development, which allow gene expression experiments in low resource environments with poor access to refrigeration and containment facilities. Invited speakers were: Jim Swartz (Stanford), Keith Pardee (University of Toronto), Sebastian Maerkl (EPFL), Simon Moore (Imperial College), Simon Moore (Imperial College), Maneshree Jugmohan-Naidu (South African National Department of Science and Technology), Simon Trace (Oxford Policy Management), Lara Allen (Centre for Global Equality), Fiona Robertson (University of Zimbabwe), Jake Wintermute (CRI Paris), Karen Ingram (Artist), Helene Steiner (Microsoft Research), Mario Arteaga (Universidad Veracruzana), Dan Jenkins (Science and Plants for Schools).

Objective M7: Coordination of SAW activities

Public engagement and outreach through SAW Trust activities is being coordinated by Jenni Rant and Colette Matthewman. Several activities were organised in this first year. Further details are provided in the Workpackage L progress reports.

The activities have continued to grow, year-by-year, with a large increase in involvement from OpenPlant PDRAs and OpenPlant Fund grant recipients. A successful application to Latitude Festival earned OpenPlant a 3-day exhibit at this mixed-arts festival in 2016.

2017 has seen a busy schedule of activities in schools, at science festivals, at music festivals, and on the John Innes Centre site. In addition to the SAW Trust activities, Colette has delivered activities for talented and enthusiastic young scientists, age 14+ with the Youth STEMM Award programme (http://ysawards.co.uk/). This work is feeding activities into the SynBio for Schools OpenPlant Fund project.

Objective M8: Coordination of OpenLabTool activities

OpenPlant supported OpenLabTools projects, with University of Cambridge engineering students working to produce open source scientific instrumentation to support synthetic biology. Outcomes were published at www.openlabtools.org.

In 2015 the University of Cambridge and the John Innes Centre jointly supported an iGEM team to enter the hardware track and were awarded a gold medal at the iGEM jamboree for their OpenScope, a 3D-printed microscope using a Raspberry Pi Camera. In 2016, the joint iGEM team, InstaCHLAM, focused on chloroplast transformation in *Chlamydomonas* and competed for (and won) a new Plant prize, and helped to introduce Phytobricks as an official DNA standard the iGEM competition. The 2016 Cambridge-JIC iGEM teamwas managed by Jim Haseloff and Jenny Molloy in Cambridge with help from several OpenPlant post-docs. In 2017, these resources and efforts were directed through the Biomaker Challenge programme - designed to expand participation throughout Cambridge and Norwich, and to promote interaction amonst a larger group of participants (around 100 in 2017).

Leadership group

Prof. Anne Osbourn, Norwich Director
Anne investigates plant natural products - function, synthesis and mechanisms underpinning metabolic diversification. An important advance from the Osbourn laboratory has been the discovery of gene clusters for specialized metabolic pathways in plants, a finding that has opened up new opportunities for elucidation of new pathways and chemistries through genome mining and for the development of synthetic/refactored clusters for improved/high-value plant traits. She has also developed and co-ordinates the Science, Art and Writing (SAW) initiative, a cross-curricular science education programme for enabling engagement of scientists with society.

Prof. Jim Haseloff, Cambridge Director
Jim and his lab engineered the first synthetic RNA enzymes with targeted substrate specificity, developed fluorescent proteins for plants, new misexpression systems in plants, new 3D microscopy and visualisation methods and computer models for plant morphogenesis. He has pioneered the application of Synthetic Biology approaches in plants, including new quantitative imaging techniques, genetic circuits for cell-cell communication, and adoption of lower plant systems for bioengineering.

Prof. Sir David Baulcombe, Principal Investigator
David's group was the first to identify small interfering (si)RNAs as the specificity determinant of RNA silencing and through their genetic analyses have identified many components of RNA silencing pathways. Relevant to this application the group has unravelled many aspects of the role of RNA silencing in virus defense and other aspects of genetic and epigenetic regulation. His work has been recognised through several awards including the 2008 Lasker Award for Basic Medical Science, the 2010 Wolf Prize for Agriculture and the 2012 Balzan Prize for Epigenetics.

Prof. Dale Sanders, Principal Investigator
Dale's research investigates how plant cells respond to changes in their environment and how they store the nutrients they acquire. He is a leading authority on the mechanisms for the transport of chemical elements across cell membranes in plants. These mechanisms have key roles in the control of crucial crop traits such as nutritional value of foods, seed germination, response to drought and how plants cope with toxic compounds in the soil.

Coordination group

Dr. Colette Matthewman, Manager
Colette is the Norwich-based Project Manager for OpenPlant. With a research background in the plant sciences, she has a broad overview of OpenPlant research activities, and coordinates events, training, and outreach to build new synergies and increase the impact of the centre. She is a member of an OpenPlant working group exploring new IP solutions for biotechnology and is leading a project to develop resources for school pupils to learn about synthetic biology.

Dr. Jenny Molloy, Coordinator
Jenny is the Cambridge-based Coordinator for OpenPlant and the University of Cambridge Synthetic Biology Strategic Initiative. Jenny is a molecular biologist by training and researched genetic control of mosquito populations while becoming increasingly interested in the role and impacts of open source in science. She enjoys being an enabler of open approaches and her role involves coordination of events and activities including the IP working group and OpenPlant Fund, through which the centre is developing new legal tools for sharing and a wide variety of innovative open technologies.

Dr. Susana Sauret-Gueto, Cambridge Research Manager
Susana is an experienced molecular biologist and microscopist. She has established new facilities for robotic liquid-handling and advanced microscopy in the Cambridge OpenPlant laboratory, and is coordinating the sharing of standardised Marchantia resources. These include libraries of DNA parts and transformed plant lines. With a scientific background in plant growth and development, she supports researchers and strengthens the integration of research projects. Susana is the main organiser of the ROC Group (Researchers with OpenPlant Cambridge).

Research leaders

Dr. Fernan Federici, OpenPlant International Fellow
Fernan is an assistant professor at PUC (Santiago, Chile) and manages a research group that explores spatial patterning, open science and educational efforts across Latin America. Fernan has a long association with Cambridge as a Gates Scholar and research fellow. He is continues to play a pioneering role, contributing open technologies for bioengineering, science and education across Latin America, and exploring international collaborations with OpenPlant colleagues.

Dr. Nicola Patron, Earlham Institute
Nicola is a Group Leader in Synthetic Biology at the Earlham Institute. Her work aims to develop technologies to engineer photosynthetic organisms for the biosynthesis of materials and therapeutics and to improve plants for increased production and nutritive value. Her broader interests are in understanding the function of DNA sequences and the mechanisms and consequences of gene transfer. As a SynBio LEAP fellow Nicola was recognized as an emerging leader in synthetic biology with a desire to ensure that synthetic biology has positive social impact; she is interested in the complex questions of ownership and intellectual property that surround genetic sequences and biomolecules.

Dr. Jim Ajioka, University of Cambridge
Jim's lab works on large scale DNA assembly of synthetic circuits in Gram positive bacteria and protozoan biology. He leads a Wellcome Trust programme to build and employ novel biosensors, using Synthetic Biology techniques. Jim's lab is also funded by the EPSRC for foundational work such as generalised codon optimisation, robust switches and counters and big DNA manipulation. The lab's work on big DNA extends to the collaboration with the Haseloff lab on plant plastids.

Prof. Sarah O'Connor, John Innes Centre
Sarah uses transcriptomic and genomic data to elucidate the alkaloid pathways of Madagascar Periwinkle, a medicinal plant that produces compounds that are used to treat a variety of cancers and other diseases. Plants synthesize thousands of complicated molecules that they use to protect themselves from predators, attract pollinators and communicate with other plants. Thousands of years ago, humans realized that many of these plant-derived molecules also have a powerful impact on human health and well-being. Advances in genomic and transcriptomic sequencing have rapidly advanced understanding of the complex metabolic pathways that produce these high-value chemicals.

Prof. Rob Field, John Innes Centre
Rob has 30 years' experience in glycobiology and associated (bio)chemistry. His interests lie in understanding and exploiting carbohydrate recognition, in the design of enzyme inhibitors as probes plant and microbial metabolism, and for the development of lectin-binding anti-adhesive agents to impact on cell adhesion by microbial pathogens (trypanosomes, Campylobacter, flu virus). These activities are underpinned by synergistic synthetic chemistry and synthetic biology efforts aimed at providing new routes to scalable bespoke carbohydrate production.

Prof. Paul Dupree, University of Cambridge
Paul studies plant cell wall polysaccharide synthesis, structure and function. These carbohydrates have important functions in the human diet, agriculture, bioenergy, paper and packaging and for building construction using timber. He has developed a range of innovative techniques for quantitative analysis of polysaccharides, such as PACE for studies of polysaccharide structures and enzyme activities, and DASH capillary electrophoresis of oligosaccharides using DNA sequencers. Having discovered a number of the enzymes that synthesise cell walls, he is now engineering plants to produce novel polysaccharide structures. This approach will generate plants with modified cell walls for improved material properties, and will enable producuction of high value plant products.

Prof. Giles Oldroyd, Sainsbury Laboratory Cambridge University
Giles is a leading investigator in plant-symbiotic interactions, with a particular focus on the signalling processes that allow the establishment of nitrogen-fixing and arbuscular mycorrhizal associations. His work has provided the genetic underpinnings to understand the symbiosis signalling pathway that allows rhizobial recognition in legumes and mycorrhizal associations in most plants. He leads an international programme funded by the Bill and Melinda Gates Foundation and the BBSRC that is attempting to engineer cereal recognition of rhizobial bacteria as the first step towards engineering nitrogen-fixing cereals.

Prof. Christopher Howe, University of Cambridge
Chris has long experience with the biochemistry and molecular biology of photosynthetic bacteria and chloroplasts, with a particular emphasis on electron transfer reactions. His lab has pioneered the development of 'biophotovoltaic' technology – the direct production of electricity from photosynthetic microorganisms – which underpins his contribution to OpenPlant. He has also made influential contributions to our understanding of the evolution of chloroplast genomes in organisms ranging from plants to protists. He is a scientific advisor to two local companies working in microbial biotechnology.

Prof. Cathie Martin, John Innes Centre
Cathie uses genetics, biochemistry and molecular biology to investigate the basis of cellular specialisation in plants. This includes many aspects of metabolic specialisation, particularly phenylpropanoid metabolism and its regulation. She has used this to effectively engineer the production of polyphenol bioactives in crops, demonstrating healthpromoting properties in preclinical studies. Her expertise on transcriptional regulators of metabolic pathways has been applied in a wide range of plant species, establishing effective plant production systems of natural products including natural colours and bioactives from Chinese medicinal plants.

Prof. Alison Smith, John Innes Centre
Alison (JIC) studies starch and sucrose metabolism. Her recent work is on starch degradation in Arabidopsis leaves at night, the control of flux through this pathway and its relationship to carbon availability and growth. Her lab also studies pathways of starch metabolism in crops, including potato and wheat. A major current interest is the relationship between starch synthesis and grain yield in wheat.

