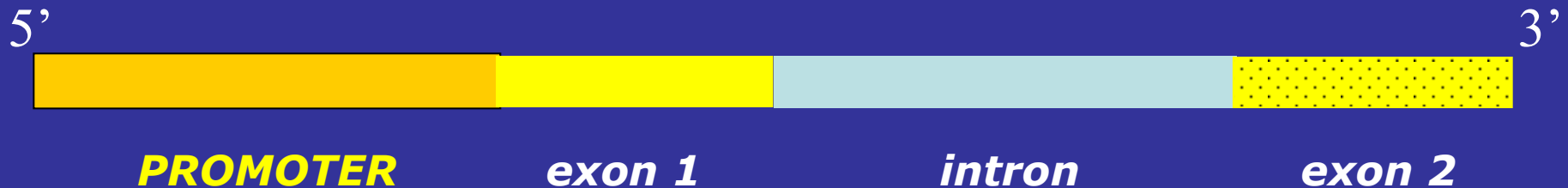


Recombinant DNA Technology

*Characterization of
transcription regulatory
sequences by exploiting
reporter genes*

Reporter gene technology

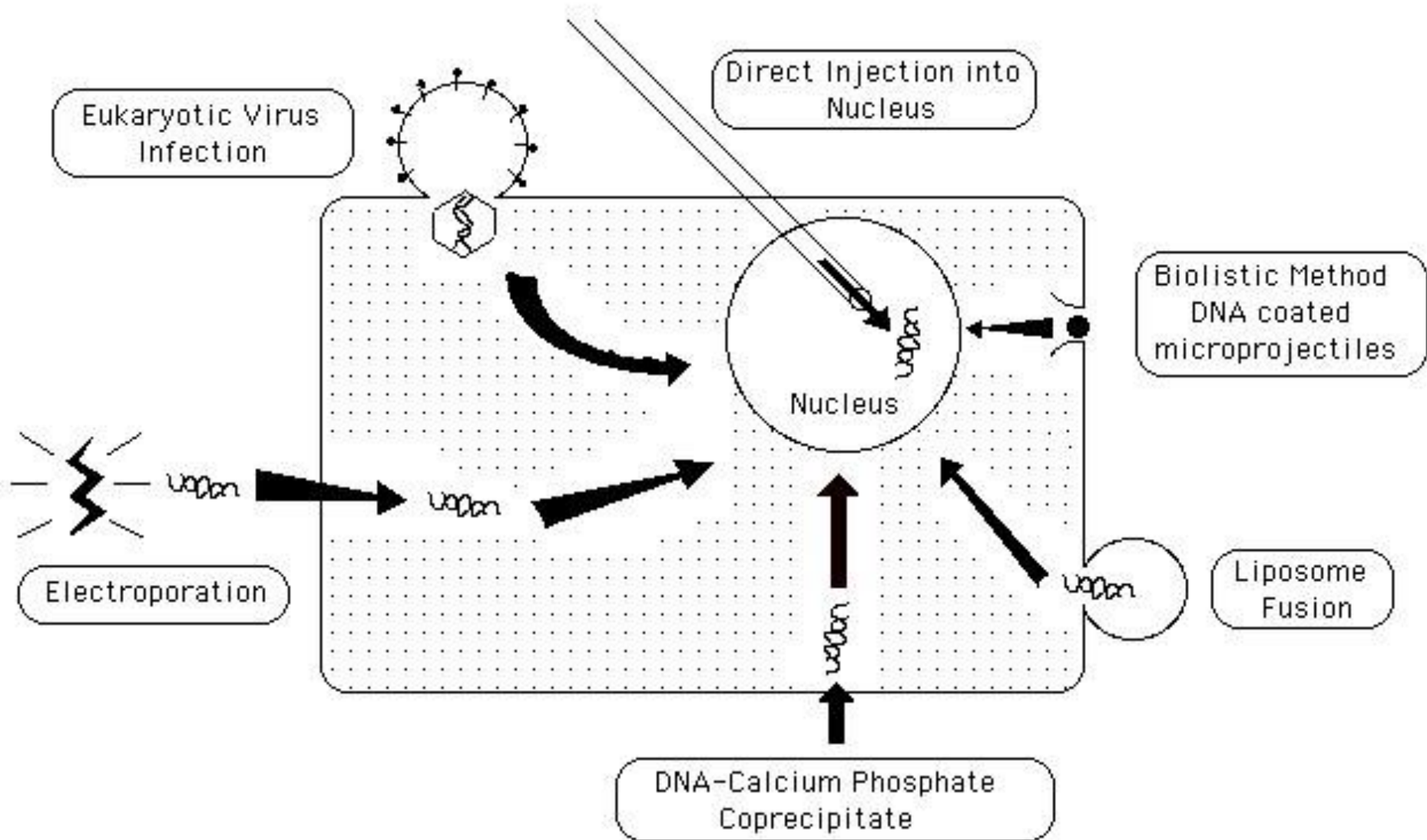
Reporter genes are nucleic acid sequences encoding easily assayed proteins. They are used to replace other coding regions whose protein products are difficult to assay.



