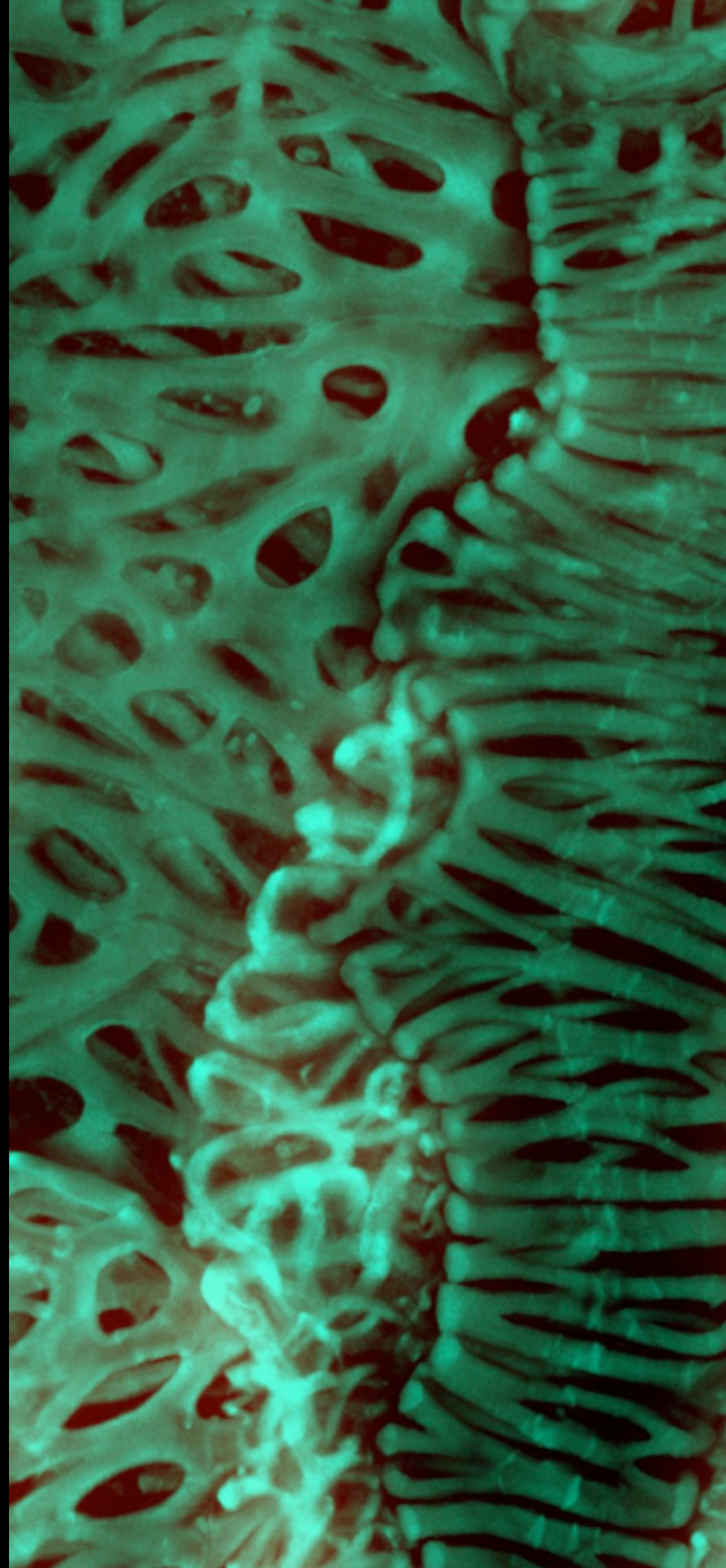
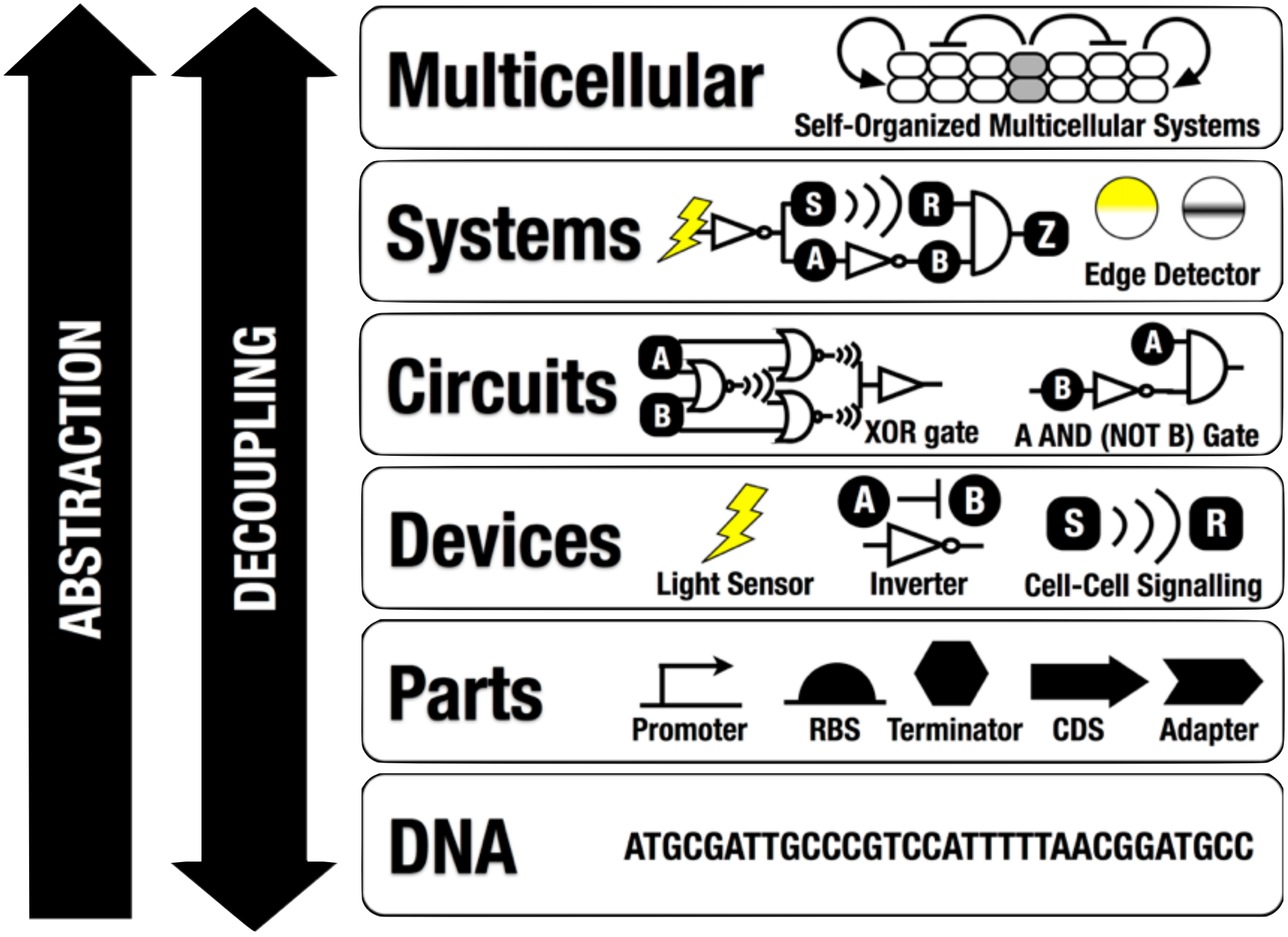
Genomics, Epigenetics & Synthetic Biology

Lecture 2:
Genetic circuits and genome scale DNA engineering

Jim Haseloff
www.haseloff-lab.org (Education)



Modular DNA parts and construction



Federici, Rudge, Pollak, Haseloff, Gutierrez, 2013

Lecture 1: Genetic modification in agriculture and the advent of Synthetic Biology.

Lecture 2: Genetic circuits and genome scale DNA engineering.

1. **BioBricks, standardisation and social engineering**
2. **Type IIS assembly and common syntax**
3. **Smart DNA registries and software tools**
4. **Chromosome engineering**
5. **Reprogramming metabolic pathways in plants**
6. **Implementing plant pathways in microbes**

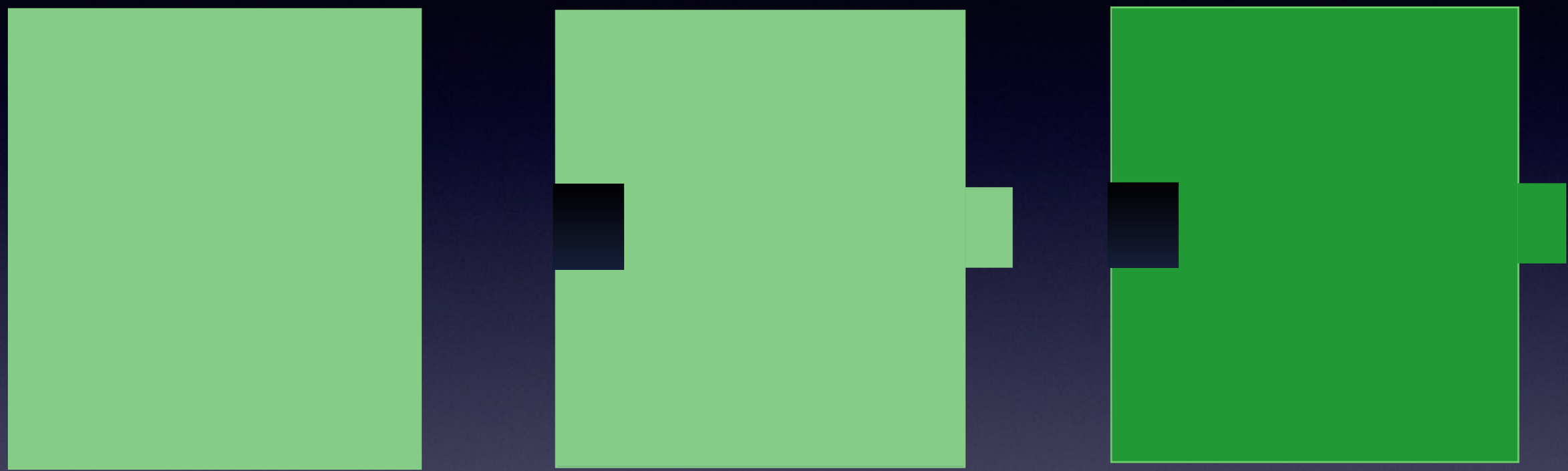
Lecture 3: Engineered logic and the control of gene expression.

Lecture 3: Self-organisation and reprogramming of multicellular systems.

Additional resources: **http://www.haseloff-lab.org** (Education)

Decoupling

Insulate design process from fabrication details



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cgccgacaagcagaagaatggaatcaaagccac
cgctagattcgtacgtgtgac

INSERT PREPARATION

Starting Material

Plasmid OR gDNA OR PCR products OR Annealed oligos OR RNA → cDNA Synthesis → cDNA

VECTOR PREPARATION

Starting Material

Multiple cloning site (MCS)

Plasmid

	Traditional Cloning (RE Digestion & Ligation)	PCR Cloning (TA & Blunt-End)	Seamless Cloning (Gene Assembly)	LIC (Ligation Independent Cloning)	Recombinational (Gateway/Creator/Univector)
DNA Preparation	RE Digestion 60 min. (Standard) 5 – 15 min. (Time-Saver)	PCR 90 min.	PCR 90 min.	PCR 90 min.	PCR 90 min.
dsDNA intermediate	OR	OR	OR	OR	
	Dephosphorylation Blunting (Optional) 30 min.	Clean Up 15 min.	Clean Up 15 min.	Clean Up 15 min.	Clean Up 15 min.
DNA End Modifications		Phosphorylation (Optional) 30 min.	Cohesive-End Formation by 5′ → 3′ exo 30 min.	Cohesive-End Formation by 3′ → 5′ exo 30 min.	RE Digest 60 min. (Standard) 5 – 15 min. (Time-Saver)
dsDNA intermediate 2	OR OR	OR			
	Gel and Column Purification 75 min.				Clean Up 15 min.
Vector & Insert Joining	Ligation Instant – 15 min.	Ligation 15 min.	Ligation Occure simultaneously with previous step	Annealing 30 min.	Site-Specific Recombination 60 min.
					Proteinase K Treatment 10 min.
	OR OR	OR			Recombination sites → 70 min.**
	Assembled vector				Holding vector → Endpoint vector
Estimated total time*	1 hr., 20 min. – 3 hr.	2 hr. – 2 hr., 30 min.	2 hr., 15 min.	2 hr., 45 min.	3 hr., 15 min. – 5 hr., 20 min.

	Restriction Enzyme (RE) Digestion	PCR
DNA Processing	RE Digestion 60 min. (Standard) 5 – 15 min. (Time-Saver)	PCR 2 hr.
		Clean Up 15 min.
	OR	
	Linear vector	
DNA End Modifications	Dephosphorylation (Optional) 30 min.	T-addition 1.5 hr.
	Clean Up 15 min OR Gel & Column Purification 75 min.	
	OR	
	Linear vector, ready for joining	
Estimated total time	20 min. – 2 hr., 25 min.	3 hr., 45 min.

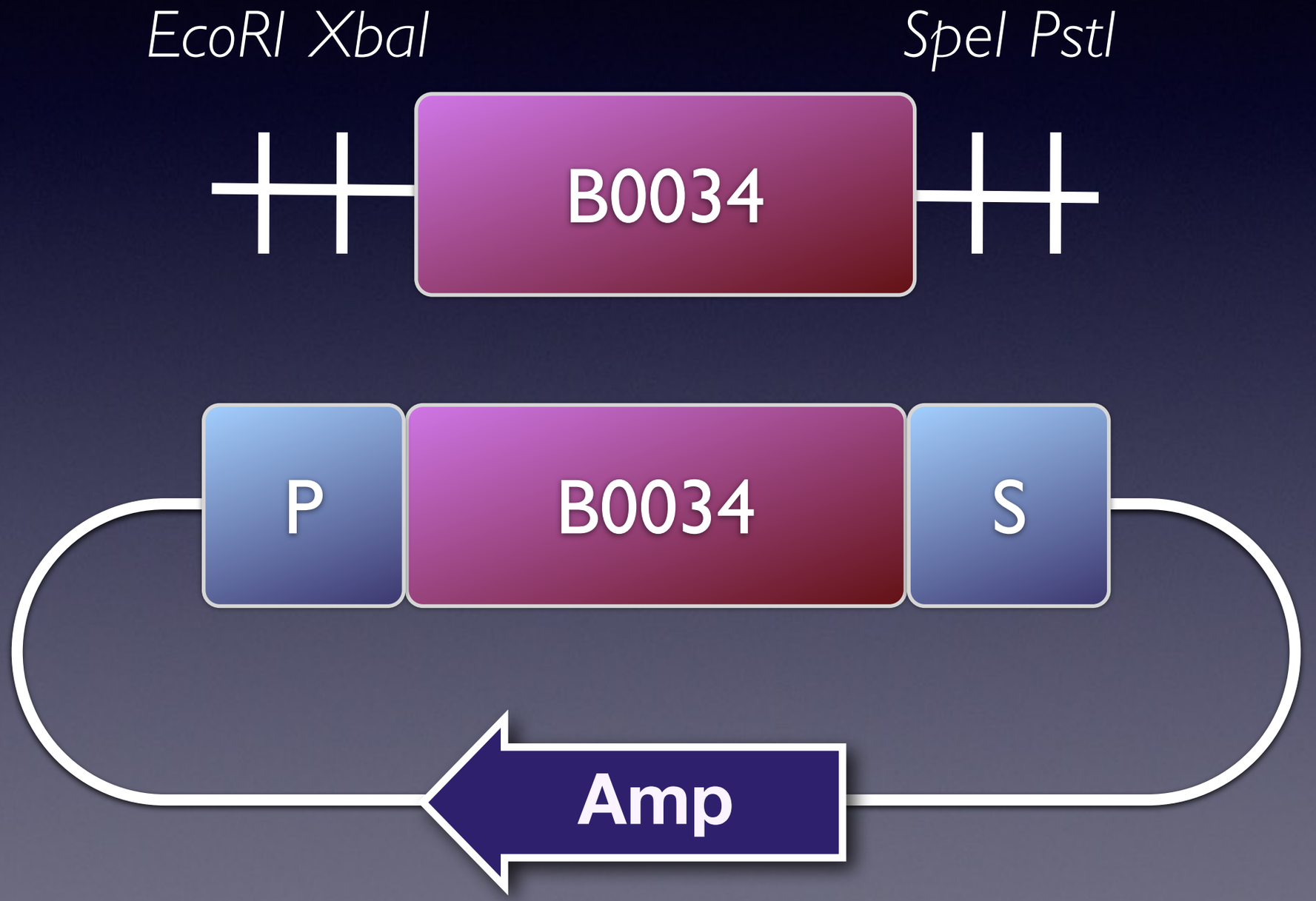
* Note that times are based on estimates for moving a gene from one plasmid to another. If the source for gene transfer is gDNA, add 2 hours to calculation for the traditional cloning method. Total time does not include transformation, isolation or analysis.
** 70 minutes for recombination occurs on second day

Transformation → DNA Isolation (Plasmid Purification) → DNA Analysis

DNA Analysis: RE Digest OR Colony PCR OR Sequencing → Protein Expression; Functional Analysis; Site-Directed Mutagenesis

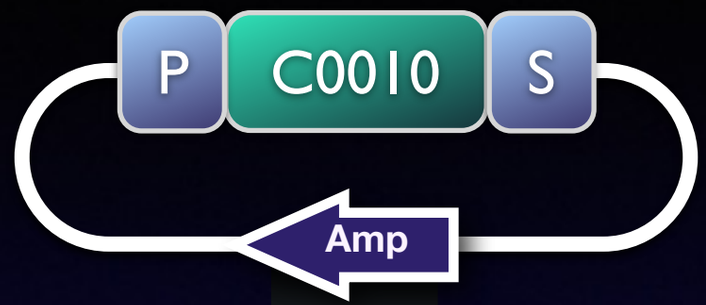
BioBricks

Standardised, interchangeable parts for Biology

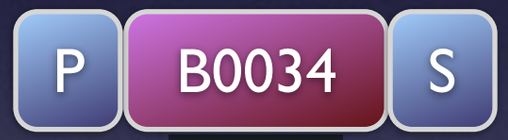


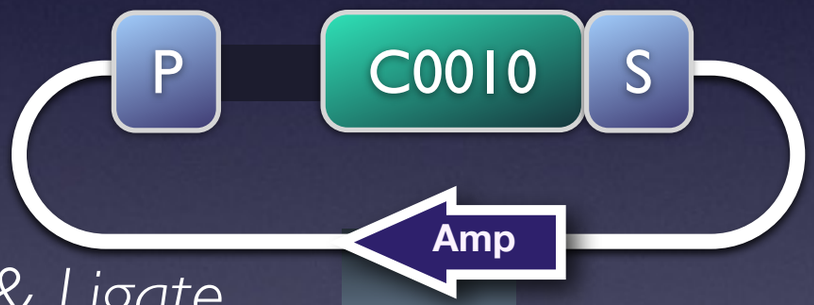
BioBrick assembly method

Cut with EcoRI & SpeI

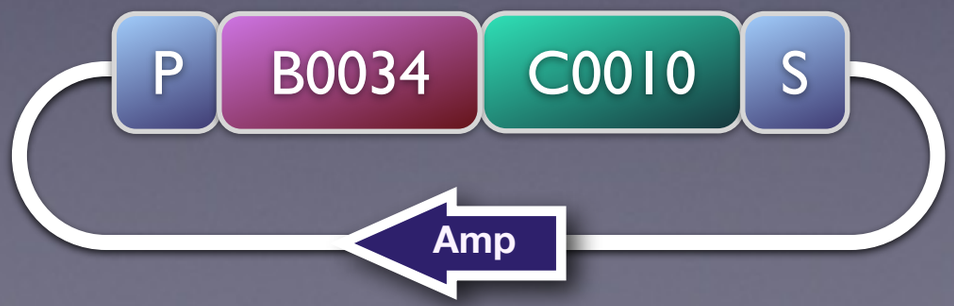


Cut with EcoRI & XbaI





Mix & Ligate



biobricks.org

The BioBricks Foundation

- Develop and implement legal strategies to ensure that BioBrick™ standard biological parts remain freely available to the public.

- Support the development of open technical standards that define BioBrick™ standard biological parts.

- Develop and provide educational and scientific materials to allow the public to use and improve existing BioBrick™ standard biological parts, and contribute new BioBrick™ standard biological parts.

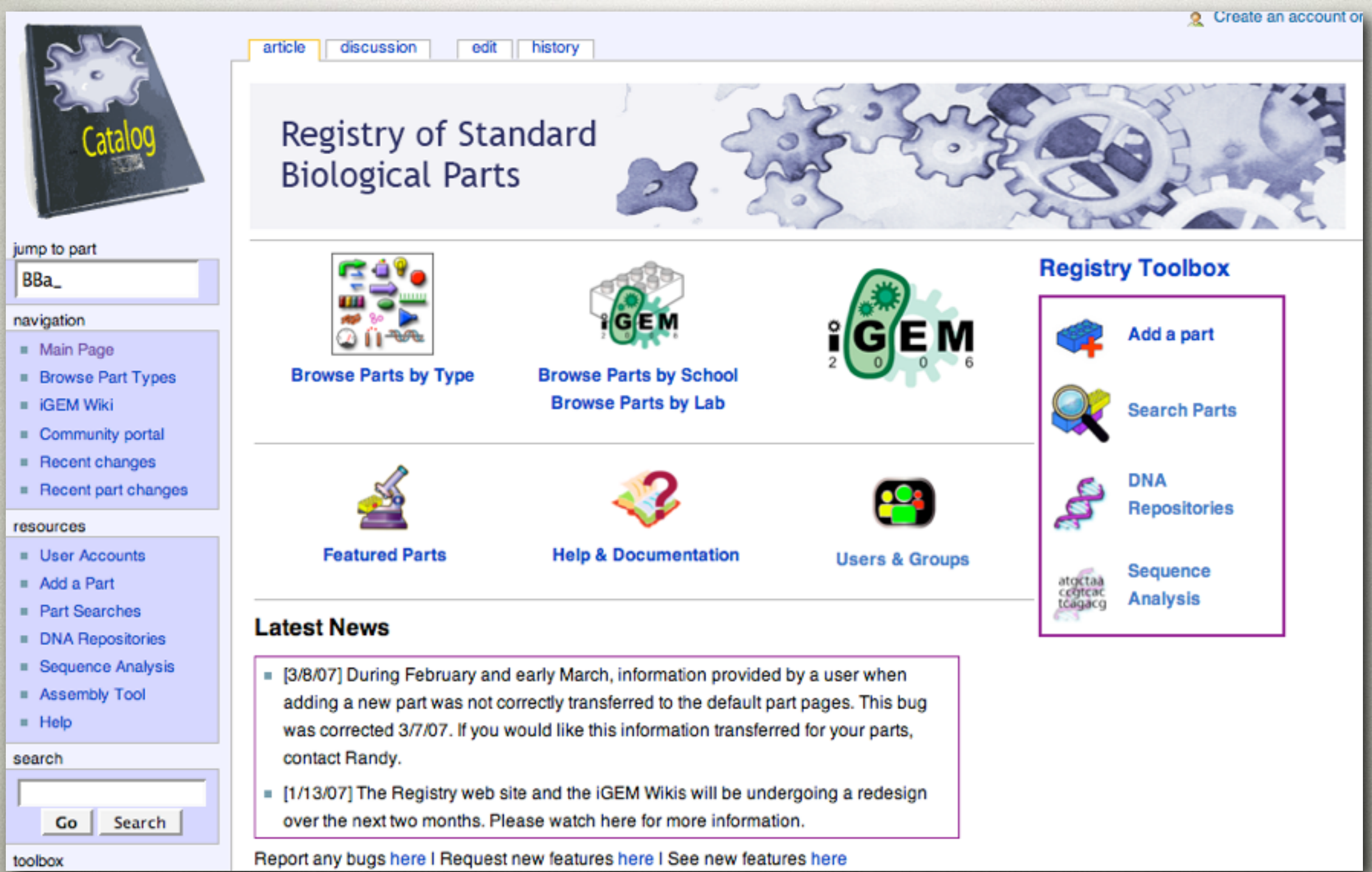
International Genetically Engineered Machine competition for Synthetic Biology

iGEM Genetically Engineered Machine competition

iGEM2016 Synthetic Biology competition: Plant Track and distribution of Common Syntax DNA parts

The Registry of Standard Biological Parts

http://parts.igem.org



Libraries of Standard Part

Cell-Cell Signalling

Cell-cell signalling devices allow communication between an individual cell and
synchronized behavior across a cell population or the communication of inform
signal and it can receive an averaged signal from all its neighbors carrying the s
cell-cell signalling are therefore a Sender device and a Receiver device. The cur
system of V. Fischeri or its analogs in other organisms (see references). Thes

Available signal senders

-?-	Name	Description	Family
A W	BBa_F1610	3OC$_6$HSL Sender Device	

Available signal receivers

-?-	Name	Description	Fa
A W	BBa_F2620	3OC$_6$HSL Receiver Device	
A W	BBa_F2621	3OC$_6$HSL Receiver Device	
A W	BBa_F2622	3OC$_6$HSL Receiver Device	

Available other signalling pa

-?-	Name	
A W	BBa_I13261	Lux Receiver
A W	BBa_I13263	Lux Receiver
A W	BBa_I13272	YFP Producer
A W	BBa_I13273	YFP Producer
A W	BBa_T9002	GFP Producer
A	BBa_I0424	I0404.I6101
A	BBa_I0426	I0406.I6107
A	BBa_I0428	I0408.I6106
A	BBa_I0466	RhlR Protein Generator
A	BBa_I13018	LuxR Cassette under Ptet (Other)
A	BBa_I13202	3OC$_6$HSL Sender Controlled by Lac Repressible Pro
A	BBa_I13207	HSL/aiiA test construct
A	BBa_I13208	aiiA (LVA-) protein generator driven by plac
A	BBa_I1466	RhlR protein generator (LVA-)
A	BBa_J13040	pOmpR dependent 3OC6HSL sender device

Available sig

-?-	Name
A W	BBa_F26
A W	BBa_F26
A W	BBa_F2

BBa_F2620

3OC$_6$HSL → PoPS Receiver

http://parts.mit.edu/registry/index.php/Part:BBa_F2620

Authors:
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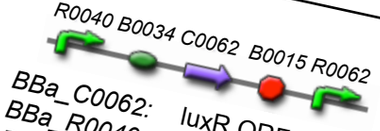
Anna Labno [labnoa@mit.edu]

Last Update: 19 October 2007

Description

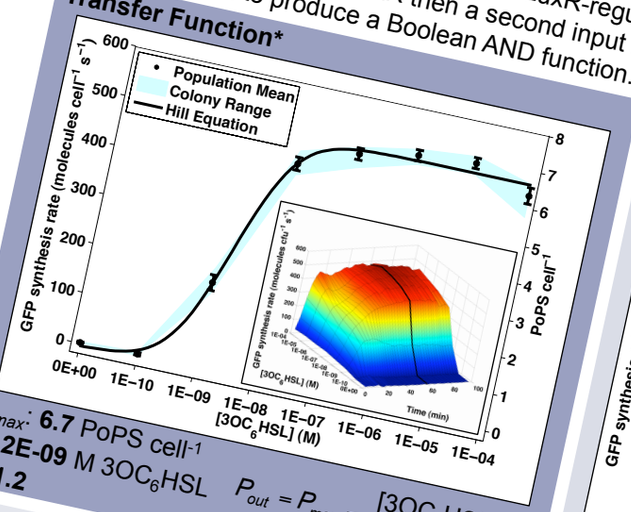
A transcription factor (LuxR, BBa_C0062) that is active in the presence of cell-cell signaling molecule 3OC6HSL is controlled by a TetR-regulated operator (BBa_R0040). Device input is 3OC6HSL. Device output is PoPS from a LuxR-regulated operator. If used in a cell containing TetR then a second input signal such as aTc can be used to produce a Boolean AND function.