Prof. Alison G Smith, University of Cambridge
Alison (CAM) focuses on metabolism of plants and algae, particularly biosynthetic pathways for high value products such as vitamins, pigments and lipids. She has been developing tools for improved genetic manipulation of microalgae, in particular by generating regulatory genetic circuits using vitamin responsive promoters and riboswitches. By taking a synthetic biology approach to generate standard parts and workflows for optimal transgene expression, the aim is to establish microalgae as suitable platforms for industrial biotechnology production. In addition, she has established the Algal Innovation Centre in Cambridge that allows scale up of algal cultivation.

Prof. Alex Webb, University of Cambridge
Alex's lab is investigating how plants measure time by studying the circadian clock. They identify how the circadian clock provides benefits to plants to maximize their growth and productivity. As part of these studies they discovered that the regulation of photosynthesis, carbon metabolism and growth are regulated by the circadian clock. They use molecular genetic, transcriptomic, imaging and physiological techniques to understand circadian mechanisms. They also develop new engineering approaches for systems biology in collaboration with Engineers. They are collaborating with Bayer to convert our biological discoveries in to real world solutions for crop improvement.

Prof. Julian Hibberd, University of Cambridge
Julian's research aims to understand how C4 photosynthesis operates and to provide insight into the molecular mechanisms driving its evolution. The group uses a mixture of wet-lab, computational and synthetic approaches to answer these questions. His work includes the demonstration that C3 plants possess characteristics of C4 photosynthesis, the identification of cis-elements that underpin the expression of multiple C4 genes in evolutionarily independent C4 lineages, and technologies to allow specific cell types to be marked and isolated in leaves of C3 species.

Dr. Sebastian Schornack, Sainsbury Laboratory Cambridge University
Sebastian studies processes underlying the interaction of microbes with plants, especially plant processes targeted by microbial effector proteins. He is credited with the discovery of DNA base-specific TAL effector repeat-binding in promoter elements of target host genes. This discovery led to generation of customised TAL based transcription modulators and nucleases with unrivalled DNA binding specificity, that are now being widely exploited in animals and plants.

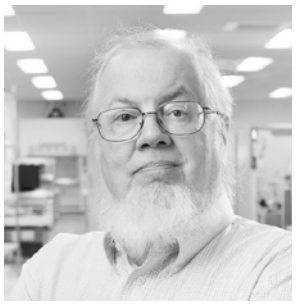
Prof. George Lomonossoff, John Innes Centre
George is a project leader in the Department of Biological Chemistry JIC, with more than 30 years post-doctoral experience working with plant viruses and plant virus-derived expression systems, including the CPMV HyperTrans™ system for which he was named BBSRC Innovator of the Year 2012. This expression system is used in over 200 academic institutions, and employed for commercial production of human vaccines. He has considerable experience of large collaborative projects and international collaborations in the field of biotechnology. He also has extensive experience in handling intellectual property issues, is a named inventor on several patents and acts as a consultant for several companies.

Dr. James Locke, Sainsbury Laboratory Cambridge University
James is an expert in mathematical modelling and single cell analysis of genetic networks. He developed the first model of the plant circadian clock, and experimental data and modelling to correctly predict a new feedback loop. He co-developed a high-throughput time-lapse single cell analysis and tracking system for bacteria, and used the system to discover a new mode of prokaryotic gene regulation; stochastic frequency modulated pulsing. He is studying stochasticity and signal integration at the single cell level in B. subtilis, plants and Cyanobacteria

Prof. Pietro Cicuta, University of Cambridge
Pietro's group uses optical tweezers, microrheology, advanced confocal microscopy and image analysis methods to address dynamics both in colloidal and cellular systems. The lab's research includes self-assembly of phospholipids, including physical properties of lipid bilayers, hydrodynamic synchronisation of motile cilia, including model colloidal systems and living ciliated cells, particularly human airways; and physical mechanisms of regulating gene expression in bacteria.

Prof. Lisa Hall, University of Cambridge
The main theme of research in Lisa's Analytical Biotechnology Group is in heterogeneous analytical systems, with a primary but not exclusive focus on molecular sensors, the latter including both chemical and biological systems. The activities are concerned with interfacing these systems and/or principles of mechanism and action, with transduction technologies to achieve diagnostic devices and monitoring capability. This research is directed towards environmental, medical and industrial application, with the group pro-active in responding to and advising industry of existing capability and future direction.

Scientific Advisory Board

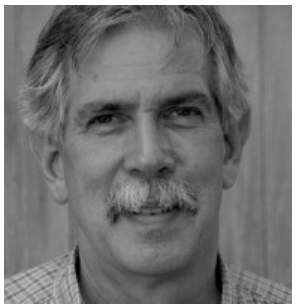


Dr. Tom Knight, Ginkgo Bioworks, Chair of Scientific Advisory Board
Tom is widely regarded as father of the Synthetic Biology field from his work at MIT, which followed seminal work in early networking technology and artificial intelligence at MIT Computer Science and AI Laboratory, a part of the MIT School of Engineering. He has an extraordinary record of serial innovation in different fields, and is orginator of the first widely used standard for DNA parts, and was a driving force behind the start of iGEM and the Registry of Standard parts, an open competition and social network for assembly and sharing of DNA parts. He now runs a prominent synthetic biology start-up in Boston, Ginkgo Bioworks - engineering high value biochemical pathways in microbes. Tom is widely respected as one of the brightest and most original thinkers in the field. He is extremely well connected, and very interested in plant work.

Dr. Tim Fell, Synthace, Co-Chair of Scientific Advisory Board
Tim is an experienced technology venture entrepreneur with an R&D background in both the physical and life sciences. Before Synthace, he was Chief Operating Officer of CellCentric, a leading epigenetics drug discovery company, Chief Technology Officer of Arrow Therapeutics and co-founder and General Manager of DNA microarray tools company, Oxford Gene Technology (Operations). Prior to this, Tim spent 13 years performing highly interdisciplinary research at the University of Oxford holding post-doctoral positions in three different departments (Biochemistry, Engineering and Materials). He has a D.Phil in Semiconductor Materials and an MBA from London Business School. Tim is the Chairman of the UK BioIndustry Association's Synthetic Biology Advisory Committee and also a member of the Synthetic Biology Leadership Council.

Prof. Christina Smolke, Stanford University
Christina is one of the few "dyed-in-the-wool" synthetic biologists exploring plant systems, outside OpenPlant. She has a very high profile in the field, with a fast-track career at Berkeley-Caltech-Stanford - working on the engineering of RNA-based control mechanisms and natural product biosynthesis. She's been president of the Society for Biological Engineering, and has string of awards to her name: NIH Director's Pioneer Award, National Institutes of Health (2012), World Technology Award in Biotechnology (Individual), World Technology Network (2009), Alfred P. Sloan Foundation Fellow, Alfred P. Sloan Foundation (2008), National Science Foundation CAREER Award, National Science Foundation (2006),

Prof. Drew Endy, Stanford University
Drew is a well-known evangelist for synthetic biology. As well as his scientific work at MIT and Stanford, Drew has provided early leadership and support for many open biotechnology programs. He was the co-founder of the iGEM competition, OpenWetWare.org, the Biofab and Bionet, efforts to share high-quality standard biological parts. Drew is founder and President of the BioBricks Foundation, which supports open technical and legal standards. He has worked with Congress, the White House, DARPA, OECD, the National Academy, etc. on policy matters. Drew is a "big ideas" person, with an exceptional track record in promoting successful international open science initiatives in synthetic biology.



Dr. David Rejeski, Woodrow Wilson Institute
David directs the Science and Technology Innovation Program (STIP) at the Woodrow Wilson Center in Washington DC, including synthetic biology (www.synbioproject.org). STIP focuses on emerging technologies and the critical choices innovation presents to public policy. He has graduate degrees in public administration and environmental design from Harvard University and Yale University and a degree in industrial design from the Rhode Island School of Design. He founded and co-directed a non-profit involved in renewable energy technologies, was head of the Future Studies Unit at the US Environmental Protection Agency, and worked at the White House Council on Environmental Quality (CEQ) and the Office of Science and Technology (OSTP) on a variety of technology, R&D, and policy initiatives.

Prof. François Kepes, Genepole, CNRS, France
François is a noted leader in the Synthetic Biology field in Europe. He studies and engineers genome architecture. For this purpose he uses various approaches including bioinformatics and molecular, systems and synthetic biology. François Képès is a Research Director at CNRS. He is the Founding director of the Epigenomics Project (Genopole), an Institute of Complex Studies that is dedicated to the emerging disciplines of Systems and Synthetic Biology. He is a Team Leader at the institute of Systems and Synthetic Biology (Genopole, CNRS, UEVE). He is a permanent Invited Professor at Imperial College London. He is a member of the National Academy of Technologies of France.

Prof. Susan Rosser, University of Edinburgh
Susan Rosser is a Professor and PI of the SynthSys-Mammalian: Edinburgh Mammalian Synthetic Biology Research Centre. Susan studied microbiology and genetics at the University of Dundee before a PhD working on the mechanisms of multiple antibiotic resistance. Susan then moved to the Institute of Biotechnology at the University of Cambridge to work on biotransformations of cocaine and high explosives. She then became a lecturer in biotechnology at the University of Glasgow before being promoted to Professor in 2012, followed by a Chair in Synthetic Biology at the University of Edinburgh.

Dr. Scott Steedman, British Standards Institute
Director at the British Standards Institution (BSI), where he is responsible for the work of the UK National Standards Body, representing the UK internationally and for advising industry and government on the role of standardization in the economy. Prior to joining BSI in January 2012, Scott spent around twenty years working on major infrastructure and building projects in the UK and around the world for consulting and contracting companies, including GIBB, Whitbybird, High-Point Rendel and Foster Wheeler Energy. Formerly a lecturer at Cambridge University, he has specialised in natural disasters, forensic engineering, risk and innovation strategy. Scott chaired the European Council for Construction, Research and Innovation for over a decade, and is former Vice President of the Royal Academy of Engineering.

Research Council Programme Managers

Dr. Rowan McKibben, BBSRC
Head of Science Strategy: Exploiting New Ways of Working at the Biotechnology and Biological Sciences Research Council.

Dr. Jamie Parkin, BBSRC
Strategy and Policy Manager at the Biotechnology and Biological Sciences Research Council, covering Synthetic Biology, Systems Biology, Genomics and Mathematical Biology.

Rebecca Leithall EPSRC
Senior Portfolio Manager for Balancing Capability, Infrastructure, Sensors and Instrumentation. Engineering and Physical Sciences Research Council.

