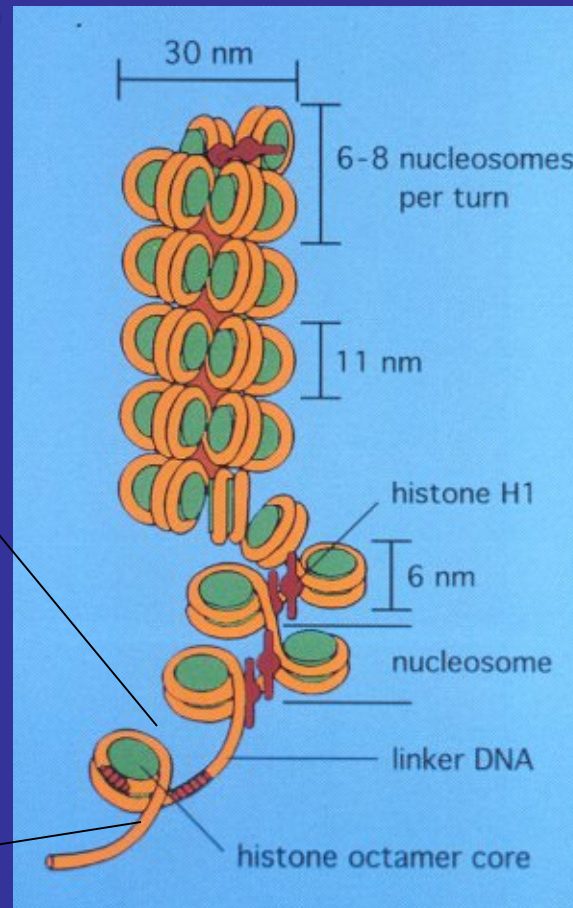
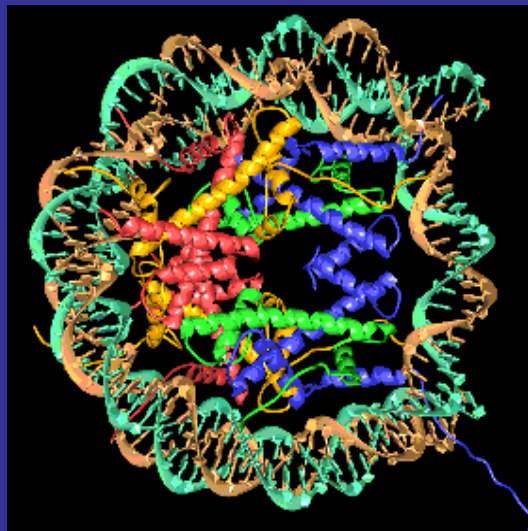


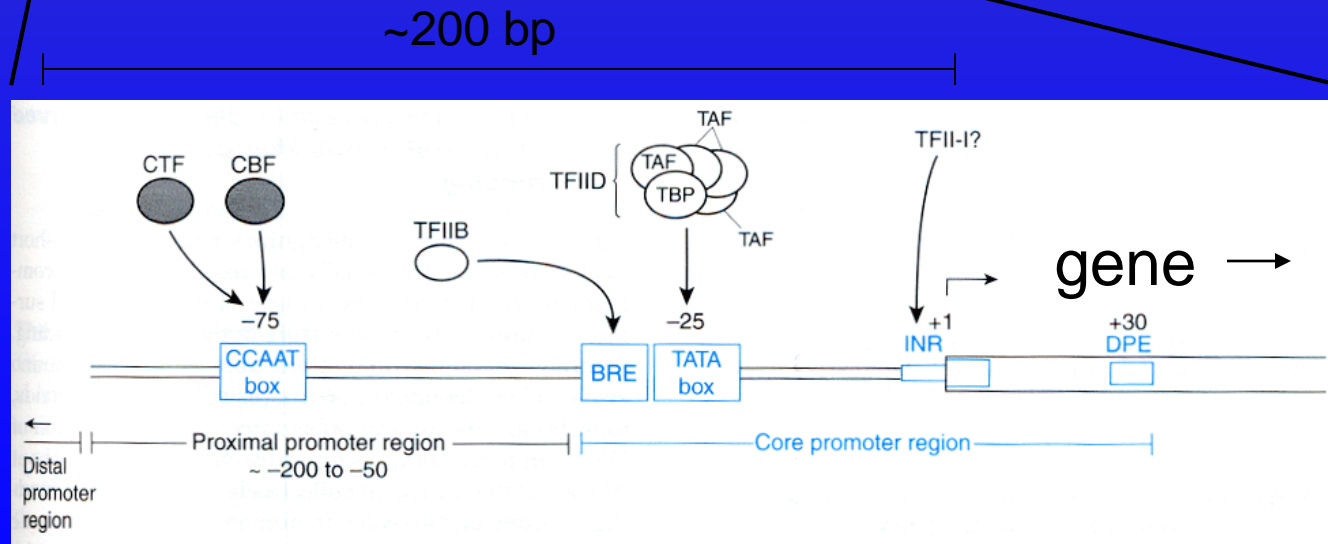
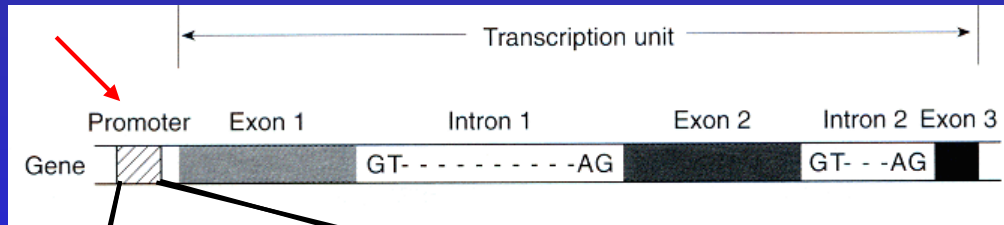
# *Recombinant DNA Technology*

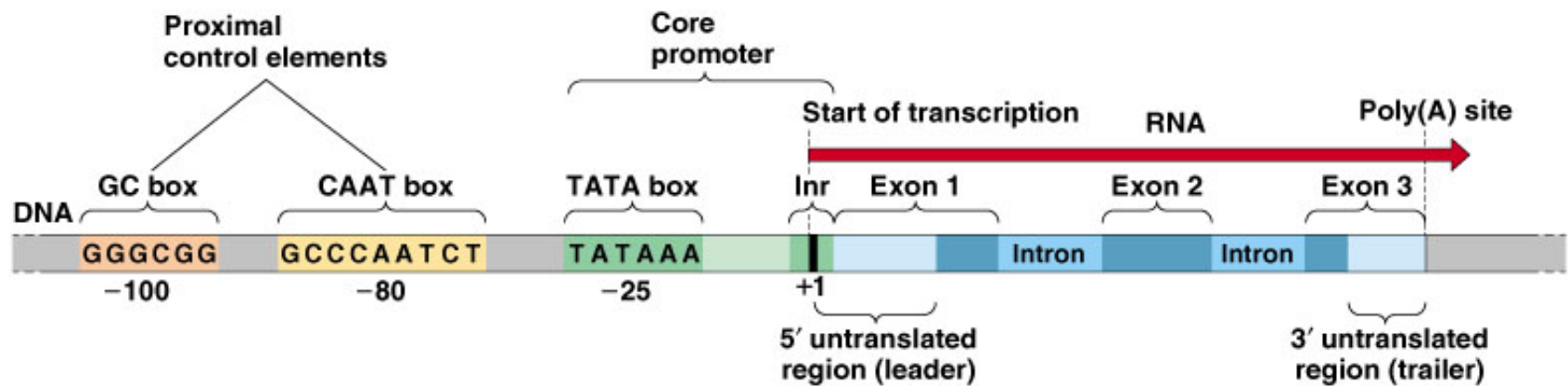
*Characterization of  
transcription regulatory  
sequences by exploiting  
reporter genes*