Parts



BBa_C0062: luxR ORF
BBa_R0040: LuxR-regulated operator
BBa_R0040: TetR-regulated operator

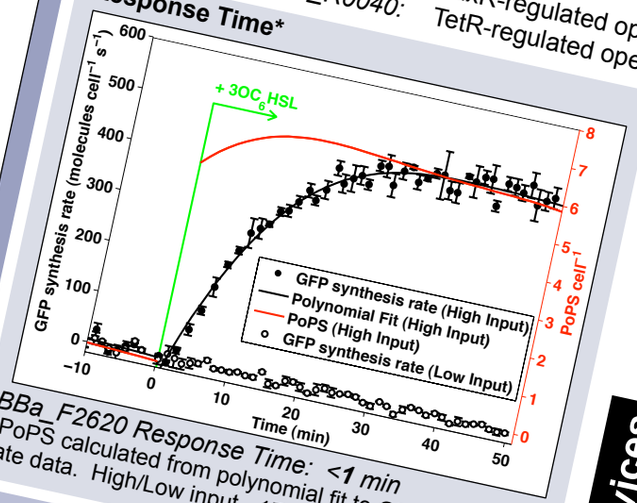
Transfer Function*



P_{max}: **6.7** PoPS cell^{-1}
K: **2E-09** M 3OC$_6$HSL
n: **1.2**

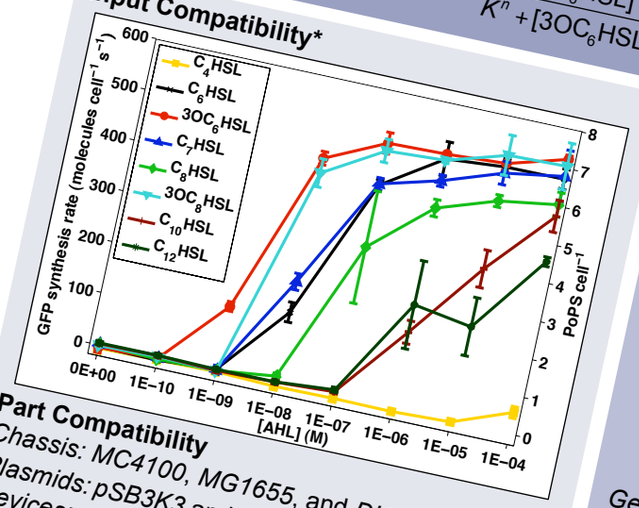
$$P_{out} = P_{max} \frac{[3OC_6HSL]^n}{K^n + [3OC_6HSL]^n}$$

Response Time*

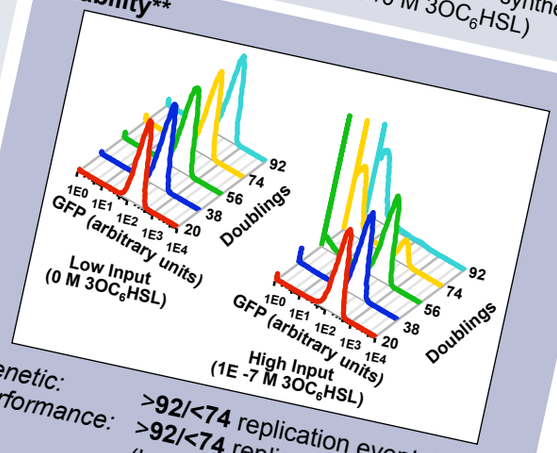


BBa_F2620 Response Time: ***<1 min***
(PoPS calculated from polynomial fit to GFP synthesis rate data. High/Low input - 1E-7/0 M 3OC$_6$HSL)

Input Compatibility*



Stability**



Part Compatibility
Chassis: MC4100, MG1655, and DH5α
Plasmids: pSB3K3 and pSB1A2
Devices: E0240, E0430 and E0434
Crosstalk with systems containing C0040

Transcriptional Output Demand (low/high input)
Nucleotides: **0.2xNt / 6xNt** nucleotides cell^{-1} s^{-1}
Polymerases: **4.4E-3xNt / 1.5E-1xNt** RNAP cell^{-1}
(**Nt** = downstream transcript length)

Genetic: **>92/<74** replication events**
Performance: **>92/<74** replication events**
(low/high input)

Conditions (abridged)
Output: PoPS measured via *BBa_E0240*
Culture: Supplemented M9, 37ºC
Vector: pSB3K3
Chassis: MG1655
**Equipment:* PE Victor3 plate reader
***Equipment:* BD FACScan cytometer

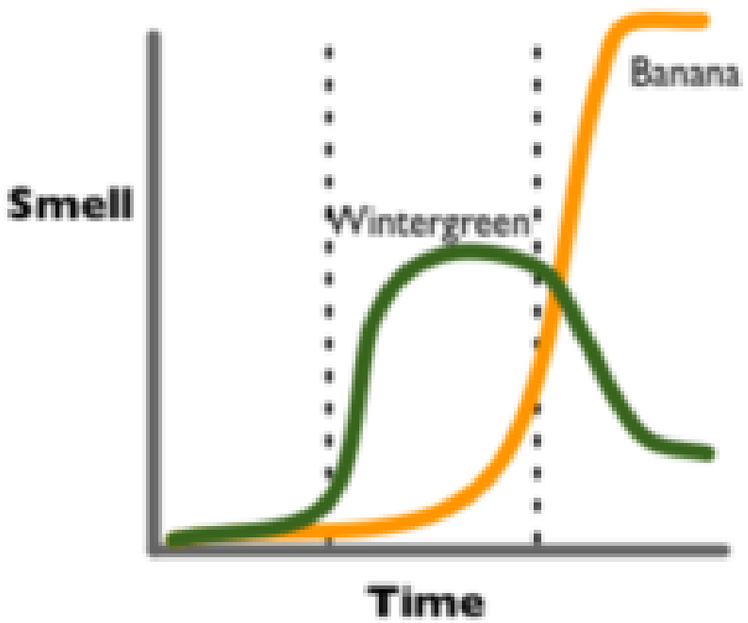
Registry of Standard Biological Parts
making life better, one part at a time

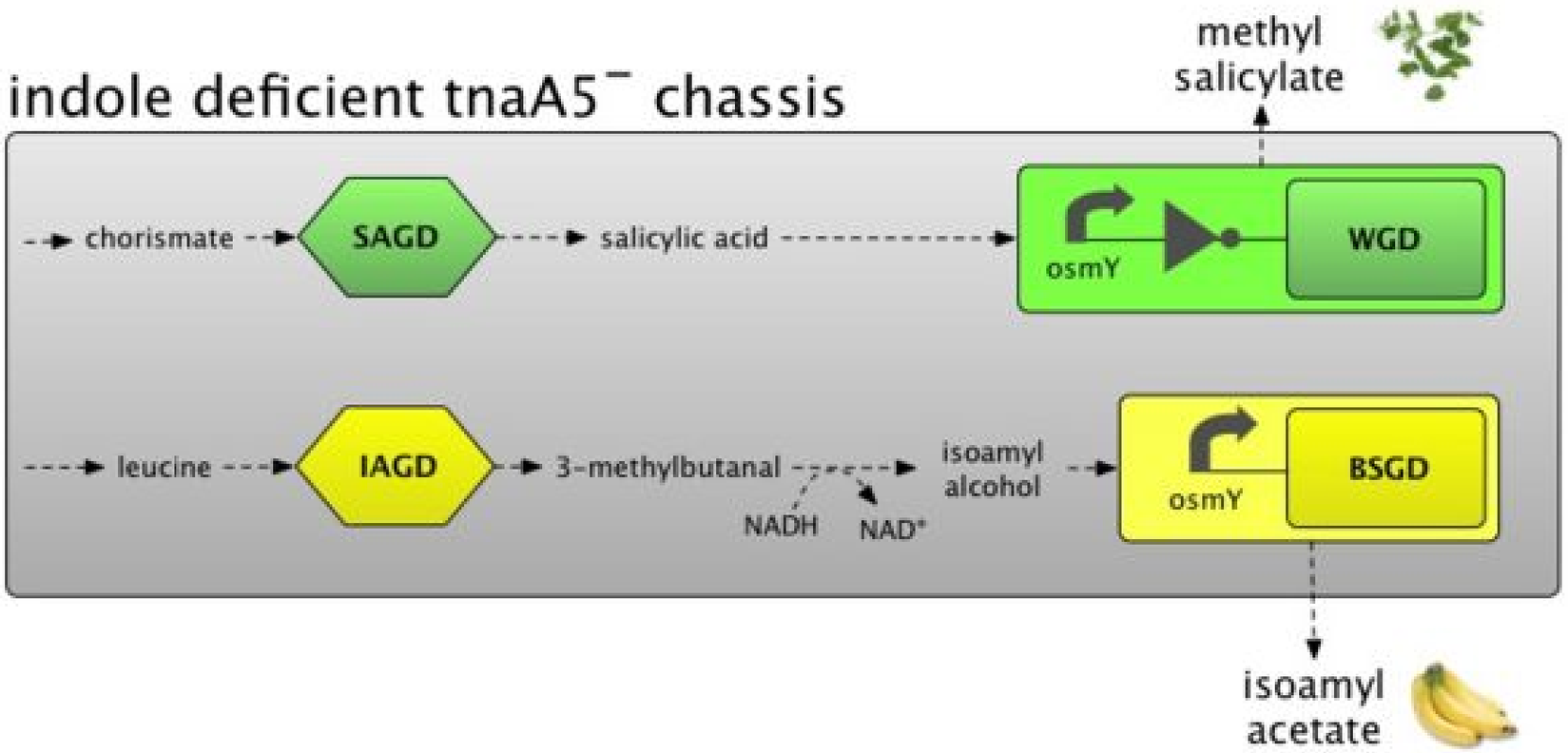
License: Public

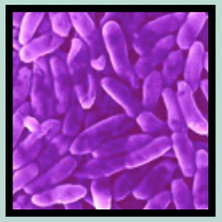
Signaling Devices

Bioproduction of scents

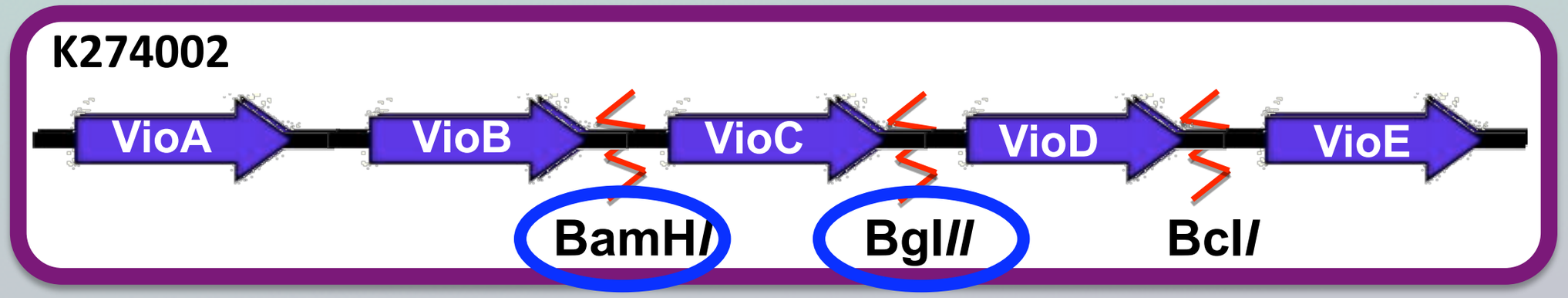
2006







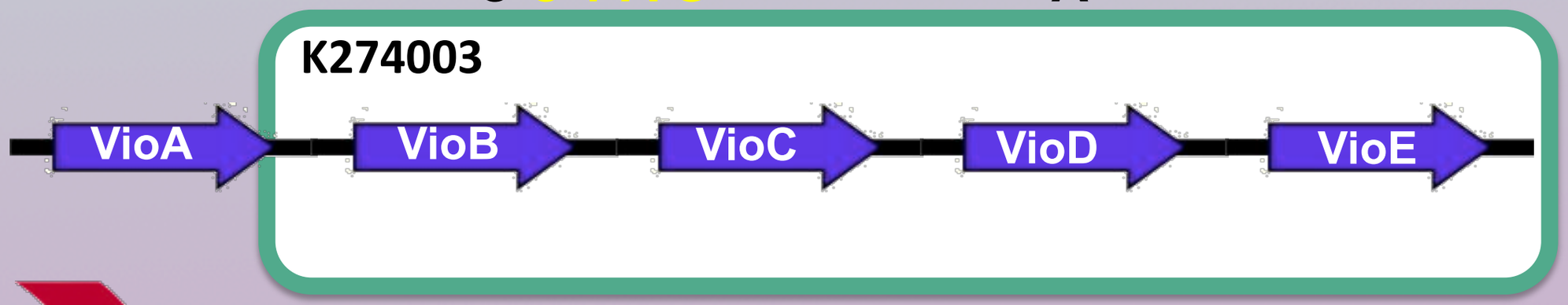
Violacein: *Design & Synthesis*



VCG
K274002

G G A T C T

C C T A G A



GCG
K2742003

DNA 2.0

BioBricks: *Colour Generators*

VCG
K274002

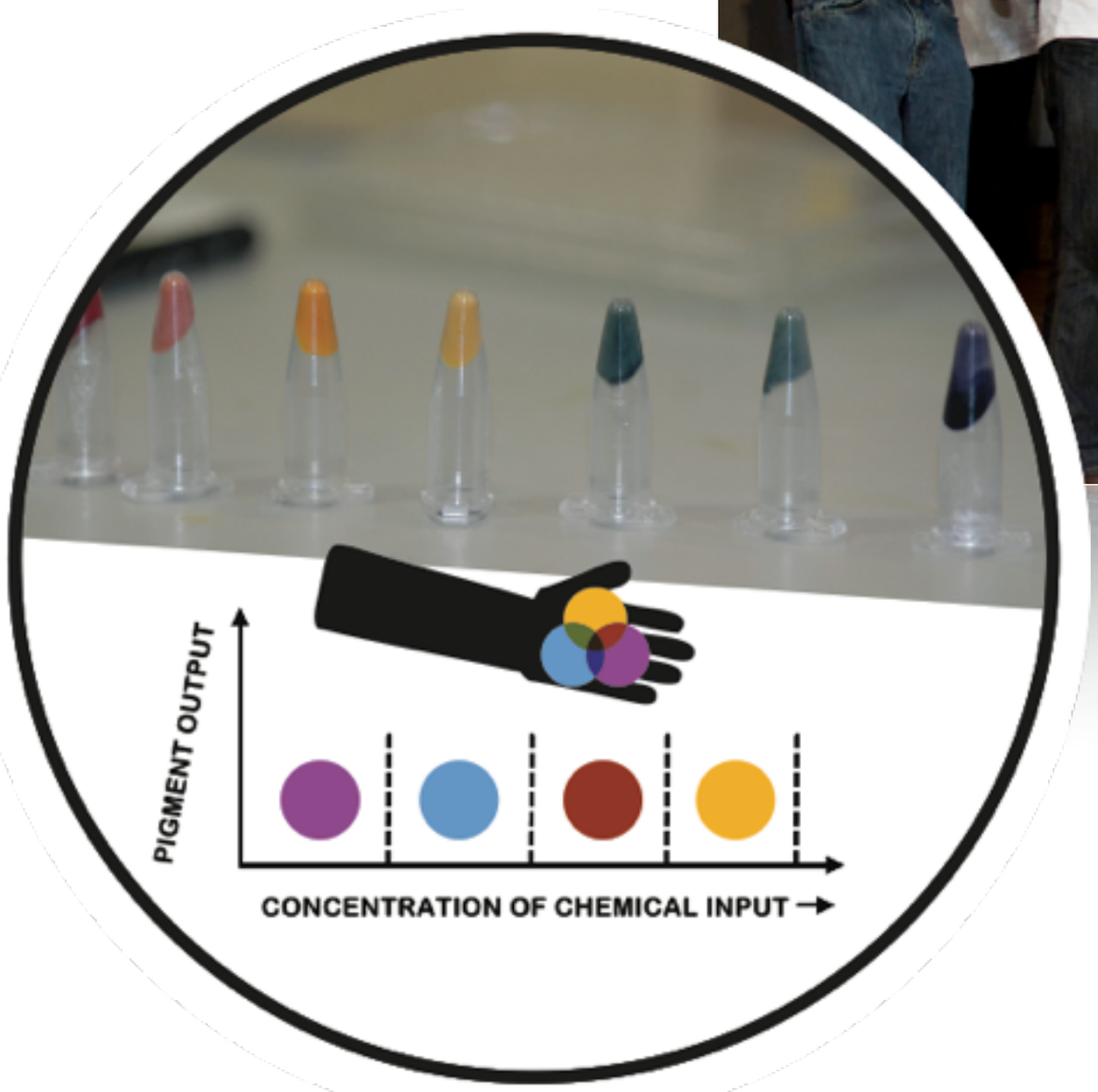
BCG
K274001

RCG
K274100

GCG
K2742003

OCG
K274200

iGEM 2009
Grand Prize



E.chromi

Type IIS DNA assembly protocols:

Golden Gate MoClo ENSA Golden Braid:

adopted by the plant research community

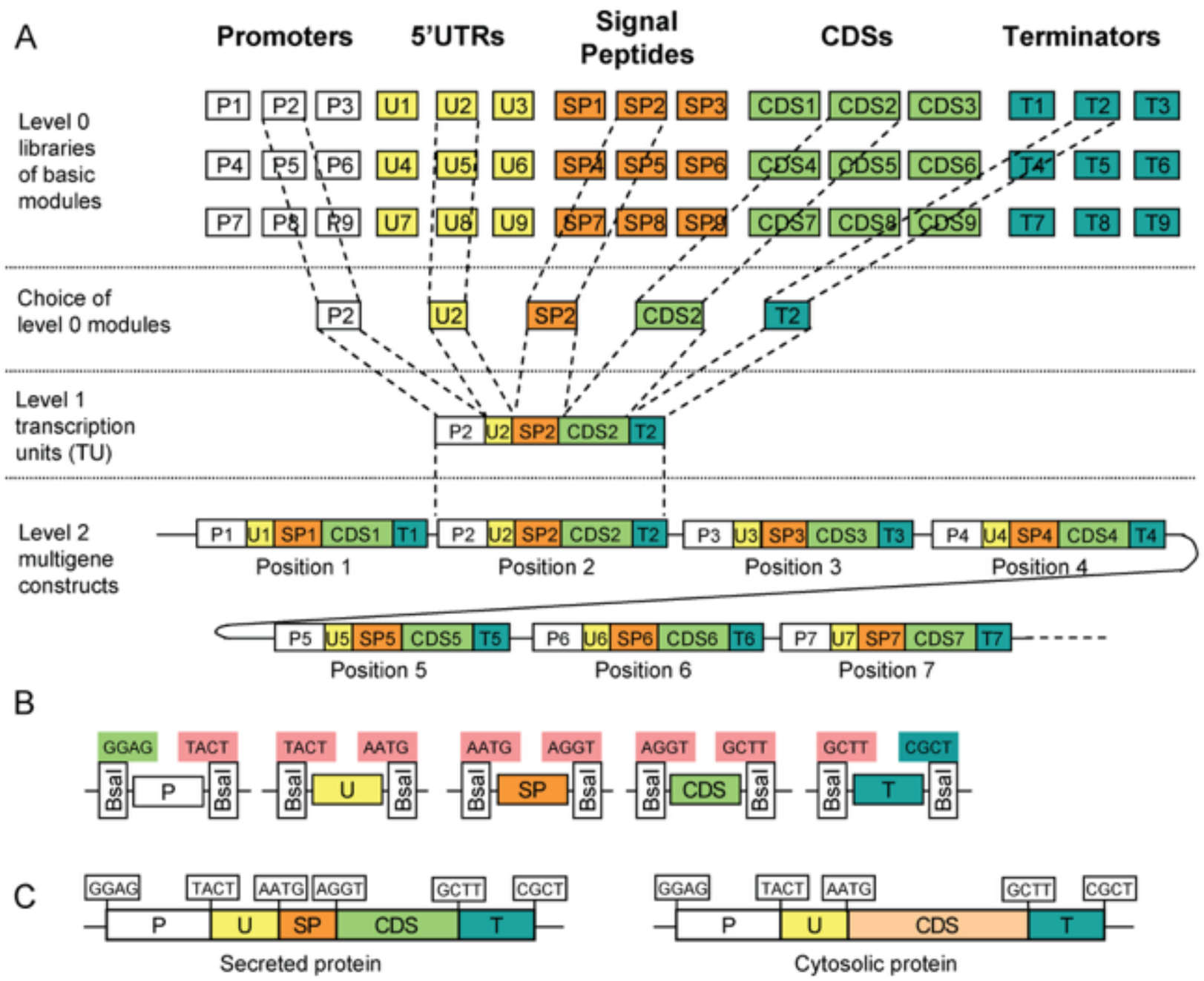
OPEN ACCESS Freely available online

PLoS one

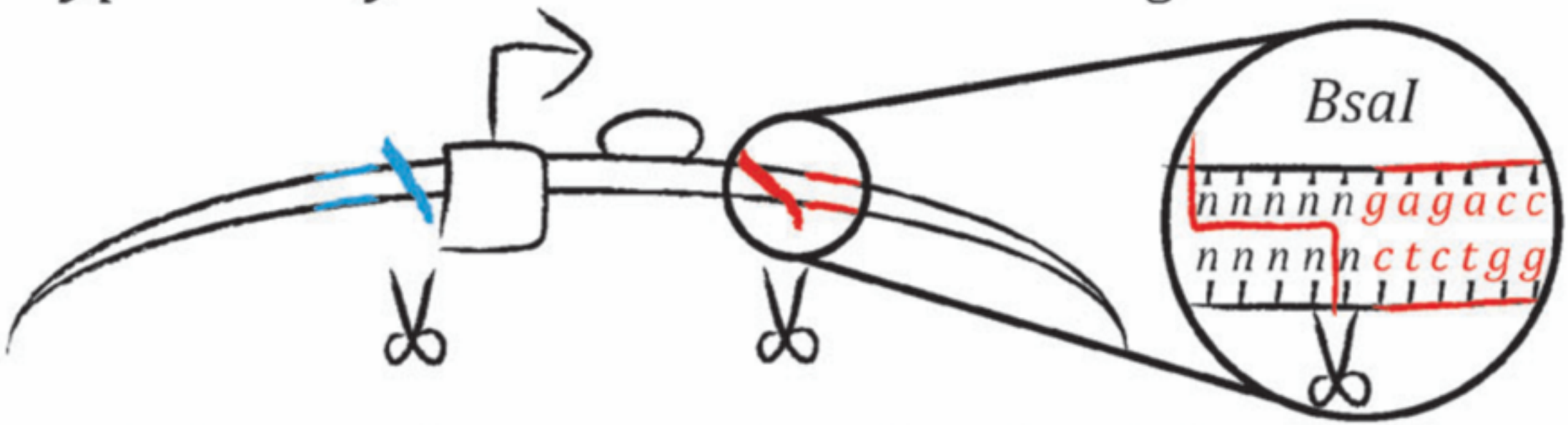
A Modular Cloning System for Standardized Assembly of Multigene Constructs