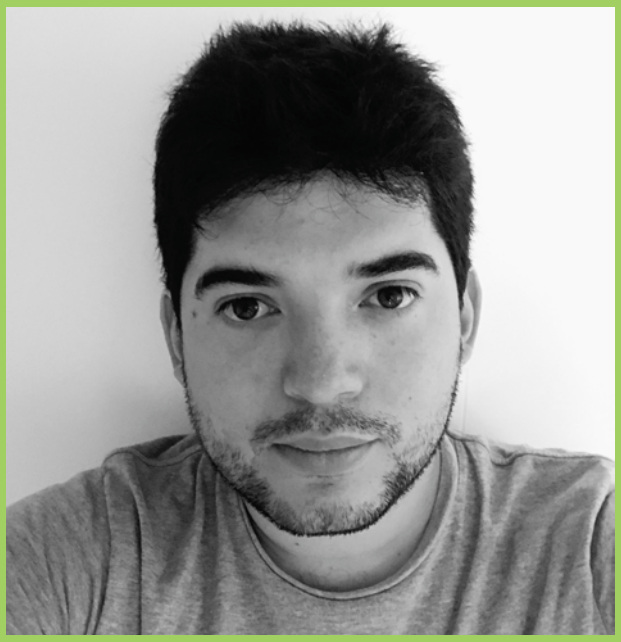
Research Associates

Dr Philip Carella
Postdoctoral Fellow, Schornack Lab, Sainsbury Laboratory, University of Cambridge

I recently completed my PhD in Dr. Robin Cameron's lab (McMaster University, Canada), where I studied phloem-mediated long-distance immune signalling induced by a bacterial pathogen in Arabidopsis thaliana. Feeling a need to branch out a little, I joined Dr. Sebastian Schornack's group (Sainsbury Laboratory, University of Cambridge, UK) to study interactions between filamentous microbes and non-vascular early land plants. Our goal is to identify core developmental processes required for the colonization of early land plant tissues by filamentous microbes and to understand how these processes evolved into the defense and symbiotic programs employed by higher plants. Our work will generate transcriptomics data, fluorescent marker lines and microbe inducible promoters for cell biology, and other molecular-genetic tools that will enable the OpenPlant community to explore early land plant biology.

Dr Eftychis Frangedakis
Postdoctoral Fellow, Haseloff Lab, University of Cambridge

Eftychis did his PhD at Oxford University focusing on the evolution of developmental mechanisms in land plants. During his doctoral research he developed a strong interest and fascination for bryophytes. He then moved to the University of Tokyo to work with the least studied group of bryophytes, hornworts. After a short detour in Hong Kong he is now back to the UK working on the development of new synthetic biology tools in Marchantia. In particular, he is developing tools for engineering the chloroplast genome, where work with the liverwort allows the benefits of single-cell handling through spores, facile transformation and regeneration, and access to a full set of genetic and optical tools for manipulation and quantitative screening of the organism.



Dr Henry Temple
Postdoctoral Fellow, Dupree Lab, University of Cambridge

Plant cell walls represent the most abundant renewable source on the planet, but only a small fraction of this biomass is used by humans. With ongoing interest in use of cell wall polysaccharides, we are just starting to understand their biosynthesis in plant cells. Synthesis of polysaccharides occur mainly through the activity of glycosyltransferase (GTs) enzymes which transfer an activated sugar in the form of a nucleotide-sugar onto a specific growing polysaccharide acceptor. I have great interest in the different processes that govern cell wall biosynthesis. In my Master's and PhD thesis I worked on characterisation of Golgi localised nucleotide sugar transporters (NSTs) responsible for the incorporation of substrates used by GTs enzymes. Now I'm working in Professor Dupree's laboratory as a Postdoctoral Research Associate on a very exciting project, where our goal is to manipulate polysaccharides synthesis by developing genetic tools expressing different GT activities (and other required activities) under tissue specific promoters to evaluate whether it's possible to engineer polysaccharide synthesis, proportions/structures and assess the consequences of these changes

Marta Tomaselli
PhD student, Haseloff Lab, Schornack lab, University of Cambridge

I did my bachelor and master in Biotechnology in Pisa, where I discovered how fascinating plants can be. In the past, I have worked with CRISPR/Cas9 systems in two different plant models: *Arabidopsis thaliana* and *Marchantia polymorpha*. These were my first experiences related to synthetic biology and they really got me involved. I started as an OpenPlant PhD student at the University of Cambridge in 2016. During a rotation in the Haseloff Lab, I developed optical clearing techniques for microscopy of Marchantia gemmae. These tools allow 3D reconstruction of the plant tissue. In my second rotation in the Schornack lab, I focused focused on plant-pathogen interactions: looking for pathogen-responsive promoters in Marchantia. These sequences can be exploited to generate new reporter lines.
In the future, I wish to continue working with Marchantia and exploit this plant as a model to implement new synthetic circuits. I think that the OpenPlant Community is a great resource for a PhD student, since a lot of different topics are covered by senior researchers, who can help answer questions and provide suggestions about your own project.

Dr Bruno Martins
Postdoctoral Fellow, Locke Lab, University of Cambridge

I am a post-doctoral researcher in James Locke's group at the Sainsbury laboratory. I am interested in how cells discriminate between different environmental states, integrate dynamic outputs from different gene circuits, and make decisions. In my current research, I use a combination of theory and time-lapse microscopy experiments to understand the dynamical coupling of the cyanobacterial circadian clock to other networks, in both endogenous and synthetic systems.
Before coming to Cambridge, I did a PhD in Peter Swain's lab at the University of Edinburgh. In my PhD I used mathematical modelling to gain insight into two simple, yet ubiquitous, sensing and transductions mechanisms: allosteric sensing and phosphorylation-dephosphorylation cycles. I studied the input-output dynamics of these mechanisms in terms of the fundamental constraints inherent in their design.

Dr Francisco Navarro
Postdoctoral Fellow, Baulcombe Lab, University of Cambridge

Fran's work focuses on the function of small RNA (sRNA) molecules and their use as regulatory elements in synthetic gene circuits. sRNA molecules most likely evolved as a defense mechanism against viruses and retro-transposons, and were co-opted for fine-tuning of gene expression. Their small size and predictable targeting rules make them perfect tools for regulating gene expression in synthetic gene circuits. This project is carried out in the green alga *Chlamydomonas reinhardtii*, which is amenable to genetic manipulation and possesses a sRNA pathway that resembles that of higher plants. Chlamydomonas provides a testbed for plant RNA-based genetic devices.
Fran completed his PhD in the laboratory of Prof. Jose Manuel Siverio (University of La Laguna, Spain), studying nitrate assimilation in *Hansenula polymorpha*, a methylotrophic yeast with important biotechnological applications. This was followed by a postdoc in the laboratory of Sir Paul Nurse, at The Rockefeller University, USA, and the London Research Institute, on cell size control and regulation of gene expression by RNA-binding proteins in *Schizosaccharomyces pombe*.

Linda Silvestri
Research Technician, Haselhoff Lab, University of Cambridge

As the Research Technician for the Haseloff group, I work closely with Susana Sauret-Gueto, Research Lab Manager, to ensure the smooth running of the lab. I am responsible for *Marchantia polymorpha* tissue culture and am working on the standardisation of existing protocols for the propagation, transformation and short and long term storage solutions, including cryopreservation.
This work will enable and facilitate the high-throughput screenings of Marchantia lines, such as the Enhancer Trap lines; a project on which several lab members collaborate. A summer student joined us for 8 weeks to work on this project and I helped with her supervision and provided laboratory training.

Dr Lukas Müller
Postdoctoral Fellow, Webb and Haseloff Labs, University of Cambridge

I'm interested in the circadian clock and its effect on physiological and agricultural performance in plants. In the OpenPlant project I am investigating the circadian clock in Marchantia polymorpha and analyze the regulation of clock behavior and outputs in this relative of early land plants. In particular, I am focusing on the primary metabolism as an excellent proxy for systemic processes and vegetative growth.

I apply fluorescent imaging tools with computational time-lapse analysis to obtain cell-specific read-outs for the whole plant in real-time. This data is intended to set the stage for both physiological engineering and systems biology approaches. Part of my project is to engineer fluorescent proteins that are standardised and improved reporters for dynamic changes in gene expression.

Dr Ingo Appelhagen
Postdoctoral Fellow, Martin Lab, John Innes Centre

I am a molecular biologist, with a background in plant transcription factors, flavonoid biosynthesis, natural colours and metabolic engineering. In Cathie Martin's lab at the John Innes Centre, we have recently developed novel suspension cultures from engineered tobacco plants, to obtain stable sources of natural colourants. These cultures can produce exceptionally high levels of red to purple anthocyanin pigments, and allow a scalable constitutive year-around production under controlled conditions.

Intense blue colours are rare in nature and difficult to reproduce in pigment formulations, which is the main reason why almost all blue food colourants are synthetic dyes. Our project aims to investigate the structural properties of anthocyanin preparations that confer strong and stable blue colours and to select for anthocyanins with improved stability as reliable natural colourants. Our goal is to extend our plant cell culture approach to develop the first production platform for blue anthocyanin colourants, to replace synthetic food dyes.

Louis Wilson
PhD student, Dupree lab, University of Cambridge

I started as an OpenPlant PhD student at the University of Cambridge in September 2016. I am interested in all parts of plant biochemistry, but my projects tend to focus on the characterization and manipulation of enzymes and catalytic pathways.
In my first rotation project, I worked with Prof. Alison G Smith in Cambridge on metabolic gene clusters, developing methods for the expression of higher plant clusters in algae and yeast, and the detection of potential clusters endogenous to algae themselves. I am working with Paul Dupree to study and engineer cell wall-modifying enzymes for improved crops, food and materials. I have been using OpenPlant heterologous expression systems and a transient expression construct from the Lomonossoff lab to assess the stability of glycosyltransferases *in vitro*, with the aim of finding better enzymes for further study and exploitation. Increasing our understanding of these enzymes may ultimately permit the creation of designer fibres and saccharides, as well as being able to manipulate the properties of plant cell walls.

Dr Susana Sauret-Gueto
Research manager, Haseloff Lab, University of Cambridge

Dr. Susana Sauret-Gueto is an experienced molecular biologist and microscopist, with a scientific background in plant growth and development. In the OpenPlant Cambridge laboratory, she coordinates the establishment of semiautomated workflows to accelerate the generation and characterisation of genetically engineered Marchantia lines. Susana is establishing a new facility for robotic liquid-handling around the Echo acoustic liquid handler, and an advanced microscopy facility. The microscopy hub includes a Keyence digital microscope for real-time 3D reconstruction of Marchantia plants, as well as a series of fluorescent microscopes with different resolution capabilities, including a Leica SP8 confocal microscope. She is specially interested in the sector analysis project in order to dissect gene function and autonomy at the cell and tissue level. Susana is also the main organiser of the ROC Group (Researchers with OpenPlant Cambridge), which brings together synthetic biology researchers from across Cambridge.

