

Reporter genes in plants - worksheet

Gene fusions, GUS, GFP and microscopy.

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Web resources at: http://www.plantsci.cam.ac.uk/Haseloff/teaching/Index_teaching.htm

Library/Internet exercise:

Answer the following questions:

1. Draw a diagram of a typical protein-coding plant gene. The annotation should include the following listed elements, and indicate their typical positions within the gene. Briefly describe the properties of the elements.

promoter

upstream regulatory sequences

enhancer

silencer

RNA polymerase initiation site

intron

exons

UTRs

START codon

STOP codon

polyadenylation site

2. What are transcription factors, and how do they interact with genes?

3. What is a reporter gene, and how is it detected?

4. Draw a schematic view of the differences between plant transformation vectors that might be used to produce protein fusions, transcriptional fusions and for enhancer detection in plants.

5. Why are these different gene fusions useful?

6. Both green fluorescent protein (GFP) and β -glucuronidase (GUS) are widely used as reporter genes in plants. Describe major advantages of GFP over GUS, and vice versa.

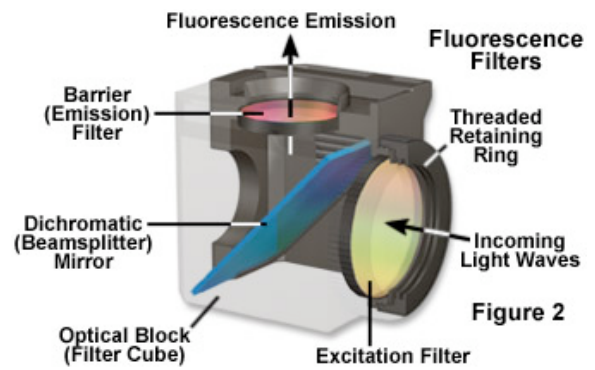
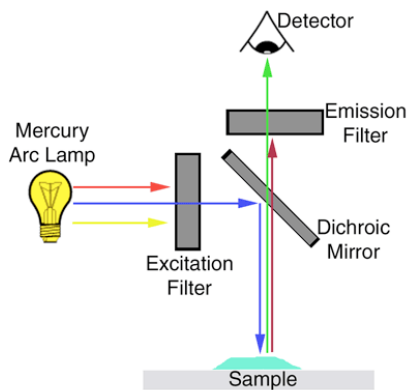
7. As a keen plant biologist, you wish to construct your own fluorescent microscope for work with a variant of green fluorescent protein. The excitation and emission spectra of the protein are shown below. You have access to a box of filters that transmit or reflect light in the following bands:

Bandpass Filter 1: 350-460nm; Filter 2: 450-490nm; Filter 3: 515-560nm, Filter 4: 550-570nm

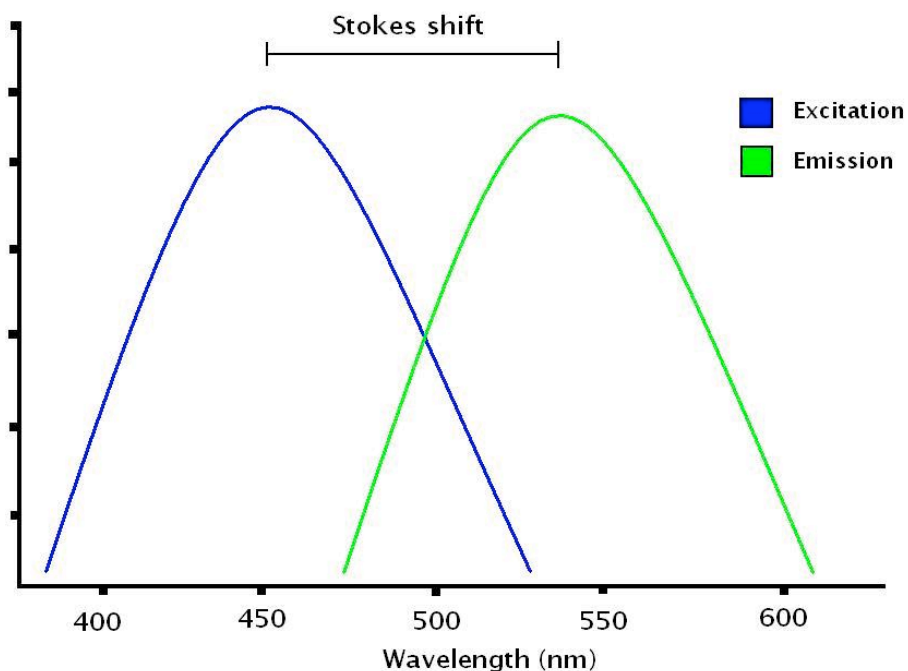
Beamsplitter Mirror 1: 460nm; Mirror 2: 500nm; Mirror 3: 580nm; Mirror 4: 595nm