*How can we demonstrate whether a given sequence has a functional role in transcription?? Once the promoter has been individuated, how can we identify the short regulatory sequences?*



# TRANSCRIPTION IS FINELY REGULATED

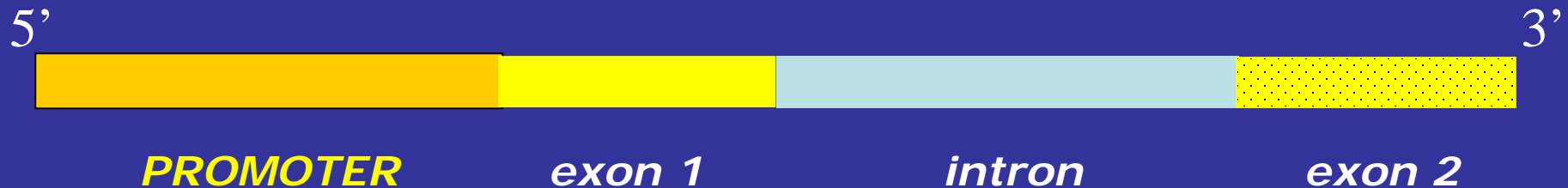




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# *Reporter gene technology*

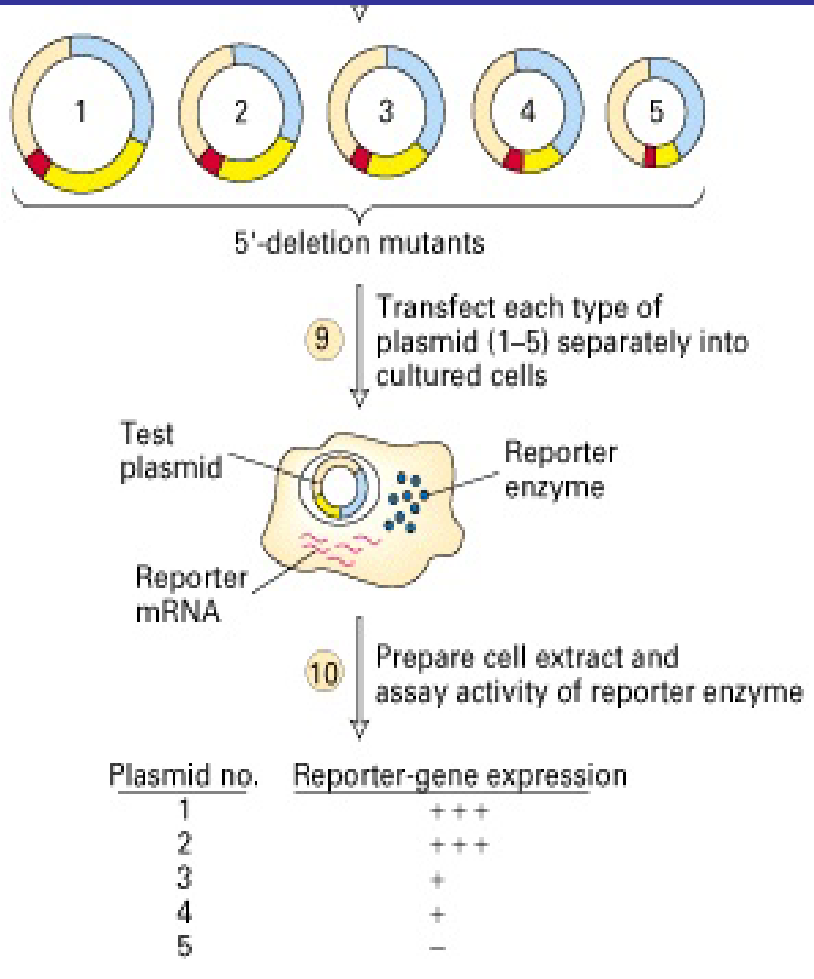
**Reporter genes** are nucleic acid sequences encoding easily assayed proteins. They are used to replace other coding regions whose protein products are difficult to assay.



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*



# Reporter Assay

1. Measures gene expression or transcriptional activity
2. Assay of transcription factors.
3. DNA promoter assay
4. Confirmation of transgenesis

# ***Choice of Reporter genes***

## **CAT (chloramphenicol acetyltransferase)**

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

## **GAL ( $\beta$ -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



# CAT: chloramphenicol acetyltransferase

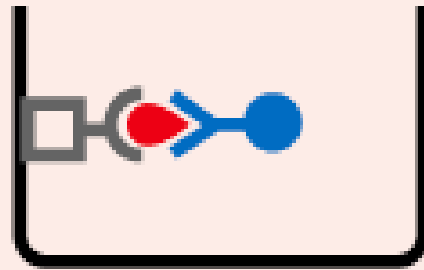
1. 1st reporter gene used to monitor transcriptional activity in cells
2. Bacterial enzyme that transfers acetyl groups from acetyl-CoA to chloramphenicol, detoxifying it
3. Reaction quantified by monitoring acetylation of radiolabeled substrates ( $^{14}\text{C}$ -chloramphenicol) or by ELISA (non-radioactive)

