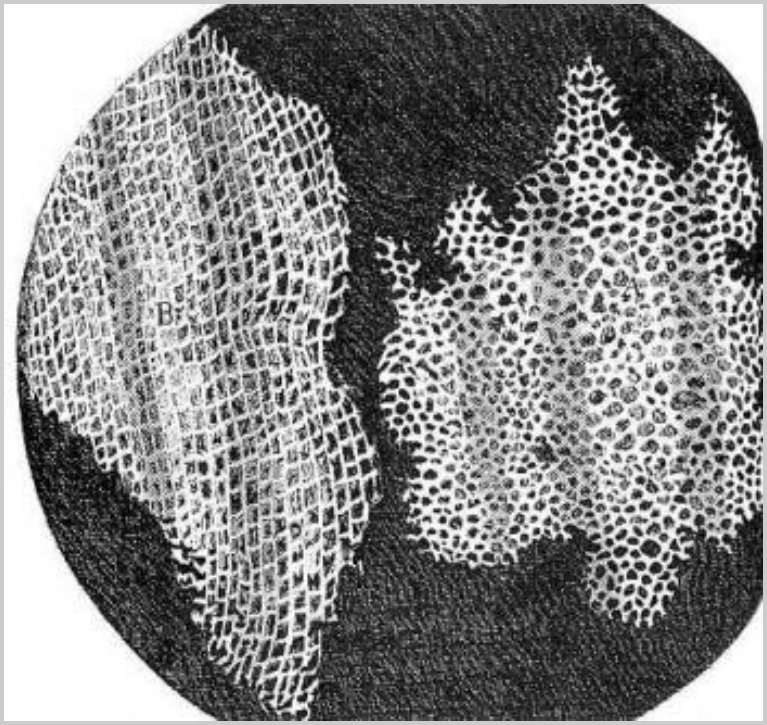
PLANTS MAKE US HAPPY

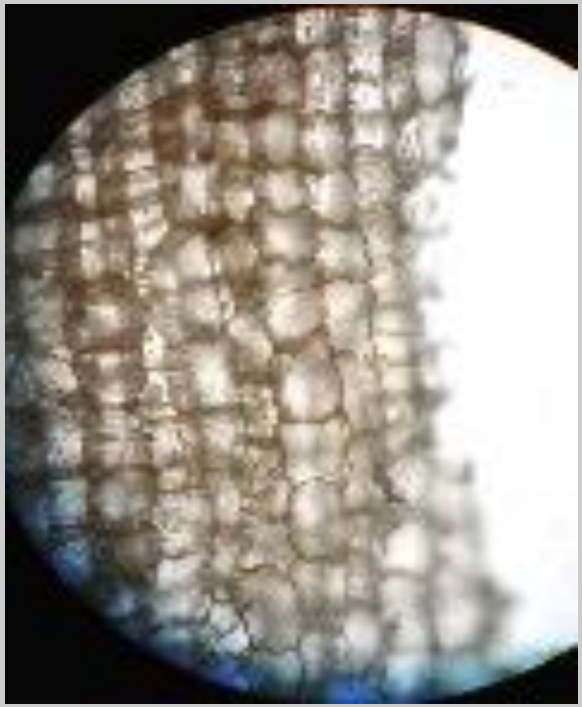
People at work who can see plants report significantly greater job satisfaction than those who can't.

Dravigne, A., Waliczek, T.M., Lineberger, R.D., Zajicek, J.M. (2008) The effect of live plants and window views of green spaces on employee perceptions of job satisfaction. HortScience 43: 183–187. Photo credit: tom donald

Cells were first observed in plants.



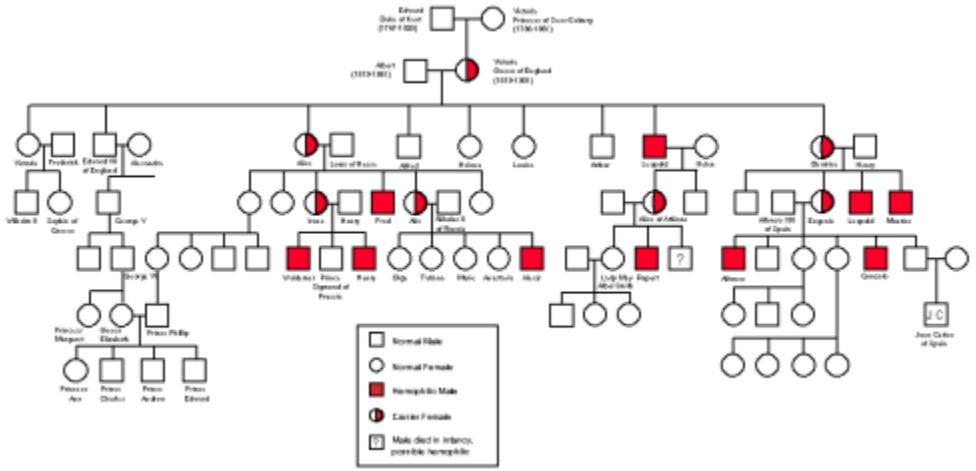
Drawing of cork by Robert Hooke, discoverer of “cells”



Photograph of cork cells

Photo credit: ©David B. Fankhauser, Ph.D

Mendel's observation of peas revealed the laws of inheritance



Experimental biology

Observation >

Description>

Question?>

Manipulation = Experiment >

Answering question (Understanding ?)

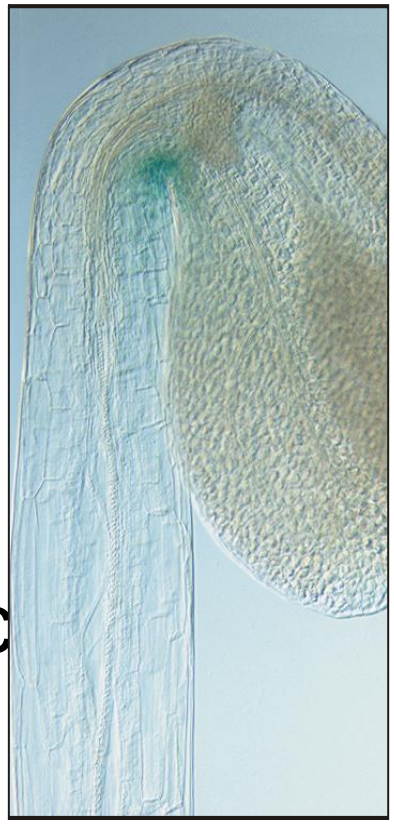
↓

More questions

Experimental biology

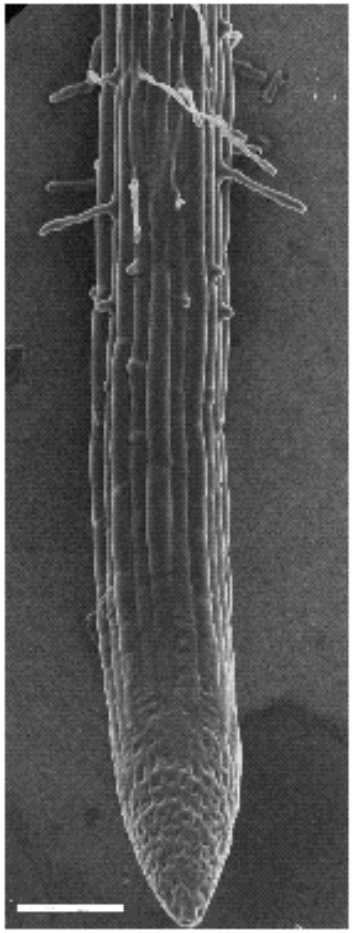
Observation > Manipulation > Understanding