Ernst Weber, Carola Engler, Ramona Gruetzner, Stefan Werner, Sylvestre Marillonnet*

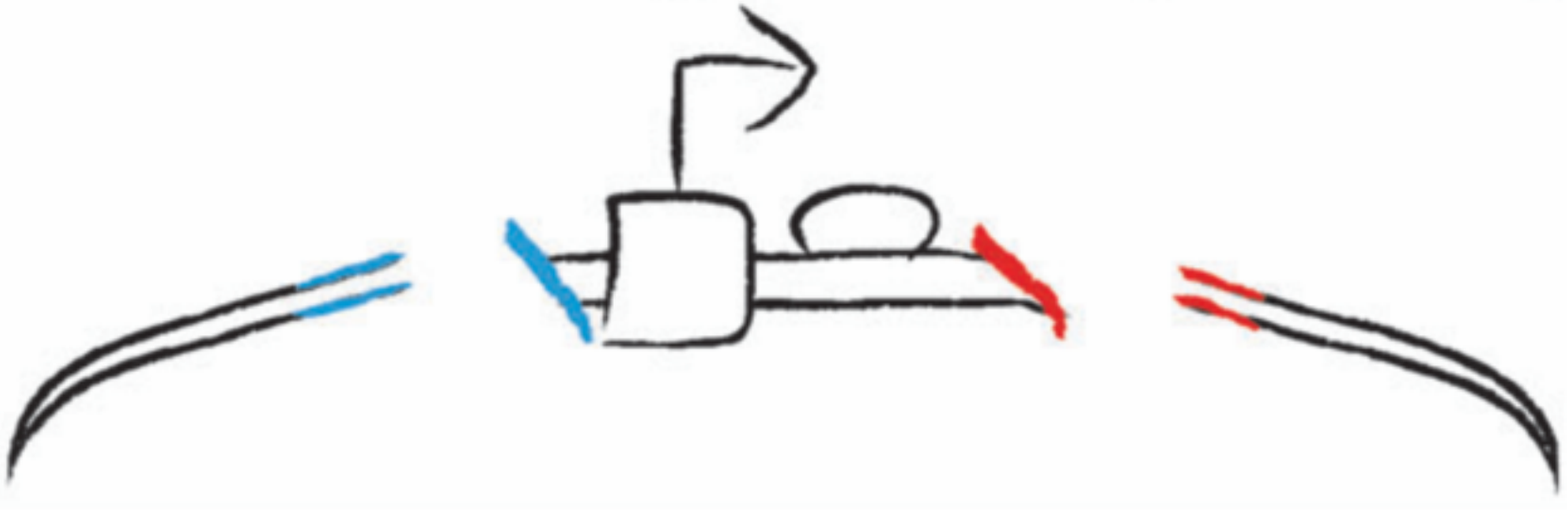
Icon Genetics GmbH, Halle/Saale, Germany



D *type IIs enzymes cut outside their recognition site*

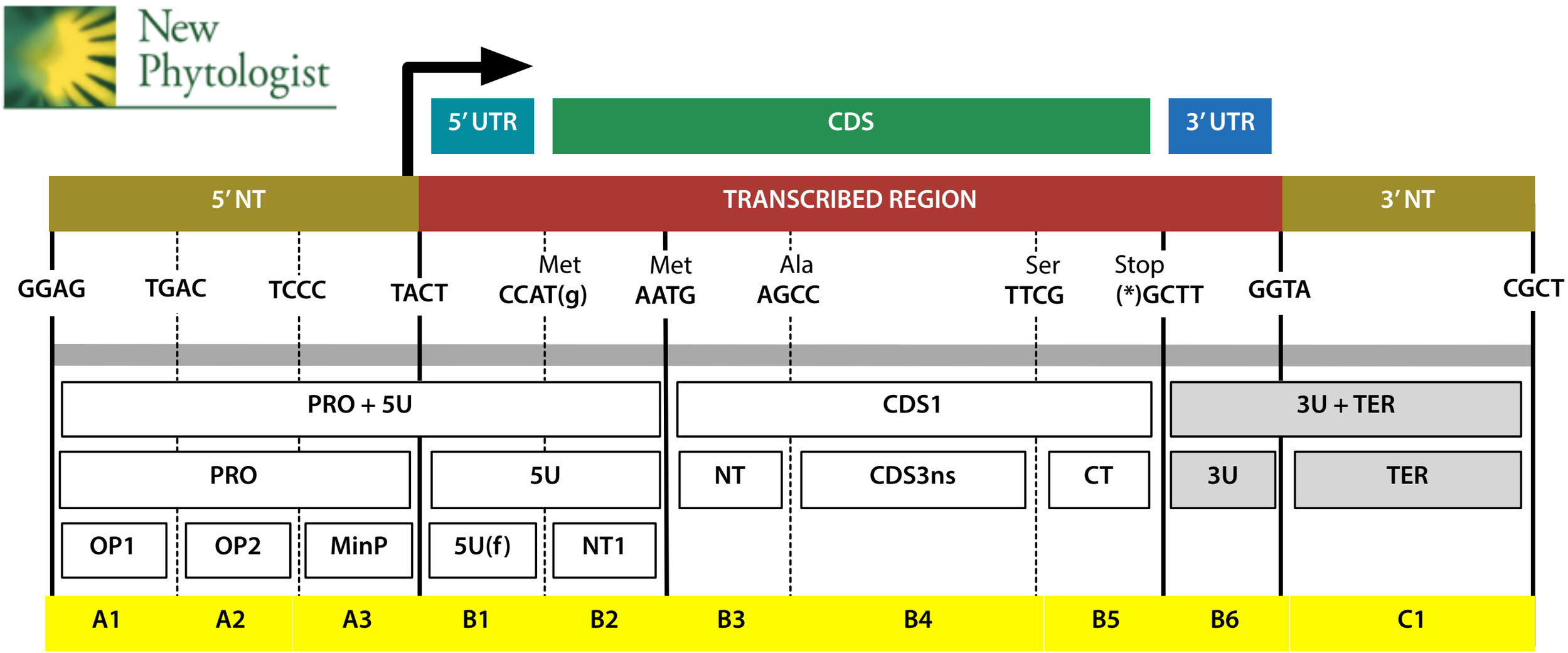


restriction overhangs can therefore be defined



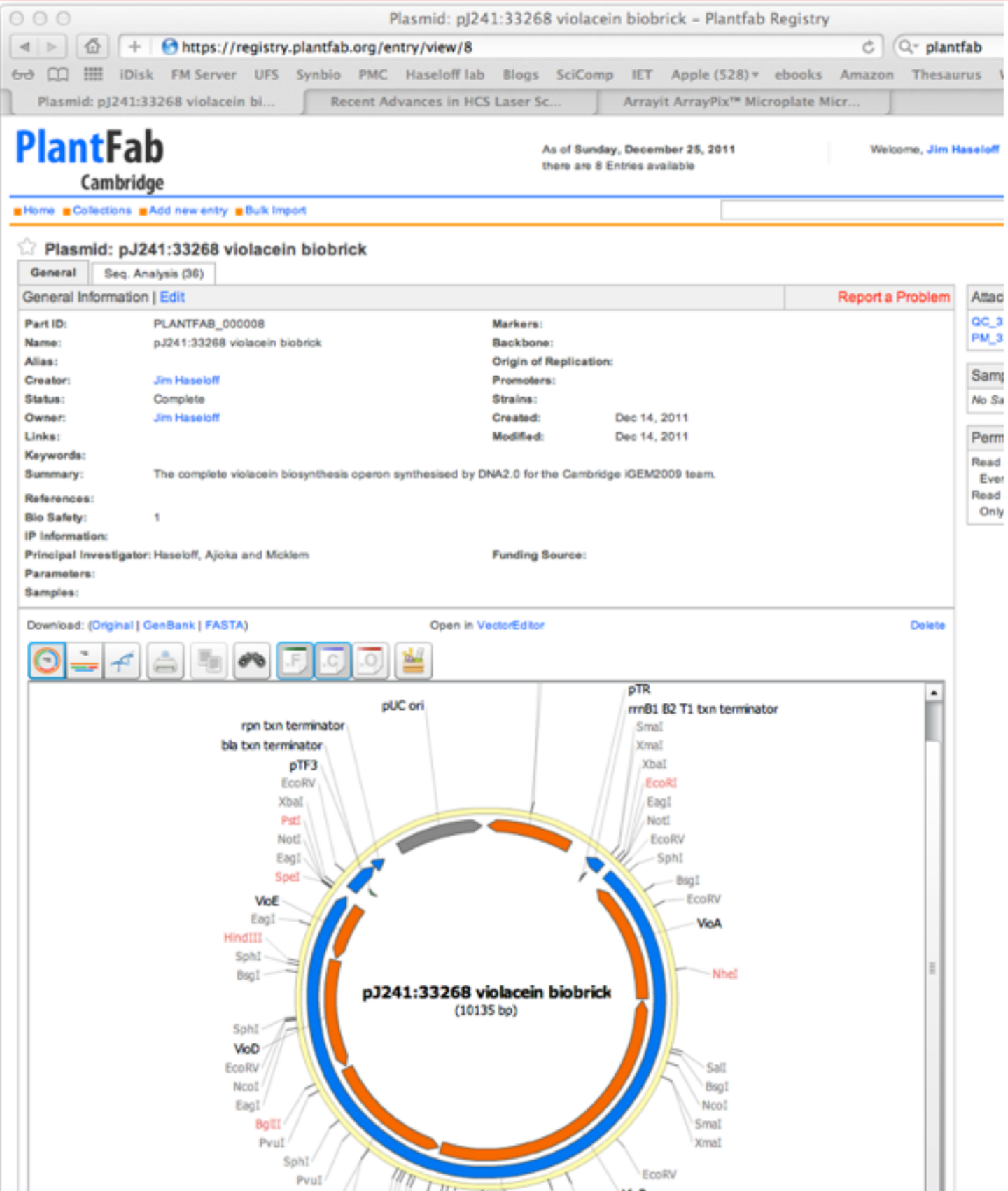
A common syntax for plant DNA parts

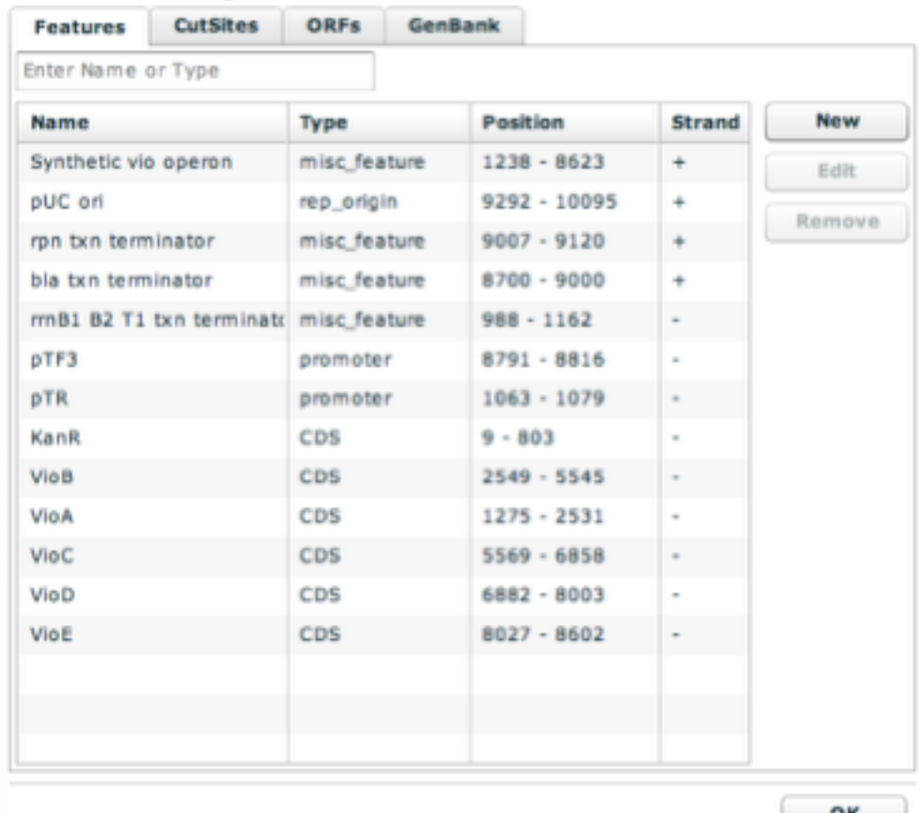
Based on Golden Gate standard assembly and type IIs restriction enzyme splints.



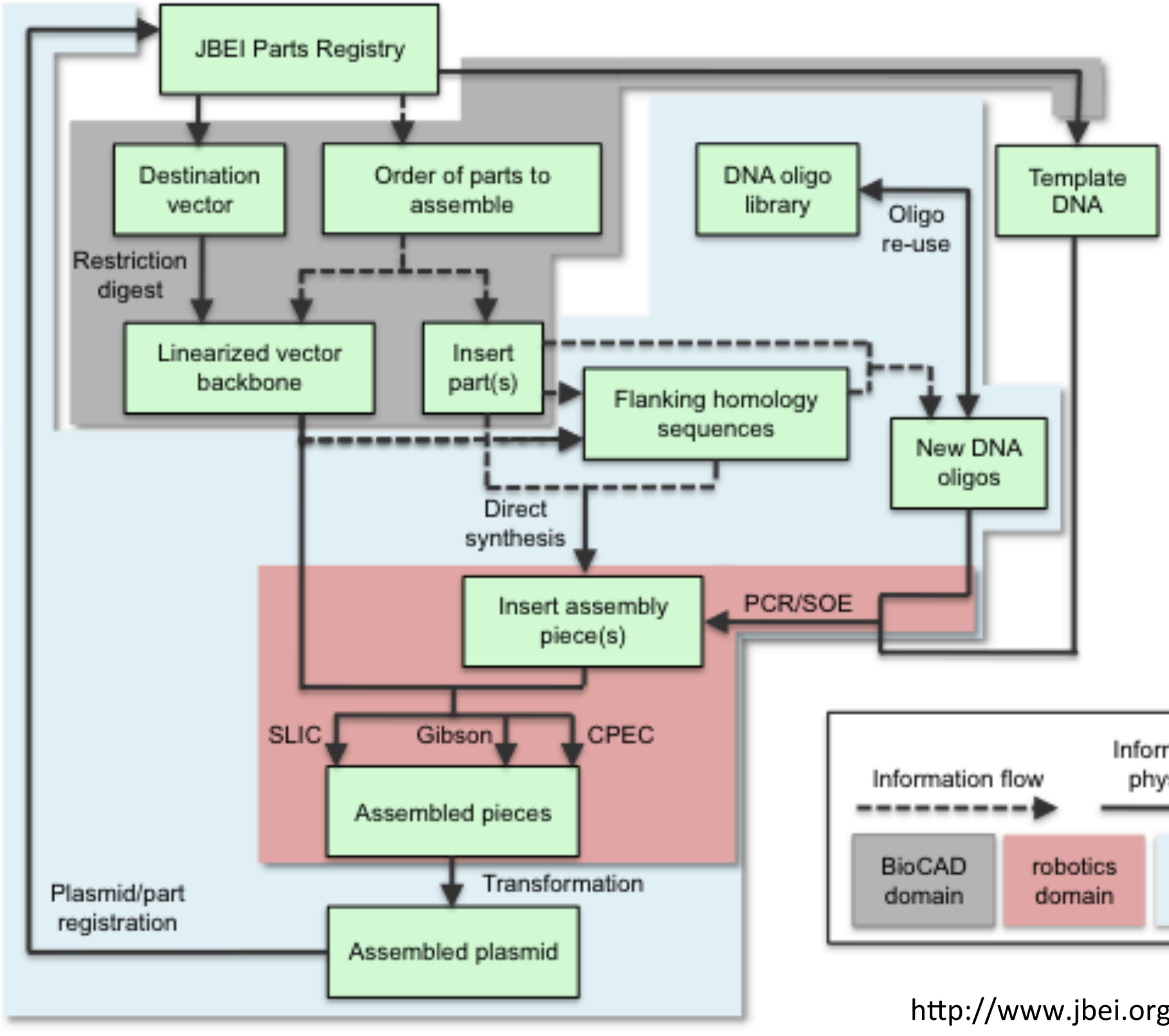
NPH-L-2015-19556.R1 Standards for Plant Synthetic Biology: A Common Syntax for Exchange of DNA Parts
by Patron, Nicola; Orzaez, Diego; Marillonnet, Sylvestre; Warzecha, Heribert; Matthewman, Colette; Youles, Mark; Raitskin, Oleg; Leveau, Aymeric; Farre-Martinez, Gemma; Rogers, Christian; Smith, Alison; Hibberd, Julian; Webb, Alex; Locke, James; Schornack, Sebastian; Ajioka, Jim; Baulcombe, David; Zipfel, Cyril; Kamoun, Sophien; Jones, Jonathan; Kuhn, Hannah; Robatzek, Silke; Van Esse, H Peter; Oldroyd, Giles; Sanders, Dale; Martin, Cathie; Field, Rob; O'Connor, Sarah; Fox, Samantha; Wulff, Brande; Miller, Ben; Breakspear, Andy; Radhakrishnan, Guru; Delaux, Pierre-Marc; Loque, Dominique ; Granell, Antonio; Tissier, Alain; Shih, Patrick; Brutnell, Thomas; Quick, Paul; Rischer, Heiko; Fraser, Paul; Aharoni, Asaph; Raines, Christine; South, Paul; Ané, Jean-Michel; Hamberger, Björn; Langdale, Jane; Stougaard, Jens; Bouwmeester, Harro; Udvardi, Michael; Murray, Jim; Ntoukakis, Vardis; Schafer, Patrick; Denby, Katherine; Edwards, Keith; Osbourn, Anne; Haseloff, Jim Haseloff

Development of smart registries for DNA parts





Smart Registry for DNA parts



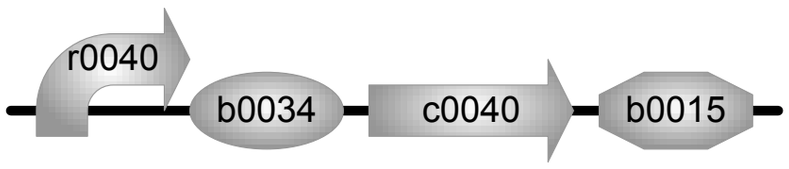
JBEI Inventory for Composable Elements (ICE)

Open source registry software for DNA parts

Nathan Hillson
Tim Hamm

http://www.jbei.org/fuels-synthesis/synth.shtml

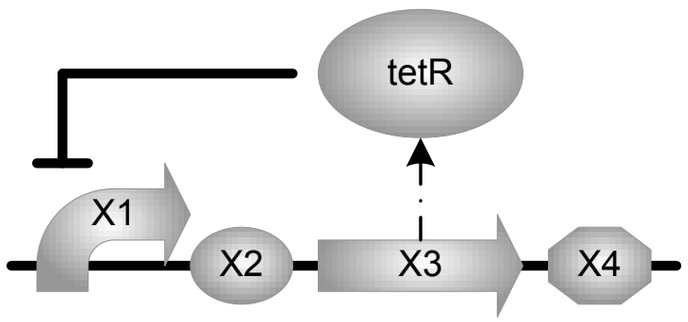
Software for compilation of DNA circuits



r0040:prom; b0034:rbs; c0040:pcr; b0015:ter

Specific set of parts:

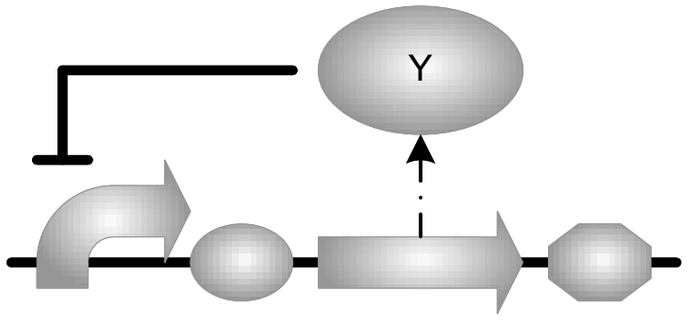
[r0040; b0034; c0040; b0015]



X1:prom<neg(tetR)>; X2:rbs; X3:pcr<codes(tetR)>; X4:ter

tetR negative feedback

[r0040; b0034; c0040; b0015]



prom<neg(Y)>; rbs; pcr<codes(Y)>; ter

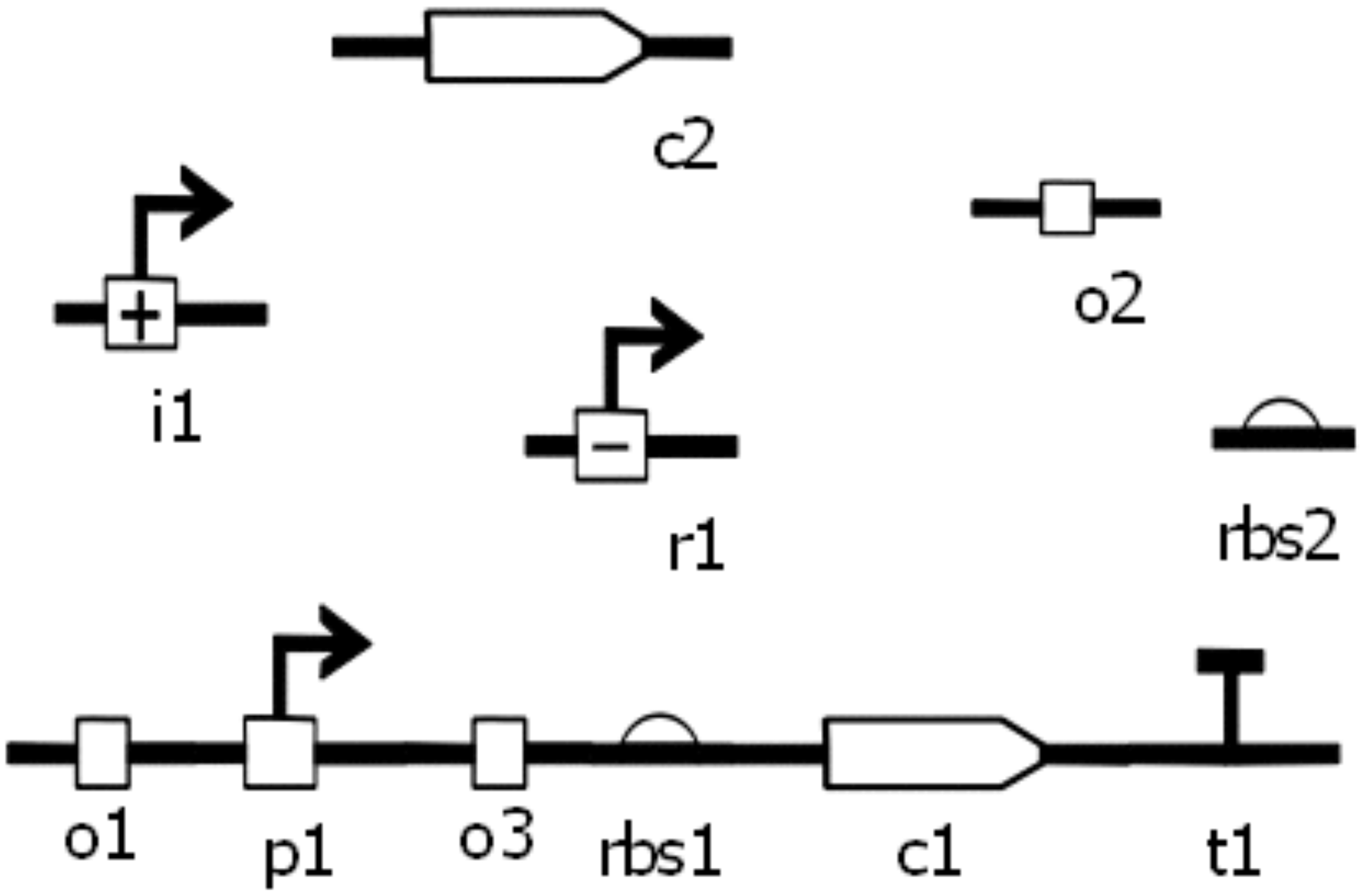
Any negative feedback:

[r0051; b0034; c0051; b0015]
[r0040; b0034; c0040; b0015]
[i0500; b0034; c0080; b0015]
[r0011; b0034; c0012; b0015]

Systems Biology Markup Language (SMBL), Synthetic Biology Open Language (SBOL), Microsoft GEC

SBOL

Synthetic Biology Open Language: is a software standard for the electronic exchange of specifications and descriptions of genetic parts, genetic devices, genetic modules, genetic systems, and engineered genomes.



