Part 1B Plant & Microbial Sciences Practical

Reporter genes in plants - solution sheet

Gene fusions, GUS, GFP and microscopy.

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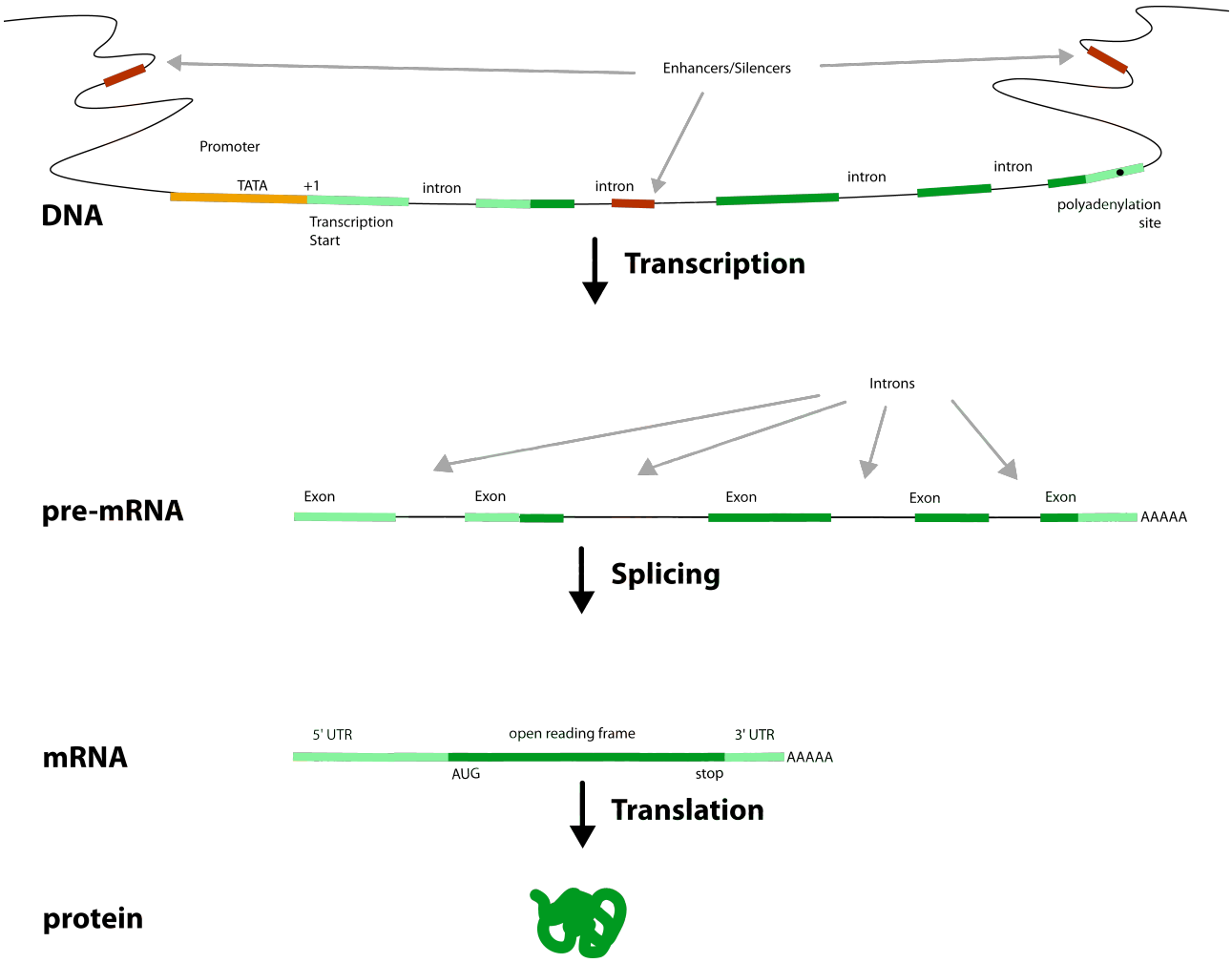
Web resources at: http://www.plantsci.cam.ac.uk/Haseloff/