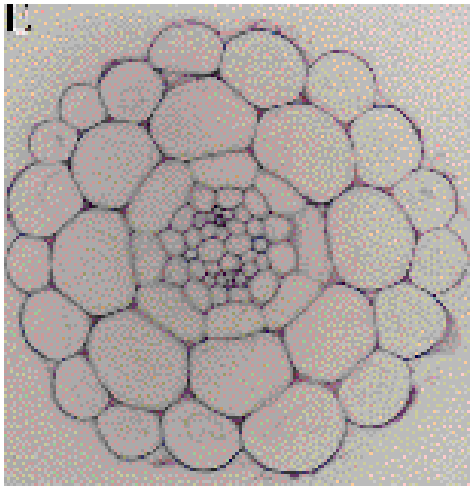
Money > Applications > Publishing

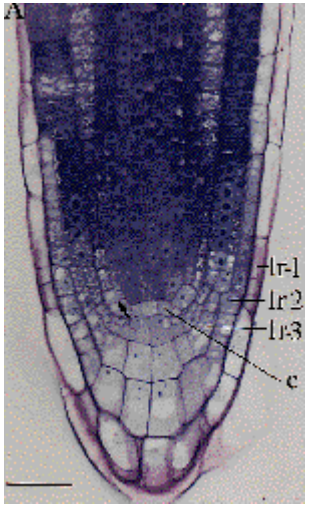


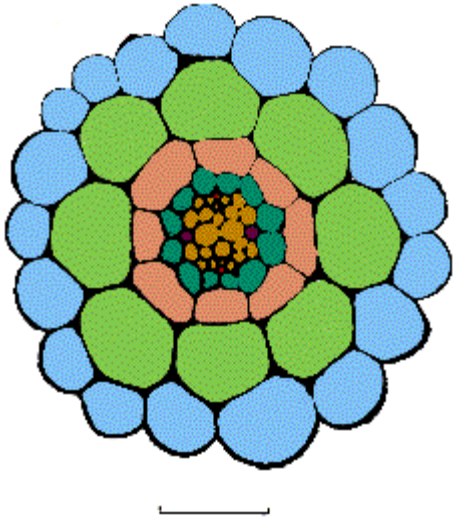
- Anatomy
- Physiology (spray and pray)
- Chemistry (identification of signals)
- Biochemistry (protein isolation/struc
- Genetics (genes/mutants)
- Cell biology (subcellular structures)
- Molecular biology (gene manipulation)

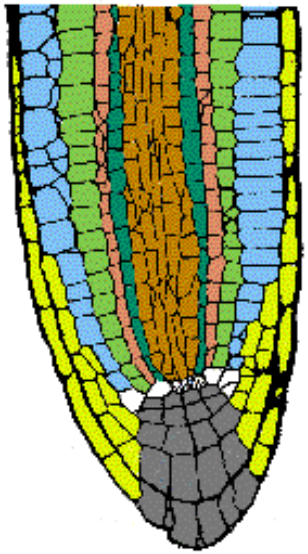
Anatomy





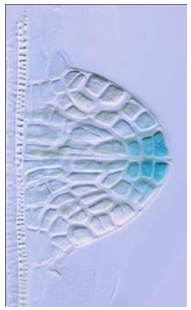






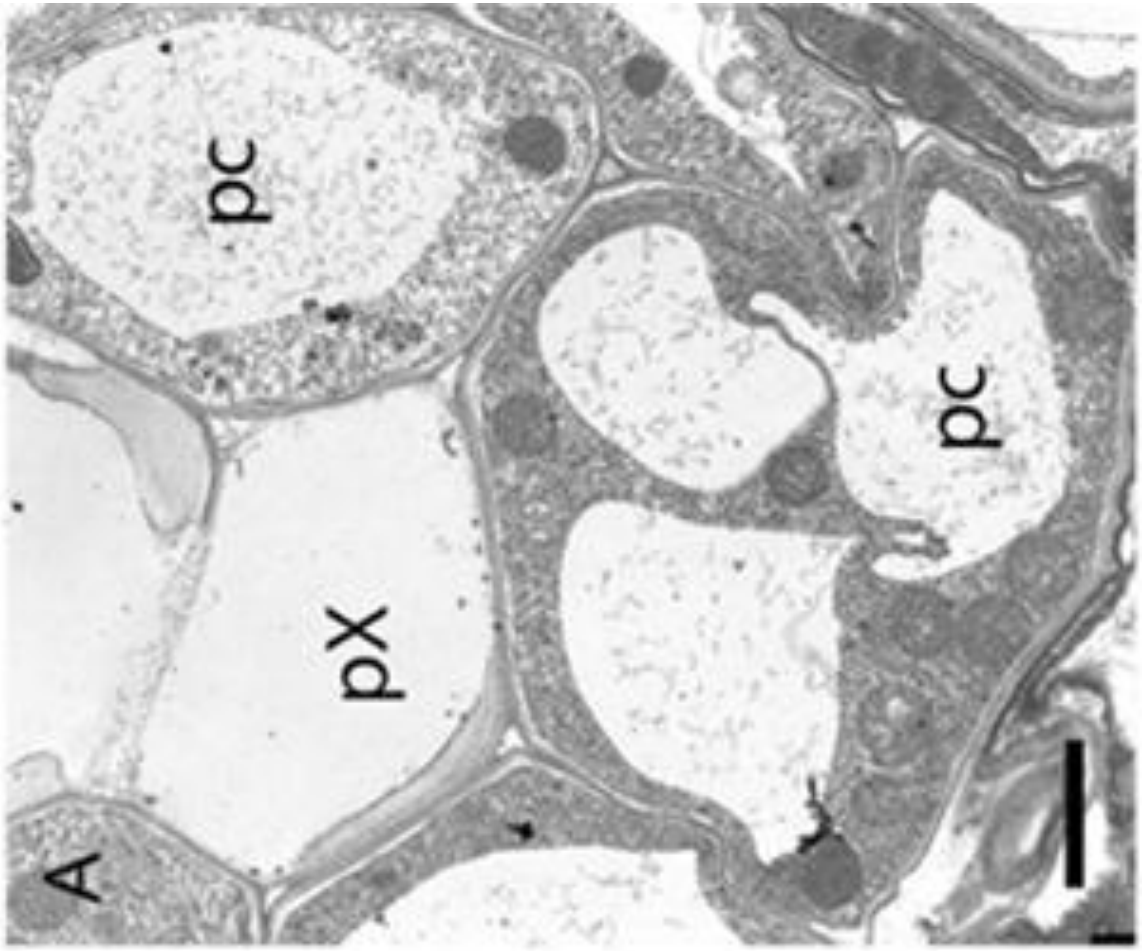
Following process in time and with markers

EMERGENCE



MI

Cell biology (subcellular structures)