# CAT assay: ELISA

1



2



3



 Anti-CAT

 CAT

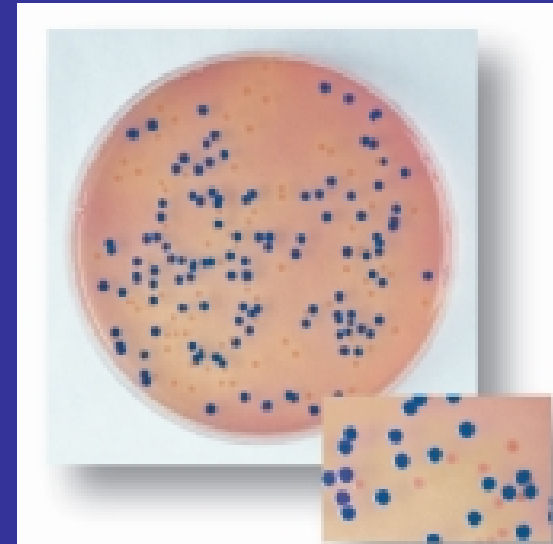
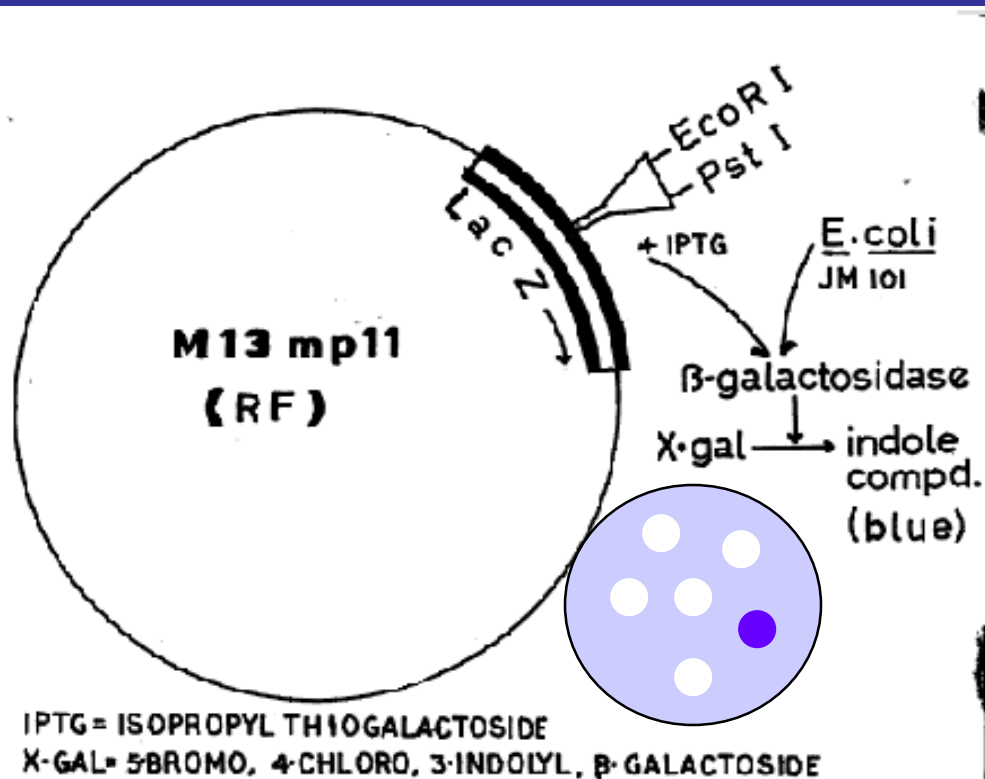
 Anti-CAT-DIG

 Anti-DIG-POD  
Fab fragment

 ABTS substrate

# $\beta$ -gal ( $\beta$ -galactosidase):

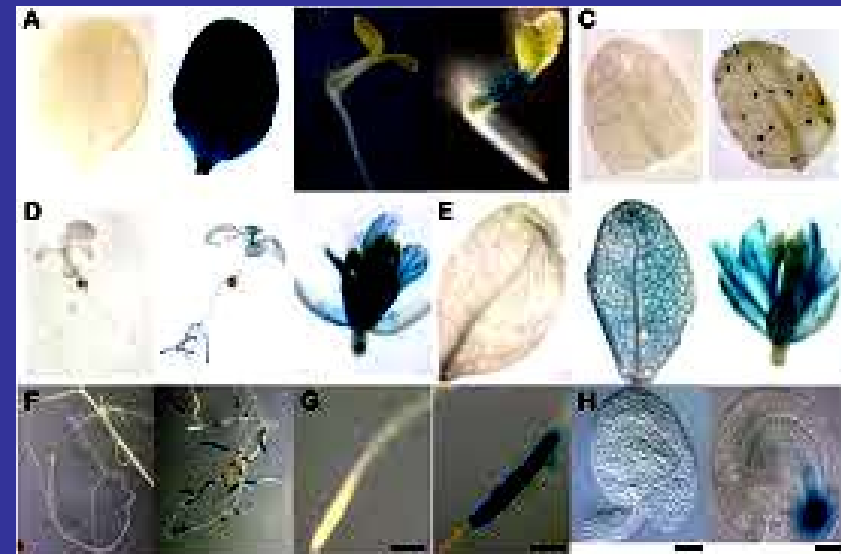
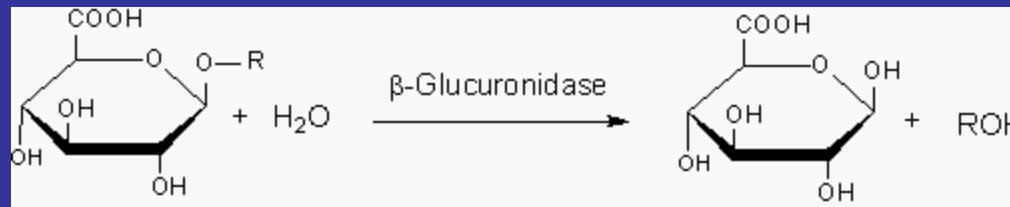
- *E. coli* enzyme (encoded by *lacZ*) that hydrolyzes galactosidase sugars such as lactose
- Many assay formats: colorimetric, fluorescent, chemiluminescent



# GUS Reporter Gene System

GUS encodes the beta-glucuronidase enzyme from *E. coli*.

An active enzyme may be detected using X-gal, which forms an intense blue product after cleavage by  $\beta$ -galactosidase



# Luciferase:

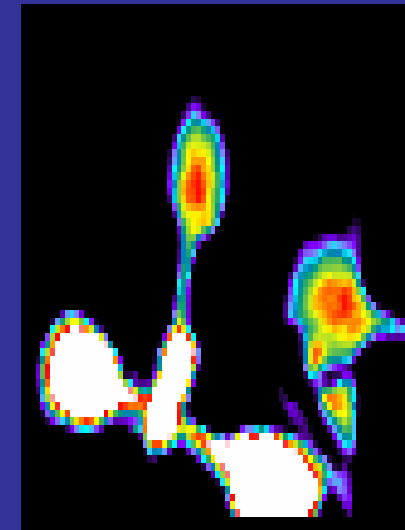
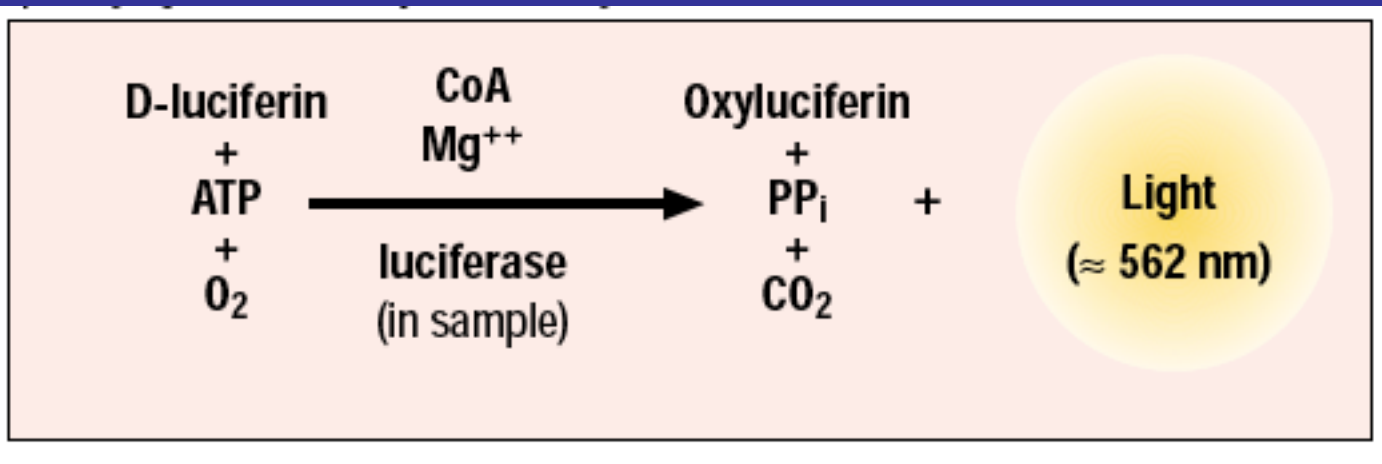