Longpass Filter 1: >470nm; Filter 2: >530nm; Filter 3: >580nm; Filter 4: >635nm

You need to construct a suitable filter block for imaging GFP. What are the roles of the different types of filter in the microscope lightpath? What filters will you choose for the (i) excitation filter, (ii) beamsplitter and (iii) emission filter. Explain why you chose this combination?



Excitation, Emission and Stokes shift



Microscopy weblinks

Molecular Expressions website featuring our acclaimed photo galleries that explore the fascinating world of optical microscopy. One of the Web's largest collections of photo-micrographs, and a lot of tutorials including diagrams and interactive Java applets:

<http://micro.magnet.fsu.edu/index.html>

Laser Scanning Confocal Microscopy

<http://micro.magnet.fsu.edu/primer/techniques/confocal/index.html>

Comparing wide-field and confocal microscopy techniques. Interactive Java applet.

<http://micro.magnet.fsu.edu/primer/virtual/confocal/index.html>

<http://www.olympusconfocal.com/java/confocalvswidefield/index.html>

Olympus and Nikon educational sites for confocal Microscopy

<http://www.olympusconfocal.com>

<http://www.microscopyu.com/>

Microscopy Primer: Physical and geometric optics of the microscope and their implications for practice. http://www.microscopy-uk.org.uk/full_menu.html

Including the Micropolitan Museum - <http://www.microscopy-uk.org.uk/micropolitan/index.html>

Fluorescent proteins

2008 Nobel prize for Chemistry - Fluorescent proteins

http://nobelprize.org/nobel_prizes/chemistry/laureates/2008/press.html

Fluorescent protein variants

<http://www.olympusconfocal.com/applications/fpcolorpalette.html>

Chromophore maturation in fluorescent proteins

<http://www.olympusconfocal.com/java/fpfluorophores/index.html>

Plant anatomy

The [Photographic Atlas of Plant Anatomy](http://botweb.uwsp.edu/anatomy/) contains photographs of plant structures.

<http://botweb.uwsp.edu/anatomy/>

The [The Botanical Society of America Online Image Collection](http://www.botany.org/plantimages/) includes many photographs of plants and their anatomy. (<http://www.botany.org/plantimages/>)

An Introduction to Plant Tissues: McGraw-Hill

<http://www.mhhe.com/biosci/pae/botany/histology/html/ptmodov.htm>

Plant Study Resources: introduction to plant anatomy

<http://biology.nebrwesleyan.edu/benham/plants/index.html>

The [Revision Modules in Plant Anatomy](http://bugs.bio.usyd.edu.au/2003A+Pmodules/home.html) is an interactive site for reviewing plant structure.

<http://bugs.bio.usyd.edu.au/2003A+Pmodules/home.html>

The [Plant Anatomy Laboratory](http://www.sbs.utexas.edu/mauseth/weblab/) contains micrographs and explanatory text from the textbook "Plant Anatomy", by James Mauseth. (<http://www.sbs.utexas.edu/mauseth/weblab/>)