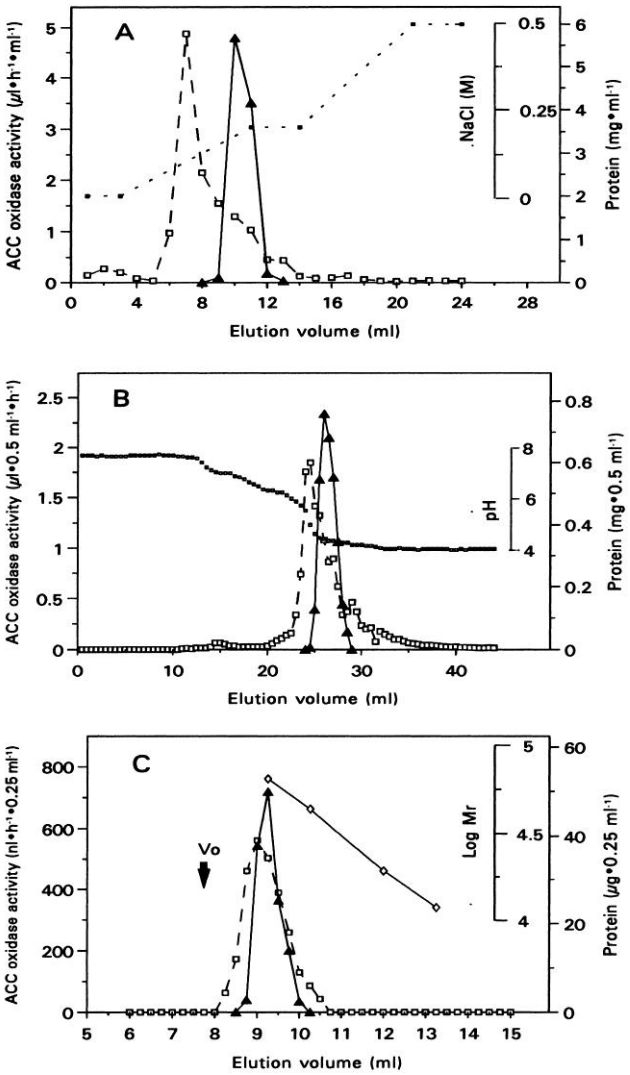


Biochemistry

1-aminocyclopropane-1-carboxylate

↓ ACC oxidase

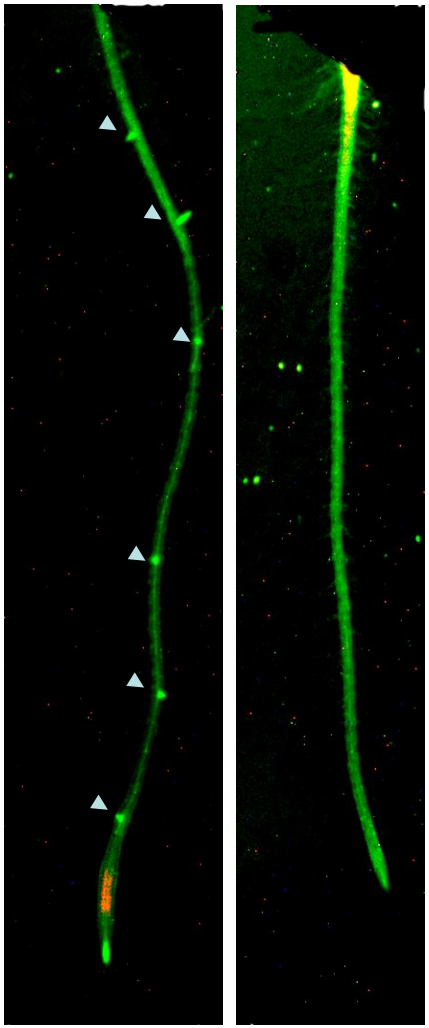
Ethylene

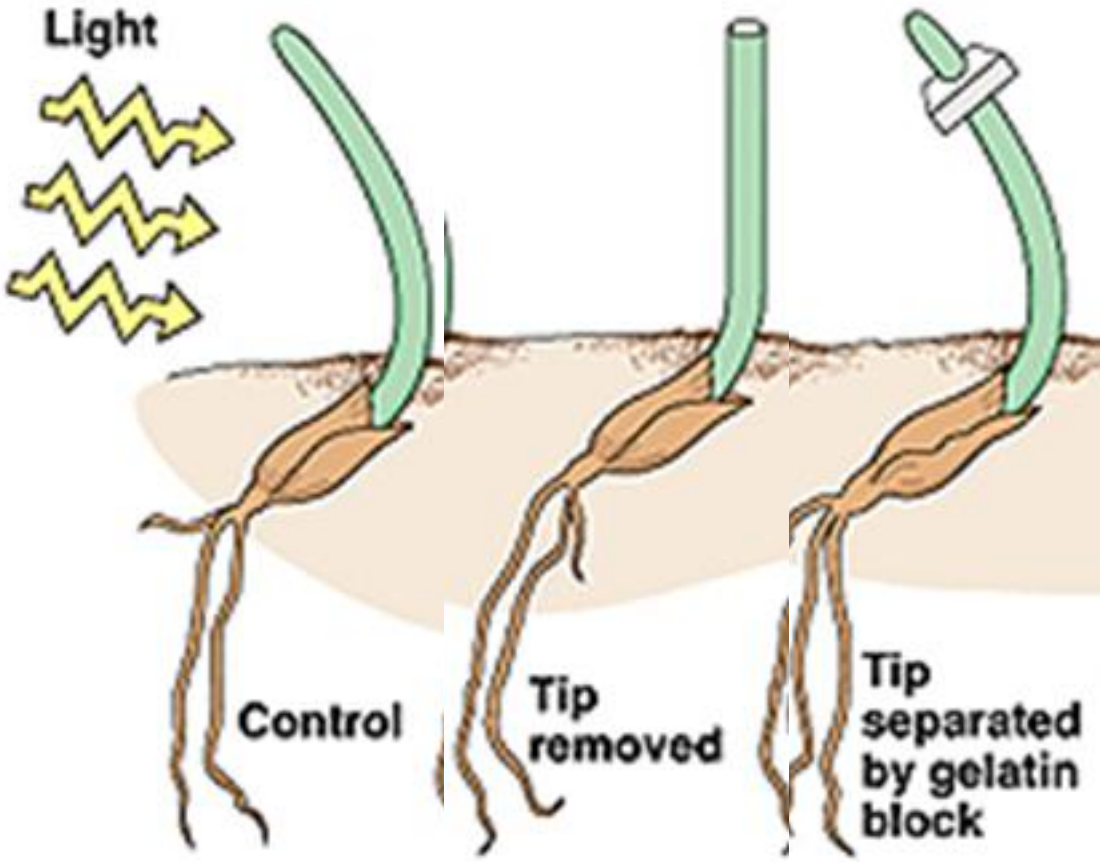


Elution profiles of the purification of ACC oxidase activity from cherimoya fruit after passage through (A) Mono Q anion exchange column, (B) Mono P chromatophocusing column, and (C) Sephadex G-75 gel filtration column (, ACC oxidase activity; , protein).

Chemistry (identification of signals)

water *extract X*





Physiology (spray and pray)

-nutrients (N,P,Ca,)
-conditions (water, light, stress)
-compounds (hormones, chemicals)

inhibitor

stimulator

Genetics (genes/mutants)

Choice of research topic?