Dr Steven Rowden
Postdoctoral Fellow, Howe Lab, University of Cambridge

I completed my Ph.D in Biochemistry in 2016 at the University of Cambridge, under the guidance of Professor Christopher Howe and Dr Andrew Spicer of Algenuity. Together with collaborators, Chris' lab has pioneered the development of biological solar cells, which are able to produce current as a result of photosynthetic activity in cyanobacteria. I then joined Professor Patricia Harvey's laboratory to work on the D-factory project, which aims to set up a sustainable CO2 algal biorefinary utilizing the algae Dunaliella. While there I also contributed to a European Commission report 'food from the oceans' as part of a high level group of scientific advisors. I have now returned to Chris Howe's laboratory as part of the OpenPlant Project. The goal of this project to create an overexpression system for transgenes that is sensitive to changed in electropotential.

Dr Aytung Tuncel
Postdoctoral Fellow, Smith Lab, John Innes Centre

I am applying the genome editing tools to generate novel, commercially or nutritionally valuable glucans in model crop species. The primary objective of my OpenPlant project is to generate potatoes that contain digestion-resistant starches with two major nutritional benefits: reduced calorie intake from consumption of chips, crisps and other potato-based foods and increased supply of complex carbohydrates to the microbiota of the lower gut that reduces risk of several diseases including colorectal cancer and type II diabetes.

More specifically, the project involves knocking out the gene(s) of starch branching enzymes I and/or II using crispr-CAS9 method thereby increasing the ratio of amylose to amylopectin (linear to branched starch chains) in tubers without significantly compromising the starch yield. The engineered starch will be less accessible to starch degrading enzymes, thus more resistant to digestion.

Dr Michael Stephenson
Postdoctoral Fellow, Osbourn Lab, John Innes centre

I am a chemist, with a background in natural product total synthesis, medicinal chemistry, and pharmacy. In the Osbourn group we are interested in plant secondary metabolites, and this places us at the very interface between biology and chemistry. I bring expertise in small organic molecule extraction, purification, and structural characterisation. This strengthens the group's ability to functionally characterise biosynthetic enzymes; something which is important for many areas of research within the Osbourn lab. As a medicinal chemist I am interested in applying these techniques to engineer chemical diversity, and to explore the structure activity relationships of bioactive triterpenes. I have been involved in isolating and characterising several novel triterpenes structures arising from co-expression of 'un-natural' combinations of biosynthetic enzymes. In addition, I have solved the structure of a number of novel and usual triterpene scaffolds, produced by oxidosqualene cyclases under investigation within the group.
UK.

Dr Ivan Reyna-Llorens
Postdoctoral Fellow, Hibberd Lab, University of Cambridge

My research involves using synthetic biology and evolution for improving agricultural traits, more specifically to improve photosynthesis. C3 photosynthesis can be very inefficient as Rubisco interacts with oxygen in a wasteful process known as photorespiration. In order to increase yields, photorespiration should be reduced considerably. Fortunately, some plants have evolved such mechanism already. C4 photosynthesis results from a series of anatomical and biochemical modifications in the leaf that lead to photosynthesis being compartmentalized between mesophyll and bundle sheath cells. This division of labour generates a CO_2 enriched environment where photorespiration is effectively abolished. C4 plants therefore produce more yield and use water and nitrogen more efficiently. In order to engineer this trait, cell specific genetic circuits need to be developed. Unfortunately there is a limited number of genetic parts driving cell specificity in leaves. My main objective in OpenPlant is to generate a library of leaf specific motifs that can be used to drive the expression of both nuclear and plastid encoded genes in specific compartments and specific cells of leaves.

Dr Oleg Raitskin
Post-doc, Patron Group, Earlham Institute

My project involves optimization of CRISPR/Cas9 methodology of genome editing in plants. CRISPR/Cas9 is a method of choice to perform genome engineering. There are however significant limitations which prevent broader implementation of this technology in plants.

These limitations include variable efficiency of editing at different targets, off target activity, inefficient inheritance of the created mutations, ability to edit simultaneously several targets, limited selection of targets/PAM repertoire and the need to segregate Cas9 and sgRNA from the created mutations. Numerous configurations of CRISPR/Cas9 designed to address these limitations had been published. Our aim is to establish a uniform testbed and toolkit, where many of these configurations are tested under the same conditions and their editing efficiency and off target activity will be assessed. In order to minimize variability in transgenic expression we established an editing essay in plant protoplasts.

Dr. Zhenhua Liu
Postdoctoral Fellow, Osbourn Lab, John Innes Centre

It has been estimated that plants can produce over 1 million specialized metabolites, but we know less than 0.1 % of their biosynthetic pathways. As a post-doc from Anne Osbourn group at John Innes Centre, I am employing multidisciplinary approaches across bioinformatics, genetics, and chemistry, to comprehensively understand how and why plants produce this hallmark of specialized metabolites. I am currently focusing on plants from the Brassicaceae family and systematically studying the function, evolution and biosynthesis of triterpenes from this family. I am in particular interested in pathways encoded by gene clusters. It holds great potential to mine more and novel biosynthetic pathways efficiently. However, how and why plants have evolved BGCs is still a mystery. We are aiming to gain the first understanding of their assembly, patterns of evolution and common features in a systematic fashion. This knowledge can then be used as a template guiding the research of BGCs in other types of compounds and plant families.

Dr Eva Thuenemann
Postdoctoral Fellow, Lomonossoff Lab, John Innes centre

Plants can be used as a production platform for high-value products such as vaccines, enzymes and metabolites, thereby providing a potentially fast and cost-effective alternative to other cell culture techniques. Developed within the Lomonossoff group, HyperTrans (HT) is a technology for rapid, high-level transient expression of proteins in plants. One key application of HT in the Lomonossoff group has been the production of virus-like particles for use as vaccines, scaffolds for nanotechnology and in fundamental research of virus assembly.
In addition to my research project, I was involved in the planning stages for the new John Innes Centre spin-out, Leaf Systems International Ltd, which opened on the Norwich Research Park in January 2017 and will enable translation of research to indsutry through scale-up of plant-based production of proteins and metabolites. I have also participated in various outreach activities, such as a TV interview for regional news, the Great British Bioscience Festival, JIC's Speed Science event as well as a work experience day for school children, amongst others.

Dr Benjamin Lichman
Postdoctoral Fellow, O'Conor Lab, John Innes Centre

Plants are incredible chemical factories, capable of producing a host of complex molecules that synthetic chemists struggle to produce. These compounds are produced by plants to interact with their environment, but they also have great significance for humans, as we use them for fragrances, agrichemicals and medicines. This knowledge can then be used to produce natural products and novel chemicals in microbial or plant based platforms. I am currently working with catnip and catmint (*Nepeta cataria* and *N. mussinii*), plants famous for their intoxicating effect on cats. The origin of this activity is the nepetalactones, a group of volatile compounds from the iridoid family of natural products. Along with their role as feline attractants, nepetalactones have also been reported to have both insect pheromone and insect repellent properties, in some cases having activities superior to DEET. The biosynthetic origin of these compounds is currently unknown. We have been using transcriptomics and proteomics to discover enzymes in the Nepeta nepetalactone biosynthesis pathway.

Dr. Gonzalo Mendoza Ochoa
Postdoctoral Fellow, Smith Lab, University of Cambridge

I obtained my PhD in Cell Biology from the University of Edinburgh, mentored by Prof. Jean D Beggs. During this time, I was interested in the spliceosome cycle, in the connection between splicing and transcription, and also in how proofreading factors help to prevent error in splicing. I spent a significant amount of time using the auxin-inducible degron to conditionally deplete essential proteins, and finding ways to improve this depletion system to get a faster and more tightly-controlled response. My desire to embark on plant synthetic biology, while maintaining an interested in splicing and conditional expression systems, lead me to join the Plant Metabolism Group of Prof. Alison Smith in October 2017, to develop riboswitches as molecular tools to control transgene expression in algae, higher plants and other eukaryotes. The ultimate aim of this project is to develop novel inducible systems for metabolic engineering applications or as in vivo sensors of metabolites.

Mihails Delmans
PhD Student, Haseloff Lab, University of Cambridge

Mihails is a 4th year PhD student, with an Engineering background as an undegraduate. His research topic is the regulation of cell proliferation in Marchantia gemmae. In collaboration with Bernardo Pollak, he has developed an open source gene-centric database platform for managing genome data and synthetic DNA parts for Marchantia. He maintains a strong interest in enginnering approaches to biological problems, and explots his considerable expertise with electronics, optics and 3D printing to build and modify instrumentation for observing Marchantia cell dynamics.

His PhD research combines the construction of new marker genes, expression in Marchantia gemma, quantitative imaging and software analysis in order to map the dynamics of growth in gemmae. He has found evidence of long distance control of cell proliferation which can be deregulated by surgical manipulations.

Dr. Jenni Rant
SAW Trust Coordinator

Whilst training as a PhD student and working as a plant pathologist at the John Innes Centre, Jenni became interested in science communication and spent time out of the laboratory volunteering for the Science Art and Writing (SAW) Trust (reg charity no.1113386). Twelve years on and she has transitioned to running SAW fulltime as a social enterprise specialising in working with researchers on the design of innovative outreach activities. SAW delivers cross-disciplinary projects, providing accessible and inclusive starting points for people with varied interests and learning styles to explore scientific concepts and cutting edge research themes. SAW works in partnership with OpenPlant to deliver a range of activities, including workshops in schools, with adult groups, exhibits at science festivals and music festivals. We have also worked with SynthSys and the UK Centre for Mammalian Synthetic Biology at the University of Edinburgh to train scientists, teachers, writers and artists in the delivery of SAW workshops. See www.sawtrust.org for more information about work with OpenPlant.

Dr. Trinh-Don Nguyen
Postdoctoral Fellow, O'Connor Lab, John Innes Centre

T. Don Nguyen was an OpenPlant postdoctoral researcher in Dr Paul O'Maille's lab, where he characterised and systematically mutated enzymes involved in the generation of sesquiterpenes. He has now joined the O'Connor lab where he is researching the biosynthesis of complex plant metabolites, including the anticancer drug vinblastine, and is investigating routes for the production of these molecules.

Dr Orr Yarkoni
Postdoctoral Fellow, Ajioka Lab, University of Cambridge

Orr has been involved in Synthetic Biology for better part of the last decade. His PhD work at Newcastle University focused on facilitating bio-electronic interface via engineered pathways as part of a larger collaborative grant to create a bio-robotic hybrid device. More recent work at the University of Cambridge focused on developing a field-use whole-cell Arsenic Biosensor for deployment in South Asia.
Relatively new to work with plants, he worked in OpenPlant to reengineer the *Marchantia polymorpha* plastid. The main focus of his contribution to Open Plant was to reconstruct the entire 120kb plastid genome in a way that makes it easier to manipulate, facilitating future work on plastid transformation in Marchantia and, in time, other plants. He also worked together with Haydn King from the Ajioka Lab on creating a codon optimised reporter toolkit for use in the Marchantia plastid, consisting of a 13 fluorescent reporters across a wide spectrum ranging from near UV to near infrared.