Transfection of Euk. cells

- Introduction of DNA into prokaryotic (and yeast) is cells termed **Transformation**
- Transformation of animal cells refers to changes in growth characteristics of cells in culture
- **Transfection** is term used for uptake of foreign DNA into eukaryotic cells resulting in inherited change

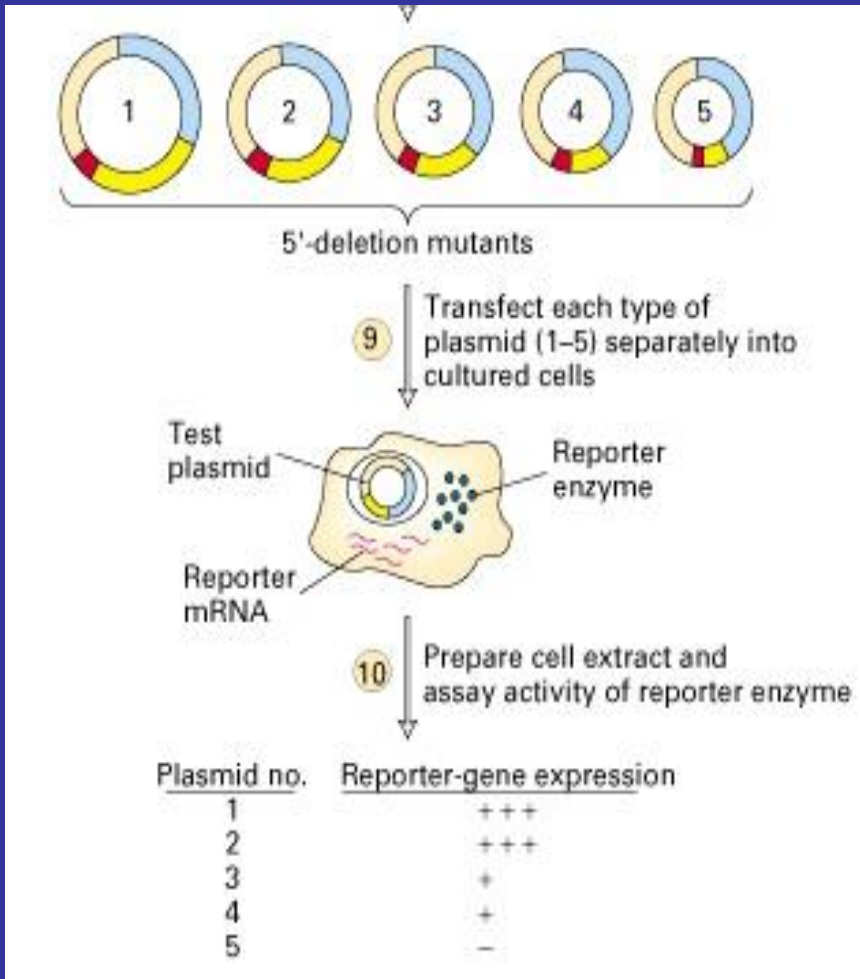
Transfection Methods



Design and engineer reporter gene construct
i.e. clone reporter gene downstream of the promoter of interest