SBOL Compliant Software Tools

TinkerCell - Synthetic biology CAD tool offering drawing and quantitative simulation

Clotho - Biological engineering tool set for development and management of biological systems

GenoCAD – Design of synthetic DNA sequences based on grammatical models of genetic part

GD-ICE - Laboratory registry software to track and search composable DNA constructs

Device Editor - Graphical user interface for J5. J5 is a bioCAD tool that provides rule-based validation of genetic designs that are constructed using SLIC, Gibson, Golden-gate, or CPEC assembly methods.

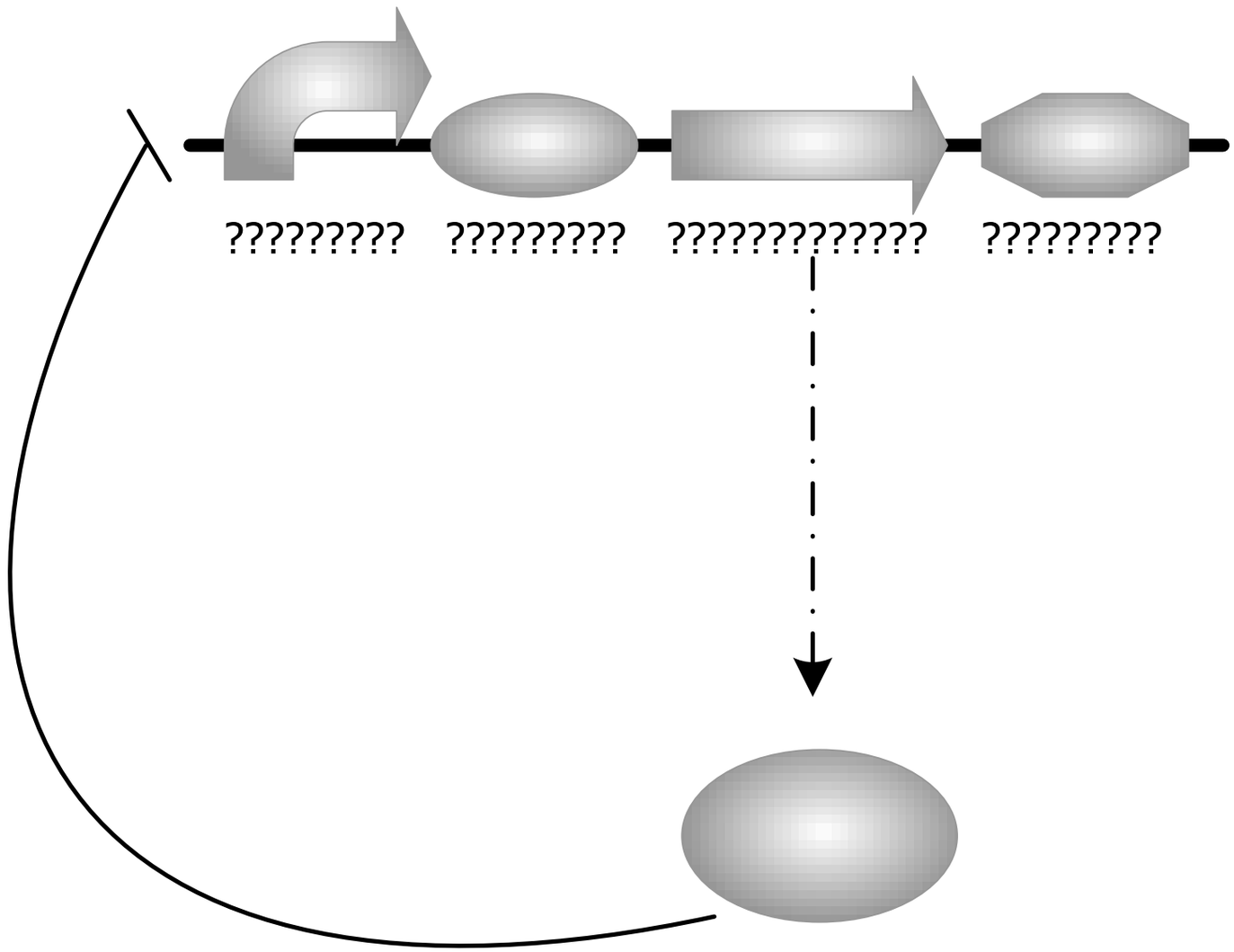
SBPkb - Knowledgebase of standard biological parts using all the data from the Registry of Standard Biological Parts at MIT.

iBiosim - Analysis tool for design of genetic circuits and discovery of their connectivity from experimental data

Electronic Datasheets - Human- and machine-readable datasheets for the standard biological parts being developed at the BIOFAB

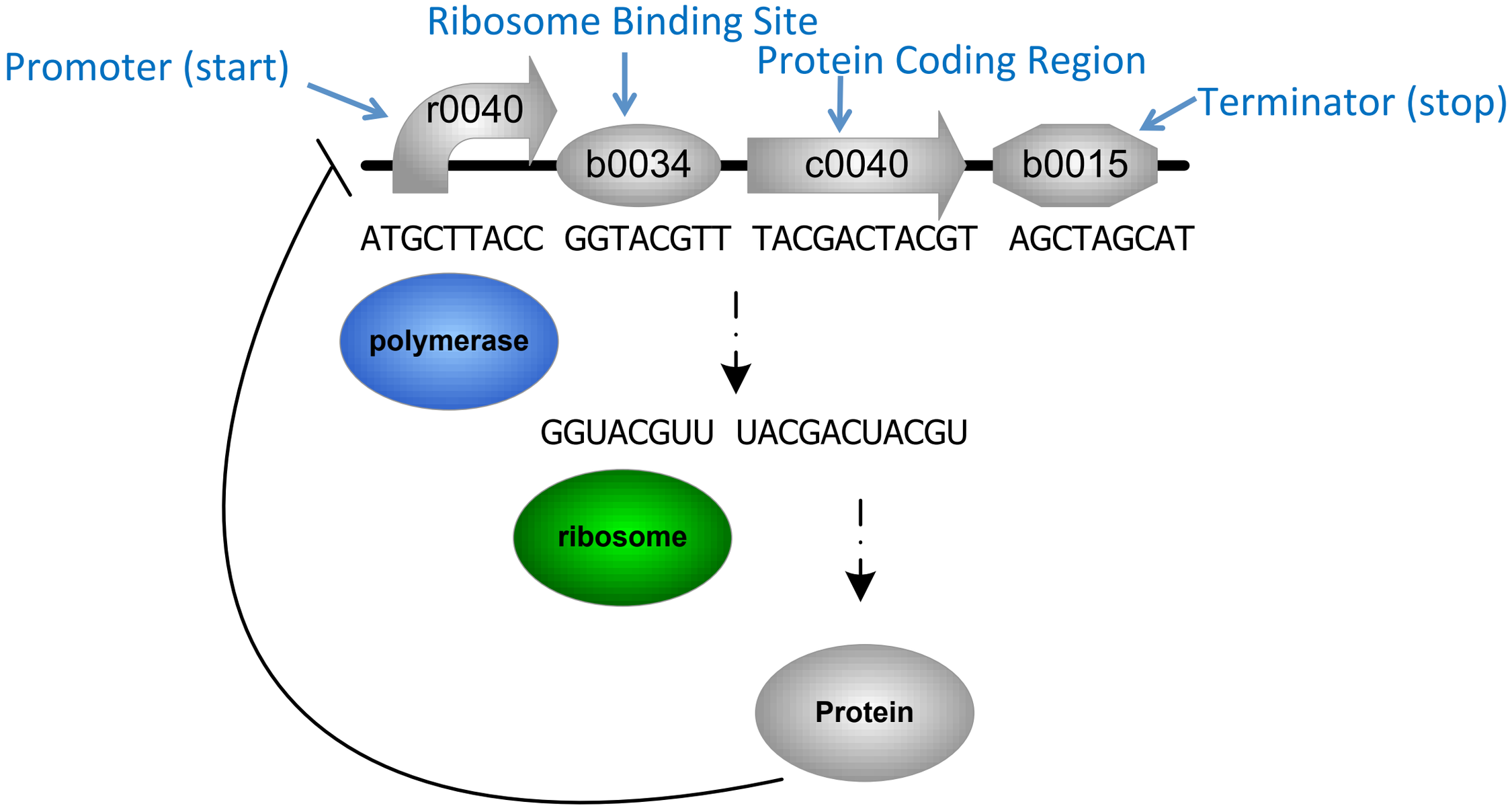
High-Level DNA Language

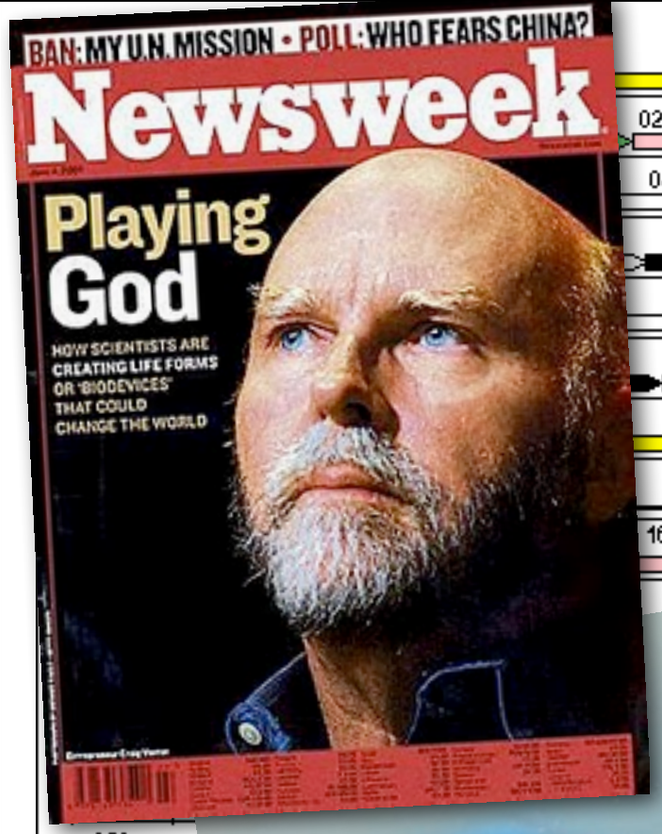
Given a design, automatically determine the DNA

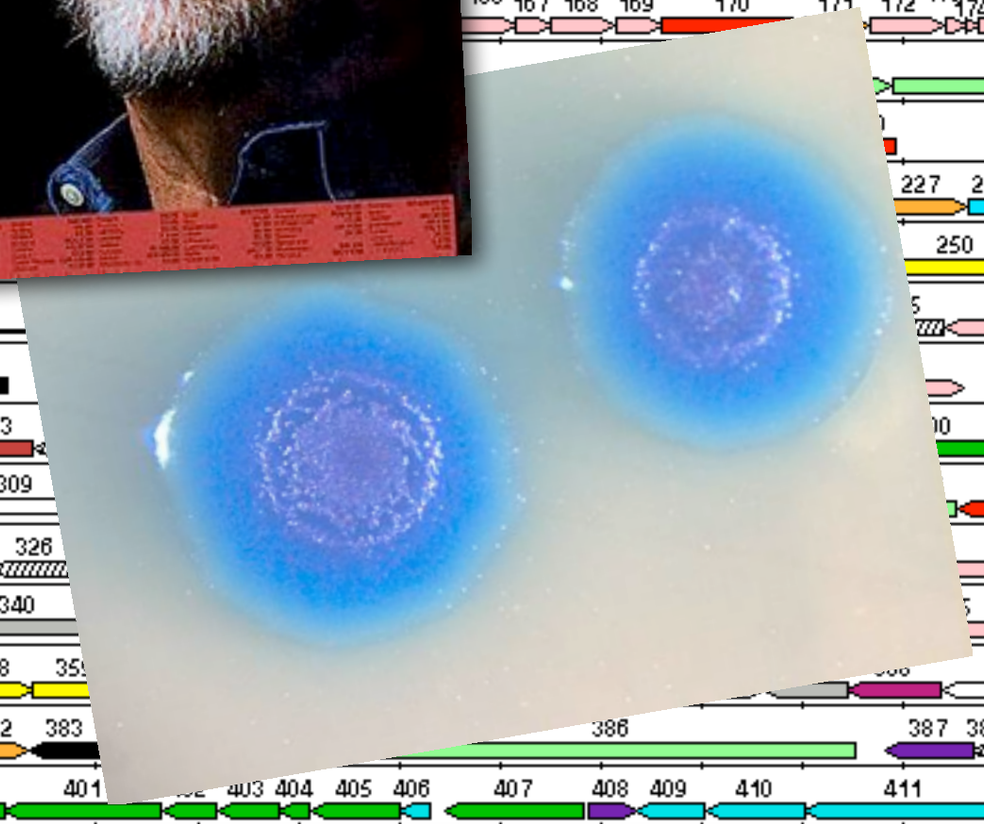


Low-Level DNA Language

A simplified view of DNA instructions

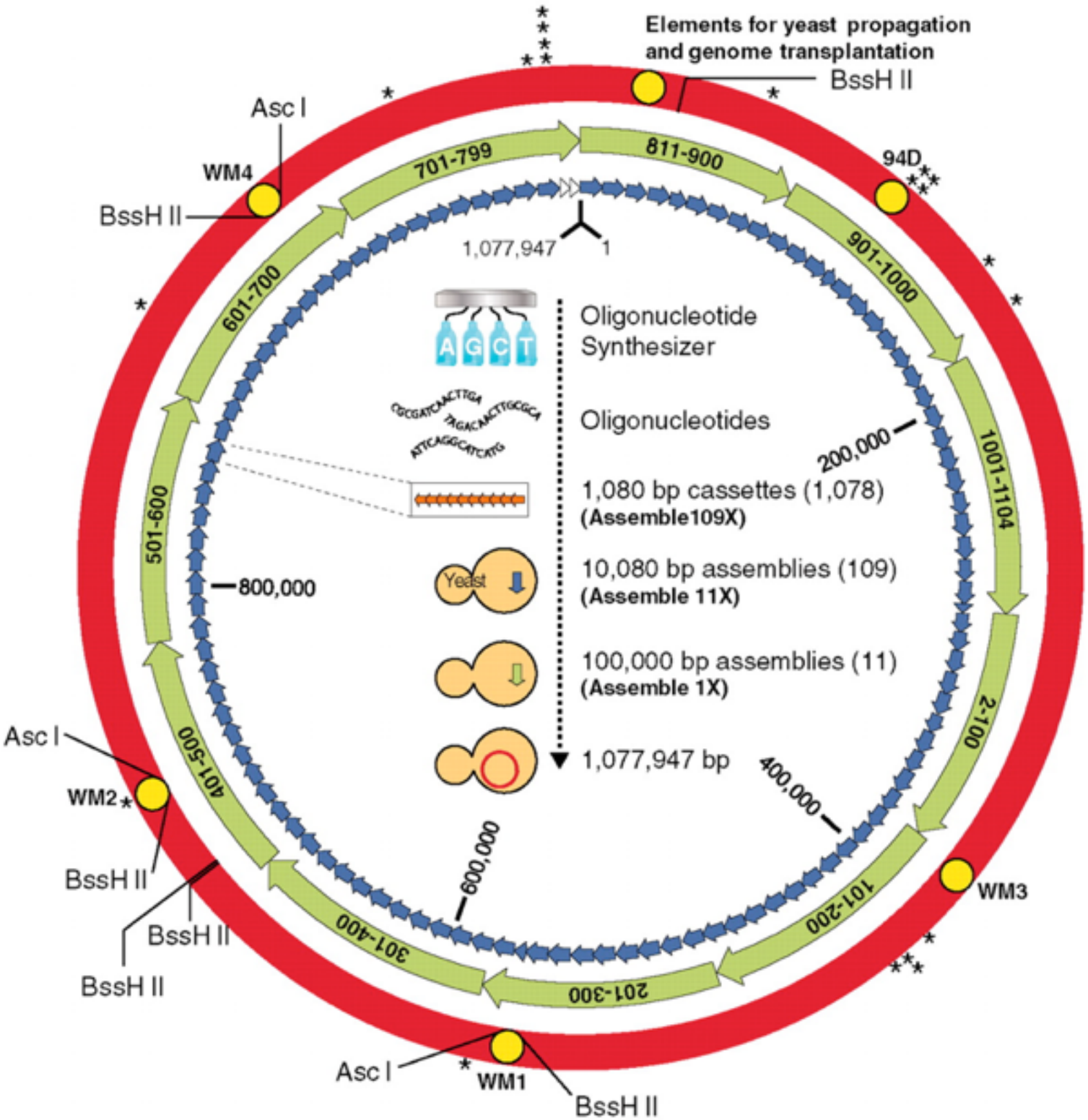






2008 - DNA synthesis of the first bacterial genome...

Chemical synthesis of a Bacterial Genome: *Mycoplasma mycoides*, JCVI 2010



Assembly of *M. mycoides* genome

1. Overlapping oligonucleotides (including yeast vector, *lacZ*) recombined to make 1080 bp cassettes (orange arrows).

2. In sets of 10, the cassettes recombined to produce 109 ~10kb assemblies (blue arrows)

3. In sets of 10, the ~10kb assemblies recombined to produce 11 ~100kb assemblies (green arrows)

4. These 11 assemblies were recombined to the final genome, sMmYCp235 synthetic genome to create JCVI-syn1.0 cell line

Gibson assembly
Breakthrough in large-scale, rapid DNA assembly

Enzymatic assembly of DNA molecules up to several hundred kilobases

Daniel G Gibson[1], Lei Young[1], Ray-Yuan Chuang[1], J Craig Venter[1,2], Clyde A Hutchison III[2] & Hamilton O Smith[2]

We describe an isothermal, single-reaction method for assembling multiple overlapping DNA molecules by the concerted action of a 5′ exonuclease, a DNA polymerase and a DNA ligase. First we recessed DNA fragments, yielding single-stranded DNA overhangs that specifically annealed, and then covalently joined them. This assembly method can be used to seamlessly construct synthetic and natural genes, genetic pathways and entire genomes, and could be a useful molecular engineering tool.

Nature Biotechnology, May 2009

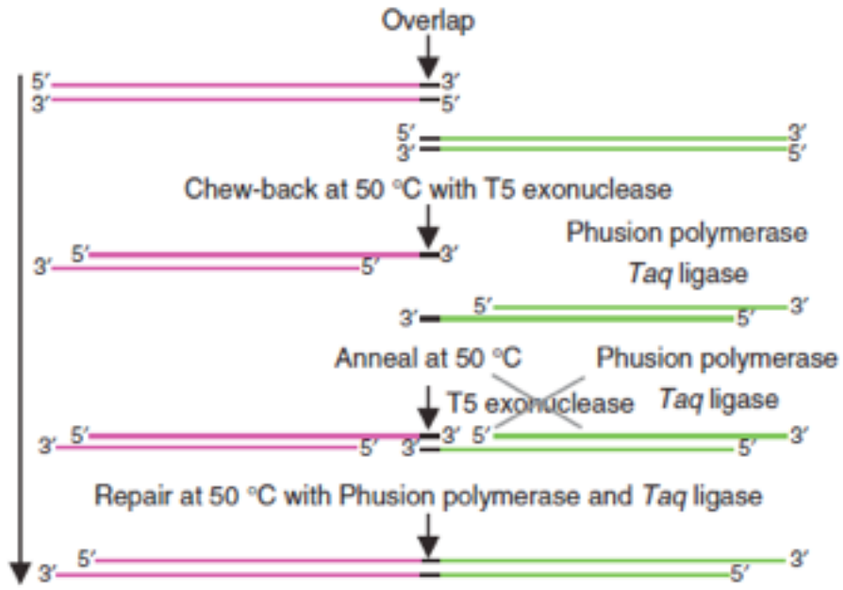
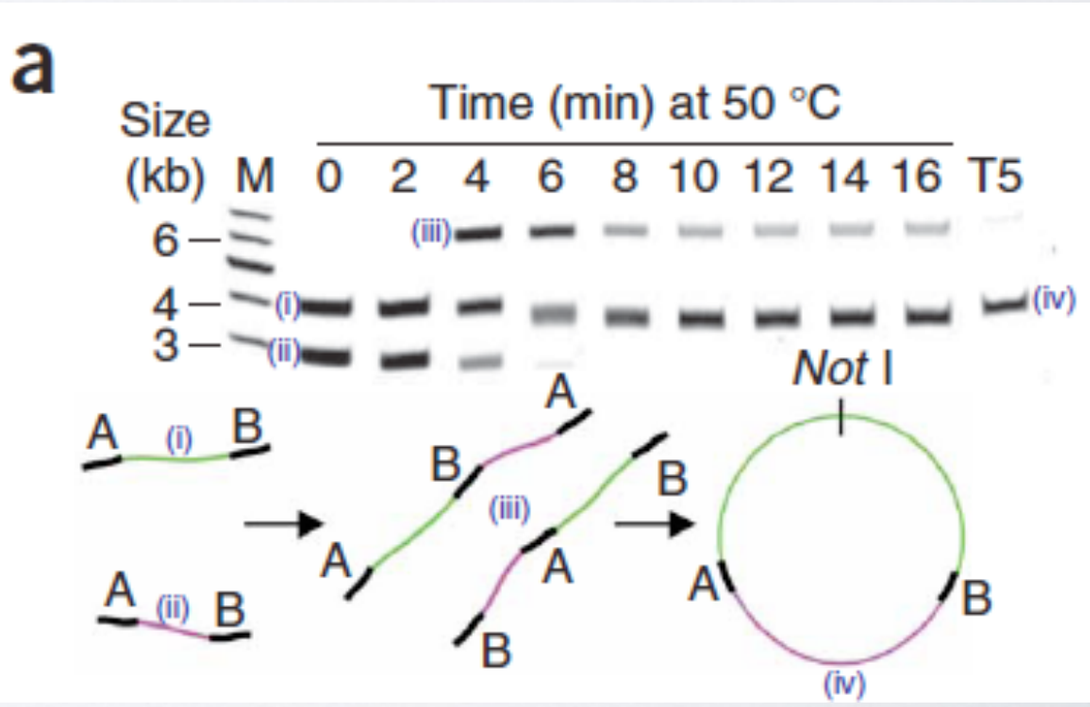


Figure 1 | One-step isothermal *in vitro* recombination. Two adjacent DNA fragments (magenta and green) sharing terminal sequence overlaps (black) were joined into a covalently sealed molecule in a one-step isothermal reaction. T5 exonuclease removed nucleotides from the 5′ ends of double-stranded DNA molecules, complementary single-stranded DNA overhangs annealed, Phusion DNA polymerase filled the gaps and *Taq* DNA ligase sealed the nicks. T5 exonuclease is heat-labile and is inactivated during the 50 °C incubation.