Dr Noam Chayut
Postdoctoral Fellow, Martin Lab, John Innes Centre

I am interested in the interface between applied plant breeding and plant metabolism. In my master's thesis we used classical breeding of passionfruit with the goal of releasing new varieties, now used by farmers. In my PhD thesis we studied carotenoid metabolism in melons and established a molecular marker now used routinely by melon breeders. More importantly, we suggested a novel non-transgenic path toward pro-vitamin A carotenoid biofortification of food crops. The objective of the current OpenPlant project is to develop pre-breeding lines of beetroot for the production of L-DOPA.
L-DOPA is used to treat Parkinson's symptoms; however, the current costs of chemical synthesis make it unavailable for deprived populations worldwide. L-DOPA, a product of tyrosine hydroxylation, is an intermediate metabolite in biosynthesis of violet and yellow betalain pigments, in Beta vulgaris (table beet). L-DOPA natural steady state levels are very low, usually undetectable. We intend to block the turnover of L-Dopa in beetroot to allow its accumulation to levels that could enable low-tech accessible production in a plant system.

Bernardo Pollak
PhD graduate, Haseloff Lab, University of Cambridge, now:
JCVI, San Diego, USA.

A PhD student at Plant Sciences in the Haseloff lab, with a BA in Biochemistry from Pontificia Universidad Católica de Chile, Bernardo is now a postdoctoral fellow at teh JCVI, San Diego. He studied the molecular genetics involved in meristem establishment and maintenance in the simple plant model system *Marchantia polymorpha*. I have experience with next-generation sequencing technologies, bioinformatics, genome assembly, microscopy and genome editing. Recently, I have developed Loop assembly, a novel recursive method for rapid construction of gene circuits.
As a side-project, I collected marine water samples from around the world and have isolated several strains of bioluminescent bacteria (>15) from the Pacific, Atlantic, Mediterranean and Caribbean oceans. He performed a preliminary characterization of these strains and found differences of brightness of about 100-fold in comparison to *Vibrio fischeri*, and constructed new bioluminescent reporter genes.

Dr. Yang Zhang
Postdoctoral Fellow, Martin Lab, John Innes Centre, now:
Principal Investigator, College of Life Sciences, Sichuan University, China

During his time as an OpenPlant postdoctoral researcher in Prof. Cathie Martin's group, Yang worked on the regulation of plant metabolic engineering and natural product production, paticularly flavanoids and anthacyanins. His work in China focusses on understanding and characterising the biosynthesis and regulation of important plant secondary metabolites, from model plants to medicinal plants. He also investigates the biological roles of important metabolites, as well as employ different bio-technologies to produce valuable compounds in plants.

Dr. Thomas Meany
Postdoctoral Fellow, Haseloff-Hall Labs, University of Cambridge, now: Chief Executive Officer at Cell-Free Technology Ltd., Cork, Ireland

Thomas Meany worked on Raman spectroscopy approaches for quantitative imaging of secondary metabolite proudtion in plants. Following this, he co-founded startup company Cell-Free Technology Ltd together with Ian McDermott (Chief Scientific Officer). They have been awarded funding from the accelerator programme RebelBio and SOS Ventures to take cell-free technology out of the lab and into the world.

Dr. Tim Rudge
Postdoctoral Fellow, Cicuta Lab, University of Cambridge, now:
Assitant Professor, PUC Santiago Chile.

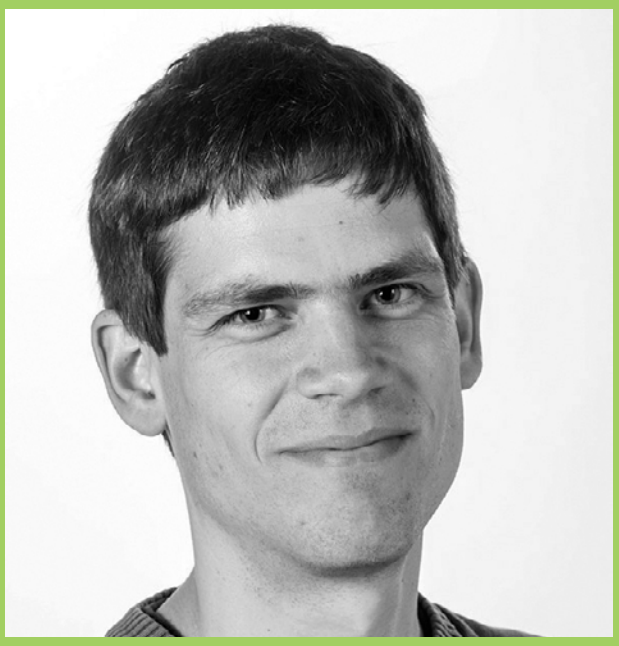
Tim is an experienced engineer and programmer, turned biologist. He has worked live imaging and GPU-accelerated computer models to integrate cellular growth, biophysics and genetics. Simple microbial systems can be genetically marked or reprogrammed, and followed by high-resolution microscopy to explore and validate cellular models. He is the primary developer of the CellModeller software.

He has recdently taken up a faculty position in the Engineering Department at PUC and manages a research group that explores spatial patterning and microbial cell dynamics and self-organisation.

Dr. Pierre-Marc Delaux
Postdoctoral Fellow, Oldroyd Lab, John Innes Centre, now:
CNRS research associate (Chargé de Recherche 2ème classe),
Laboratoire de recherche en sciences végétales (LRSV), France.

As an OpenPlant post-doctoral researcher, Pierre-Marc Delaux worked in the lab of Prof. Giles Oldroyd, establishing *Marchantia polymorpha* as a model system and test bed for understanding plant-fungi and plant-microbe interactions. His research continues to focus on understanding mycorrhizal symbiosis and cell signaling: By studying the evolution of a

Dr Hanz-Wilhelm Nützmann
Postdoctoral Fellow, Osbourn Lab, John Innes Centre, now:
Royal Society University Research Fellow, Department of Biology & Biochemistry, University of Bath, UK.



Worked tin OpenPlant o improve our understanding of the transcriptional control of plant metabolic gene clusters, with a focus on chromatin related regulatory processes that govern the expression of gene clusters.

Hans-Wilhelm Nützman's current research programme aims to understand the regulation of metabolic gene clusters, to provide fundamental new insights into genetic control of plant specialised metabolism, unveil novel avenues to rationally interfere with pathway regulation, contribute to a better understanding of the principles of gene-order dependent regulation of eukaryotic genes, ameliorate the design principles of synthetic multi-gene cassettes, and ultimately, underpin human interest in food security, higher-value natural products and synthetic biology.

OpenPlant outcomes:

Further funding

Bill and Melinda Gates Foundation - Engineering Nitrogen Symbiosis for Africa Phase 2 - Prof Giles Oldroyd (JIC/ SLCU) as Project leader - $17,468,091

National Institutes of Health (NIH) – An integrated pipeline for accelerated natural product discovery (2015-20) – Prof Anne Osbourn (JIC) as Co-I with Prof Christina Smolke (Stanford University) as PI - $12,100,000

National Institute For Health Research - Grant no. 16-107-04 - UK Vaccine Network project: A platform for the rapid development and production of candidate vaccines, analytical and diagnostic standards by plant-based transient expression for response to global viral threats. 2017-2018 - Prof George Lomonossoff (JIC) as PI - £1,988,841

ERA CoBioTech - Sustainable Bioproduction of Pheromones for Insect Pest Control in Agriculture - Dr Nicola Patron (EI) - €1,861,000

EPSRC - EP/R014000/1 - Low-cost Cell-extract Viral Diagnostics - Dr Jim Ajioka (UCam) & Prof Jim Haseloff (UCam) with collaborators from Imperial College and CSIR (South Africa) - £1, 500, 000

BBSRC Super Follow on Fund grant - BB/R005508/1 - Engineering Quillaja saponin biosynthesis pathways for bio-production of QS-21. 2018-2020 - Prof Anne Osbourn (JIC) as PI - £1,538,805.

EC H2020 Grant - Grant no. 774078 - Building the product pipeline for commercial demonstration of Plant Molecular Factories. 2017-2021 - Prof George Lomonossoff (JIC) as partner - £631,808

BBSRC - BB/N019466/1 - Targeted gene knockouts in crops – Prof Wendy Harwood (JIC) & Dr Nicola Patron (EI) - £603,560

BBSRC Responsive mode grant - BB/R00160X/1 - Exploiting the power of heterologous expression in plants to discover new virus structures. 2017-2020 - Prof George Lomonossoff (JIC) as Co-I - £563,036

BBSRC Industrial Partnership Award - BB/P010490/1 - An improved bioproduction system for proteins and small molecules – Dr Nicola Patron (EI) & Prof Sarah O'Connor (JIC) with Leaf Systems as industrial partner- £459,000

ERACoBioTech via BBSRC - BB/R021694/1 - MicroalgaE as Renewable Innovative green cell facTories (MERIT) - Prof AG Smith (UCam), with O Kruse (Bielefeld, Germany) as Coordinator - Total of €2.1M, £368K to Cambridge (80% fEC)

Shuttleworth Fellowship - The Open Bioeconomy - Dr Jenny Molloy (UCam) - £267,931

The University of Cambridge - Synthetic Biology Strategic Research Initiative, chaired by Prof Jim Haseloff and Dr Jim Ajioka - £165,000

Royal Society - RGF\EA\180002 – Dr Sebastian Schornack (SLCU) - £92,500

Wellcome Trust ISSF Junior Interdisciplinary Fellowship for Dr Tom Meany, working with Prof Lisa Hall and Prof Jim Haseloff - £100,000

Wellcome Trust ISSF Junior Interdisciplinary Fellowship for Dr Emma Talbot, working with Prof Pietro Cicuta and Prof Jim Haseloff - £100,000.