1. **Draw a diagram of a typical protein-coding plant gene.**



promoter: a general term that refers to sequences required to promote transcription, usually upstream of the gene, and includes a conserved region adjacent to the start site for transcription (-30 TATA box region), which forms the binding site for RNA polymerase.

upstream regulatory sequences: identified binding sites for regulatory proteins, upstream of coding sequence.

enhancer: DNA segment that binds proteins capable of activating transcription at a distance from the RNA polymerase binding site, and can be positioned upstream, downstream or intragenically. Enhancer complex interacts with RNA polymerase binding site, and forms a DNA loop.

silencer: DNA segment that binds proteins capable of silencing transcription at a distance from the RNA polymerase binding site, and can be positioned upstream, downstream or intragenically. Silencer complexes may cause altered chromatin conformation or supress transcription directly.

RNA polymerase initiation site: Start site for RNA transcription (+1), aproximately 30bp from TATA box.

intron: transcribed RNA segment that is removed from the pre-mRNA, characterised by conserved splice junctions, and elevated A:U content especially in dicots.

exons: spliced RNA segments that make up the final mRNA sequence, generally have higher G:C content.

UTRs: 5' and 3' **U**n**t**ranslated **R**egions outside the mRNA coding region.

START codon: initiator methionine codon AUG (mostly).

STOP codon: terminator codon, generally UGA, UAG or UAA.

polyadenylation site: site for endonucleolytic cleavage of pre-mRNA, generally marked by a sequence similar to AAUAAA, where cleavage occurs about 20 nt downstream and results in enzymatic addition of a poly adenine tract (~200 nt).

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

Transcription factors are proteins that regulate gene activity, and bind to DNA sequences, either directly or as part of a protein complex. They regulate the initiation of transcription by RNA polymerase. Often comprise modular DNA binding and activator (or repressor) domains. The latter interact with the RNA polymerase complex.

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

A reporter gene simply produces a product that can be easily assayed, and acts as a surrogate for the gene of choice. For example, a reporter gene may consist of a test promoter fused to the coding region for a fluorescent protein. Fluorescence is produced only where/when the promoter is active, and can be directly imaged.

Fluorescent proteins: detected by fluorescence microscopy or macroscopic imaging.

Enzyme markers e.g. GUS: detected by histochemistry or enzyme assay using chromogenic or fluorogenic substrates.

Photoproteins e.g. firefly luciferase or aequorin: detected by sensitive photodetectors such as photmultipliers or CCDs.

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.**

The key distinction between these vectors is the degree to which part of the gene is supplied by the reporter construct, and how much is supplied by the fused plant sequences. see answer to first question, and examples below:

From: *Gene Traps: Tools for Plant Development and Genomics*, Patricia S. Springer, The Plant Cell 12:1007-1020 (2000)