Large DNA fragment assembly via homologous recombination in yeast

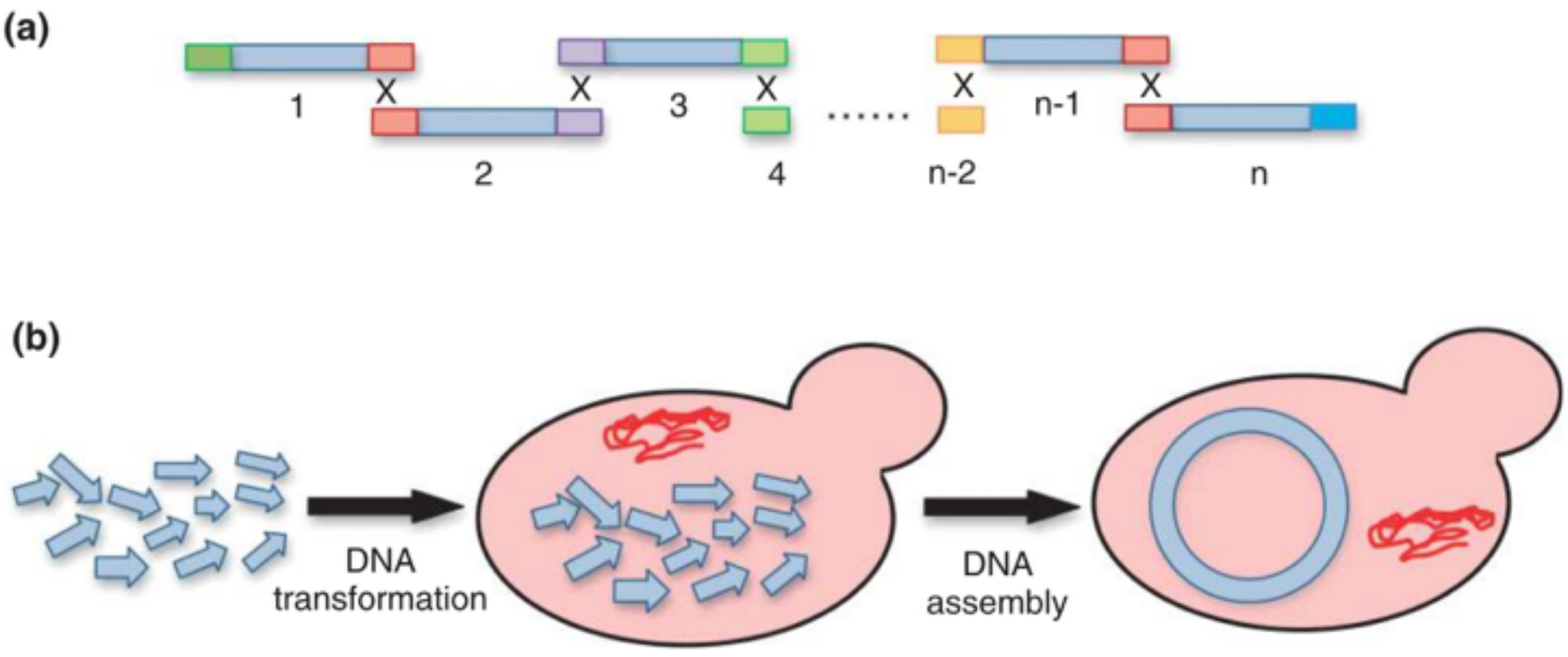
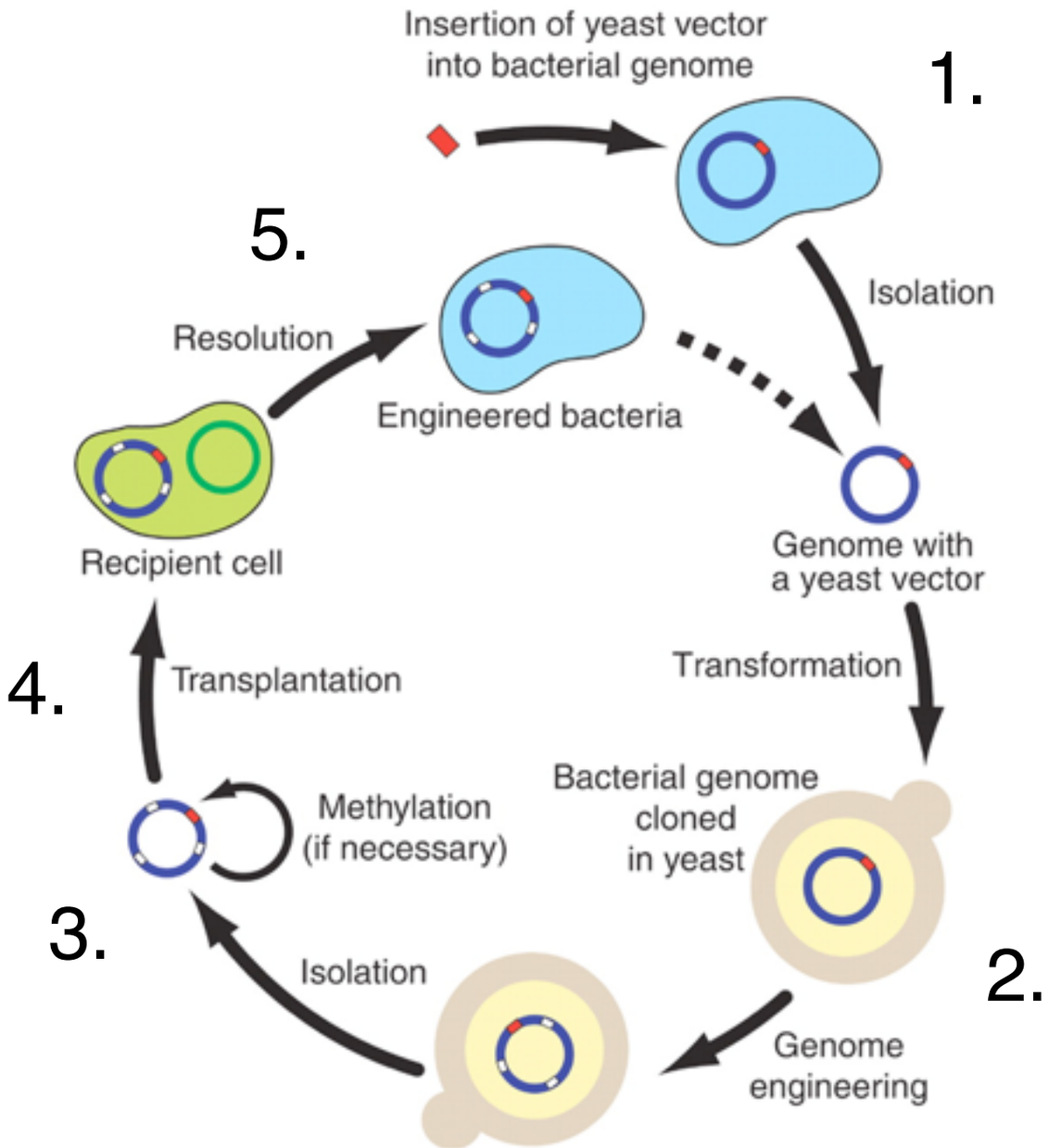


FIGURE 2 | Synthesis of large DNA molecules in yeast. (a) Yeast homologous recombination mechanism. DNA fragments sharing an overlap region at 3′- and 5′-ends with the neighboring DNA fragments can be assembled into a single larger DNA molecule. (b) Construction of a synthetic *M. genitalium* genome. Twenty-five different overlapping DNA segments (blue arrows, 17–35 kb each) composing the genome were co-transformed into yeast followed by assembly of the entire genome in a single step.

Creating Bacterial Strains from Genomes that have been cloned and engineered in yeast



Lartigue et al. (2009) Science 325: 1693-1696

Transplantation of *M. mycoides* genome via yeast engineering

1. Insertion of yeast vector into bacterial genome
2. Transform yeast with entire genome, engineer in yeast
3. Isolate DNA, protect (methylation)
4. Transform recipient bacteria
5. Resolve (loss of recipient genome)

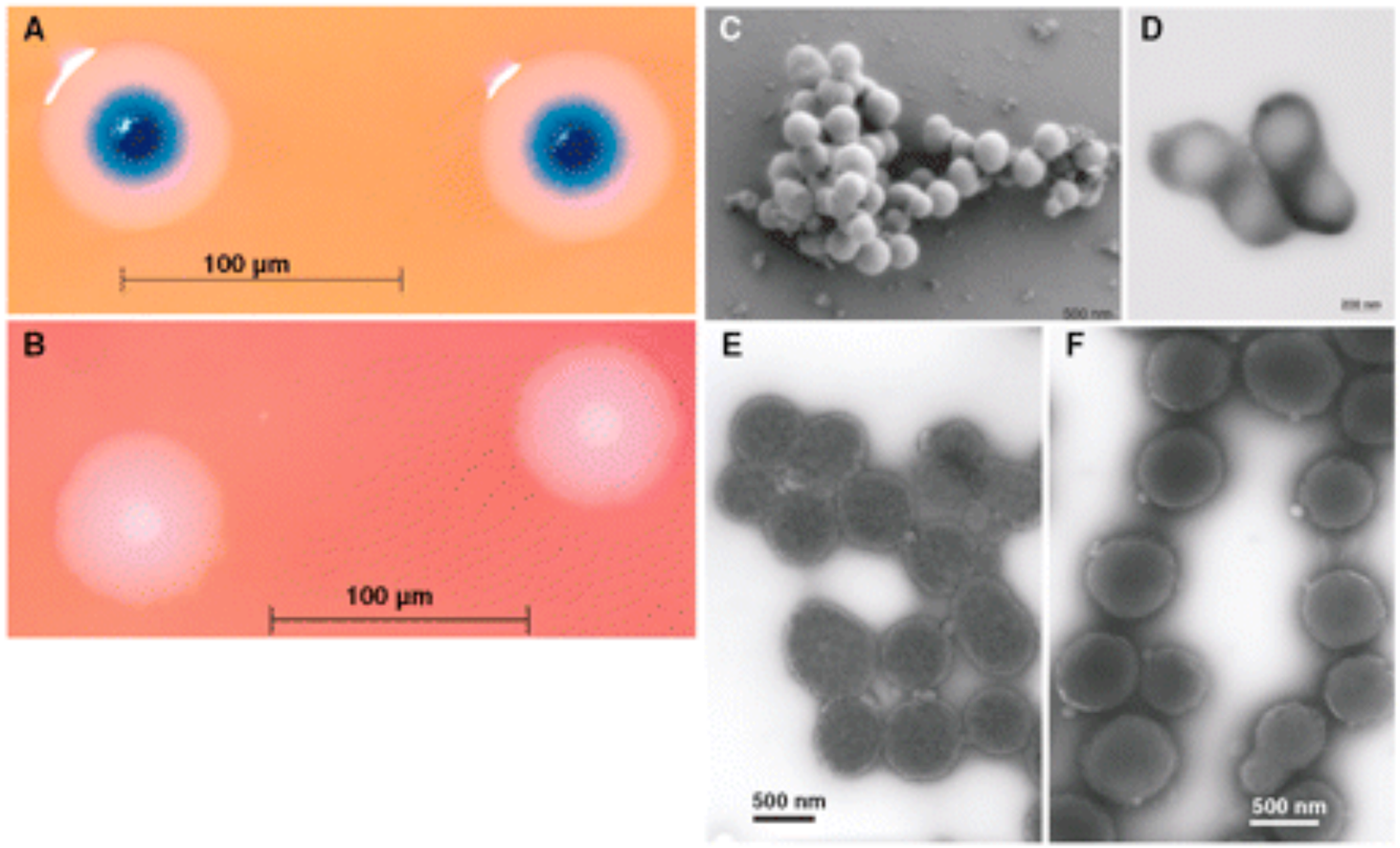
Transplantation of a synthetic bacterial genome: *Mycoplasma mycoides*, JCVI 2010

Genome transplantation

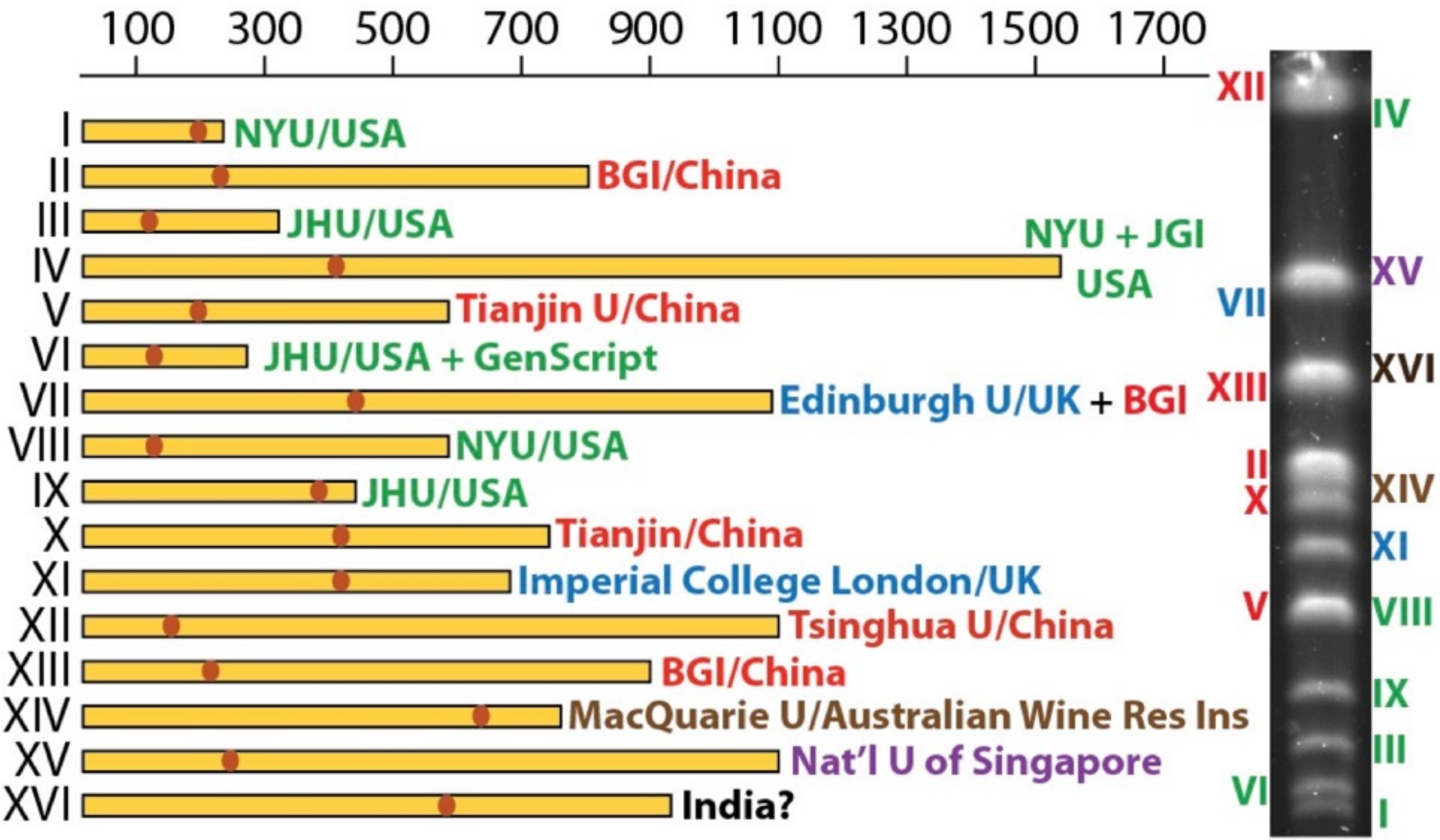
DNA from the final assembly in yeast sMmYCp235 synthetic genome was transplanted into a *M. capricolum* cell to ultimately produce JCVI-syn1.0

A&B. WT *M. mycoides* colonies are white, JCVI-syn1.0 are blue (*lacZ*/beta galactosidase + Xgal). “Fried egg” morphologies characteristic of mycoplasma species.

C,D,E & F. Electron micrographs of cells. Both WT and JCVI-syn1.0 show the same morphology



SC2.0 Synthetic Yeast Genome Project



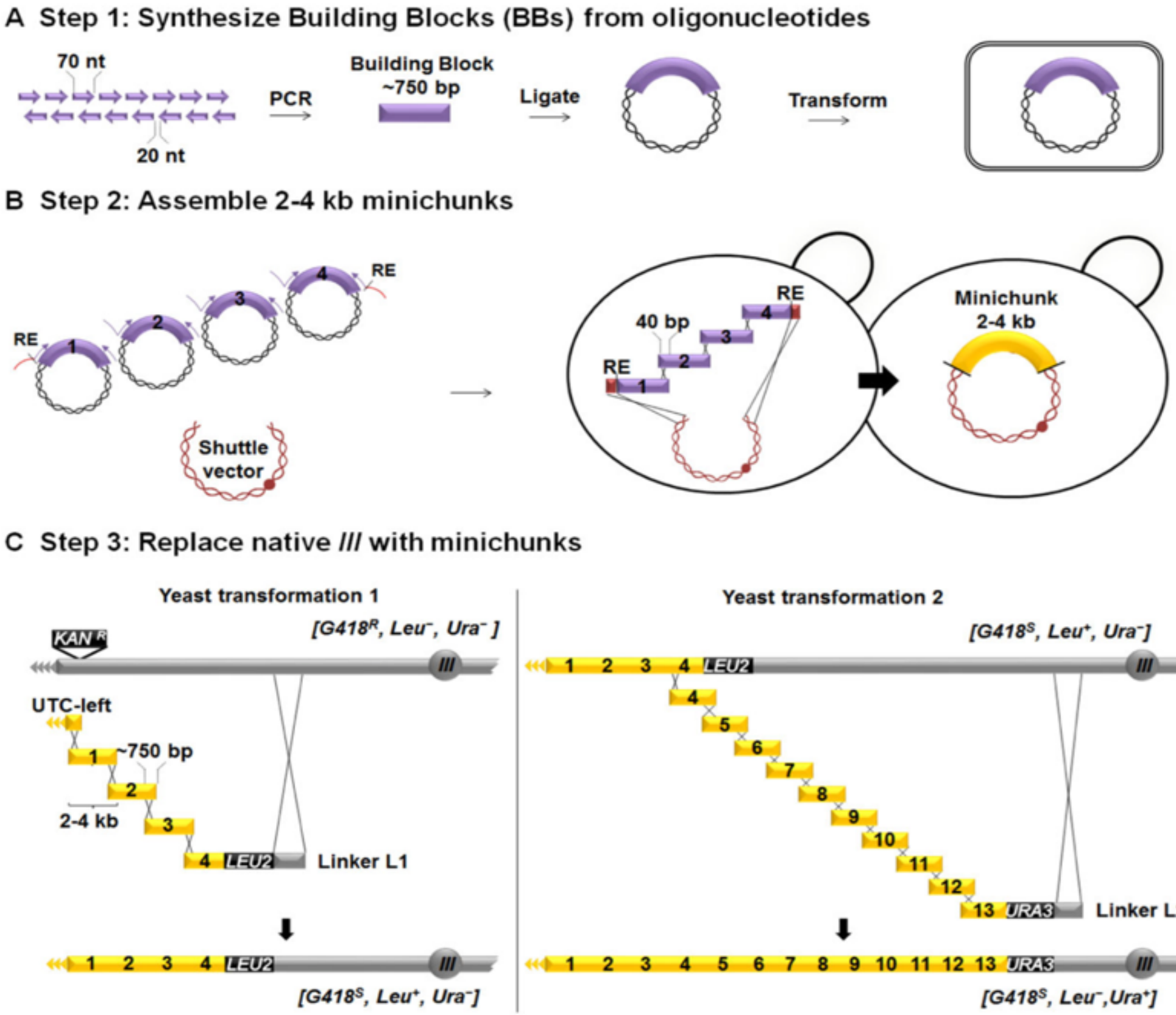
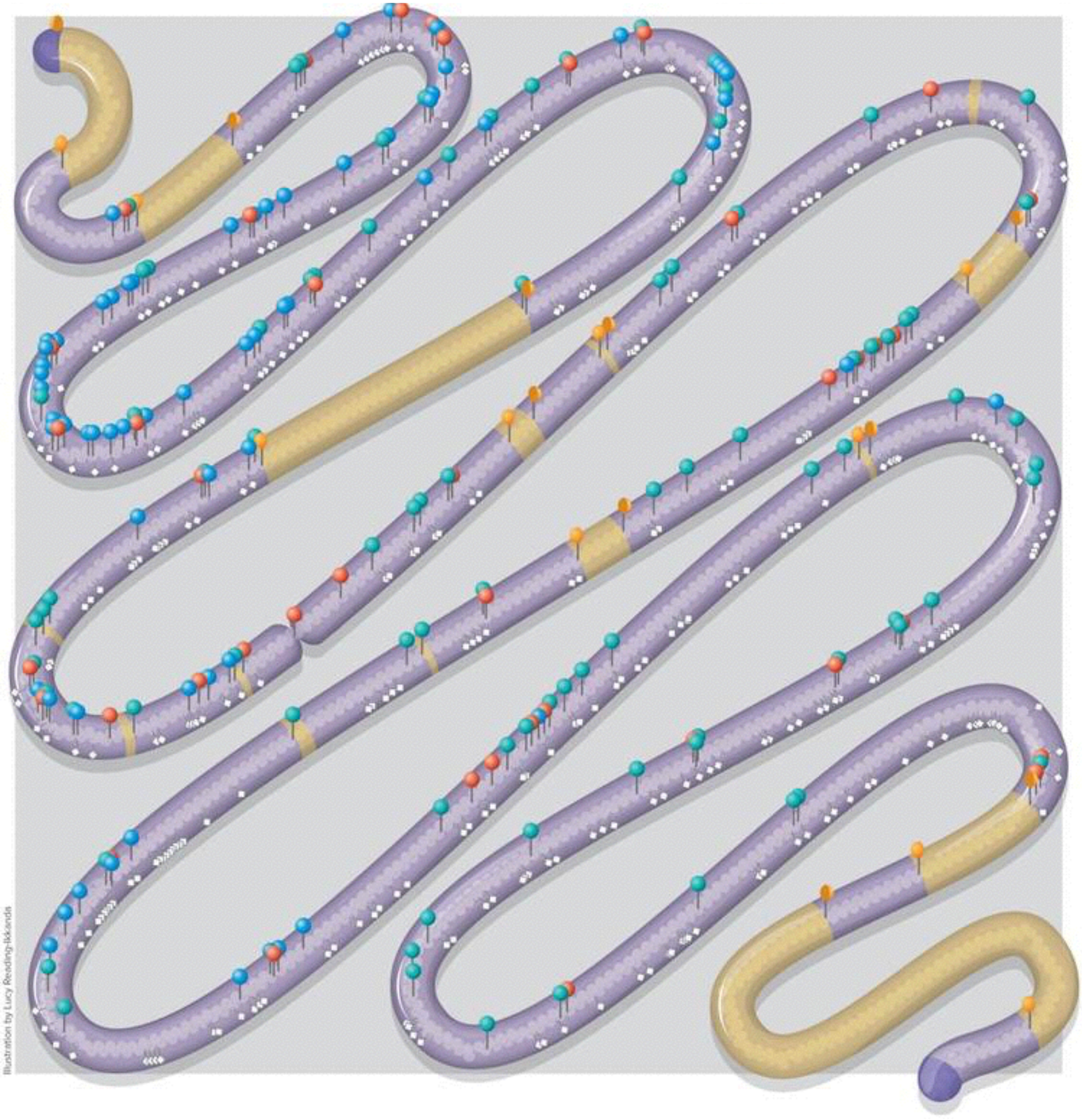


Fig. 2. SynIII construction

SynIII: refactored synthetic chromosome III for yeast



Multi-scale DNA assembly methods

Table 2. Technical specifications of several DNA assembly methods.

Assembly methods	Fragment overhangs	Typical number of fragments for assembly	Demonstrated size of assembled construct (kb)	Efficiency	References
Gibson	40 bp overlaps	c. 4	900	90% (for 3 fragments)	Gibson *et al.* (2009)
In-fusion	>15 bp overlaps	2–3	c. 5	>60% (for 2 fragments) <40% (for 3 fragments)	Sleight *et al.* (2010)
USER	7–12 bp overlaps, must contain one dU at the base	3–7	c. 8	>90% (for up to 7 fragments)	Jensen *et al.* (2014) *Lund et al.* (2014)
CPEC	15–25 bp overlaps	>4	c. 8	95–100%	Quan and Tian (2009)
MoClo	4 bp overlaps and recognition site for type IIS restriction enzyme	c. 10	33 (in three rounds)	>90%	Engler *et al.* (2009) *Weber et al.* (2011)
LCR	Fragments must be 5′-phosphorylated, 60- to 90-bp-long bridging oligos are also needed	>10	20	>90% (up to 6 fragments) c. 75% (12 fragments)	de Kok *et al.* (2014)
In vivo recombination in *S. cerevisiae*	>40 bp overlaps	>10	>20	>90% (up to 6 fragments) c. 75% (12 fragments)	de Kok *et al.* (2014)