Introduce into cells
Transfection
Stable or transient

Assay activity of reporter genes
e.g. luciferase



Choice of Reporter genes

CAT (chloramphenicol acetyltransferase)

Transfers radioactive ^{14}C acetyl groups to chloramphenicol.
Detection by thin-layer chromatography and autoradiography or EISA

GAL (β -galactosidase)

Hydrolyzes colourless galactosides to yield coloured products.
Assay change/production of colour

LUC (luciferase)

Oxidizes a luciferin emitting photons. Count photons by luminometer or photon-counting camera. **Different luciferases available**

SEAP (secreted human placental alkaline phosphatase)

highly-sensitive bioluminescent alkaline phosphatase assay

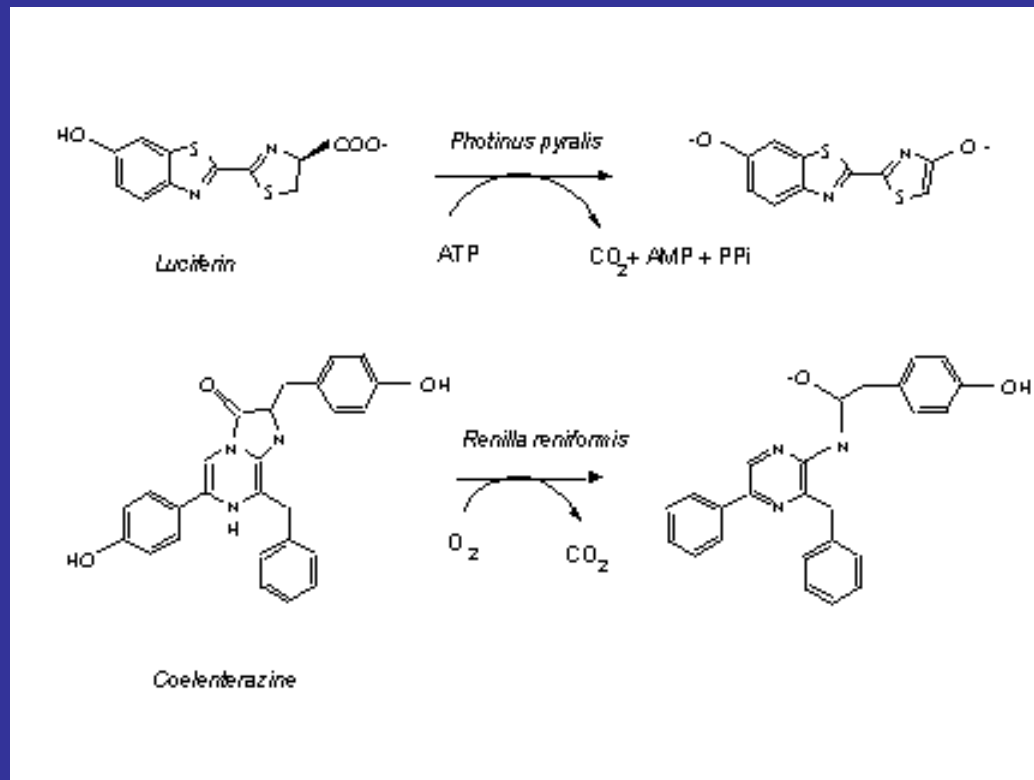
GH (Growth hormon)