Proof of Concept award from the High Value Chemicals from Plants BBSRC Network in Industrial Biotechnology and Bioenergy (NIBB) - Small molecule-mediated manipulation of specialized metabolism in plant cell cultures - PI Professor Anne Osbourn (JIC); co-I Dr Doug Cossar (Croda) - £45,679 (80% FEC)

BBSRC GCRF Workshop Grant - Practical Synthetic Biology: Plants, Agriculture and the Environment - OpenPlant and Earlham DNA Foundry - £39,250

BBSRC Follow on Fund Innovation Fellow Funds - Prof Anne Osbourn - £20,000

John Innes Centre Knowledge Exchange and Commercialisation Innovation Funds (competitively awarded, by internal peer review). 2014-5 - The healthy chip -
Prof Wendy Harwood (JIC) & Prof. Alison Smith (JIC) - £15,726

University of Cambridge Strategic Research Initiative Small Grant (competitively awarded, by internal peer review) - Biomaker Challenge: a novel mechanism of student engagement in applied interdisciplinary research - Prof Jim Haseloff (UCam) & Dr Jenny Molloy (UCam) in collaboration with CamBridgeSens - £10,000

Proof of Concept award from the High Value Chemicals from Plants BBSRC Network in Industrial Biotechnology and Bioenergy (NIBB) - Engineering enhanced content of aromatic amino acids in tomatoes for improved bioactive content - Prof Cathie Martin with industrial partner Persephone Bio - £20,000

Synbio LEAP Catalyst Grant – OpenSource Foundational Tools for Plant Biotechnology - Dr Nicola Patron (EI) - $10,000

BBSRC GCRF IAA - Exploring paper-based synthetic biology for global health - Prof Jim Haseloff (UCam) - £9,500

John Innes Centre Institute Strategic Funds (competitively awarded, by internal peer review). 2018 - Starch analysis on high-amylose potatoes - Prof. Alison Smith (JIC) - £5,316

University of Cambridge Strategic Research Initiative Small Grant (competitively awarded, by internal peer review) - Exploring paper-based synthetic biology for global health - Prof Jim Haseloff (UCam) & Dr Jenny Molloy (UCam) - £5,000

University of Cambridge Strategic Research Initiative Small Grant (competitively awarded, by internal peer review) - Implementing open source synthetic biology for global challenges: convening scientists, social scientists and practitioners - Prof Jim Haseloff (UCam) & Dr Jenny Molloy (UCam) - £5,000

University of Cambridge Wellcome Trust ISSF Workshop Grant (competitively awarded, by internal peer review) - Responsible and open innovation with large bioresources: goals, challenges and proposals - Dr Jenny Molloy (UCam) in collaboration with Dr Kathy Liddel (UCam) - £4,000

University of Cambridge Centre for Research in Arts, Social Sciences and Humanities Faculty Research Group Award (competitively awarded, by internal peer review) - Open Intellectual Property Models of Emerging Technologies - Dr Jenny Molloy (UCam) as Co-Convenor and Prof Jim Haseloff as Advisor in collaboration with Dr Frank Tietze (UCam), Dr Lara Allen (Centre for Global Equality) and others - £1,500

Biochemical Society Scientific Outreach Grant - "The Transcription - Translation Machine" - Dr Jenni Rant (SAW) & Dr Colette Matthewman (JIC) - £940

University of Cambridge EPSRC GCRF Workshop Grants (competitively awarded, by internal peer review) - Programmable biology in the test tube - Prof Jim Haseloff (UCam) & Dr Jenny Molloy (UCam) - £500

Influences on policy

Bioengineering Horizon Scan (2017) - Regulation & Governance - International
Members of OpenPlant co-organised and were involved in a horizon scanning exercise led by the University of Cambridge Centre for the Study of Existential Risk. The group convened at a one day workshop to rank the top 20 emerging issues in biological engineering. This led to a publication in eLife, a press briefing at the Science Media Centre in London and an appearance on BBC Radio 4 Inside Science by Dr Jenny Molloy (UCam). Publication: Wintle BC, Boehm CR, Rhodes C, Molloy JC, Millett P, Adam L, Breitling R, Carlson R, Casagrande R, Dando M, Doubleday R, Drexler E, Edwards B, Ellis T, Evans NG, Hammond R, Haseloff J, Kahl L, Kuiken T, Lichman BR, Matthewman CA, Napier JA, ÓhÉigeartaigh SS, Patron NJ, Perello E, Shapira P, Tait J, Takano E, Sutherland WJ. (2017). **A transatlantic perspective on 20 emerging issues in biological engineering.** *Elife; 6. pii: e30247. doi: 10.7554/eLife.30247.*

Design goals for an Open Material Transfer Agreement (2015-2016) - Legal & Tech Transfer - International
OpenPlant convened an international IP Working Group consisting of over 20 attendees who laid out the challenges and opportunities for open approaches to material transfer and the design goals for an Open MTA. This led to a report that is available via the OpenPlant website (https://www.openplant.org/openmta/) and the development of the OpenMTA (see below).

Development of the Open Material Transfer Agreement (2016-2017) - Legal & Tech Transfer - International
The OpenMTA was developed as a legal tool for sharing biological materials such as plasmids with no restriction on redistribution or commercial use. The agreement and implementing letter are now available online along with video case studies of use (http://www.openmta.org). OpenMTA has been presented and published in technology transfer forums and at international and institutional meetings e.g. SB 7.0, NIAB Innovation, AUTM, Praxis Unico Blog, La Pontificia Universidad Católica de Chile, MIT, Stanford, University of Tasmania, Global Community Biotechnology Summit. The authors have also engaged in numerous discussions with researchers, institutions and funders on openness, sharing and the practicalities of implementation of the Open MTA. Publication: Kahl L, Molloy J, Patron NJ, Matthewman C, Haseloff J, Grewal D, Johnson R, Endy D (2018) **Expanding options for material transfer via the OpenMTA**. *In review*

Opinions on Synthetic Biology: GM foods and the application of the precautionary principle in Europe (2017) - Regulation & Governance - International
Prof Dale Saunders and Dr Colette Matthewman coordinated the submission of written evidence submitted by the John Innes Centre for the European Commission's consultation.

Plant Genetic Resources and Sustainable Development Goals (2016) - International Development - International
Dr Nicola Patron contributed to a workshop funded by the Rockefeller Foundation that engaged with policy, non-profit and civil society groups to draft a report on the intersection between plant genetic resources and the SDGs. Publication: Marden E, Welsch E, Mozaferi J, Deera J, Halewood M, Sabran M, Patron N, Mooney P, Kersey P, Wenzl P, Kurtz B, Sackville-Hamilton R, Bastow R, Dorius S, Foston S, Dias S, McCouch S, Chiurugwi T, Powell W, Micheals F (2016) Plant Genetic Resources and Sustainable Development Goals: Needs Rights and Opportunities, *Rockefeller Foundation Bellagio Center, Nov 2016*

Global Gardens workshop (2017) – International development – International
Prof Anne Osbourn (JIC), Dr Colette Matthewman (JIC), Dr Jenni Rant (SAW Trust) and social scientist Dr Nick Lee (Warwick Integrative Synthetic Biology Centre) designed and delivered an interdisciplinary workshop to explore issues around global genetic plant resource sharing.

The workshop, which was held at the John Innes Centre, was advertised to the public. Participants took part in a practical science exercise to extract chemicals from plants. They discussed case studies that illustrated the history, sourcing and use of high value products from plants (drugs, sweeteners, flavourings). Following that, they extracted DNA from plants and learned about the genetic code. They then reviewed and responded to the theme creatively by writing poetry and generating artwork. Participants commented that discussions "stretched my thinking about the ethics of biopiracy" and "changed my view of plants". A write-up of this workshop, at which Katie Beckett (Access and Benefit Sharing Project Manager for the UK) was also present, is being prepared for submission to an international science journal, and further workshops with different audiences are being planned in other locations across the UK and internationally for 2018/19.

Contributions to Synthetic Biology Scoping Study for the International Treaty for Plant Genetic Resources for Food and Agriculture (2017) - Regulation & Governance - International

Prof Anne Osbourn, Prof Jim Haseloff, Dr Nicola Patron, Dr Colette Matthewman and Dr Jenny Molloy participation in interviews by Margo Bagley (Emory University), Eric Welch (ASU) and Todd Kuiken (North Carolina State University) on behalf of the UN International Treaty for Plant Genetic Resources for Food and Agriculture: Synthetic Biology Scoping Study. This had with a focus on how de-materialization and digitization of data may affect the structure, function and viability of the Treaty and will be reported back to the UN in a final report to inform future policy.

Contributions to Proportionate and Adaptive Governance of Innovative Technologies (PAGIT) project (2017) - Regulation & Governance - International

Dr Colette Matthewman (JIC) contributed to workshops as part of the Proportionate and Adaptive Governance of Innovative Technologies (PAGIT) project. Based on several activities, including the workshop discussions, the PAGIT project published of a report: Tait J, Banda G, and Watkins A (2017) A Framework to Guide Policy and Regulatory Decision Making. *http://innogen.ac.uk/reports/1222*

Hosting UK-Canada discussions on synthetic biology and public policy (2015) - Governance - International

OpenPlant researchers at the John Innes Centre hosted a visit by a mission of Canadian authorities and researchers. The work of OpenPlant was presented and the delegates discussed UK-Canada Synthetic Biology Collaborations, New Horizons for Synthetic Biology Research, Public Policy and Industry.

Contributions to UK Synthetic Biology Leadership Council (2012-present) - Governance - National

Prof Dale Sanders (JIC) is a member of the Synthetic Biology Leadership Council which is the co-ordinating body for the UK's interests in the rapidly developing field of synthetic biology. In 2016 the SBLC developed the SynBio Strategic Plan, Biodesign for the Bioeconomy which will inform future national policy and funding. This followed an extensive consultation period with the support of the SynBio Special Interest Group, the research councils and Innovate UK.

Contribution to Developing a Research Agenda for Extreme Bio-Risks (2017) - Regulation & Governance - International

Dr Jim Ajioka (Ucam) and Prof Jim Haseloff (UCam) attended an international workshop hosted by the Centre for the Study of Existential Risk in Cambridge. The workshop designed a research programme which seeks to develop a systematic approach to analysing potential extreme risks arising from developments in synthetic biology, which will inform realistic and timely regulatory and policy development.

Contributions to the Synthetic Biology Leadership Council Science and Technology Subgroup (2015 - present) - Governance - National

Prof Anne Osbourn (JIC) and Prof Jim Haseloff (UCam) are active members of the Synthetic Biology Leadership Council Science and Technology Subgroup, which monitors, enhances and integrates work and facilities across the UK. The Subgroup reports to the SBLC and therefore informs government funding and policies in a range of areas.

Contributions to the BBSRC Industrial Biotechnology and Bioenergy Strategy Board (2016 - present) - Industrial Strategy - National

Sarah O'Connor (JIC) is a member of the BBSRC Industrial Biotechnology Strategy Board, which provides BBSRC with external and independent input to the development of strategy in the IBBE research area.

Putting synthetic biology on the Agenda for the Royal Society of Chemistry (2017) - Research Policy - National

Prof Rob Field (JIC) has been working to put synthetic biology on the agenda for the Royal Society of Chemistry. For example, Prof Pam Silver (Harvard) has been invited to speak at the Chemistry-Biology Interface Division annual meeting in May 2018.