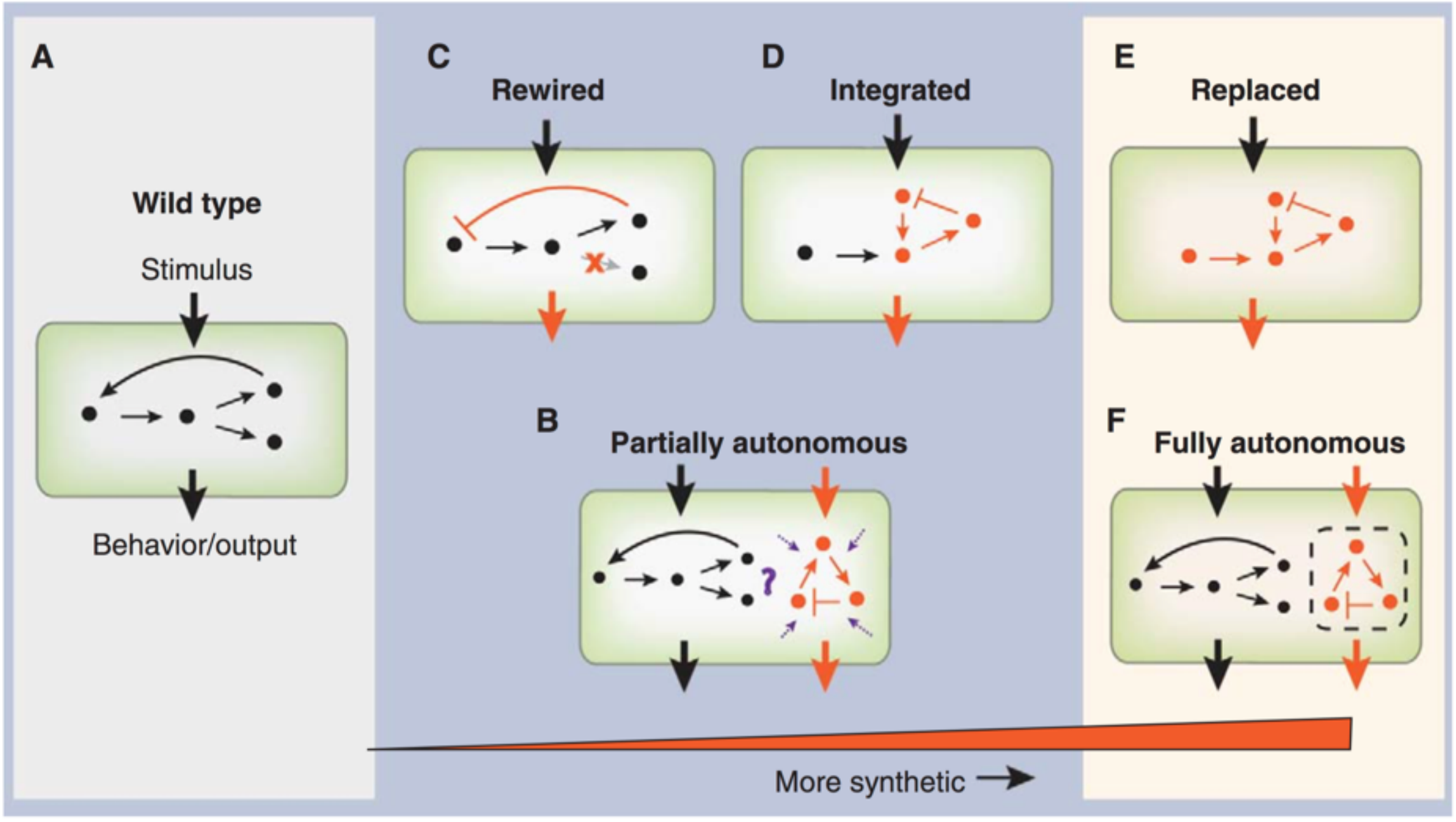
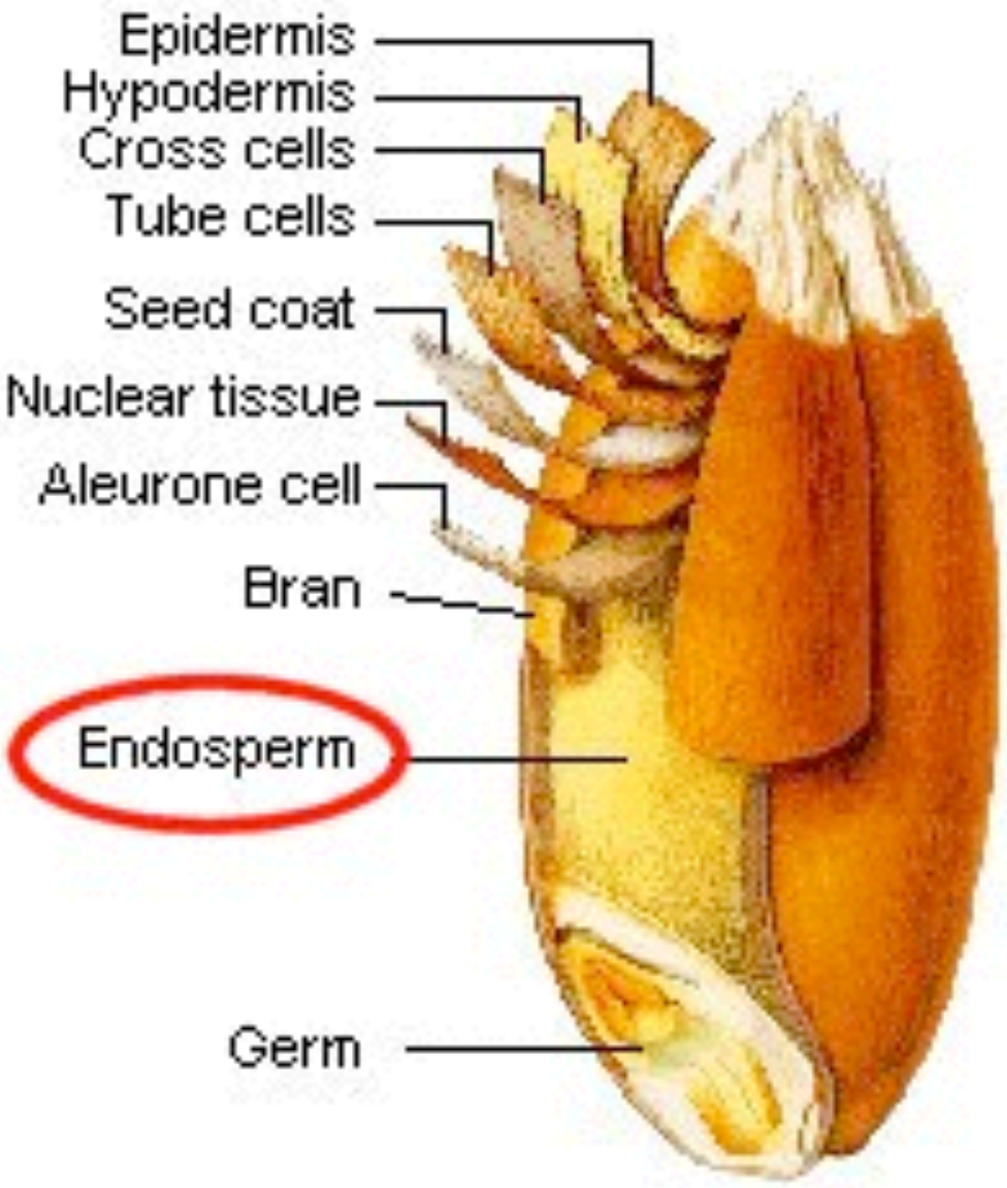


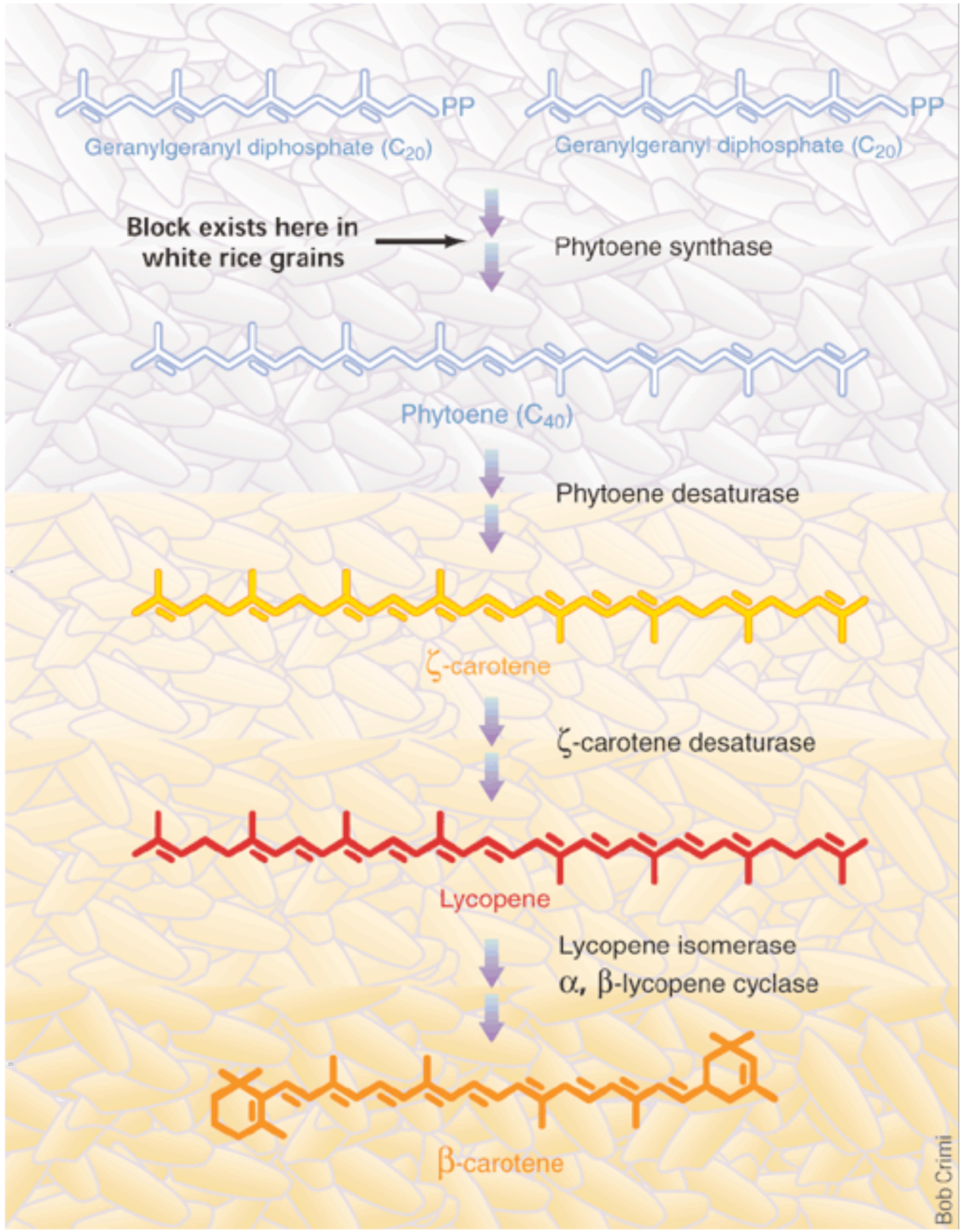
Fig. 1. A continuum of synthetic biology. Wild-type cells (**A**) can be subject to two basic types of synthetic manipulation. (**B**) Autonomous synthetic circuits, consisting of ectopic components, may be introduced into the cell. Such circuits process inputs and implement functions (orange arrows) separate from the endogenous circuitry (black). However, unknown interactions with the host cell may affect their function (purple arrows). (**C**) An alternative is to rewire (orange lines) the endogenous circuits themselves to have new connectivity. (**D**) Extending this line of synthetic manipulation, synthetic circuits could be integrated into appropriately rewired endogenous circuitry to act as sensors and to implement additional functionality. Ultimate goals of this program are to be able to design and construct (**E**) synthetic circuits that can functionally replace endogenous circuits or (**F**) fully autonomous circuits that operate independently of the cellular mileu.

Golden rice

Rice plants have beed developed containing two genes that carry out the four steps required for the production of beta-carotene in rice endosperm. Endosperm is the nutritive tissue surrounding the embryo of a seed and makes up the majority of the rice grain that we eat. The resulting plants appear normal except that after milling (to remove the brown bran), their grain is a golden yellow color due to the presence of provitamin-A.

Dr. Ingo Potrykus of the Swiss Federal Institute of Technology in Zurich and Dr. Peter Beyer of the University of Freiburg in Germany





Golden Rice 2

Engineered metabolic pathway with
Maize phytoene synthase
37 µg carotenoids per gram

Nature Biotechnology **23**, 429 - 430 (2005)

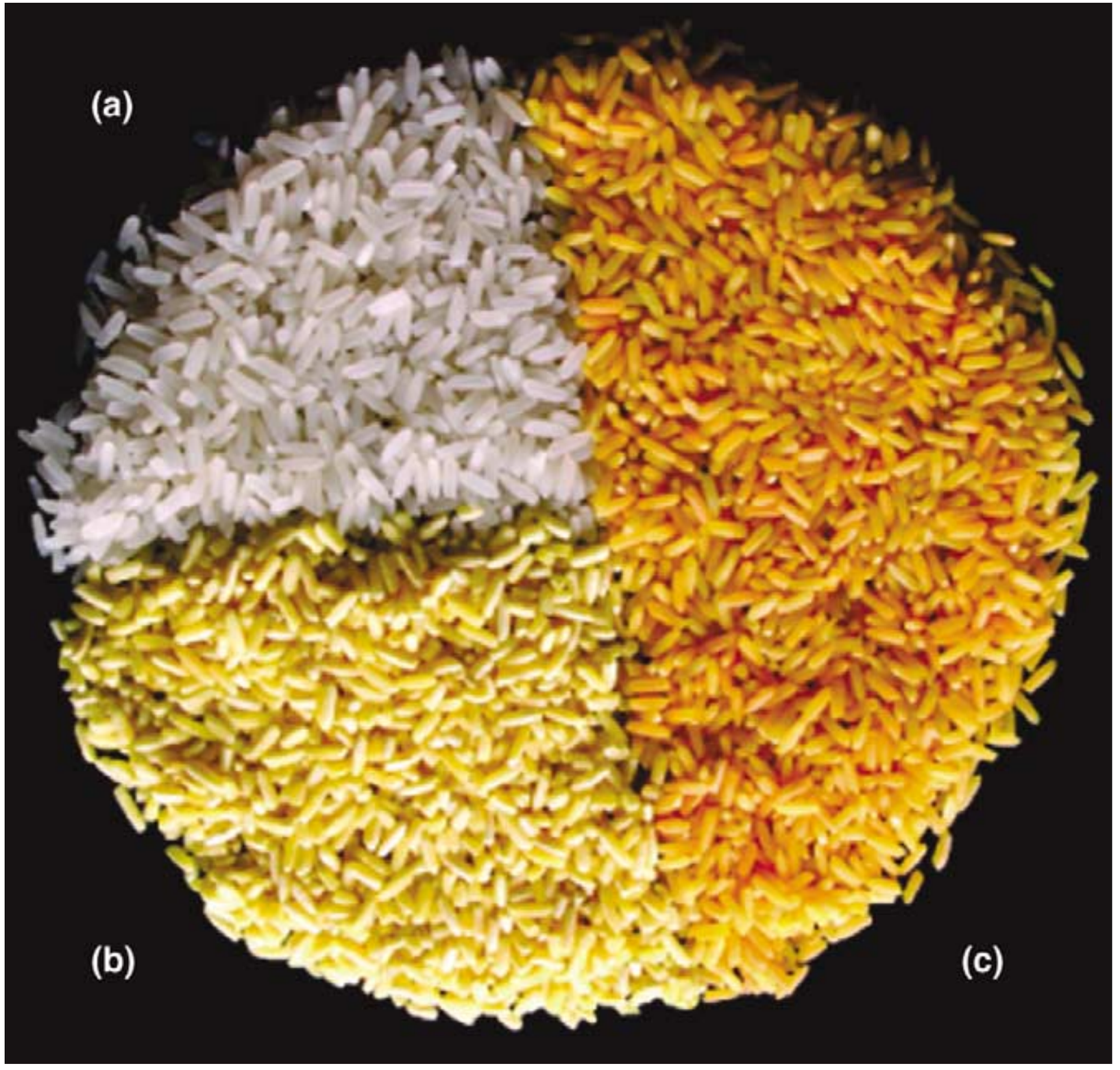
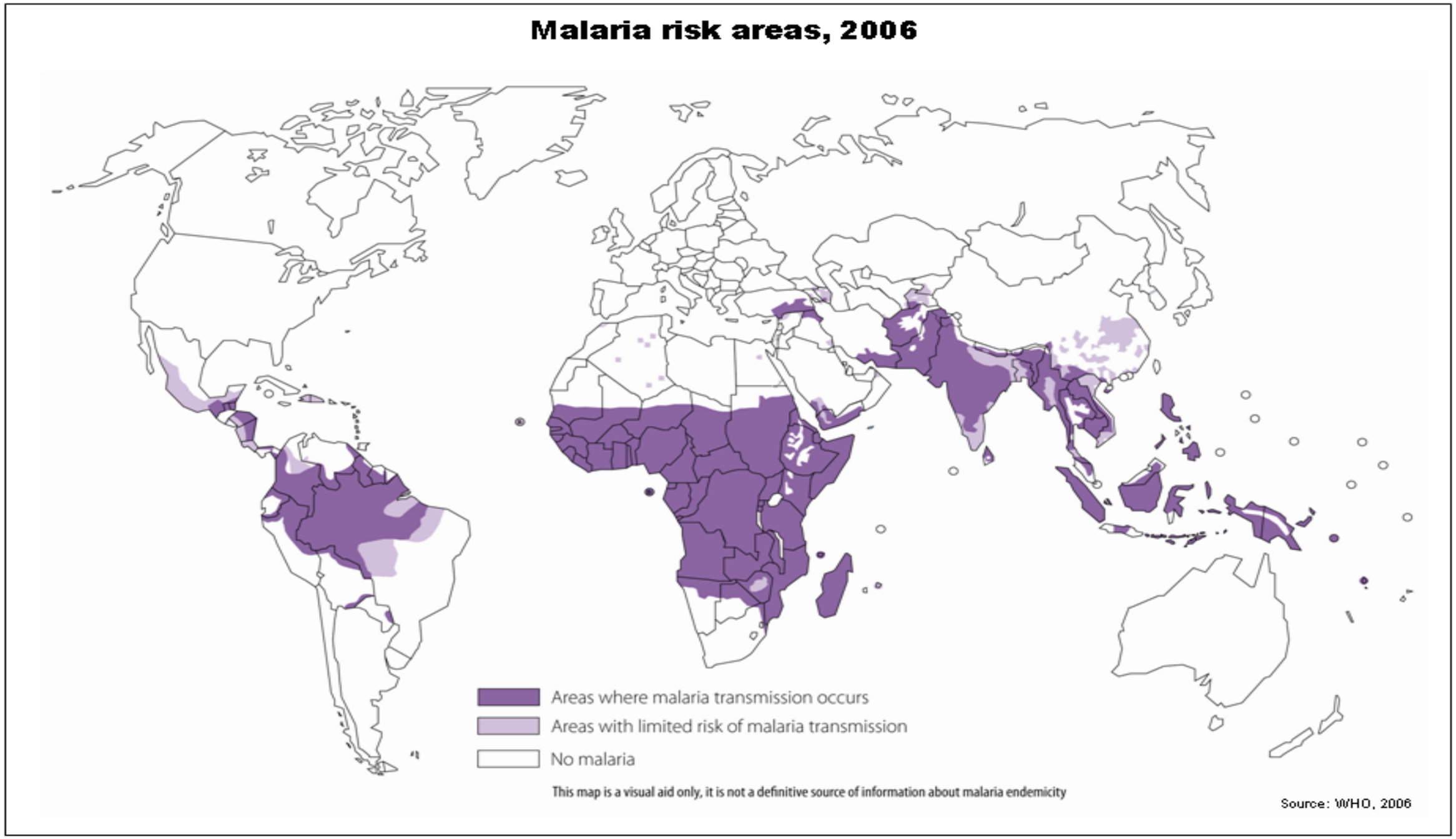


Figure 3. Golden Rice colors. **(a)** Wild-tape rice; **(b)** GR1, expressing the phytoene synthase from daffodil along with CRTI; **(c)** GR2 expressing the phytoene synthase from maize along with CRTI. Photograph courtesy of Aron Silverstone.

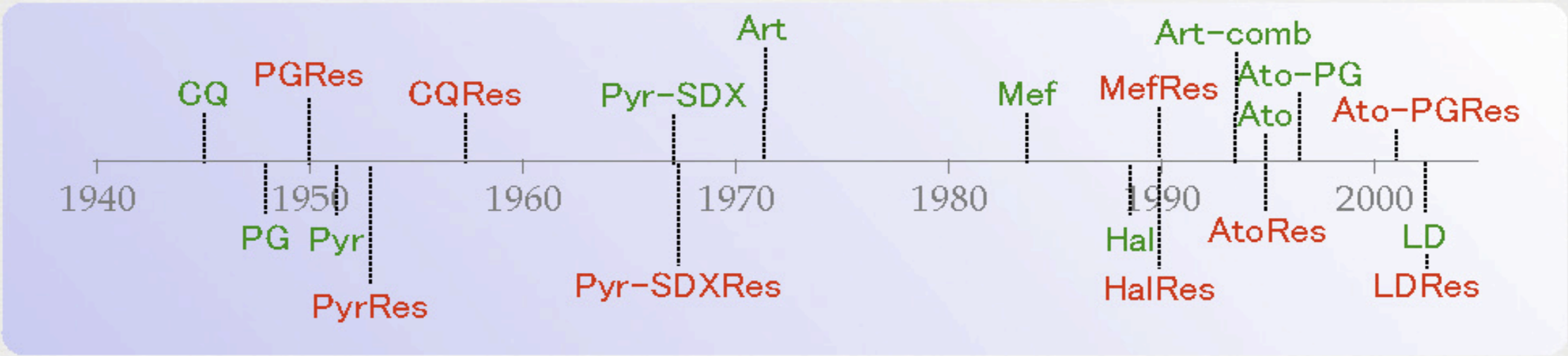
Malaria kills ~1m annually and threatens 300-500m



The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.

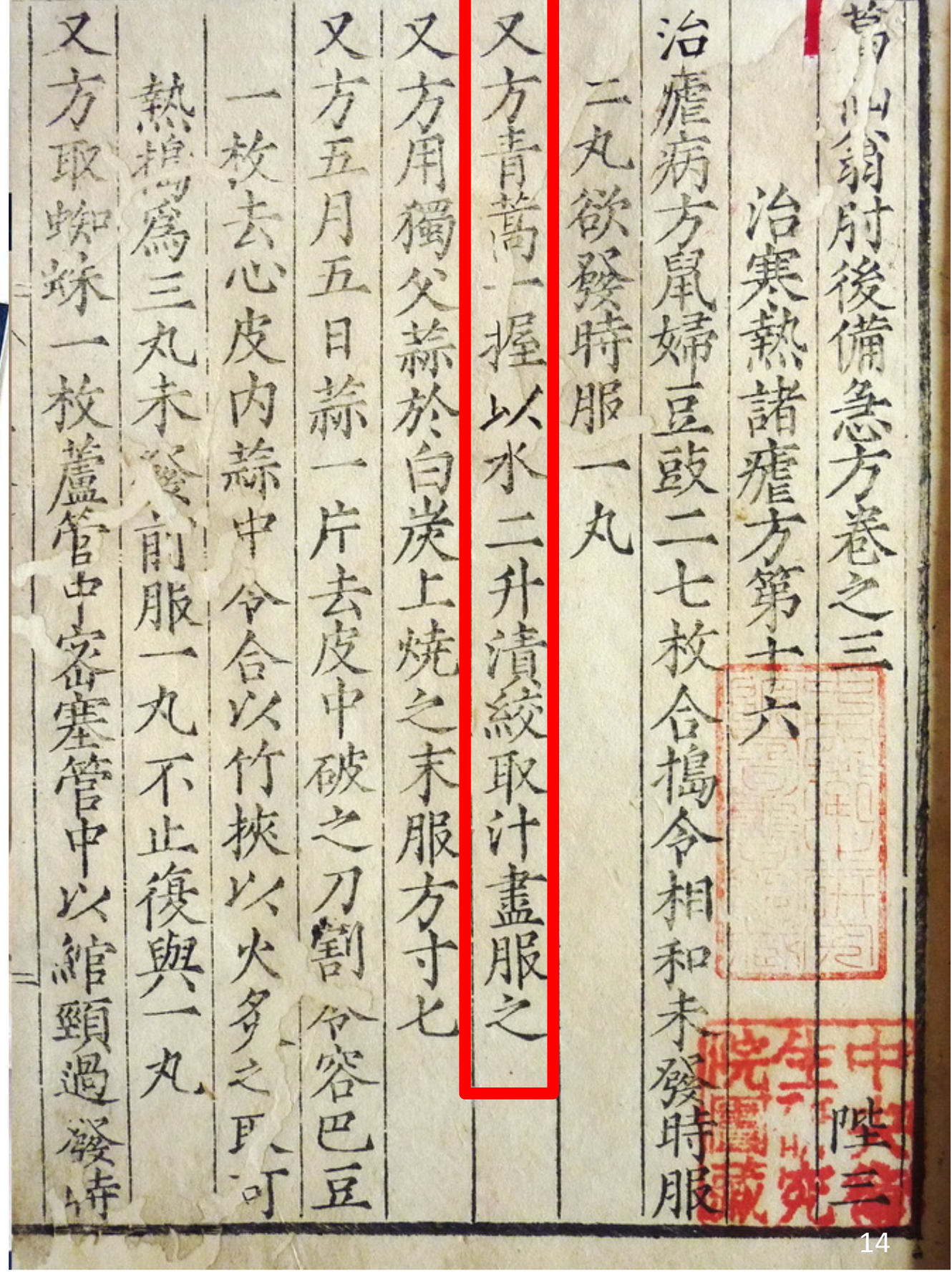
World Health Organization

DRUG RESISTANCE



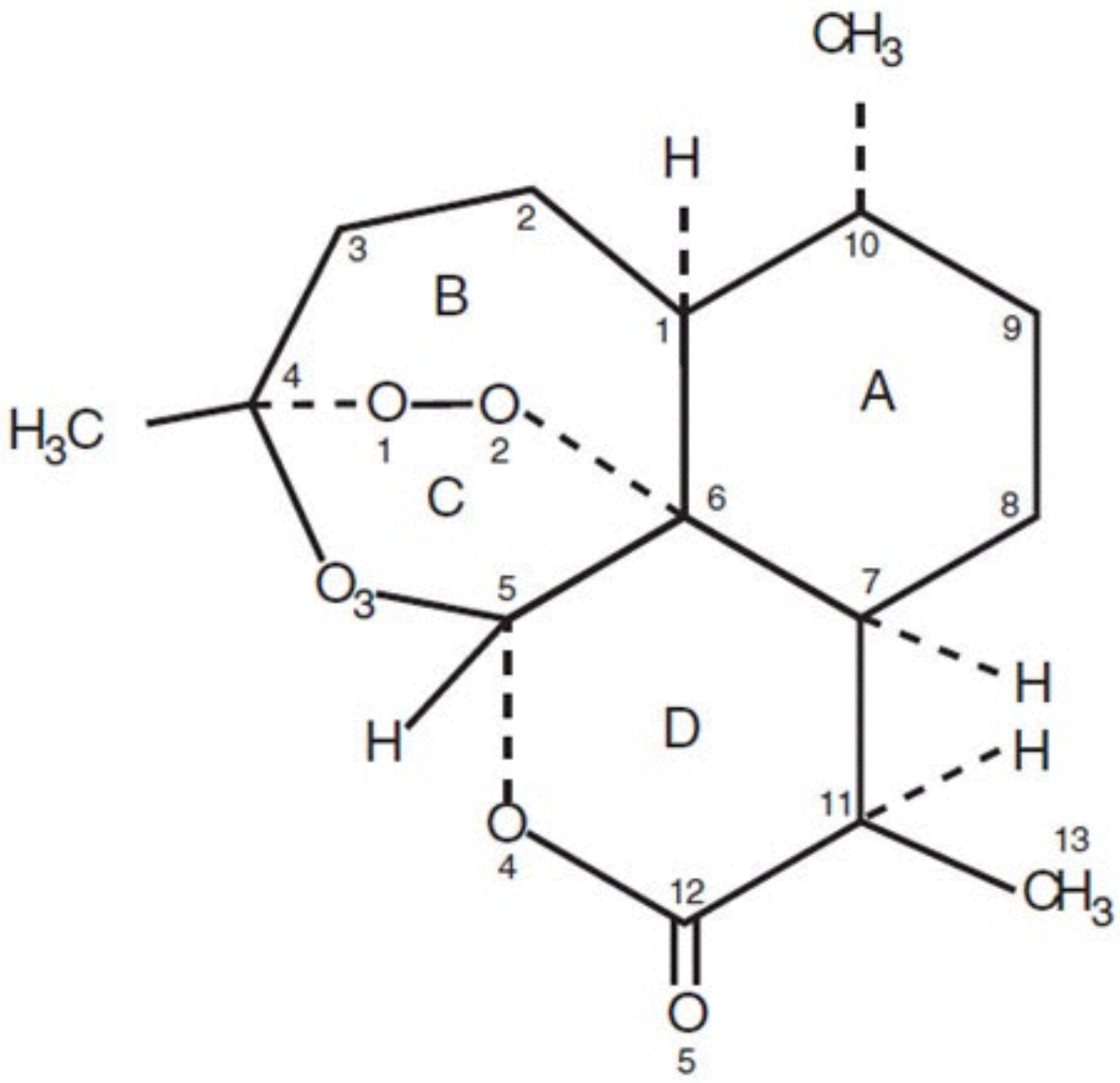
CQ:	chloroquine	Art:	artemisinin
PG:	proguanil	Art-com:	artemisinin combinations
Pyr:	pyrimethanine		
Pyr-SDX:	pyrimethanine-sulfadoxine		
Mef:	mefloquine		
Hal:	halofantrine		
Ato:	atovaquone		
Ato-PG:	atovaquone-proguanil		
LD:	LapDap		