Secreted and detected by ELISA

Co-transfection of reporter genes

Dual Luciferase Assay system - Promega

Clone promoter of interest in front of firefly luciferase and use Renilla luciferase as an internal control
co-transfect and assay



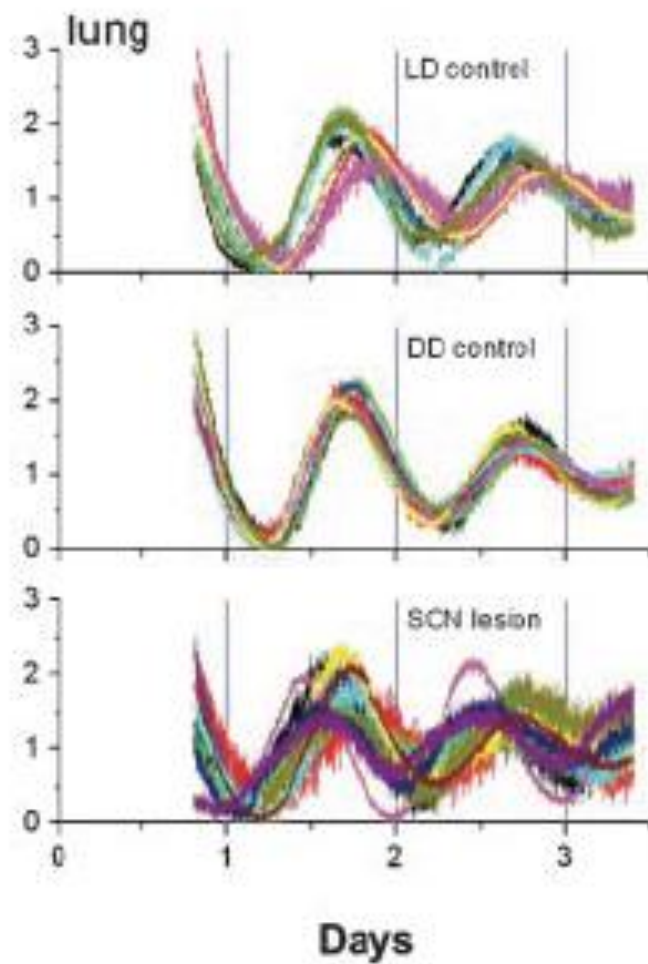
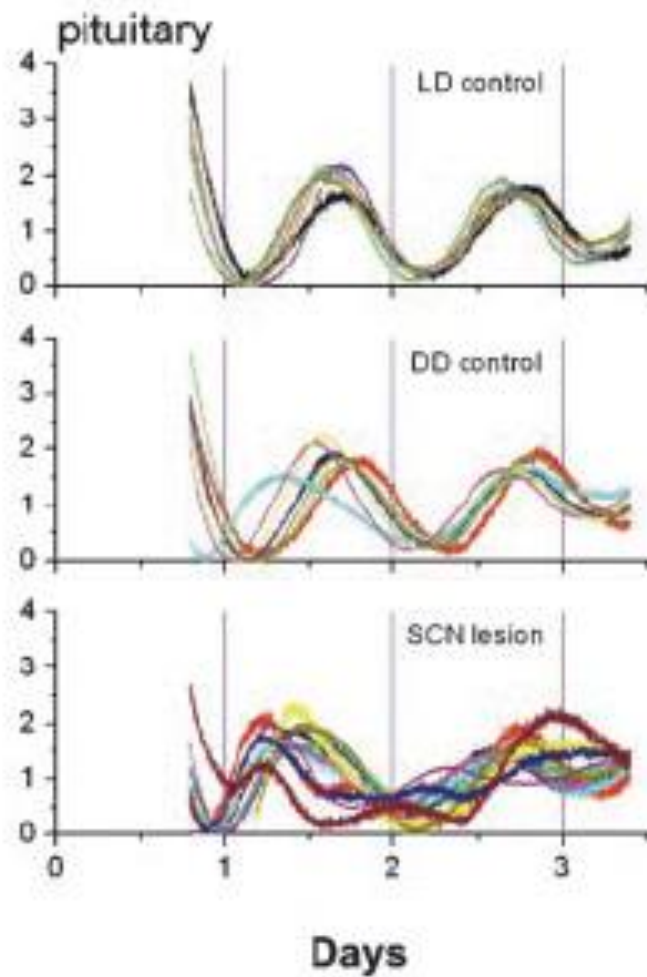
Renilla luciferase
driven by constitutive
promoter