-Model system

-Biological process

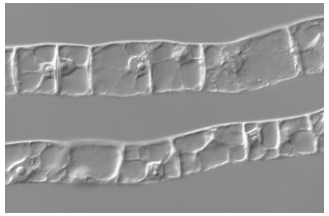
-Gene/Gene family

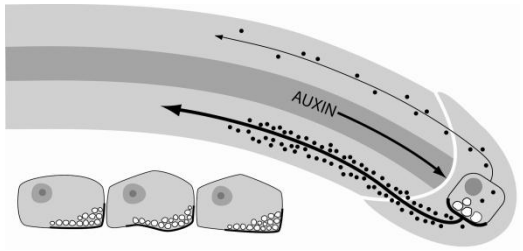
-Signaling pathway

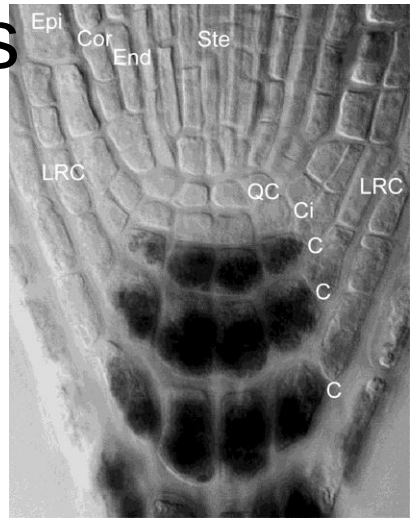
-Available methods

-„Trendy topic“

- Serendipity





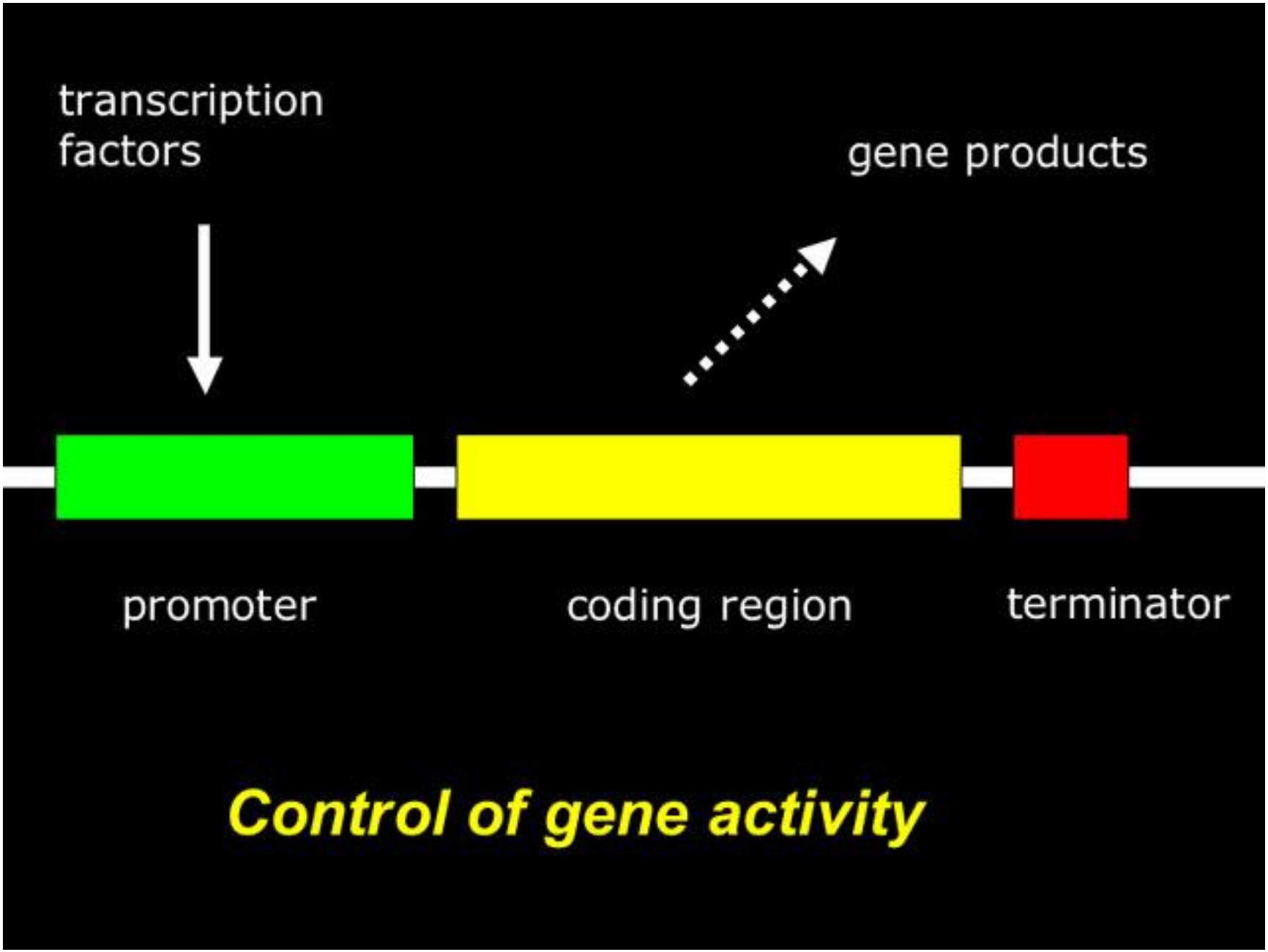


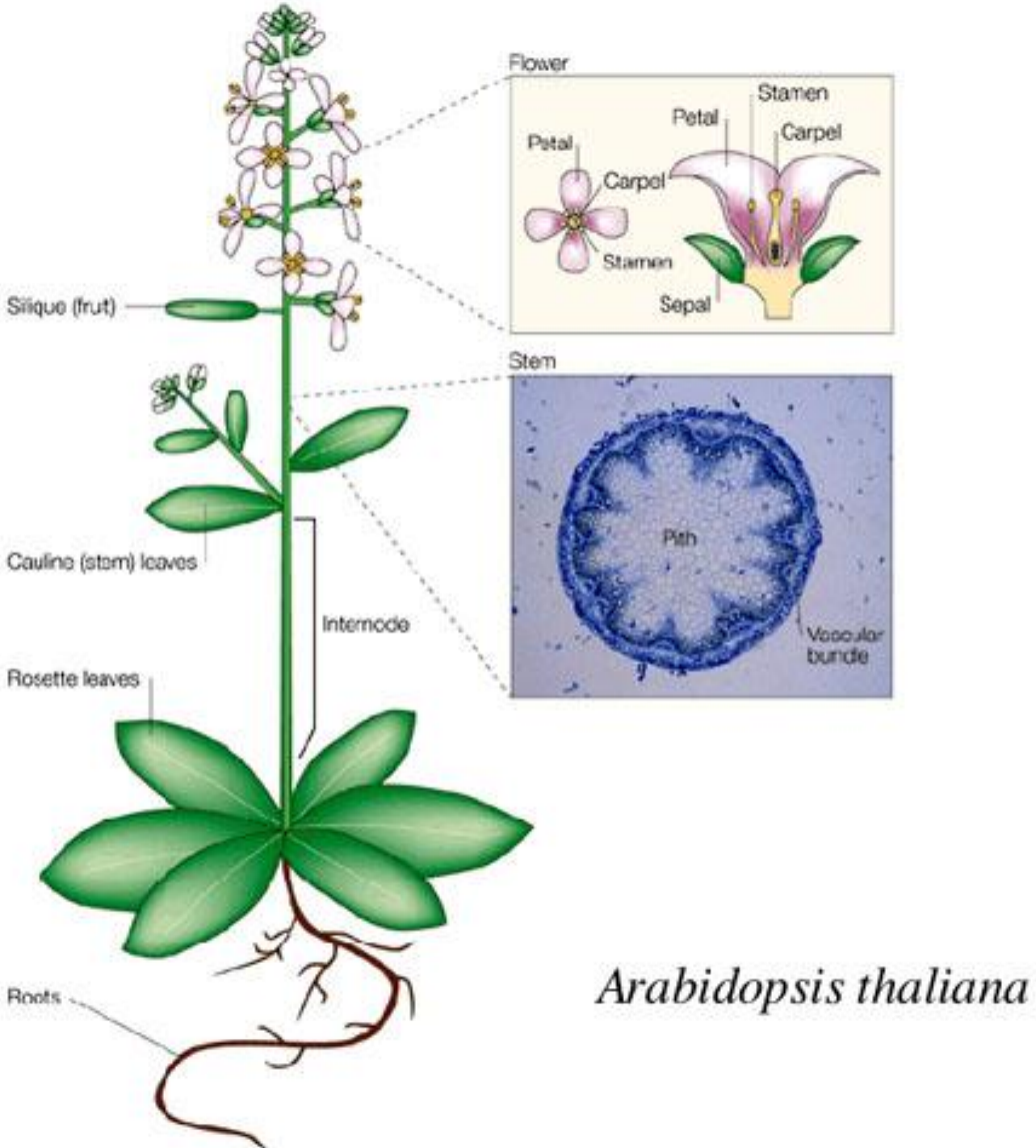
GENES

↓

Molecular biology

gene manipulation, tools to study gene functions


transcription factors
gene products
promoter
coding region
terminator
Control of gene activity