Jacob Koella, Imperial College

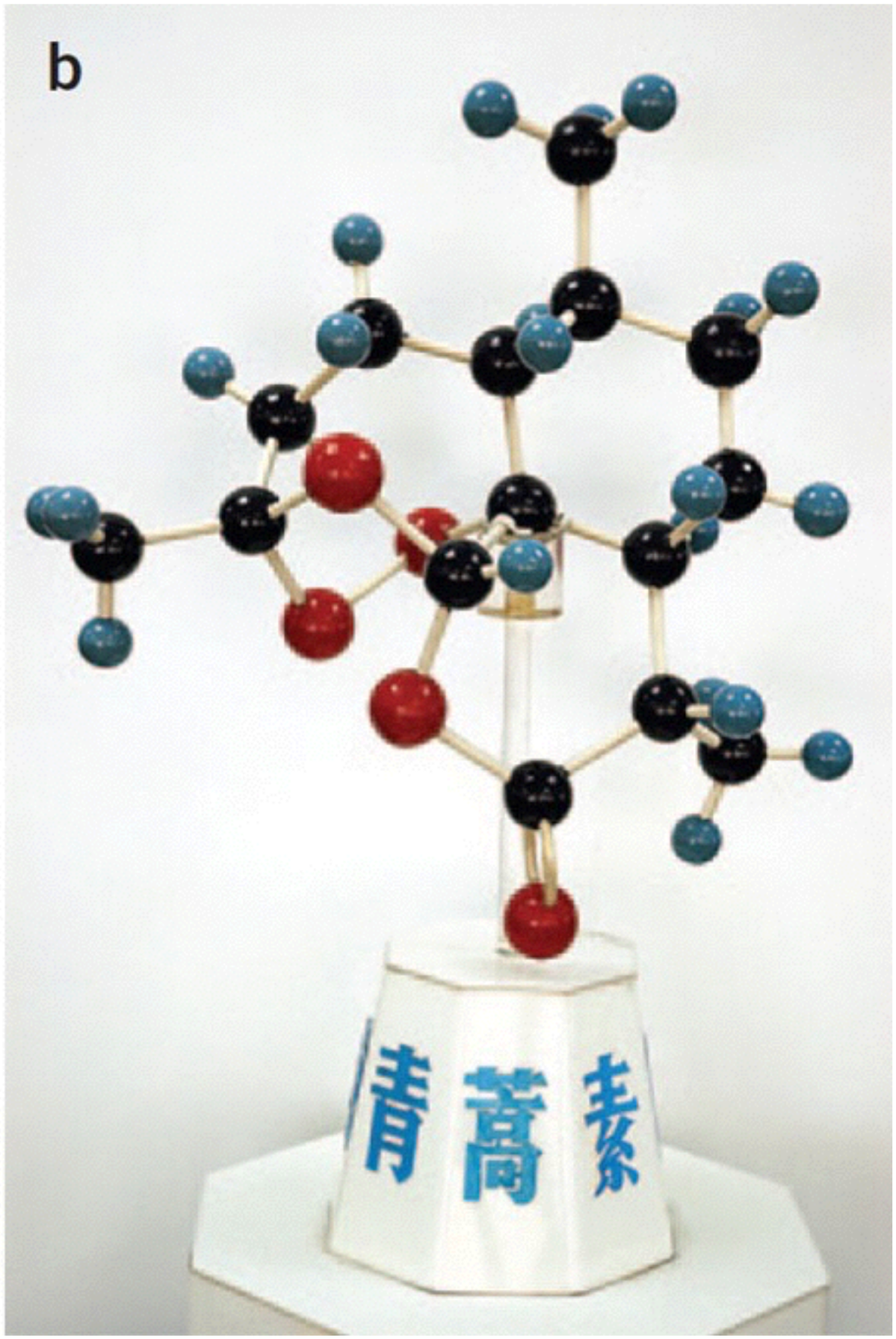


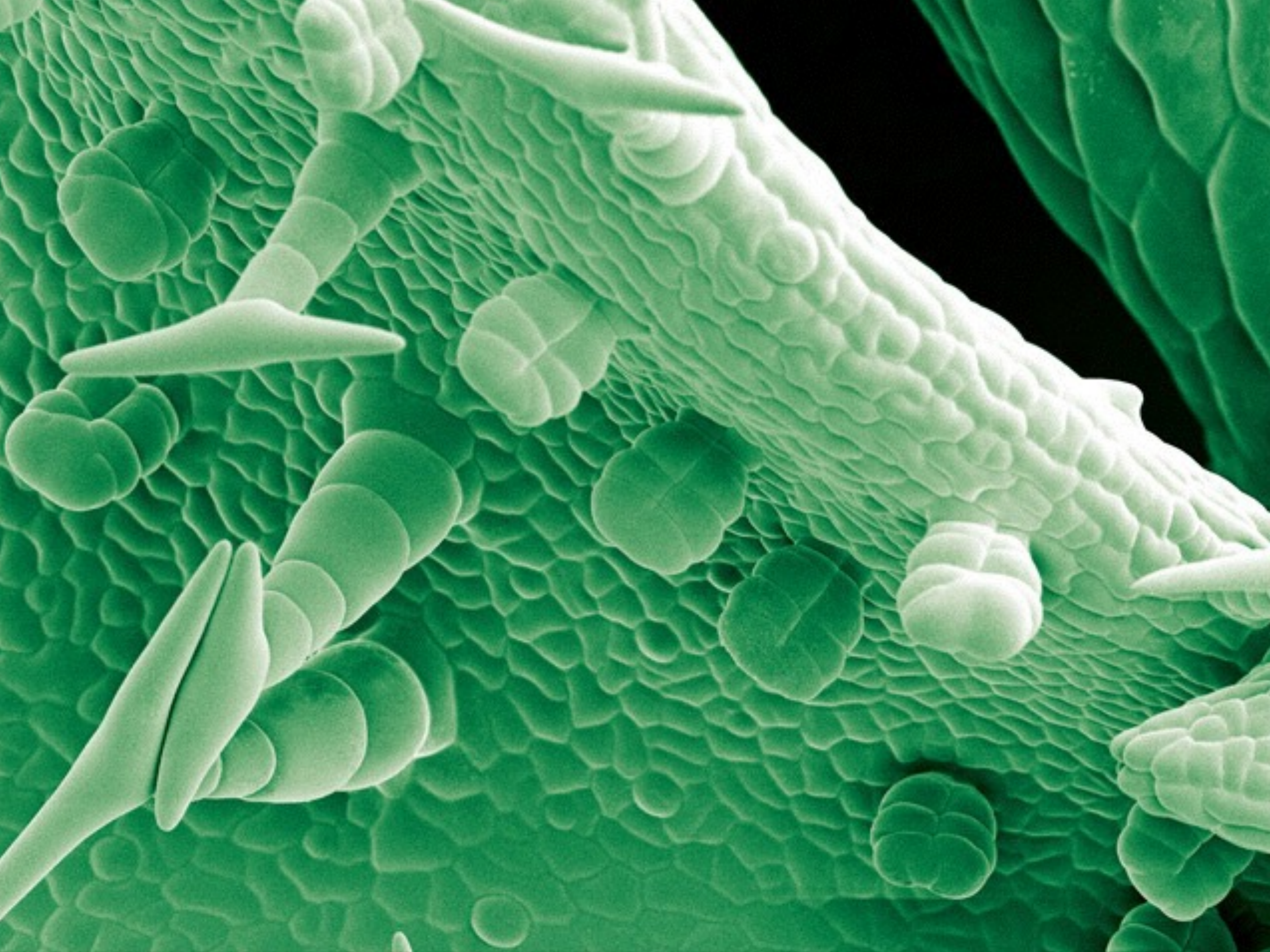
(**a**) Ming dynasty version (1574 CE) of the handbook. (**b**) “A handful of qinghao immersed with 2 liters of water, wring out the juice and drink it all” is printed in the fifth line from the right. (From volume 3.)

a



b





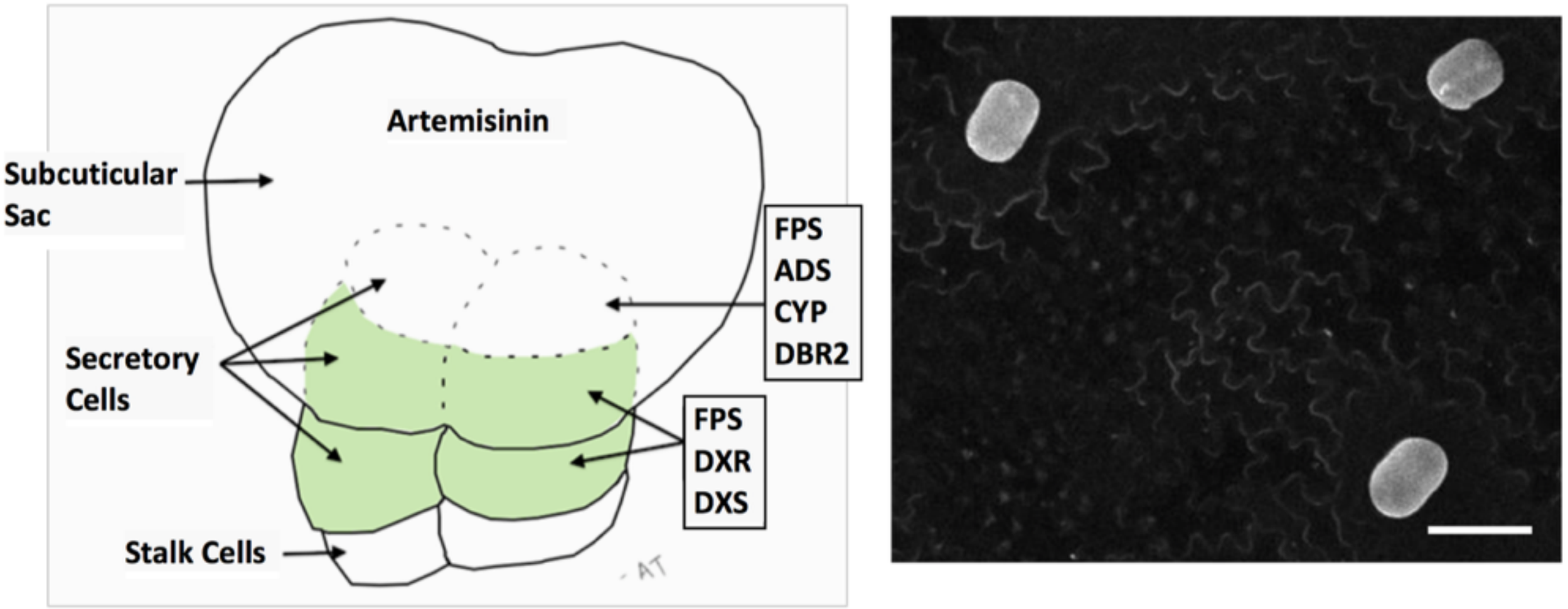
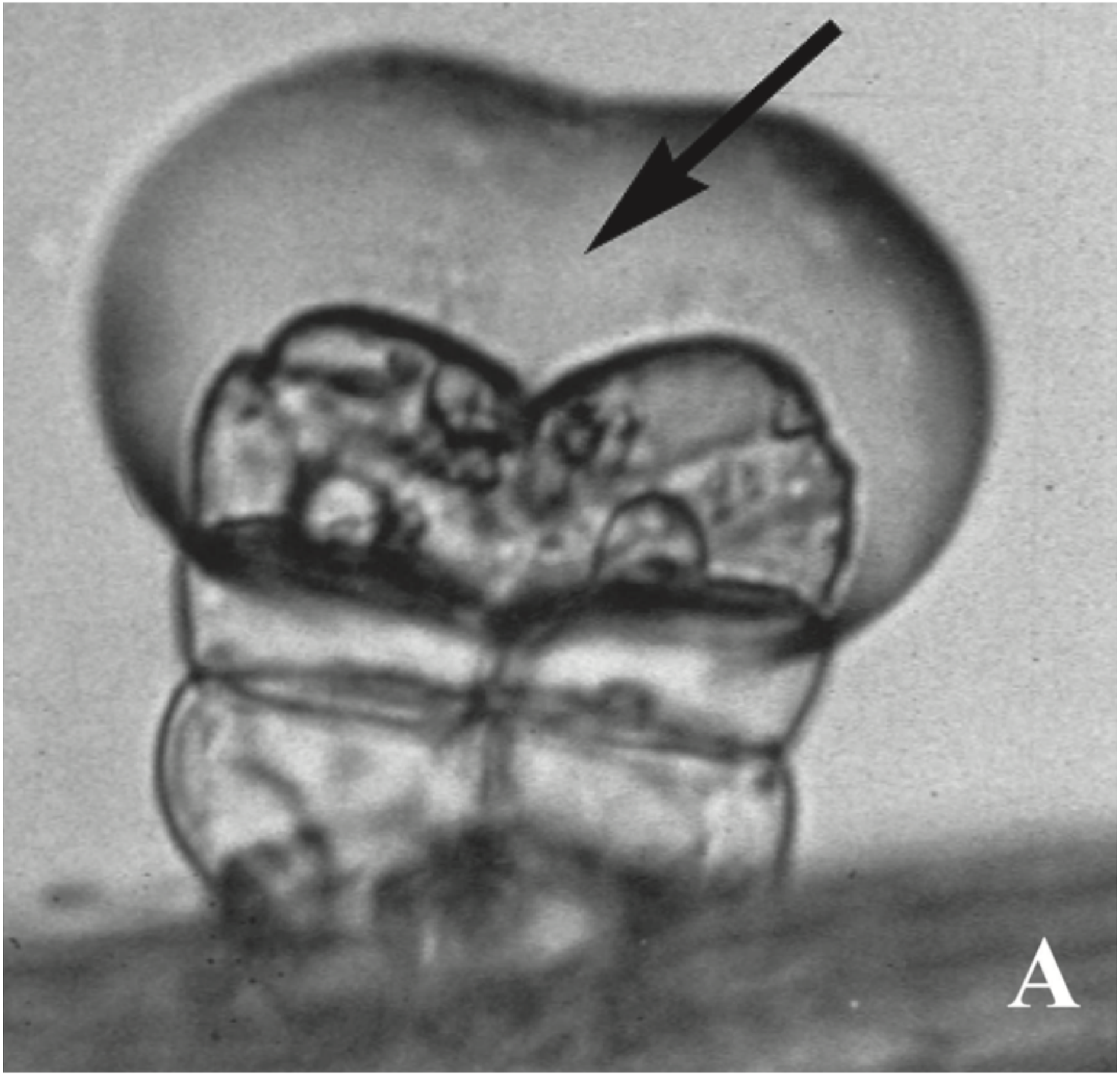


Figure 1.2: Schematic (left) and photo (right) of *Artemisia annua* trichomes. The trichomes on the right autofluoresce when visualized using UV fluorescence microscopy; bar = 100 µm. ADS, amorphadiene 4,11 synthase; CYP, cytochrome P 450 CYP71AV1; DBR2, double bond reductase 2; DXR, 1-deoxyxylulouse 5-phosphate reductoisomerase; DXS, 1-deoxyxylulose 5- phosphate synthase; FPS, farnesyl diphosphate synthase.


A

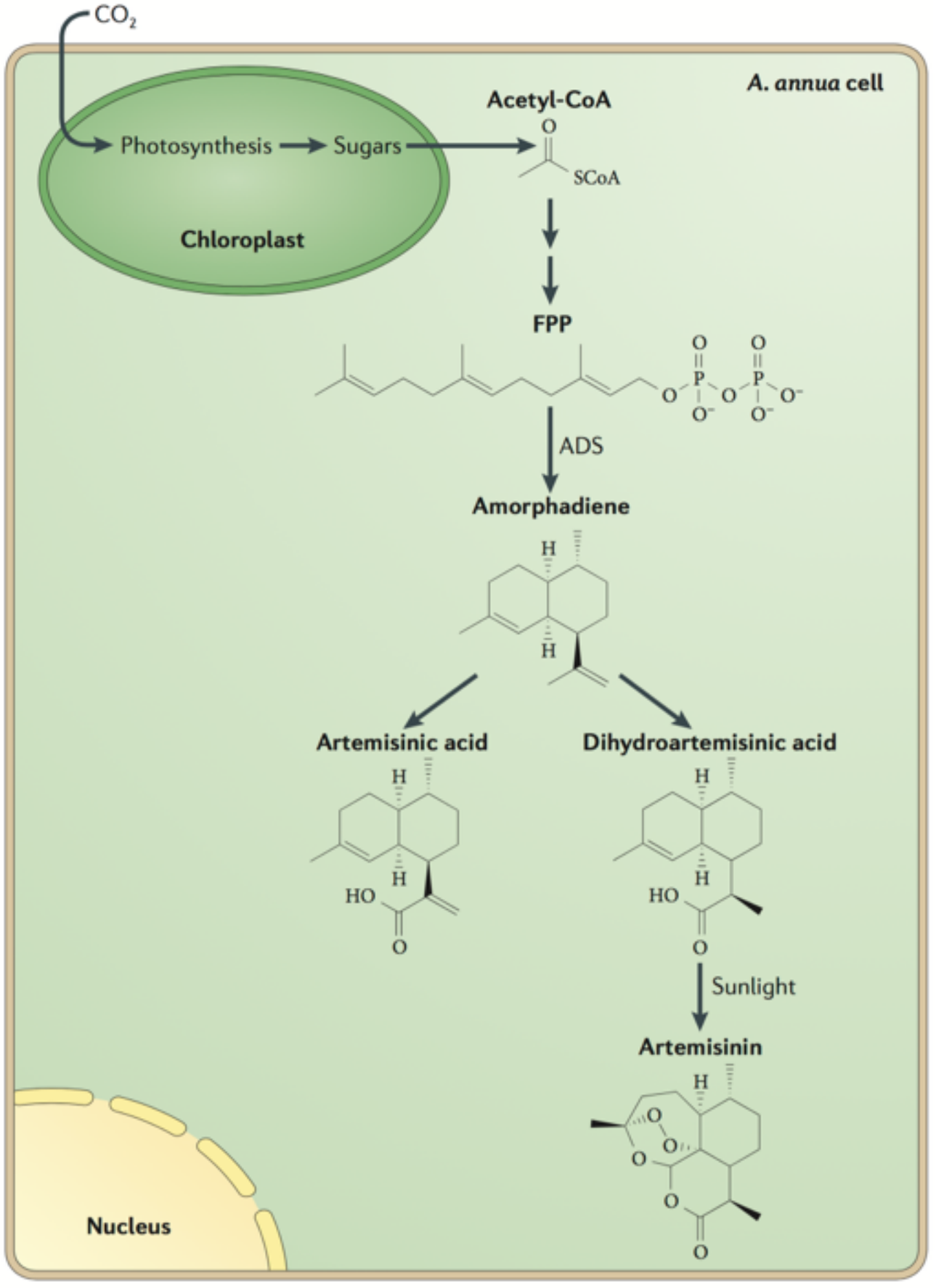
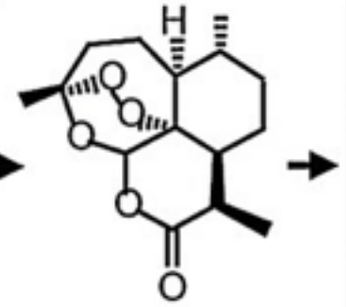


Figure 1 | **Artemisinin biosynthesis pathway in the plant *Artemisia annua*.** Sugars

Plant-derived Artemisinin

Cultivation

Extraction

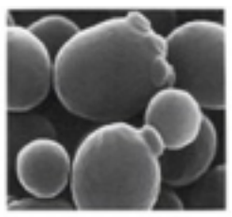


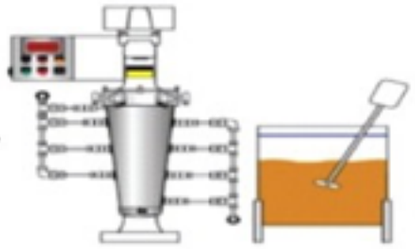
Artemisinin

14-18 Months

Weeks

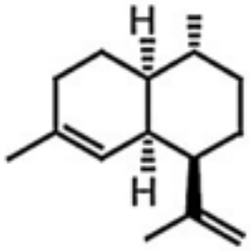
Fermentation



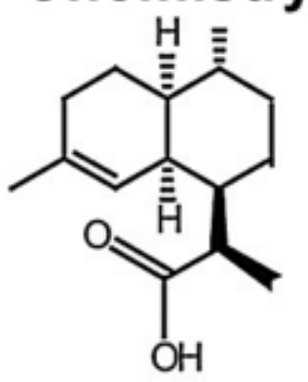


Yeast + Sugar

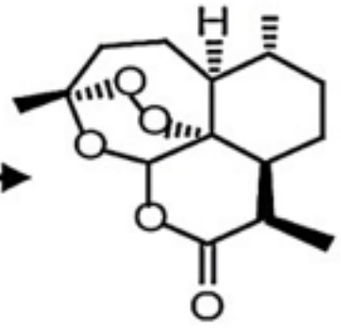
Chemistry



Amorphadiene



DHA



Artemisinin

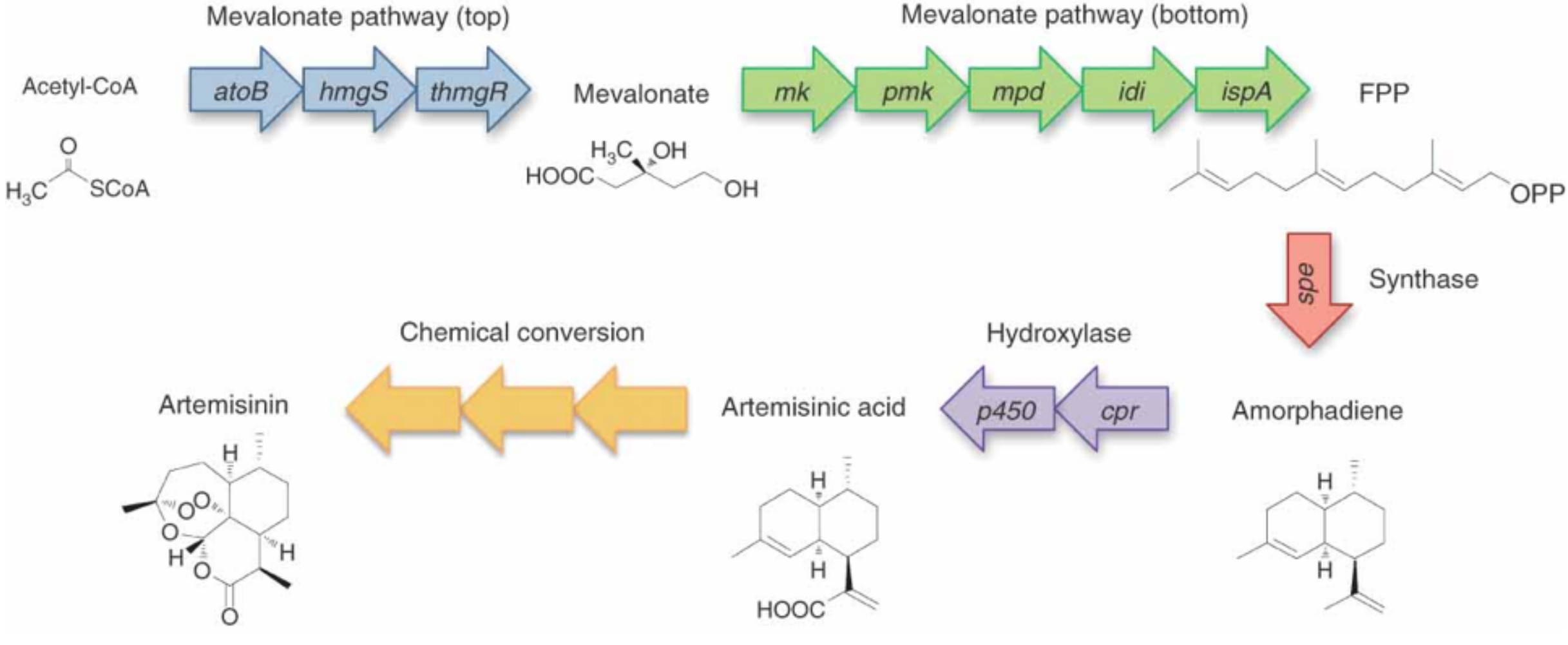
Semisynthetic Artemisinin

nature Vol 440|13 April 2006|doi:10.1038/nature04640

LETTERS

Production of the antimalarial drug precursor artemisinic acid in engineered yeast