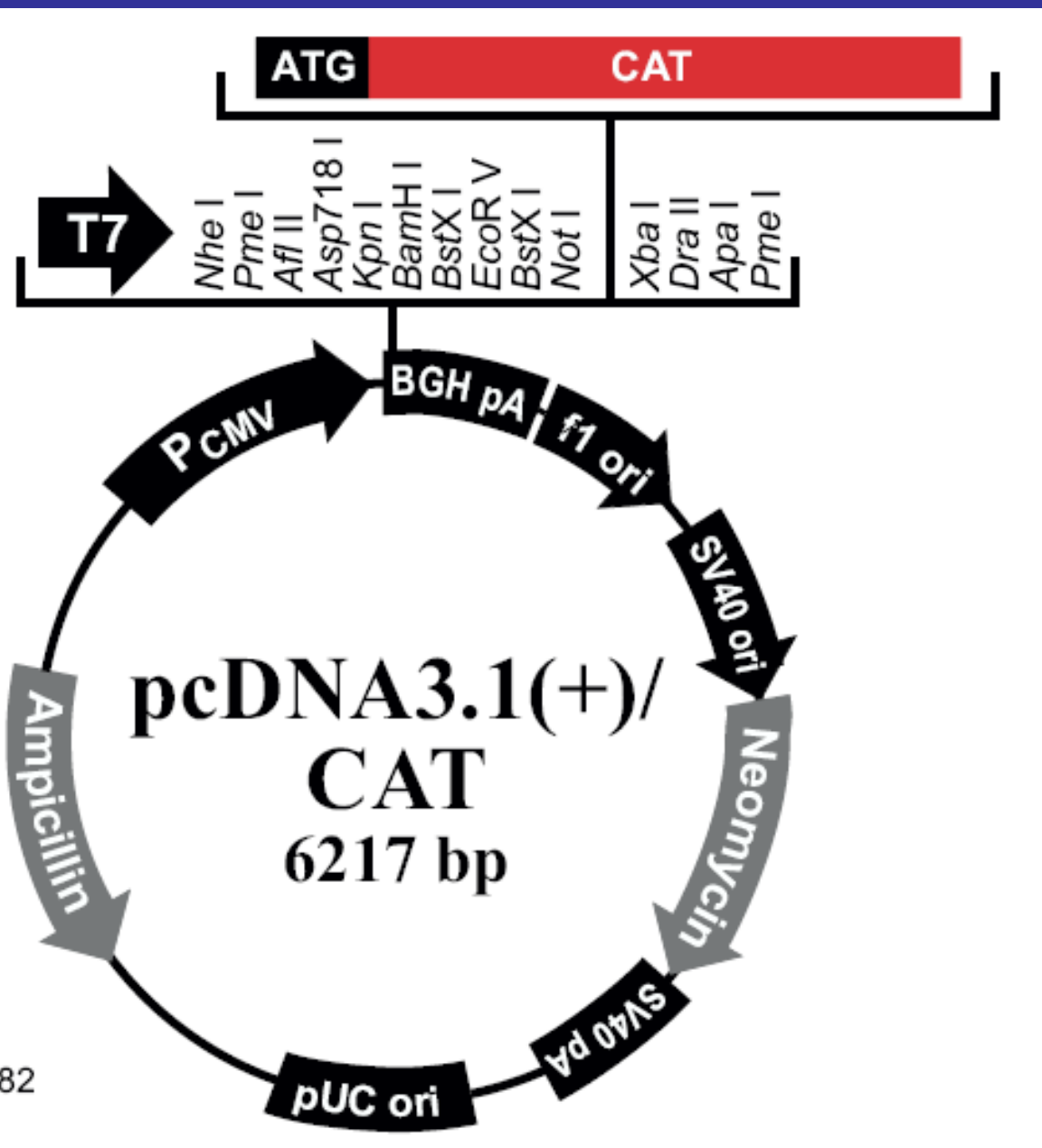
e.g. SV40 I/E