*Renilla reniformis*



*Photinus pyralis*

- Firefly (*Photinus pyralis*) luciferase
- Sea pansy (*Renilla reniformis*) luciferase
- Firefly luciferase produces light by ATP-dependent oxidation

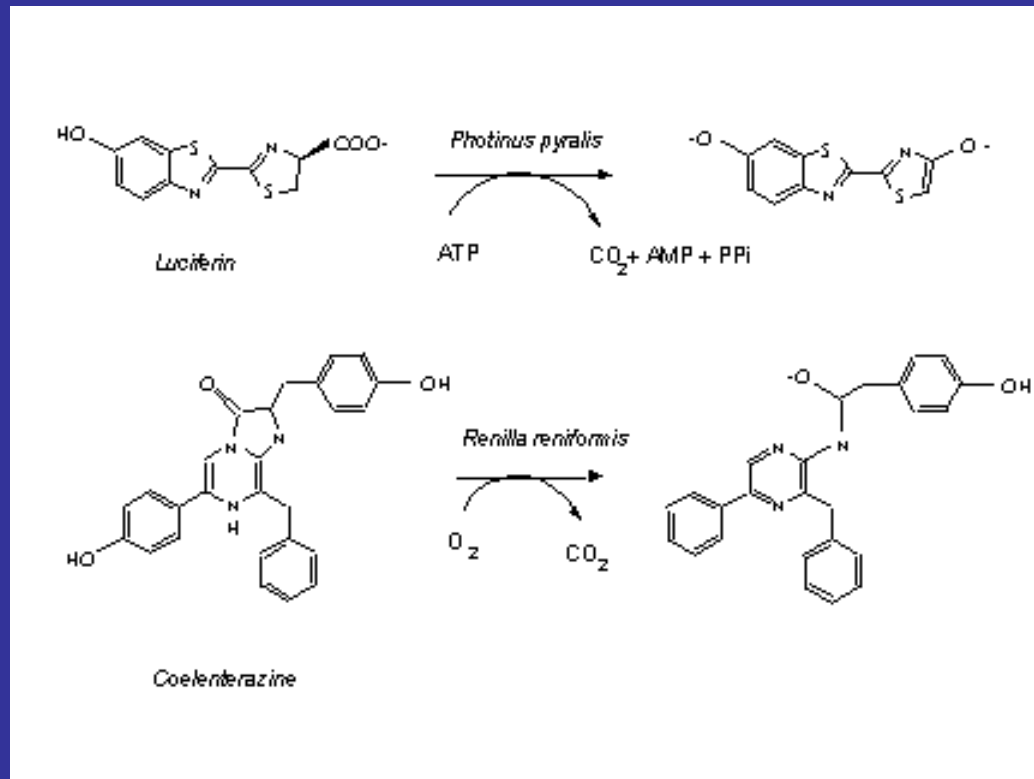


- Bioluminescence or light emission is determined by a luminometer

# 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



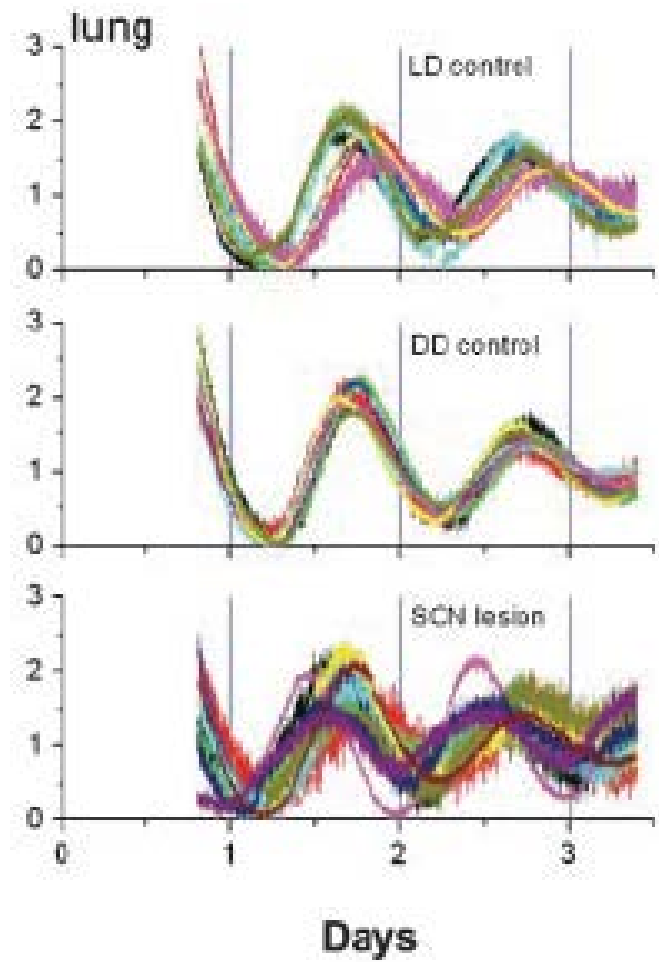
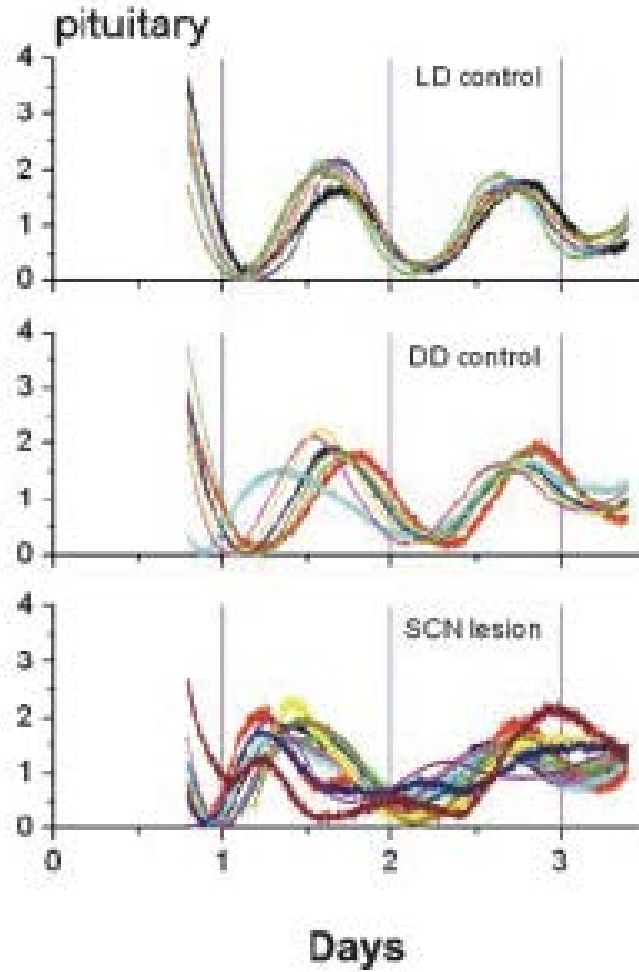
Renilla luciferase  
driven by constitutive  
promoter

e.g. SV40 I/E  
HSV TK

Period (hr)



Relative Unit of Bioluminescence



# SEAP (secreted alkaline phosphatase):

- Secreted outside the cell (can assay sample repeatedly and non-destructively by sampling culture medium)
- This protein is quantified directly by measuring the enzyme activity in the supernatant of the culture medium.
- Fluorescence and chemiluminescence assays are available for detection.