GCRF Workshop in South Africa (2017) - Research, Education & International Development - International

OpenPlant and the Earlham Foundry co-organised a public workshop in Pretoria 'Practical Synthetic Biology: fast, frugal and open technologies for education and sustainable development'with 100 attendees including government representatives, which led to the Director of Biotechnology presenting on the South African Bioeconomy Strategy at OpenPlant Forum 2017. An invitation only session followed, attended by over 20 researchers, NGOs and a policy maker from the UK, Southern, East and West Africa and Latin America. Discussion on challenges and opportunities for synthetic biology in Africa resulted in a report that was sent to BBSRC to inform future GCRF funding calls for synthetic biology 'Capacity building for the bioeconomy in Africa: harnessing fast, frugal and open technologies for education and sustainable development'. It is available online at *https://www.openplant.org/global-challenges/*

Working Group on open curriculum development for cell-free synthetic biology (2017) - Education - International
The OpenPlant working group 2017 focused on curriculum development and teaching resources for fast and frugal biotechnology. A workshop was held in July 2017 featuring a range of key player, including researchers at the cutting edge of cell-free technology, educationalists and outreach experts. The working group laid the groundwork for assembly of open and modular curriculum components that could be combined into different teaching frameworks across multiple disciplines, drawing on existing work from organisations such as BioBuilder, the National Centre for Biotechnology Education, Science and Plants in Schools and Raspberry Pi. A report is forthcoming.

European Forum Alpbach (2017) - Governance - International
European Forum Alpbach is is an interdisciplinary platform for science, politics, business and culture that addresses current socio-political questions with an audience of European policy makers, researchers and entrepreneurs. Dr Jenny Molloy (UCam) presented on OpenPlant's approach to intellectual property as a facet of responsible research and innovation on a panel at the Forum's Political Symposium entitled 'Genome editing: governing on the cusp of uncertainty'. This was organised in cooperation with Woodrow Wilson International Center for Scholars.

Open Innovation with Large Bioresources: Goals, Challenges and Proposals (2015-2016) - Innovation & Legal - National
This workshop, co-organised or attended by Dr Jenny Molloy (UCam), Dr Colette Matthewman (JIC), Dr Nicola Patron (EI) and Prof Jim Haseloff (UCam) took an interdisciplinary look at open innovation and open sharing of bioresources, comparing the approach of OpenPlant and synthetic biology with the genomics and biobank communities. Representatives from Genomics England, the UK civil service and NHS attended and the resulting report was shared widely. Publication: Liddicoat, J. and Liddell, K. (2016). **Open Innovation with Large Bioresources: Goals, Challenges and Proposals.** *https://papers.ssrn.com/sol3/papers.cfm?abstract_id=2888871*

Promotion of OpenPlant Fund as a best practice model for innovation (2016) - Research Practice - National
Dr Colette Matthewman (JIC) was invited to present the OpenPlant Fund model for open and interdisciplinary innovation at the Synthetic Biology for Growth meeting and the Synthetic Biology Leadership Council Open Meeting (Nov 2016).

Science Art and Writing projects for primary school pupils (2016 – current) – Education – International
The Science Art Writing (SAW) Trust has delivered six projects in schools on OpenPlant themes that enable children with different interests and abilities to explore scientific research concepts. These projects will form the basis for the development of lesson plans on plant science and synthetic biology that will be shared with teachers nationally and internationally.

Science Art Writing Trust training workshops for adults using Plant Natural Products (2016 - current) – Education – International
Using plant natural products as the theme, the Science Art Writing Trust has developed a workshop that trains scientists, artists, writers and teachers to use the SAW methodology to create new projects to run in schools. Following trialling at the John Innes Centre, this template has been used to deliver workshops in Edinburgh through the UK Centre for Mammalian Synthetic Biology Research for the last two years, leading to uptake of the SAW approach in schools in Scotland. Plans are in development to take the training workshop to the Warwick Integrative Synthetic Biology Centre. A training workshop for teachers has also been delivered in China.

Science Art Writing Trust and Norfolk County Council's International Collaborative Learning Conference (2017 - current) – Influencing Education – International
The Science Art Writing Trust have teamed up with Norfolk County Council's International Schools Programme to work in partnership on sharing best practice in science learning between schools locally and internationally. As part of this collaboration we are hosting an International Collaborative Learning conference on the 21st March 2018 in Norwich, where educators from around the world will come together to present and exchange ideas on innovation through cross-disciplinary learning with teachers locally.

Publication of case-study on plants as biofactories for A-level teachers' resource (2015) - Education – National
Dr Eva Thuenemann and Prof George Lomonossoff wrote a case study about the use of plants as biofactories for publication in the OCR and Edexcel A level Biology Teacher Resource Packs. *Published in Pearsons; ISBN 9781447977452; ISBN 9781447977414*.

Workshop and report for the CBD on the Nagoya Protocol and Synthetic Biology (2016) - Access and Benefit Sharing - International
In November 2016, OpenPlant co-funded and participated in a workshop with the Engineering Life project at Edinburgh University. The workshop was on "Genetic resources in the age of the Nagoya Protocol and gene/genome synthesis" and a report is being compiled to present to the Conference of the Parties (COP) at the Convention on Biological Diversity (CBD).

Intellectual Property

Patent application number 1721600.3, filed 2017 - Title: Metabolic Engineering - Inventors: Prof Anne Osbourn & Dr James Reed

No further details can be released at this time, as the information of the patent application is not yet public.

Development of technology

MarpoDB (2017). MarpoDB is a database of *M. polymorpha* genes and genetic parts, tailored for a plant synthetic biology workflow. Among its features are precompiled cross-database querying to InterPro, Pfam signatures and non-redundant Viridiplantae BLAST annotations; BLAST querying to M. polymorpha genes; sequence export in GenBank format; recoding of sequences to the common syntax for type IIS assembly and exchange of DNA parts. MarpoDB source-code is released on GitHub to promote development of computational tools for synthetic biology. Publication: Delmans, M., Pollak, B., & Haseloff, J. (2017). MarpoDB: an open registry for Marchantia polymorpha genetic parts. Plant and Cell Physiology, 58(1), e5-e5.

Loop Assembly (2017). As part of a collaboration between the University of Cambridge, Earlham Institute and the Universidad Católica de Chile, Bernardo Pollak and Fernan Federici have devised a new method for gene assembly based on two Type IIS restriction endonuceases, BsaI and SapI. Loop Assembly allows rapid and efficient production of large DNA constructs, is compatible with widely used Level zero (L0) DNA parts such as Phytobricks, and can be easily automated. Publication forthcoming, protocols and parts will be openly shared.

Establishing a plant common syntax and the PhytoBricks standard for DNA assembly (2015). With wide support from the international plant science community, OpenPlant PIs and PDRAs from all partners established a common genetic syntax for exchange of DNA parts for plants, extensible to all eukaryotes. This common syntax for plant DNA parts is at the core of RFC 106, posted at OpenWetWare, and accepted as an official standard for DNA parts in the iGEM synthetic biology competition. The PhytoBrick standard is a consolidated and consistent standard for Type IIS restriction endonuclease based assembly of DNA parts to make synthetic genes. Publication: Patron NJ, et al. (2015). Standards for plant synthetic biology: a common syntax for exchange of DNA parts. *New Phytologist 208:139.*

CellModeller (2015 - present). CellModeller is an open source Python-based framework for modelling large-scale multi-cellular systems, such as biofilms, plant and animal tissue. It was improved through OpenPlant by Dr Tim Rudge (UCam) to include a graphical user interface and is documented publicly via a website, support forum and github repository (http://haselofflab.github.io/CellModeller/). Latest features include cell-cell adhesion and cell shape, as well as algorithms for whole colony-scale segmentation from confocal microscopy datasets.

Development of a Chlamydomonas-specfic MoClo kit for efficient Golden Gate cloning (2017). Collaborative project with several European labs for the construction of MoClo kit containing more than 100 plasmids with domesticated sequences for expression in *Chlamydomonas reinhardtii*. We anticipate that this will be a very useful resource for the Chlamydomonas community.

DNA parts collections from Dr Nicola Patron deposited in Addgene (2014 - 2015)
A golden gate modular cloning toolbox for plants. ACS Synth Biol. 2014 Nov 21;3(11):839-43
pUAP1 - RFP cloning selection cassette (Synthetic): Synthetic Biology Standards for plant synthetic biology: a common syntax for exchange of DNA parts. New Phytol. 2015 Jul 14. doi: 10.1111/nph.13532.
Collection of promoters and resistance genes for transgene expression in plants: Lawrenson et al., 2015. Induction of targeted, heritable mutations in barley and Brassica oleracea using RNA-guided Cas9 nuclease. Genome Biol 30;16(1):258. doi: 10.1186/s13059-015-0826-7.

Plasmid DNA for introduction of mutations into starch-branching enzyme genes of potato by CRISPR/Cas9 (2016-7). This plasmid was used successfully to edit potato genes important for starch quality. It was made available under an MTA to the Functional Genomics Team at the James Hutton Institute, Dundee.

plantiSMASH web service (2016 - present). Prof Anne Osbourn (JIC) is collaborating with the lab of Marnix Medema (University of Wageningen) to develop and optimise computational methods for pathway discovery. This has led to the release of plantiSMASH, a customised algorithm and web service for mining for biosynthetic gene clusters in plant genomes. Publication: Kautsar, S. et al. 2017. plantiSMASH: automated identification, annotation and expression analysis of plant biosynthetic gene clusters. Nucleic Acids Res. 2017 Apr 27. doi: 10.1093/nar/gkx305.

Minimum Information about a Biosynthetic Gene cluster (MIBiG) (2017). Prof Anne Osbourn (JIC) was a co-author of the MIBiG specification, which provides a robust community standard for annotations and metadata on biosynthetic gene clusters and their molecular products. Publication: Medema MH, et al (2015). Minimum Information about a Biosynthetic Gene cluster. Nat Chem Biol. 11(9):625-31. doi: 10.1038/nchembio.1890.

Small scale vacuum infiltration for transient plant expression (2017). The Osbourn lab has developed an improved agro-infiltration methodology for production of triterpenes using the HyperTrans transient plant expression system, leading to gram-scale production of the triterpene scaffold, ß-amyrin. To increase capacity for effective leaf infiltration they designed a device for efficient vacuum infiltration of 4 – 6 plants simultaneously. Publication: Reed J. et al., (2017). A translational synthetic biology platform for rapid access to gram-scale quantities of novel drug-like molecules. *Metabolic Engineering 42, 185-193.*

Automated assembly and transient expression workflow for assessing Cas9-mediated targeted mutagenesis (2017). Dr Oleg Raitskin (EI) and Dr Nicola Patron (EI) have developed a workflow using plant protoplasts and Illumina MiSeq sequencing that is implemented at the Earlham DNA Foundry.

Dave the DNA Robot (2017). Dr Jenni Rant (SAW) and Dr Colette Matthewman (JIC) secured funding from the Biochemical Society to work with designer Molly Barratt to design and build a "Transcription-Translation machine" for education and publica engagement. The outcome was DNA Dave, an interactive robot to explain how proteins are made using instructions encoded in DNA. DNA Dave has been a great success with general public and school groups at the 2017 Cambridge Science Festival, the JIC 50th Anniversary open day, and Norwich Science Festival. Teachers are keen to take DNA Dave into the classroom, so we are now exploring possible routes to make the robot available to schools.