Dae-Kyun Ro[1]*, Eric M. Paradise[2]*, Mario Ouellet[1], Karl J. Fisher[6], Karyn L. Newman[1], John M. Ndungu[3], Kimberly A. Ho[1], Rachel A. Eachus[1], Timothy S. Ham[4], James Kirby[2], Michelle C. Y. Chang[1], Sydnor T. Withers[2], Yoichiro Shiba[2], Richmond Sarpong[3] & Jay D. Keasling[1,2,4,5]



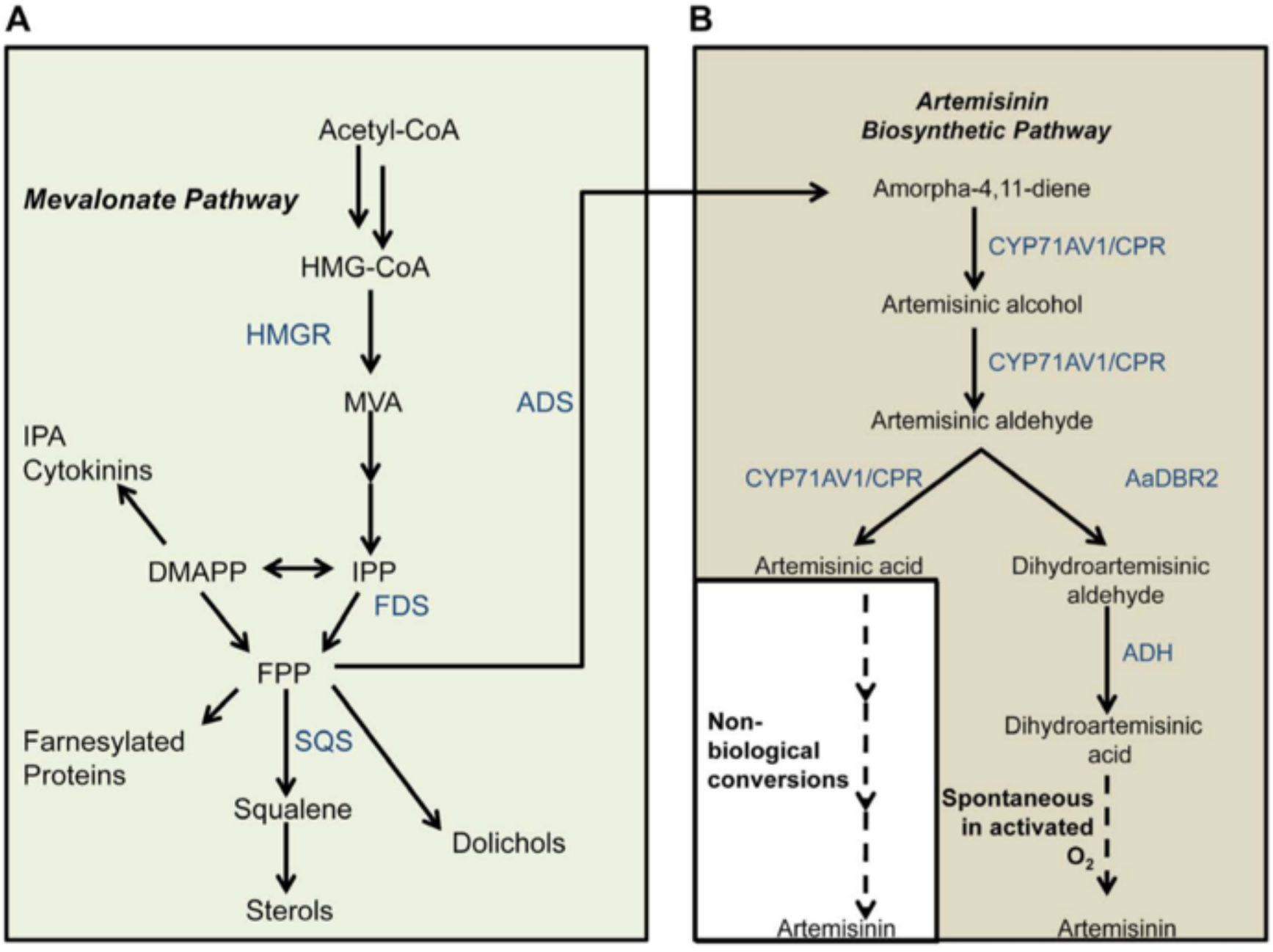
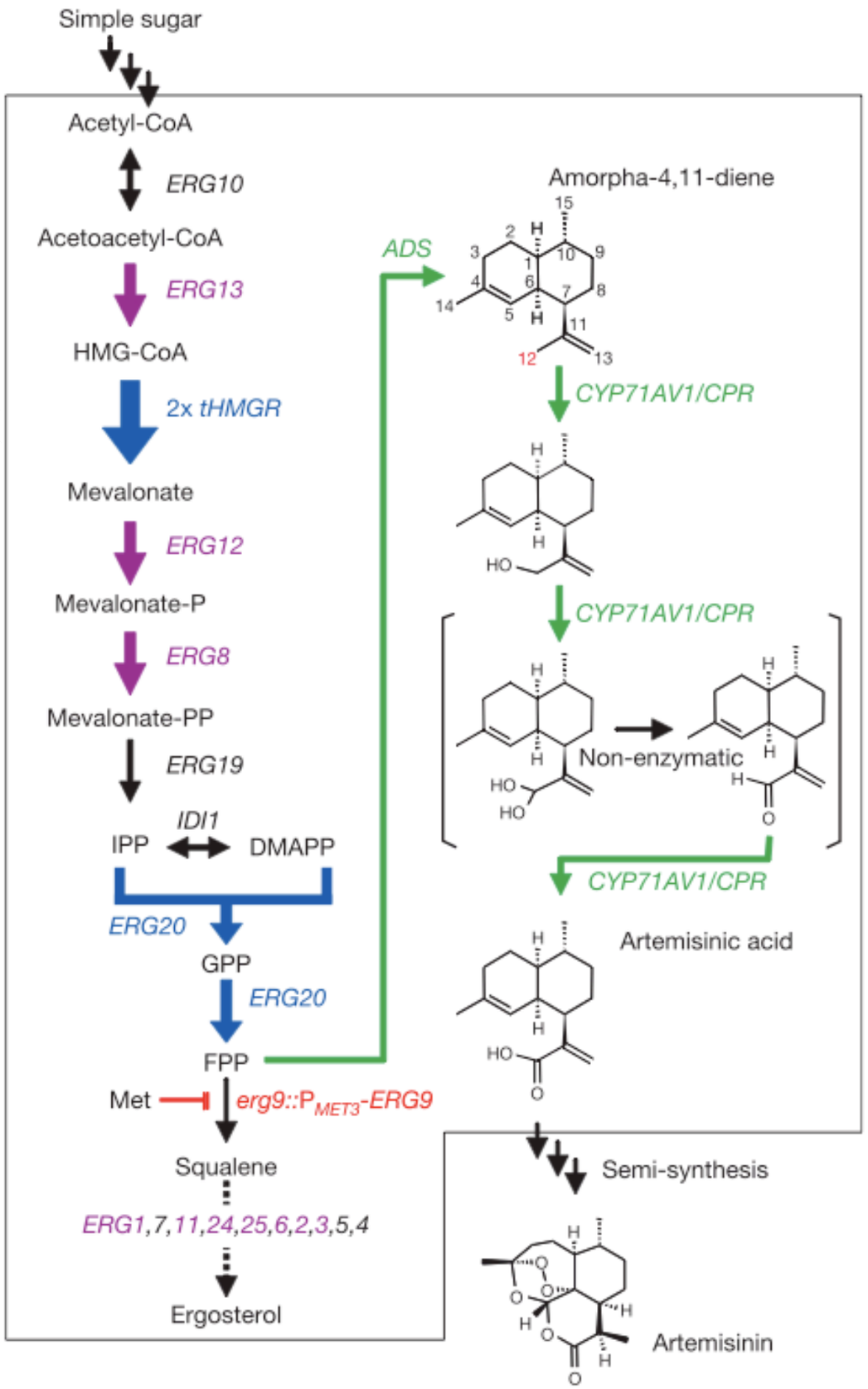


FIGURE 1 | Artemisinin precursor synthesis pathways in yeast. In the native isoprenoid biosynthesis pathway in yeast **(A)**, IPP synthesized via the MVA pathway is converted to FPP. HMGR is a key enzyme in the isoprenoid biosynthetic pathway that feeds the artemisinin precursor synthesis pathway **(B)**. Steps that are not known to be catalyzed by enzymes are depicted with dashed lines. Enzyme and metabolite name abbreviations: AaDBR2, *A. annua* double bond reductase; ADH, (dihydroartemisinic) aldehyde dehydrogenase; ADS, amorpha-4,11-diene synthase; CYP71AV1, cytochrome P450 monooxygenase; CPR, *A. annua* cytochrome P450 reductase; CoA, coenzyme A; DMAPP, dimethylallyl diphosphate; FDS, farnesyl diphosphate synthase; FPP, farnesyl pyrophosphate; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; HMGR, HMG-CoA reductase; IPA, isopentenyladenine; IPP, isopentenyl diphosphate; MVA, mevalonate; SQS, squalene synthase. Adapted from Zhang et al. (2008) and Teoh et al. (2006).

tHMGR up: 5x

ERG9 down: 2x



Overall 500x higher amorphadiene expression than previous engineered systems

Side Reaction

Regulation

Toxicity

Secretion

Heterologous Pathways

Enzymes

Product

in vivo DNA Assembly via Yeast Recombination

Homology

1 2 3 4 5 6

Assembled Genes

On Plasmid or Chromosome

in vitro DNA Assembly

Multiple Restriction Enzyme Site Guided

1 2 3 4

Plasmid

Complementary ssDNA Guided

Microbial Cell

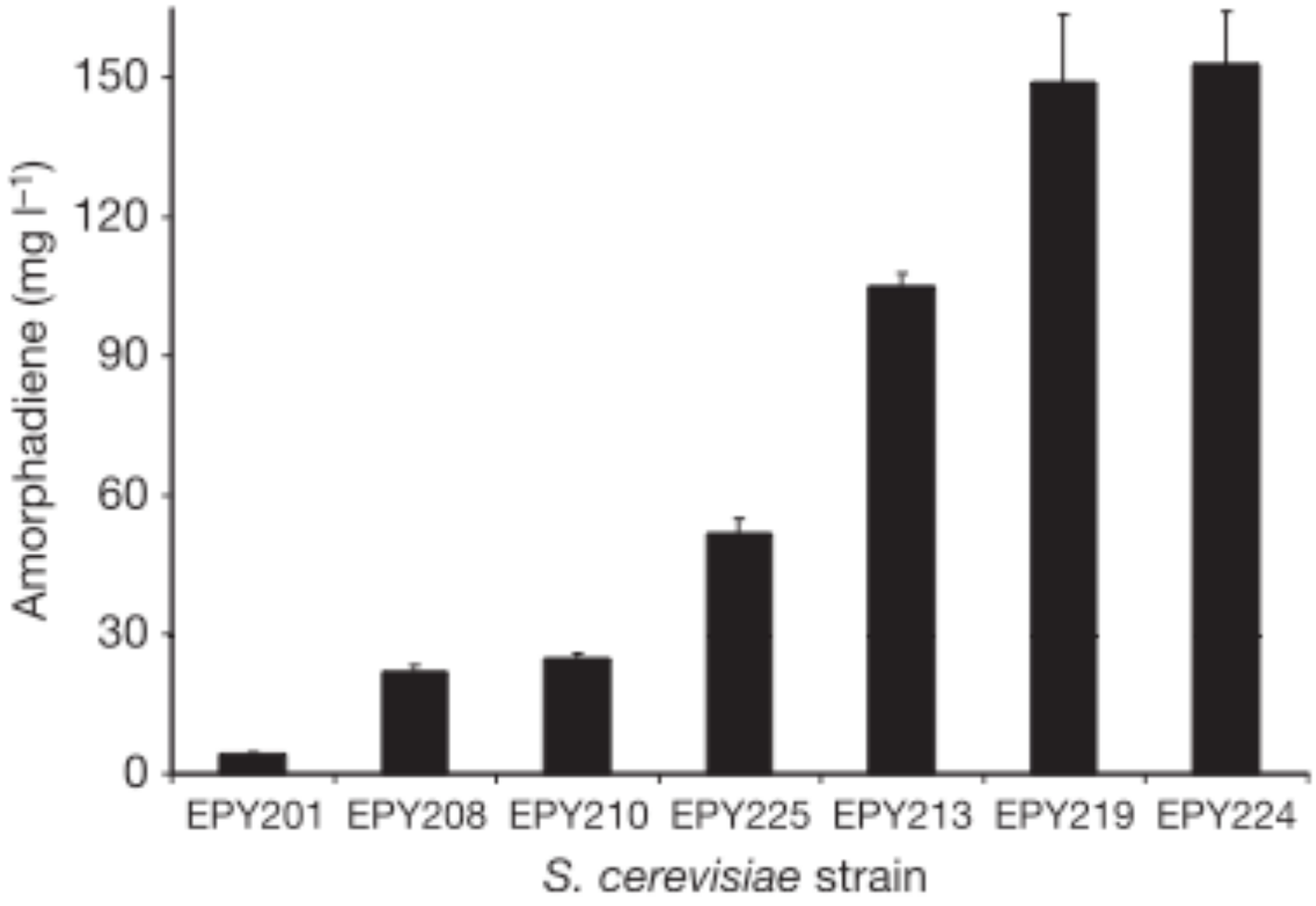


Figure 2 | Production of amorphadiene by *S. cerevisiae* strains. The various *S. cerevisiae* strains are described in the text. Cultures were sampled after 144 h of growth, and amorphadiene levels were quantified. Data, shown as total production, are mean ± s.d. ($n = 3$).

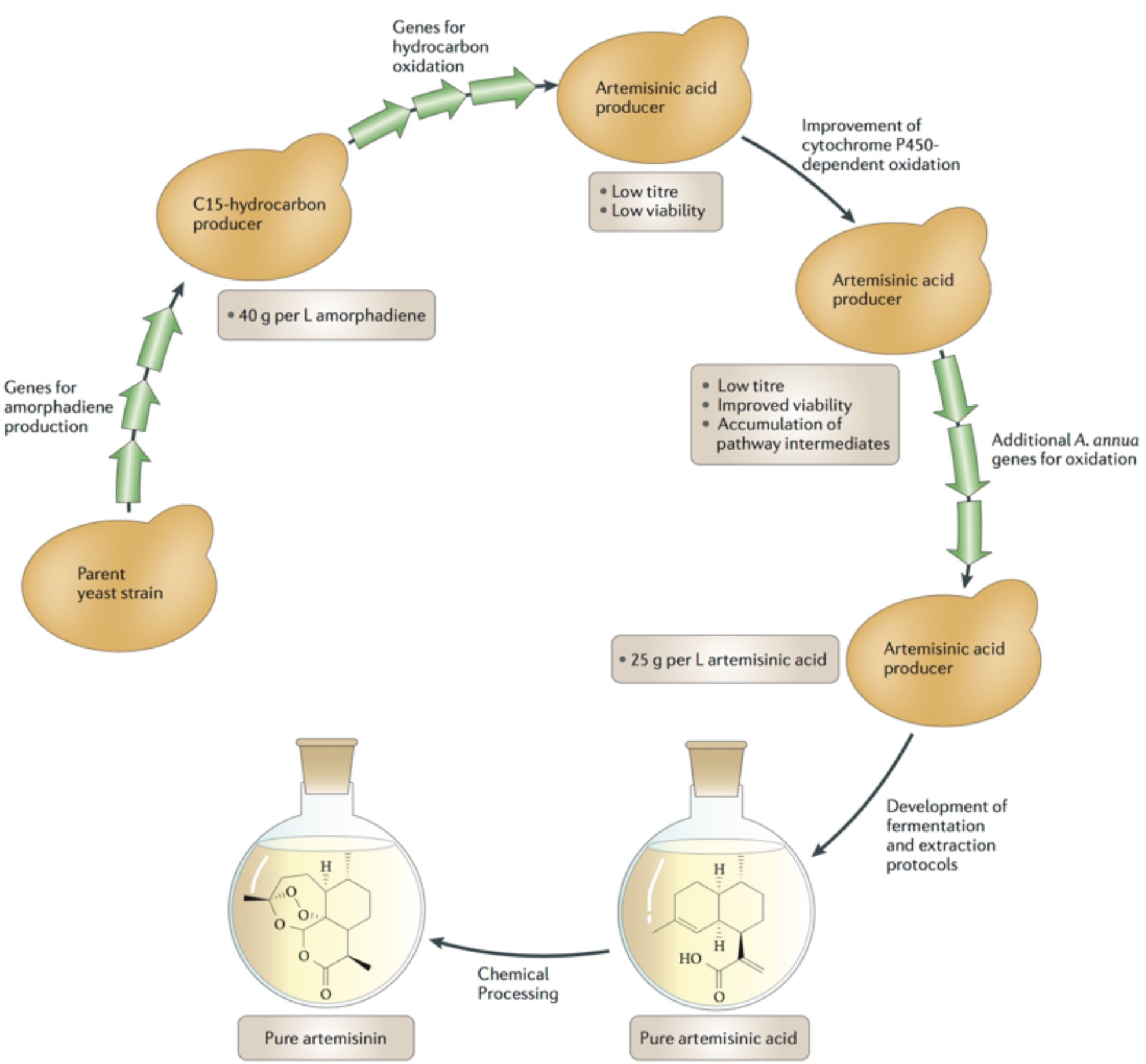
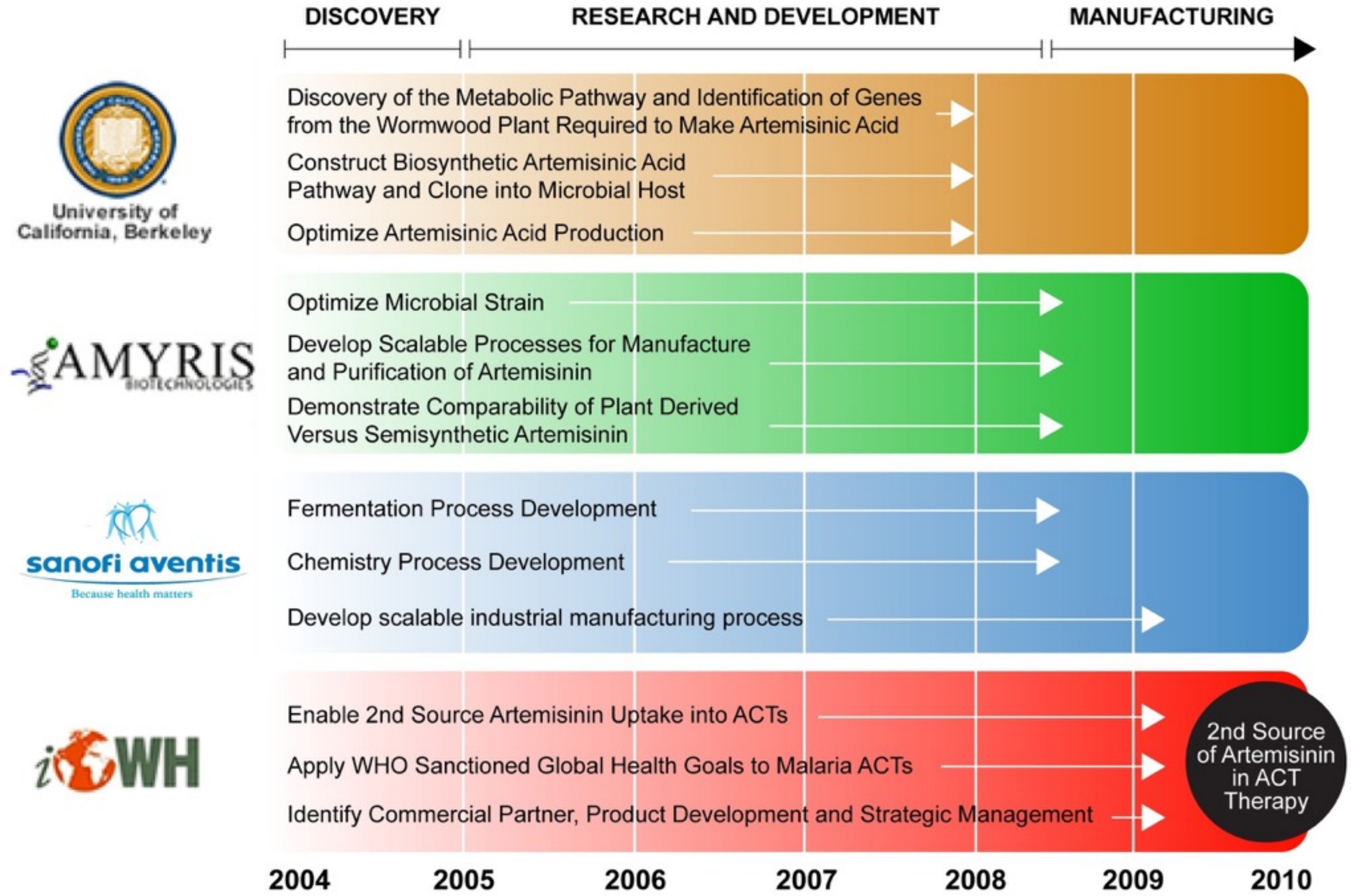


Figure 2 | **The main stages involved in the synthesis of semi-synthetic artemisinin.** The initial stage involved the


R238
R-239


DISCOVERY
RESEARCH AND DEVELOPMENT
MANUFACTURING
University of California, Berkeley
Discovery of the Metabolic Pathway and Identification of Genes from the Wormwood Plant Required to Make Artemisinic Acid
Construct Biosynthetic Artemisinic Acid Pathway and Clone into Microbial Host
Optimize Artemisinic Acid Production
AMYRIS BIOTECHNOLOGIES
Optimize Microbial Strain
Develop Scalable Processes for Manufacture and Purification of Artemisinin
Demonstrate Comparability of Plant Derived Versus Semisynthetic Artemisinin
sanofi aventis
Because health matters
Fermentation Process Development
Chemistry Process Development
Develop scalable industrial manufacturing process
iOWH
Enable 2nd Source Artemisinin Uptake into ACTs
Apply WHO Sanctioned Global Health Goals to Malaria ACTs
Identify Commercial Partner, Product Development and Strategic Management
2nd Source of Artemisinin in ACT Therapy
2004
2005
2006
2007
2008
2009
2010

Renewable biomass

MEP/MVA pathway

IPP

IDI

DMAPP

GPS

GPP(C10)

FPS

FPP(C15)

GGPS

GGPP(C20)

Steroids

SQS/SE

2,3-oxidosqualene

bAS

β-Amyrin

CYP88D6

11-oxo-β -amyrin

CYP72A154

Glycyrrhetinic acid

DDS

Dammarenediol-II

CYP716A12

Oleanolic acid

CYP716A47

Protopanaxadiol

CYP716Y1
UGT73C11

3-O-Glc-echinocystic acid

CYP716A53V2

Protopanaxatriol

UGTPg1

Ginsenoside-CK

ADS

Amorphadiene

CYP71AV1
ADH1
ALDH1

Artemisinic acid

Artemisinin

CPS/KSL

Miltiradiene

CYP76H1

Ferruginol

Tanshinones

TDS

Taxadiene

Taxol

CrtBIY

β -Carotene

CrtWZ

Astaxanthin

Lecture 1: Genetic modification in agriculture and the advent of Synthetic Biology.

Lecture 2: Genetic circuits and genome scale DNA engineering.

1. **BioBricks, standardisation and social engineering**
2. **Type IIS assembly and common syntax**
3. **Smart DNA registries and software tools**
4. **Chromosome engineering**
5. **Reprogramming metabolic pathways in plants**
6. **Implementing plant pathways in microbes**

Lecture 3: Engineered logic and the control of gene expression.

Lecture 3: Self-organisation and reprogramming of multicellular systems.

Additional resources: **http://www.haseloff-lab.org** (Education)