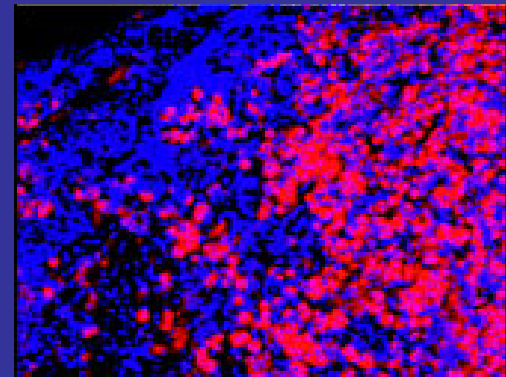
# Human Growth Hormone (hGH) Reporter Gene System

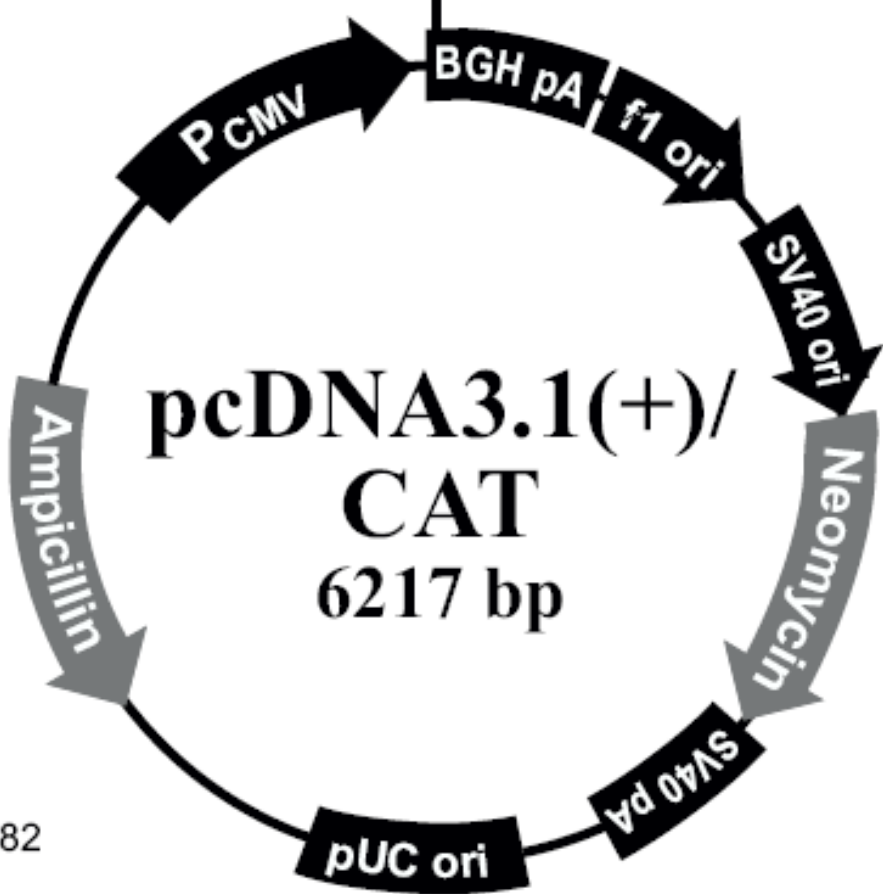
The human growth hormone (hGH) encoded reporter protein is secreted into the culture medium by transfected cells.

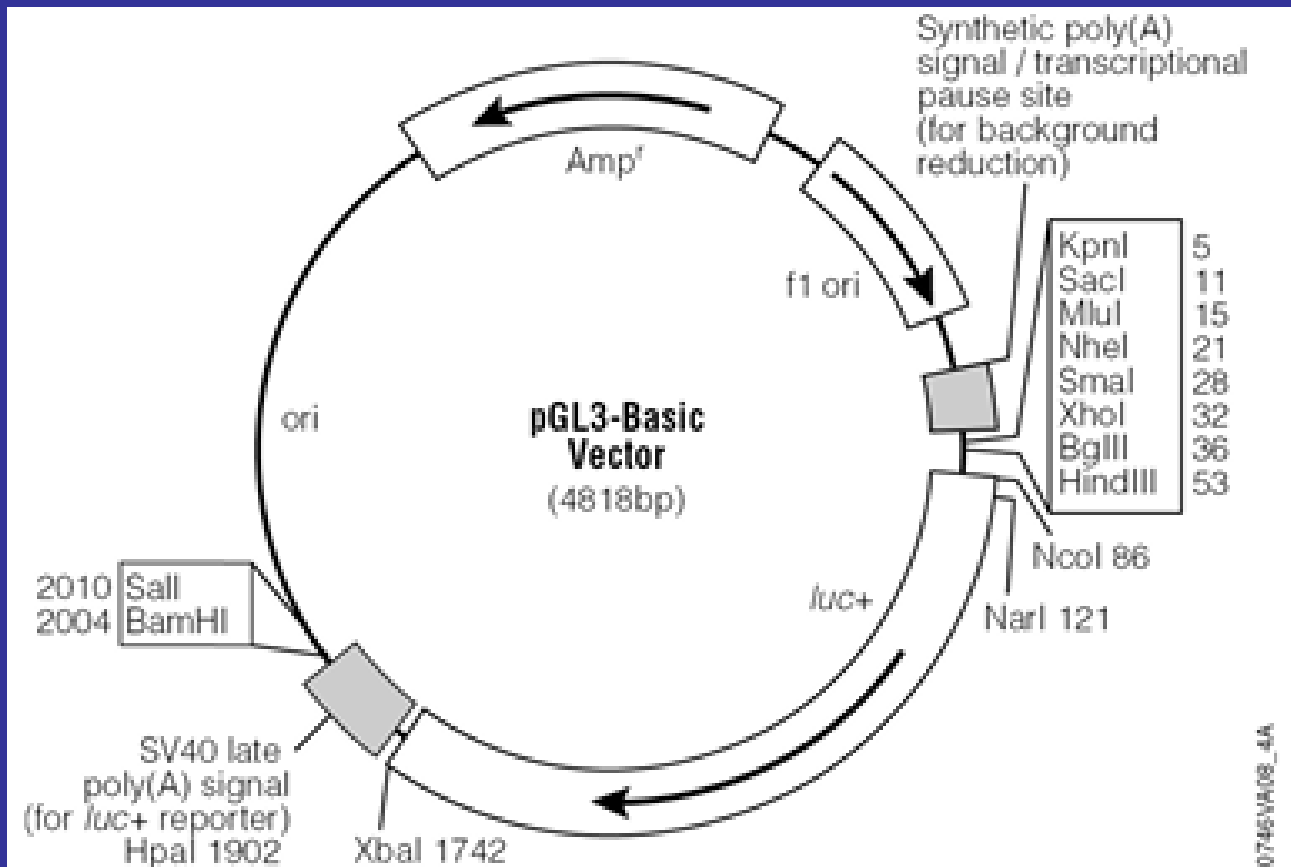
The hGH from the supernatant of the culture medium binds to the antibody on the plate.