Arabidopsis thaliana

Arabidopsis thaliana

- Small, fully sequenced genome

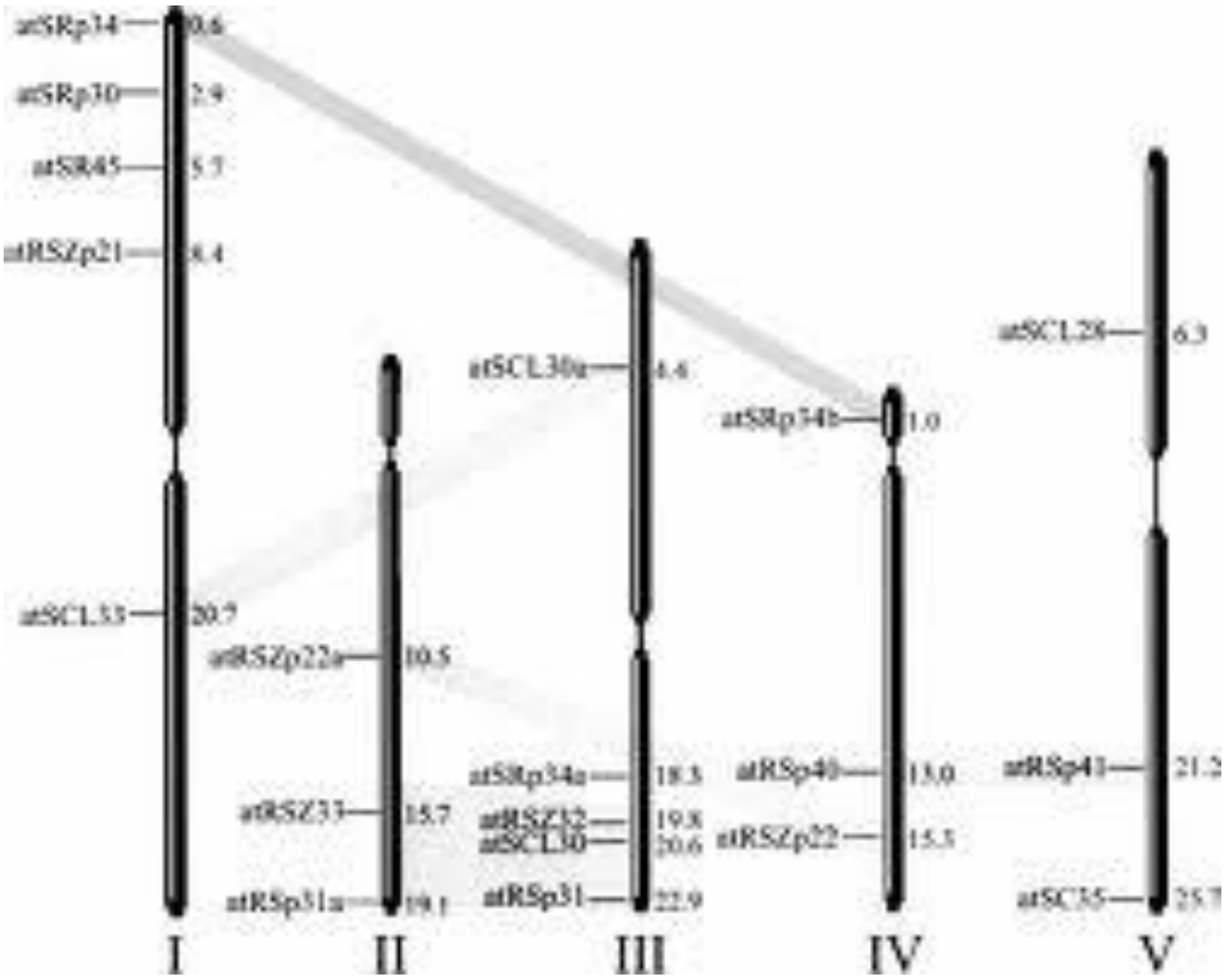
- Easy genetics (diploid/self-polinater)

- Short vegetation time

- No large space requirement

- Simple organ and tissue structure

- Many established tools and facilities
  (transformation, libraries, databases)


atSRp34
0.6
atSRp30
2.9
atSR45
5.3
atRSZp21
8.4
atSCL33
20.7
atRSZp22a
10.5
atRSZ33
15.7
atRSp31a
19.1
atSCL30a
4.4
atSRp34a
18.3
atRSZ32
19.8
atSCL30
20.6
atRSp31
22.9
atSRp34b
1.0
atRSp40
13.0
atRSZp22
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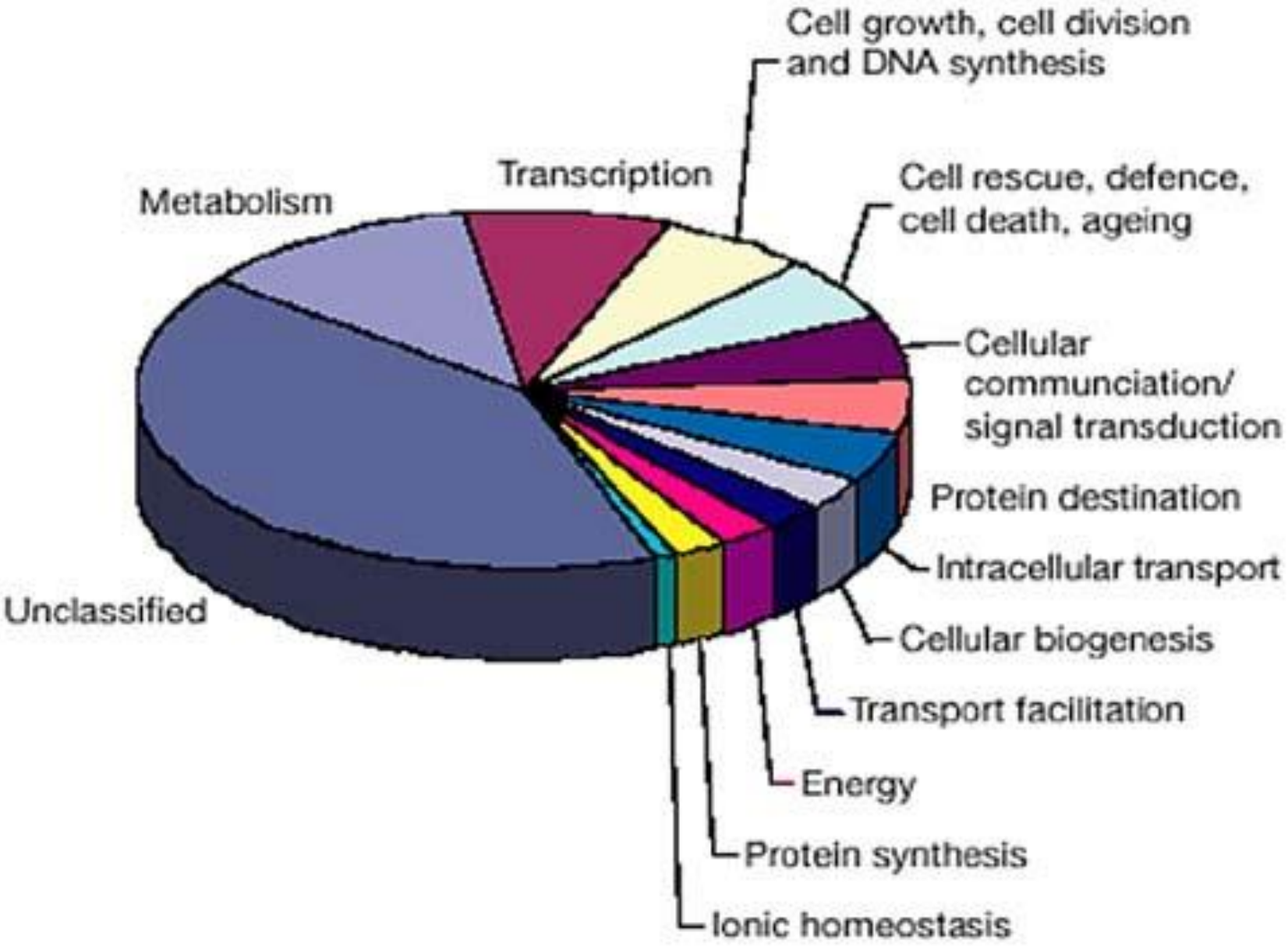
6.3
atRSp41
21.2
atSC35
25.7
I
II
III
IV
V