HSV TK

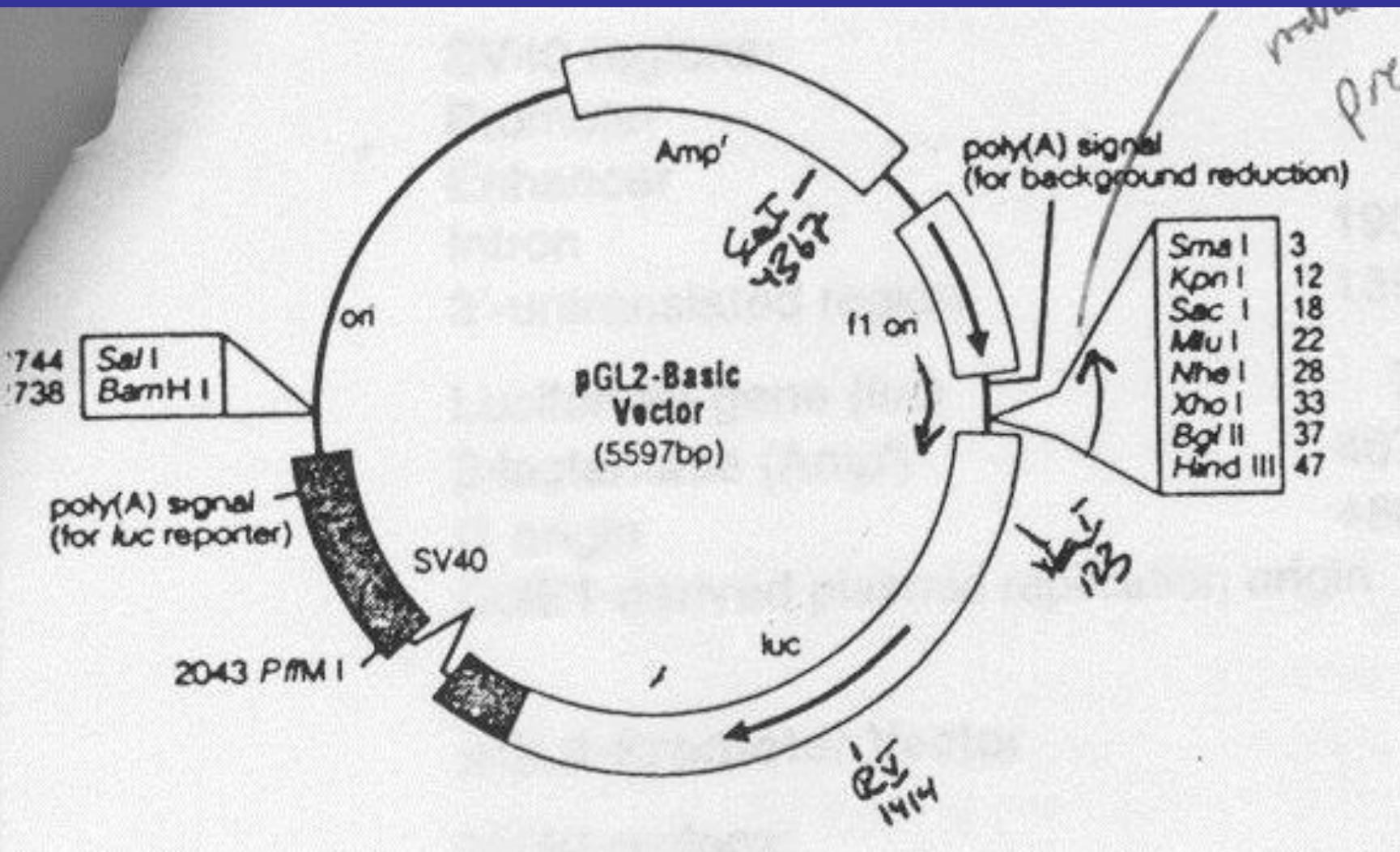
Period (hr)