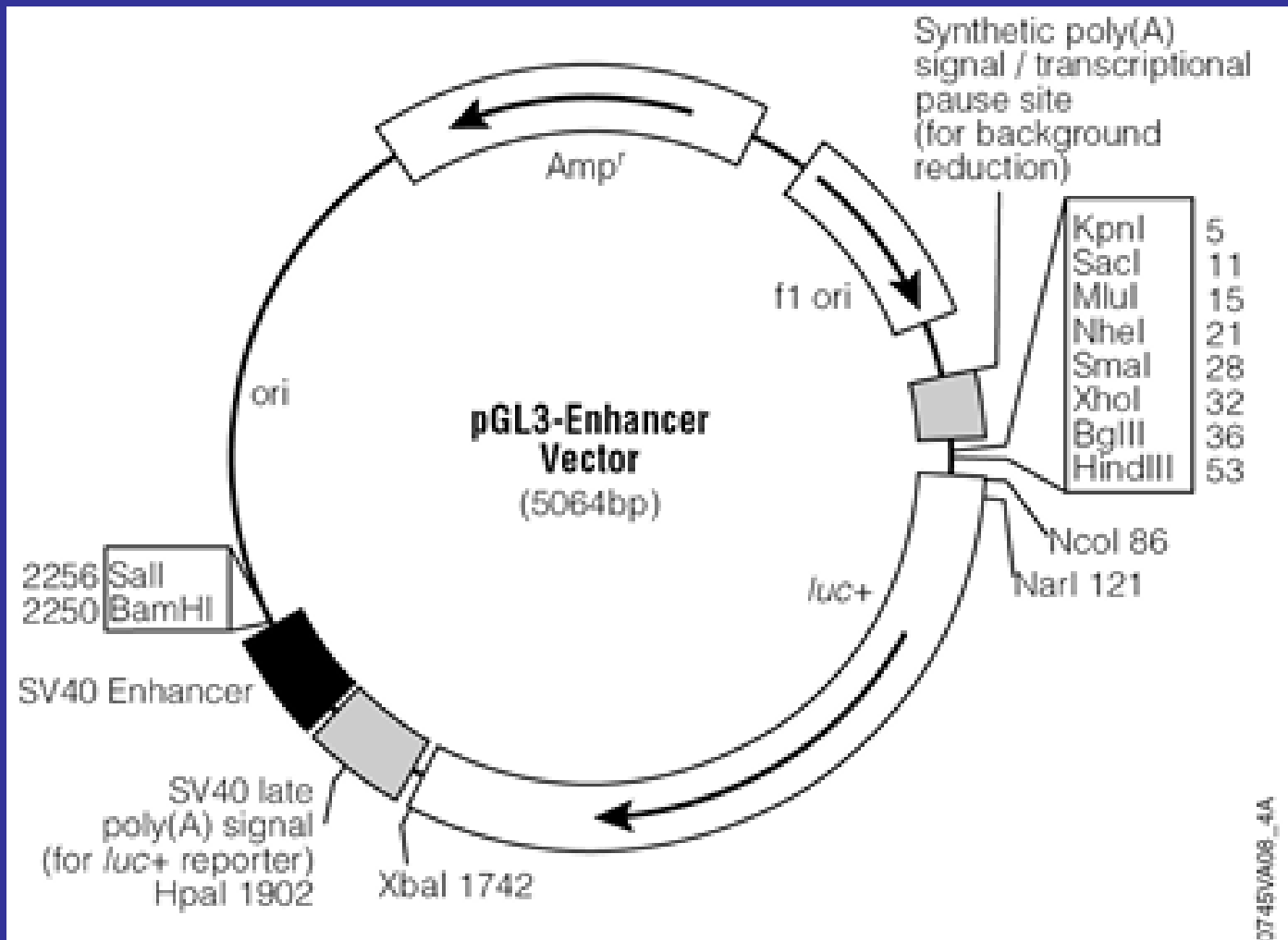
Subsequently, the bound hGH is detected in two steps via a digoxigenin-coupled anti-hGH antibody and a peroxidase-coupled anti-digoxigenin antibody.

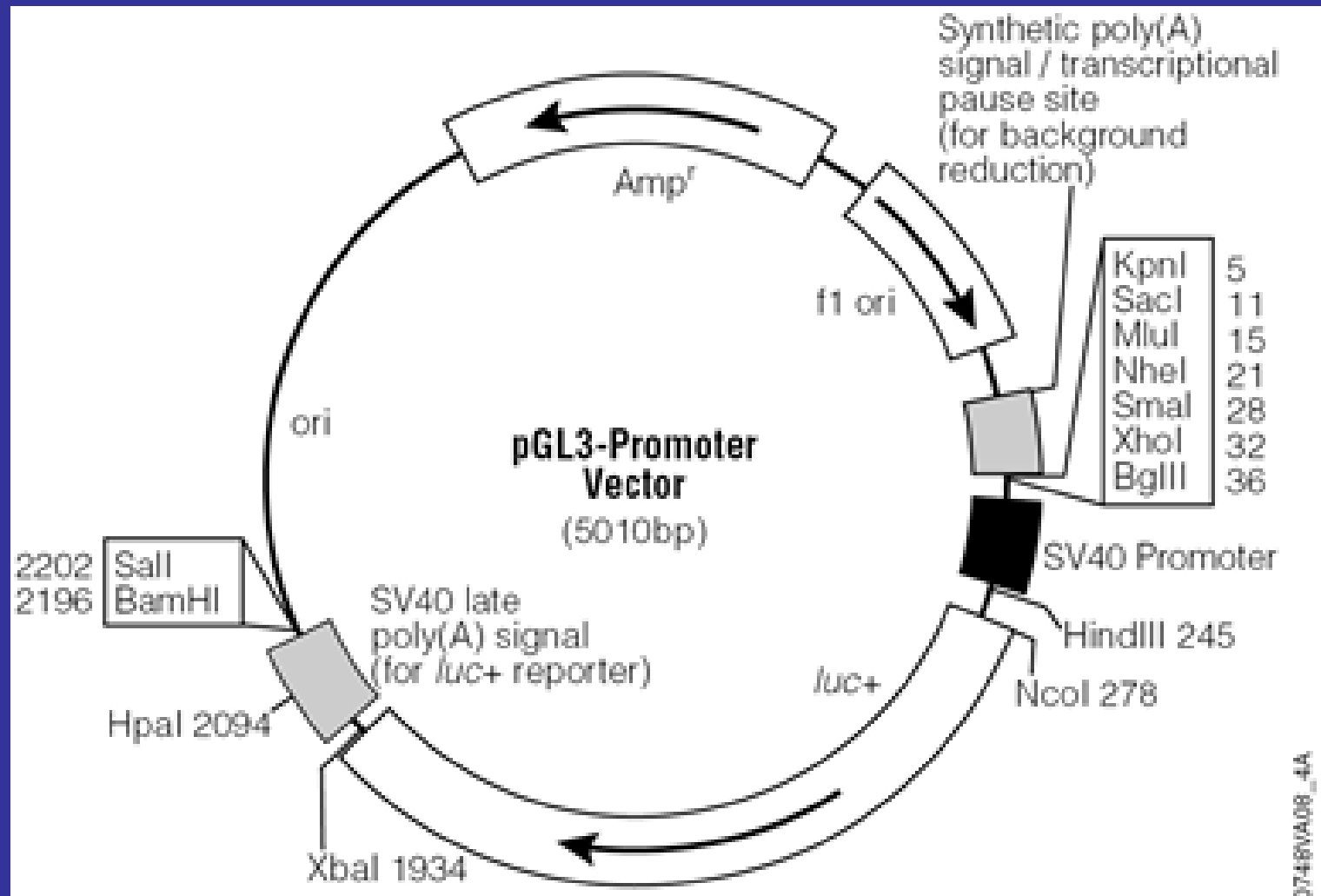
Bound peroxidase is quantified by incubation with a peroxidase substrate such as TMB (3,3',5,5'-tetramethylbenzidine)