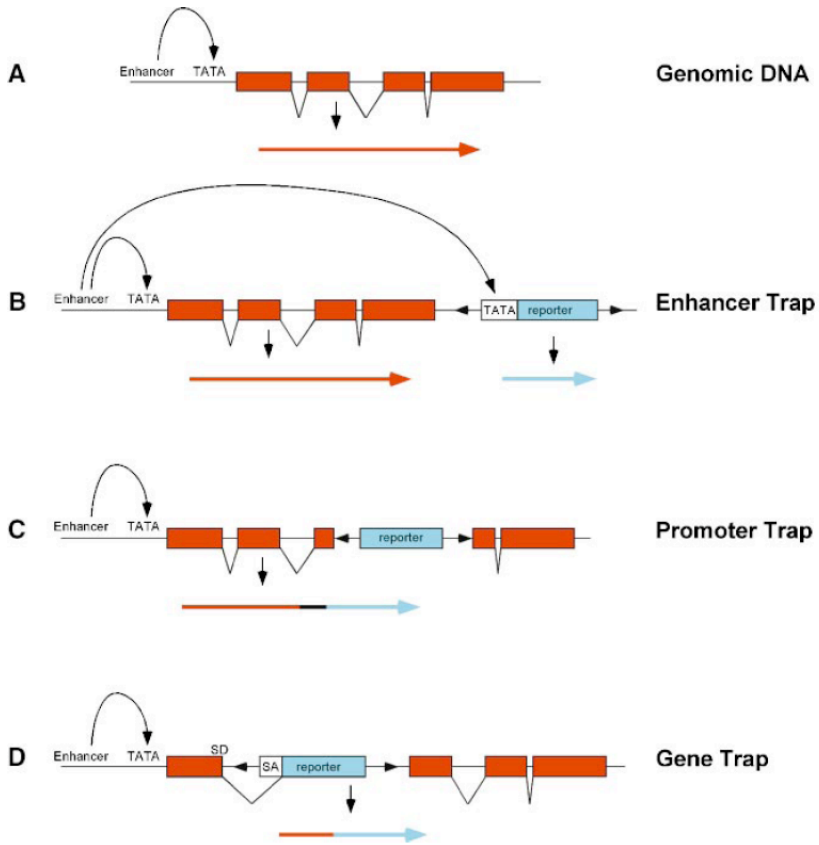


Figure 1. Structure of Enhancer, Gene, and Promoter Trap Vectors.

(A) A generic chromosomal gene with exons (boxes) and introns (lines).
(B) Enhancer trap construct. The minimal promoter of the reporter gene (TATA) is activated by a chromosomal enhancer element, resulting in expression of the reporter gene.
(C) Promoter trap construct. The promoterless reporter gene can be expressed when insertion occurs in an exon so as to result in a transcriptional fusion.
(D) Gene trap construct. The promoterless reporter gene contains splice acceptor (SA) sequences. Expression of the reporter gene occurs upon its insertion into an intron. Splicing from the chromosomal splice donor (SD) site to the SA sequence results in creation of a transcriptional fusion.
Arrows in each panel represent the transcripts that are produced as a consequence of insertion.

5. **Why are these different gene fusions useful?**

Gene Traps produce protein fusions, which can be used to produce fluorescent protein fusions for subcellular localisation studies. Promoter traps are often used for gene tagging strategies, as expression of the reporter gene is dependent on insertion directly into a transcribed region. Enhancer traps are used to provide the highest frequencies of "tagged" reporter gene expression, as enhancers will trigger reporter expression at a distance, independent of orientation or reading frame. This is useful for the production of marker plants where mutant phenotypes due to gene insertion would be undesirable.

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

GUS activity can be quantitated by fluorometric enzyme assay, or localised by cheap and simple histological techniques. Large, intact plant tissues can be stained and cleared for gross observation. Enyzmatic turnover allows very sensitive assays. GFP allows precise localisation of gene products by fluorescence microscopy. The gene products are intrinsically fluorescent and can be observed in living tissues without treatment. Different spectral variants allow the "painting" of dynamic processes *in vivo*. However high resolution observation of GFP is limited to submillimeter depths, and can be subject to problems due to autofluorescence, light scattering or pigmentation in some tissues. The two reporter genes are complementary.

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 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 filters will you choose for the following optical filters. Why choose this combination?

(i) **excitation filter:** Filter 1 or Filter 2 to allow excitation of the specimen with light in the wavelength band of 430-470nm - neither is optimal.

(ii) **beamsplitter**: Mirror 2 to allow most efficient reflection of the excitation wavelengths (peak at 450nm), and transmission of the emitted wavelengths (peak at 540nm). This mirror literally "splits" the excitation and emission spectra.

(iii) **emission filter**: Filter 2 to allow efficient removal of residual reflected light, and transmission of fluorescence emissions.