Cell growth, cell division and DNA synthesis
Transcription
Metabolism
Cell rescue, defence, cell death, ageing
Cellular communciation/ signal transduction
Protein destination
Intracellular transport
Unclassified
Cellular biogenesis
Transport facilitation
Energy
Protein synthesis
Ionic homeostasis

Genes

-choice (???)

-function - lost
- enhanced
- modulated

-expression - where (which tissue)
-control of expression (TF, upstream network)
- missexpression

-interacting network – look for partners

How to get your favorite gene?

- “Monte Carlo” candidate gene approach

- Functional complementation

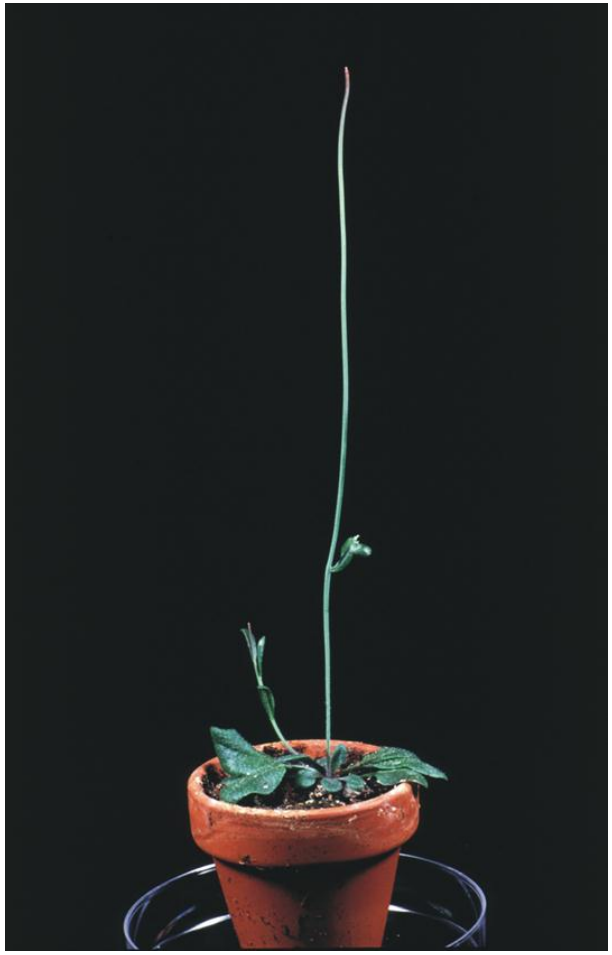
- From the protein back to the gene

- Expression

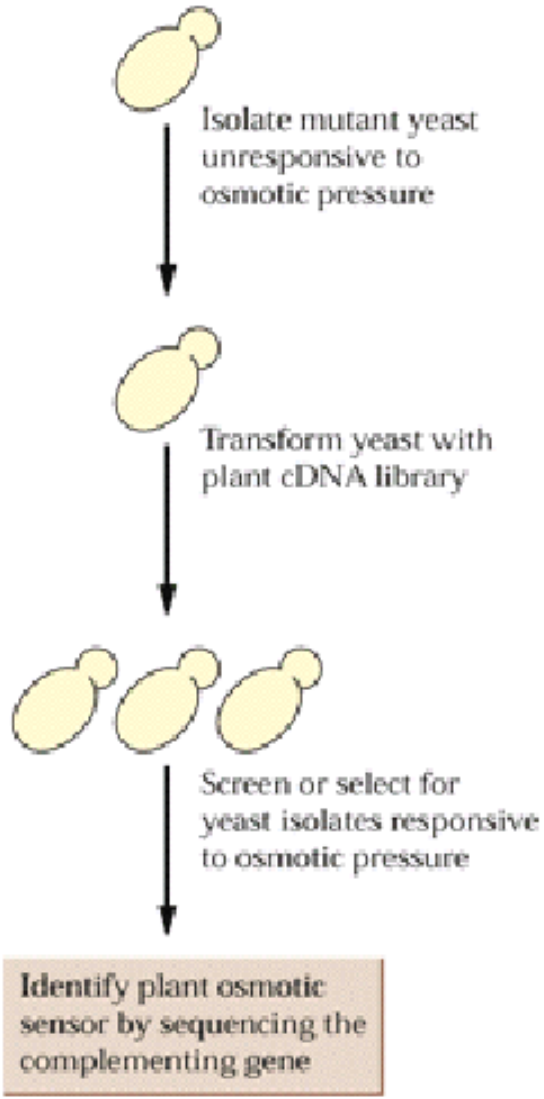
- Forward genetics

“Lottery”

- Homology to known factors
  (trimeric G-proteins)
- Interesting domains
  (kinases, phosphatases)
- „Other“ reasons
  (serendipity)



Functional complementation



Protein > gene

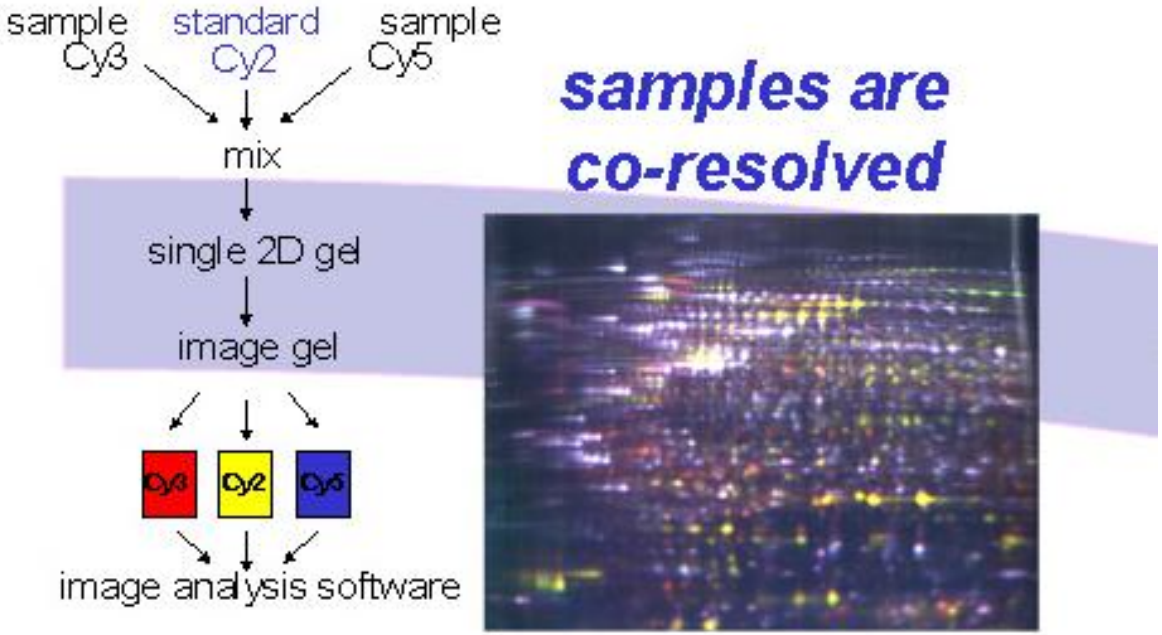
- Enzyme activity (CKX, ACS)