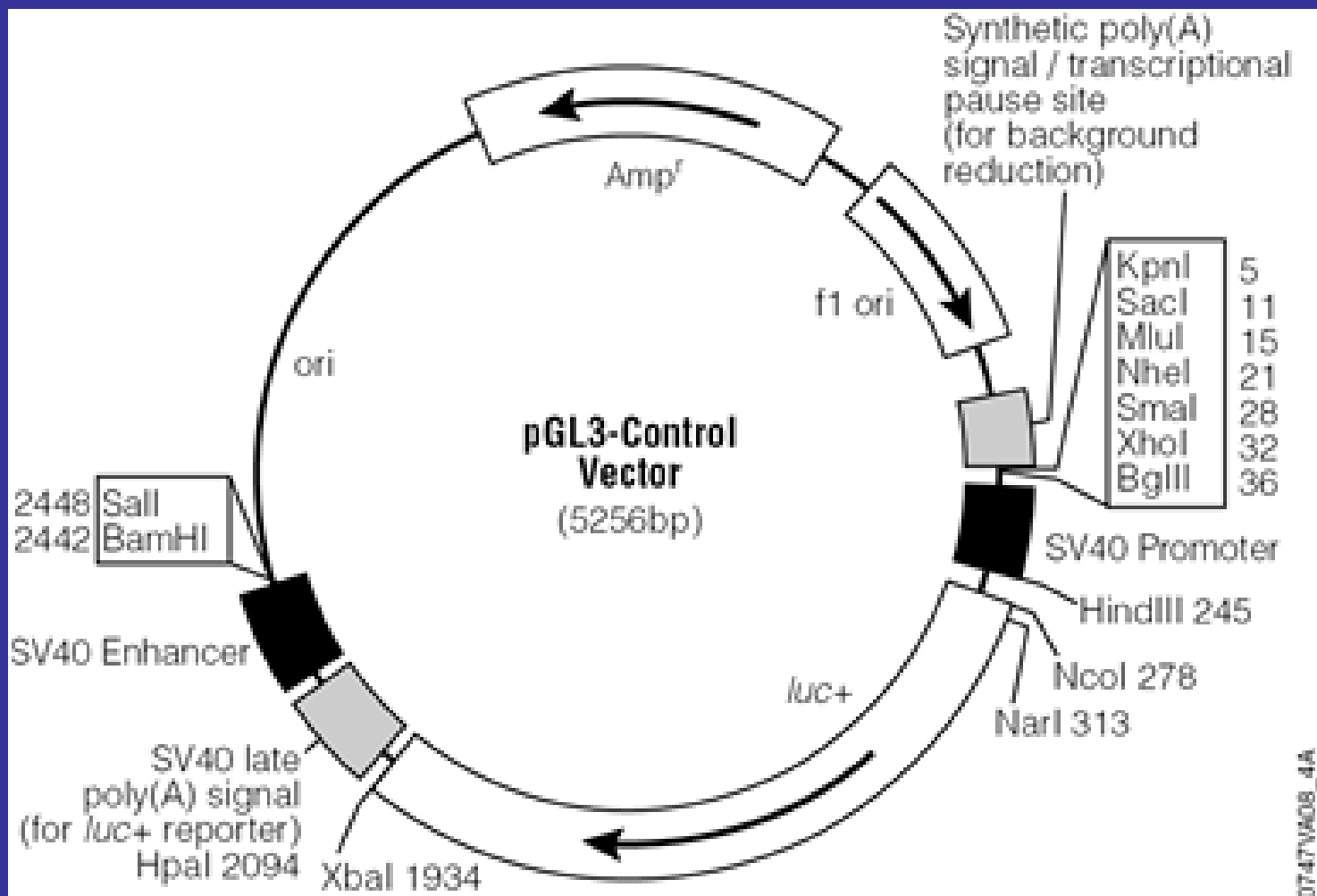






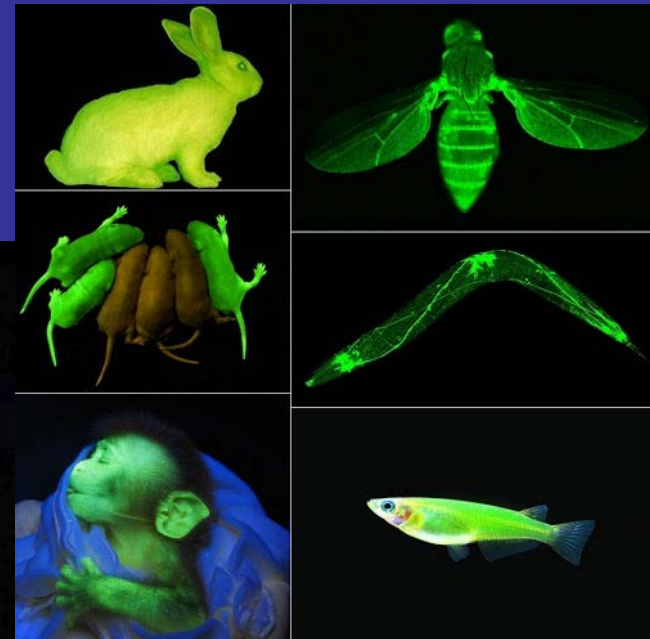
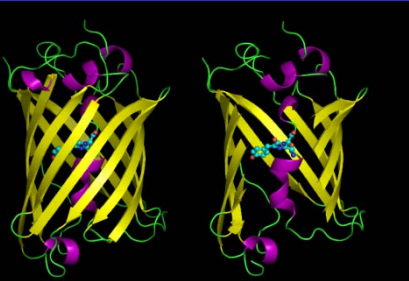






# *Green Fluorescent Protein (GFP)*

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

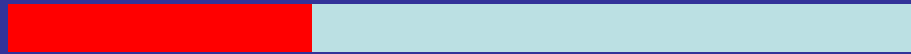


# *Use of Green Fluorescent Protein (GFP)*

- 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

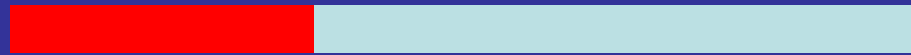
Can be used to  
visualize the  
expression of  
Gene A