C

Relative Unit of Bioluminescence







Green Fluorescent Protein (GFP)

- Gene encoding GFP isolated from the jellyfish *Aequoria victoria*
 - *GFP can be cloned and introduced into cells of other species*

Use of Green Fluorescent Protein (GFP)

- As a reporter molecule to monitor gene expression
 - Transgenic organism made with the GFP-coding sequence under the transcriptional control of the promoter belonging to the gene of interest

Gene A



Promoter

Coding region

GFP-reporter gene construct



**Promoter
for Gene A**

**Coding region for
GFP**

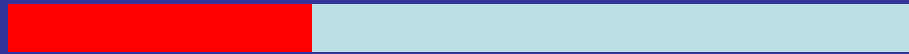
**Can be used to
visualize the
expression of
Gene A**

Promoter for Gene A regulates the expression of GFP

Use of Green Fluorescent Protein (GFP)

- As a tag to localize proteins
 - The GFP-encoding sequence is placed at the beginning or end of the gene for another protein
 - This yields a chimeric protein consisting of the protein of interest with a GFP domain attached
 - GFP-fusion protein often behaves like the original protein, directly revealing its subcellular location (Fig. 9-44)

Gene A



Promoter

Coding region

GFP-fusion protein construct

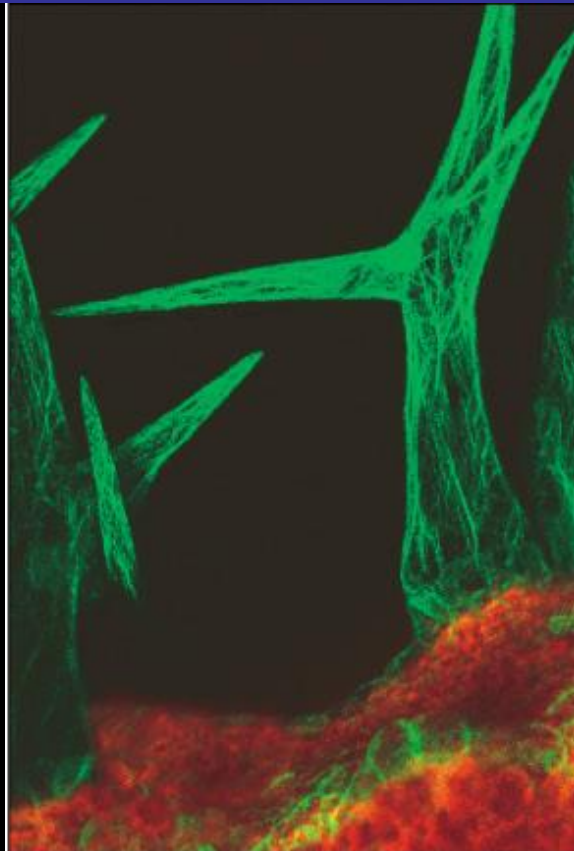


**Promoter
for Gene A**

**Coding region
For Gene A**

**Coding region for
GFP**

**Can be used to visualize the subcellular location of
the protein encoded by Gene A**



Confocal micrograph of Arabidopsis leaf surface

(Notes page)

Use of reporter proteins

e.g. yellow fluorescent protein

note that GFPs can report on protein location or movement in cells
not just act as reporters of gene activation