***S. elongatus* circadian clock model (2017)**. Dr Bruno Martins and Dr James Locke (both UCam) developed *S. elongatus* constructs for examining the circadian clock and its outputs at the single cell level. This software model was published in Molecular Systems Biology and made available in Systems Biology Markup Language (SBML) format. Publication: Martins et al. (2016). Frequency doubling in the cyanobacterial circadian clock. *Molecular Systems Biology 12(12):896. doi: 10.15252/msb.20167087*.

OpenPlant Fund Outcomes

DocuBricks (2016). Open source software to document open hardware in a modular and accessible XML format. Thirteen projects are currently live on the site and it is now a recommended repository for hardware published in the new 'Journal of Open Hardware' published by Ubiquity Press. (http://www.docubricks.com/). Project Lead: Tobias Wenzel (UCam)

Open-Pi Image (2015). This is an affordable DIY crop imaging system using a Raspberry Pi, consumer digital cameras and a 'kit drone' combined with open source software that is made available online. Components of the project are in active use in two labs where it has proved valuable and cost-effective for research use. It has been presented at the Agri-Tech East Remote Sensing and Monitoring SIG meeting. (https://www.biomaker.org/projects/open-pi-image-a-low-cost-open-source-plant-growth-imaging-and-analysis-platform). Project Lead: Alex Webb (UCam)

Open Source Autonomous Imaging Station (2017). An open source fluorescent imaging system that integrates low-cost and open-source hardware, software and genetic resources. The project was published in PLOS and was the Editor's Pick on the PLOS Open Source Toolkit Channel in Dec 2017. All hardware designs, software and protocols are openly licensed and shared. Publication: Nuñez, I., Matute, T., Herrera, R., Keymer, J., Marzullo, T., Rudge, T., & Federici, F. (2017). Low cost and open source multi-fluorescence imaging system for teaching and research in biology and bioengineering. PLOS One, 12(11), e0187163. Project Lead: Fernan Federici (UCam and PUC)

Plant-ProChip 2.0 (2017). Development of on-chip encapsulation and analysis of protoplasts isolated from the emergent plant model Marchantia polymorpha at processing rates of >100,000 protoplasts per hour. The technology demonstrated on-chip sorting of droplets containing YFP-expressing protoplasts from wild type cells using dielectrophoresis force and opens the door to droplet-based microfluidic analysis of plant cells for applications ranging from high-throughput characterisation of DNA parts to single-cell genomics. Publication: Yu, Z., Boehm, C. R., Hibberd, J. M., Abell, C., Haseloff, J., Burgess, S. J., & Reyna-Llorens, I. (2017). Droplet-based microfluidic analysis and screening of single plant cells. bioRxiv, 199992. Project Lead: Ivan Reyna-Llorens (UCam)

Synthetic gene expression system in the green alga *Chlamydomonas reinhardtii* (2016). A platform was developed by Dr Francisco Navarro and Dr Marielle Vigouroux that allows testing of the the expression of fluorescent reporters in different strain backgrounds. It includes the first Chlamydomonas-specific MoClo DNA parts following the common syntax for gene assembly in plant synthetic biology co-authored by many members of OpenPlant. Some of these parts are already being used by other researchers of the Plant Sciences department of the University of Cambridge, who have since contributed new parts to the repertoire available for Chlamydomonas research. Project Leads: Francisco Navarro (UCam) and Marielle Vigouroux (JIC)

smFISH for imaging single RNA molecules at the cellular level in *Arabidopsis thaliana* tissues and the liverwort *Marchantia polymorpha* (2017). Improvements to current smFISH technology allowed researchers to image three, rather than two, RNA targets simultaneously and led to amplified signals which were compatible with confocal imaging. RNA could also be visualized in more differentiated root cells than previously possible, e.g. root hairs. LGC are now promoting the technique at conferences and have made Dr Susan Duncan an expert collaborator. Publications: Duncan, S., & Rosa, S. (2017). Gaining insight into plant gene transcription using smFISH. Transcription, 1-5. Project Lead: Susan Duncan (EI)

Novel selection markers for plant transformation to advance live-imaging techniques (2016). The nuclear-envelope localised fluorophore dTomato, expressed under the *Lotus japonica* UBIQUITIN promoter, was characterised as a novel selection marker for live imaging in *Medicago truncatula*. Project Lead: Katharina Schiessl (JIC)

Quick analytical system for plastid genome modifications (2016). Mario Juhas developed a quick Pulsed-Field Gel Electrophoresis (PFGE)-based analytical system for plastid genome modifications. Publication: Juhas, M., & Ajioka, J. W. (2016). Integrative bacterial artificial chromosomes for DNA integration into the Bacillus subtilis chromosome. Journal of Microbiological Methods, 125, 1-7. Project Lead: Mario Juhas (UCam)

Low-cost algal bioreactors and citizen science toolkit (2016). Low-cost algal bioreactors, a website and algal density detection smartphone app were constructed as part of The Big Algal Open Experiment (http://bigalgae.com/about). The project exhibited at Latitude Festival 2016, London Zoo, New Scientist Live and developed the bioreactor design concept with the Iaac Advanced

Architecture Group in Barcelona. All designs and software are freely available online. Project Lead: Paolo Bombelli (UCam)

A cell-free sensor platform for the quantification of arsenic concentrations in drinking water (2017). A cell-free arsenic biosensor was designed by the Sensor Doctoral Training Centre 2017 cohort and coupled with an open source potentiostat and electrodes capable of detecting current changes as a result of glucose oxidase expression in response to arsenic levels. An open source data collection infrastructure was also developed. The project was documented online and a manuscript is in preparation. Project Lead: Ralf Mouthaan (UCam)

Biomaker Challenge outcomes

A low-cost, pressurized liquid chromatography system for protein purification (2017). A lower cost, modular, open-source alternative to commercial column chromatography systems for performing simple, routine purifications. Parts are largely 3D-printed with integrated sensors and automation of sample collection. Project Leads: Stéphanie Polderdijk and Wolfgang Schmied (both UCam)

Cheap Do-It- Yourself Small Volume UV Spectrometer for Nucleic Acid and Protein Quantitation (2017). An open source small volume UV spectrometer as a cheap alternative to the pre-existing versions. This uses LEDs as the UV light source, and cheap UV sensors that can be processed and analyzed via an Arduino system. The housing is 3D printed enabling users to modify the spectrometer based on their research needs. Project Leads: Joseph Wongand Dushanth Seevaratnam (both UCam)

DIY bioacoustics technology (2017). Non-invasive bioacoustic monitoring has become an increasingly effective way of monitoring ecosystem diversity and health. This team applied bioacoustics and machine learning to insect recognition and created an open source, DIY and hackable acoustic sensor for identification of various insect species. They have since found collaborators at JIC and Imperial College and received interest from other groups with complementary technologies. Project Lead: Davin Browner-Conaty (RCA)

Handheld syringe pump with heating element (2017). A handheld heated syringe pump constructed for microfluidic cell culture applications. An Arduino forms the central controlling element, setting temperature and flow rate and target volume via a touch screen. A 3D-printed structure was used to make the device light, inexpensive, and easy to replicate. Project Lead: Cornelius Bausch (UCam)

Macrophotography of fern gametophytes using a DIY focus stacking system (2017). A low-cost automated system using Arduino and open source hardware to control a camera taking stacked focus images of developing plants. The same team has since been awarded an OpenPlant Fund grant to continue technical development and create a series of training videos and resources. Project Lead: Jennifer Deegan (UCam)

Sci-Fi Cam (2017). Sci-Fi Cam is a Raspberry Pi - based camera for taking color images from a compound or stereo microscope, featuring synchronisation of images via Wi-Fi and time-lapse capability. Project Lead: Mihails Delmans (UCam)

Ultrasonic Plant Height System for High-Throughput Plant Phenotyping (2017). Plant height is a key trait studied in breeding and research programs. Measurements in the field are time-consuming as they require manual measurements with several replicates. This team built a phenotyping device that provides significant improvement in sampling time and sample size by using a low-cost ultrasonic sensor to screen the field canopy and obtain accurate height measurements. Project Lead: Ricardo H. Ramirez-Gonzalez (JIC)

Publications

Workpackage A: **Simple Plant Chassis, Tools and Gene Delivery**

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Workpackage K: OpenPlant Fund for interdisciplinary research

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Workpackage L: OpenPlant Forum: responsible innovation

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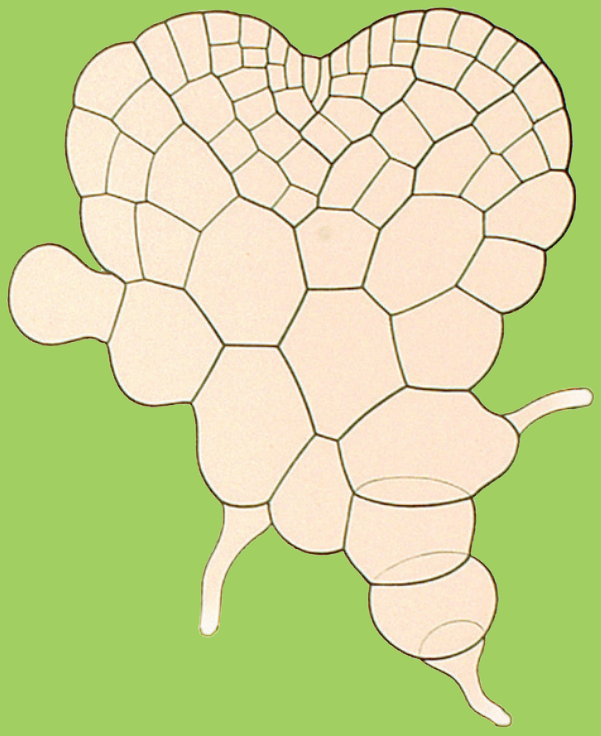

OpenPlant

OpenPlant is a joint initiative between the University of Cambridge, John Innes Centre and the Earlham Institute, funded by the BBSRC and EPSRC as part of the UK Synthetic Biology for Growth programme.

Synthetic Biology offers the prospect of reprogrammed biological systems for improved and sustainable bioproduction. While early efforts in the field have been directed at microbes, the engineering of plant systems offers even greater potential benefits. Plants are already cultivated globally at low cost, harvested on the giga-tonne scale, and routinely used to produce the widest range of biostuffs, from fibres, wood, oils, sugar, fine chemicals, drugs to food.

There is an urgent need to improve our ability to reprogram crop metabolism and plant architecture in the face of global threats from new pathogens, climate change, soil degradation, restricted land use, salinity and drought. The next generation of DNA tools for "smart" breeding of crop systems should be shared to promote global innovation and equitable access to sustainable bioeconomies.

OpenPlant is developing new tools and methods for plant synthetic biology, providing mechanisms for open sharing of standardised resources, applying these tools to world-leading projects in trait development, and facilitating interdisciplinary exchange, outreach and international development. The initiative promotes interdisciplinary exchange, open technologies and responsible innovation for improvement of sustainable agriculture and conservation.

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