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

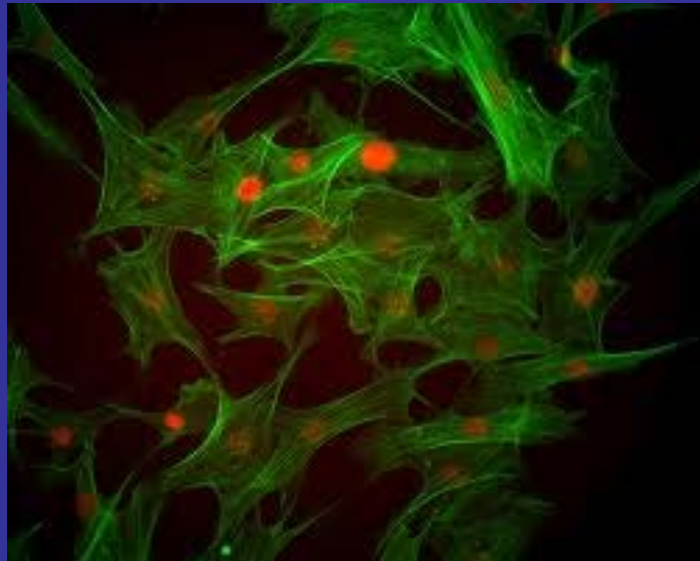
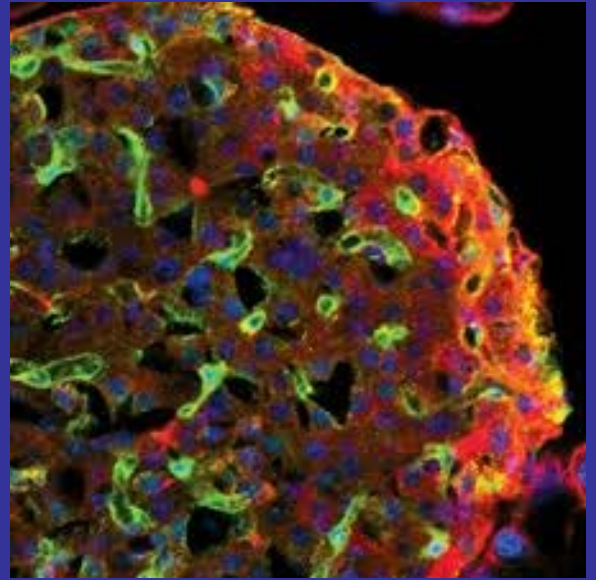
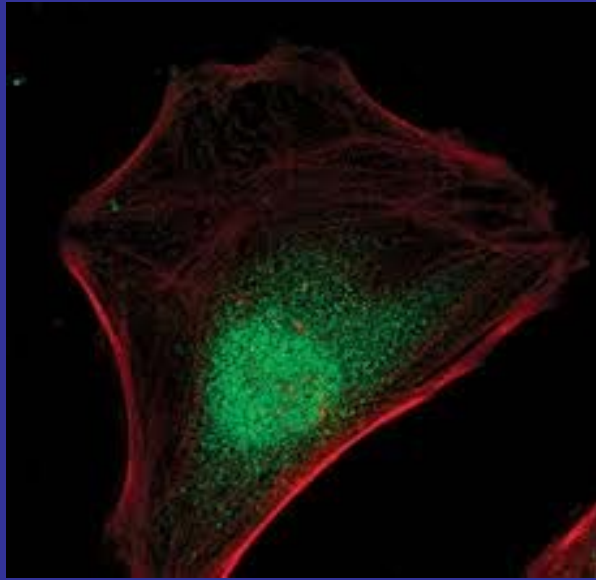
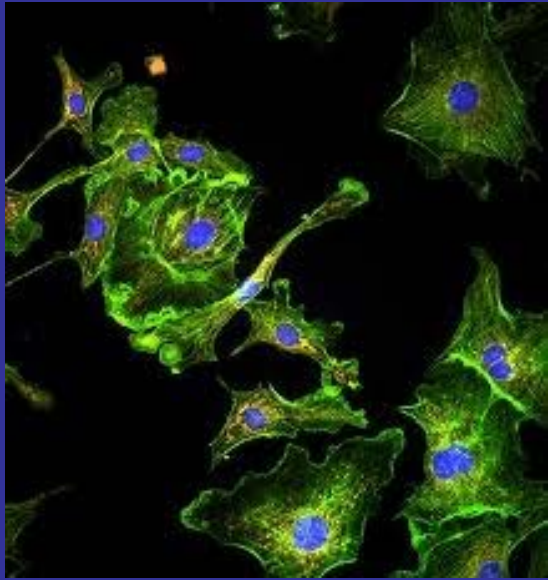


Promoter  
for Gene A

Coding region  
For Gene A

Coding region for  
GFP

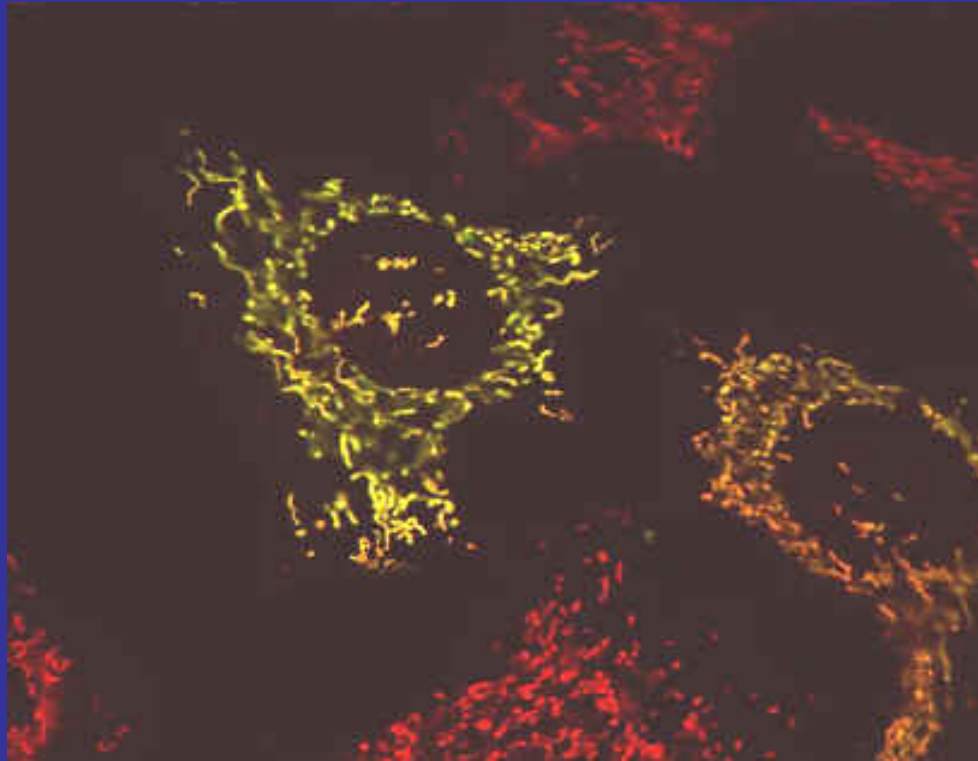
Can be used to visualize the subcellular location of the protein encoded by Gene A



## 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



- As Two-Color Splicing Reporter