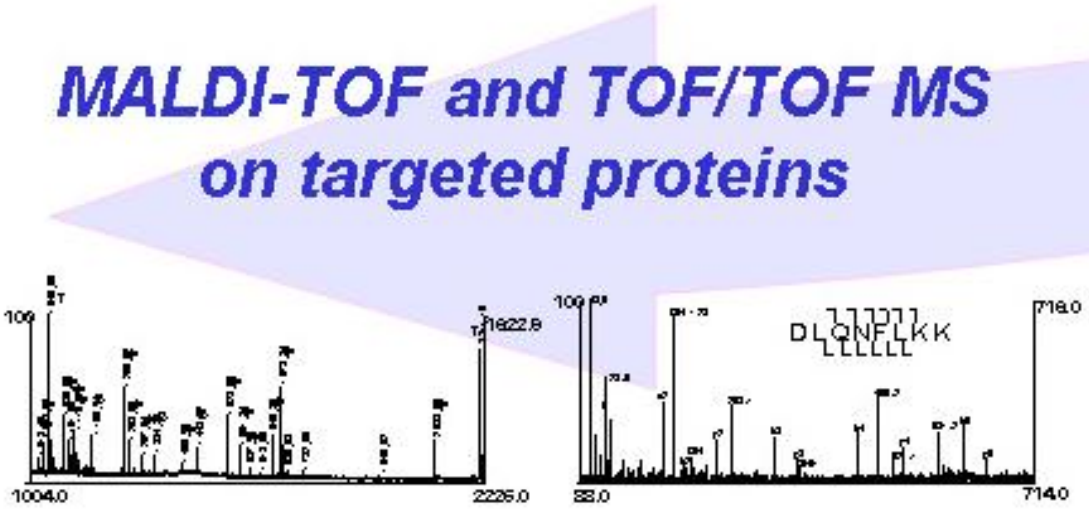
- Ligand binding (affinity chromatography, azidolabeling;
  photoaffinity label azido-[3H]IAA - auxin binding proteins ABP1, Zm-p60,
  Cytokinin – cytokinin binding protein)

- Complex members

- Proteomics approaches
  (differential display, phosphoproteomics,)

Differential Display,



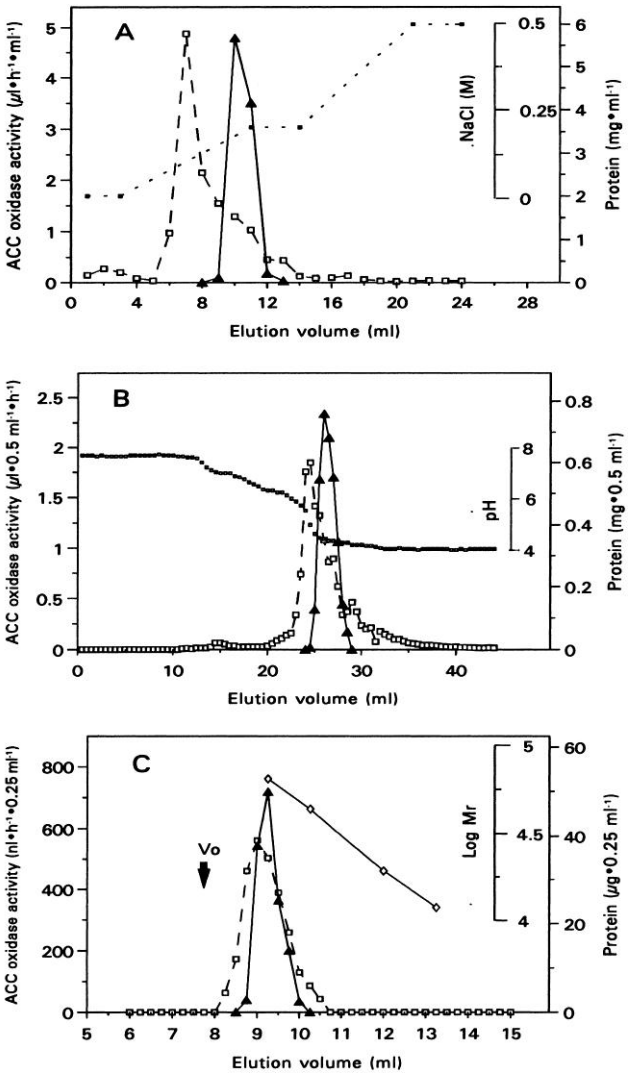


Biochemistry

1-aminocyclopropane-1-carboxylate

↓ ACC oxidase

Ethylene



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Protein > gene

-Microsequencing

- Blast search:

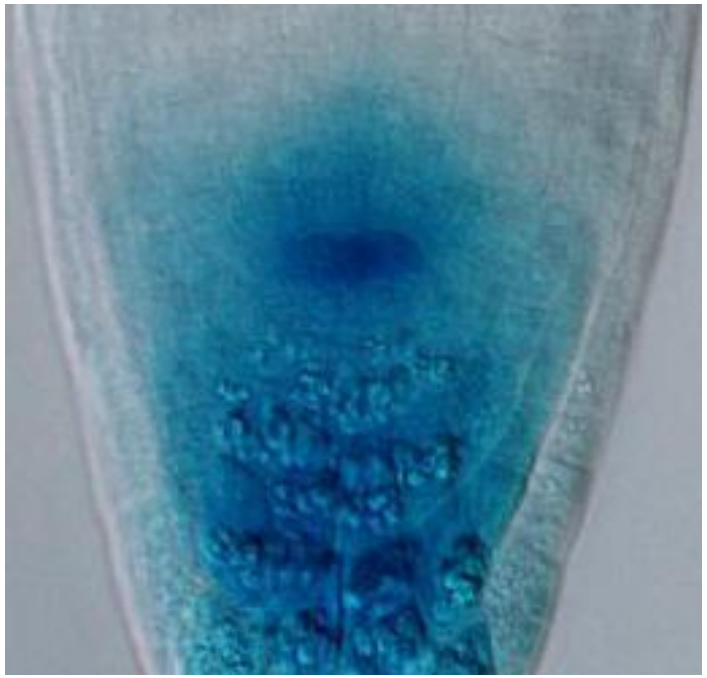
amino acid > nucleotide

- Search for a gene

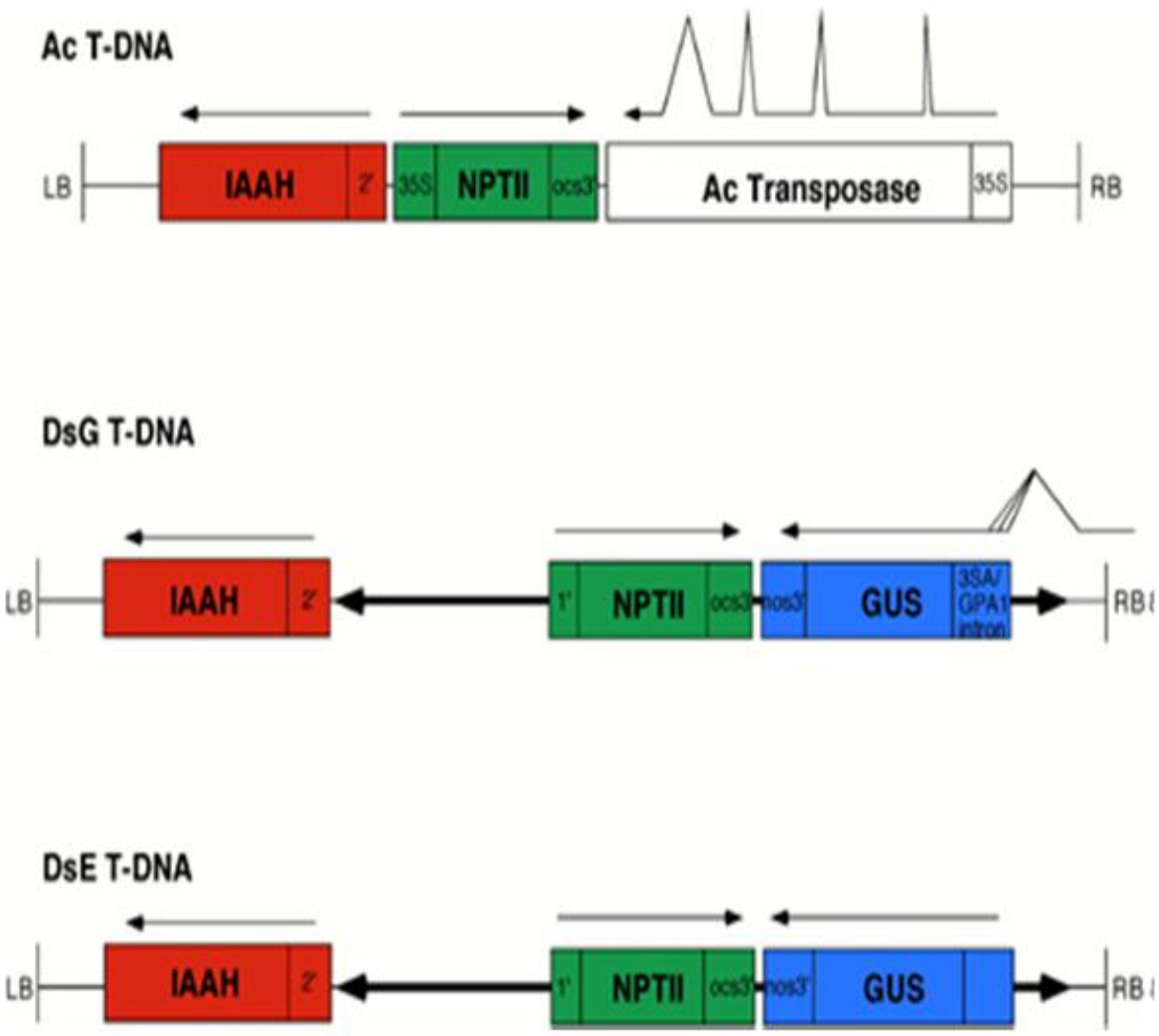
Expression pattern

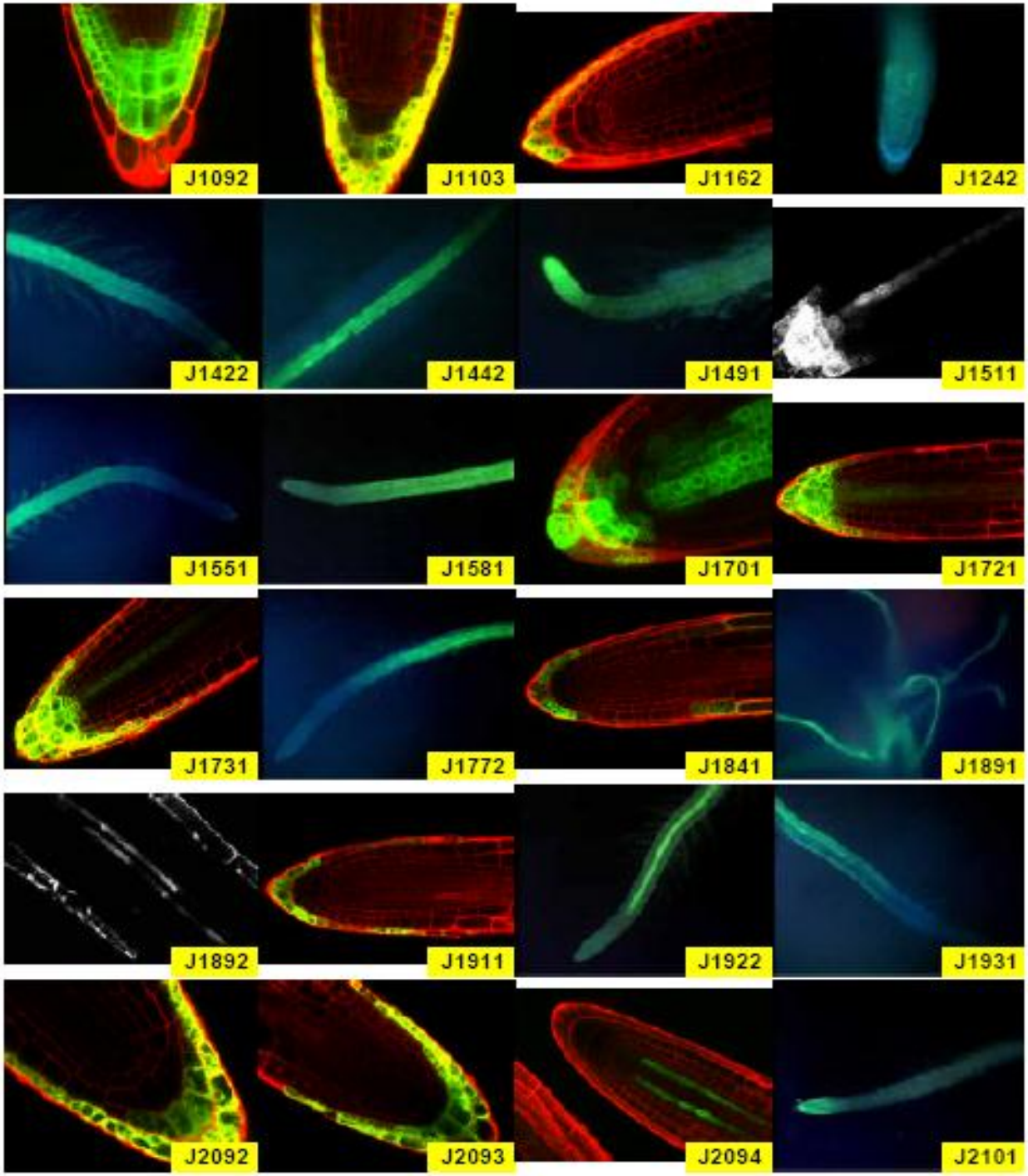
- Enhancer/Gene-trap libraries

- Differential display
  substractive hybridisation
  microarray



Gene and enhancer trap libraries

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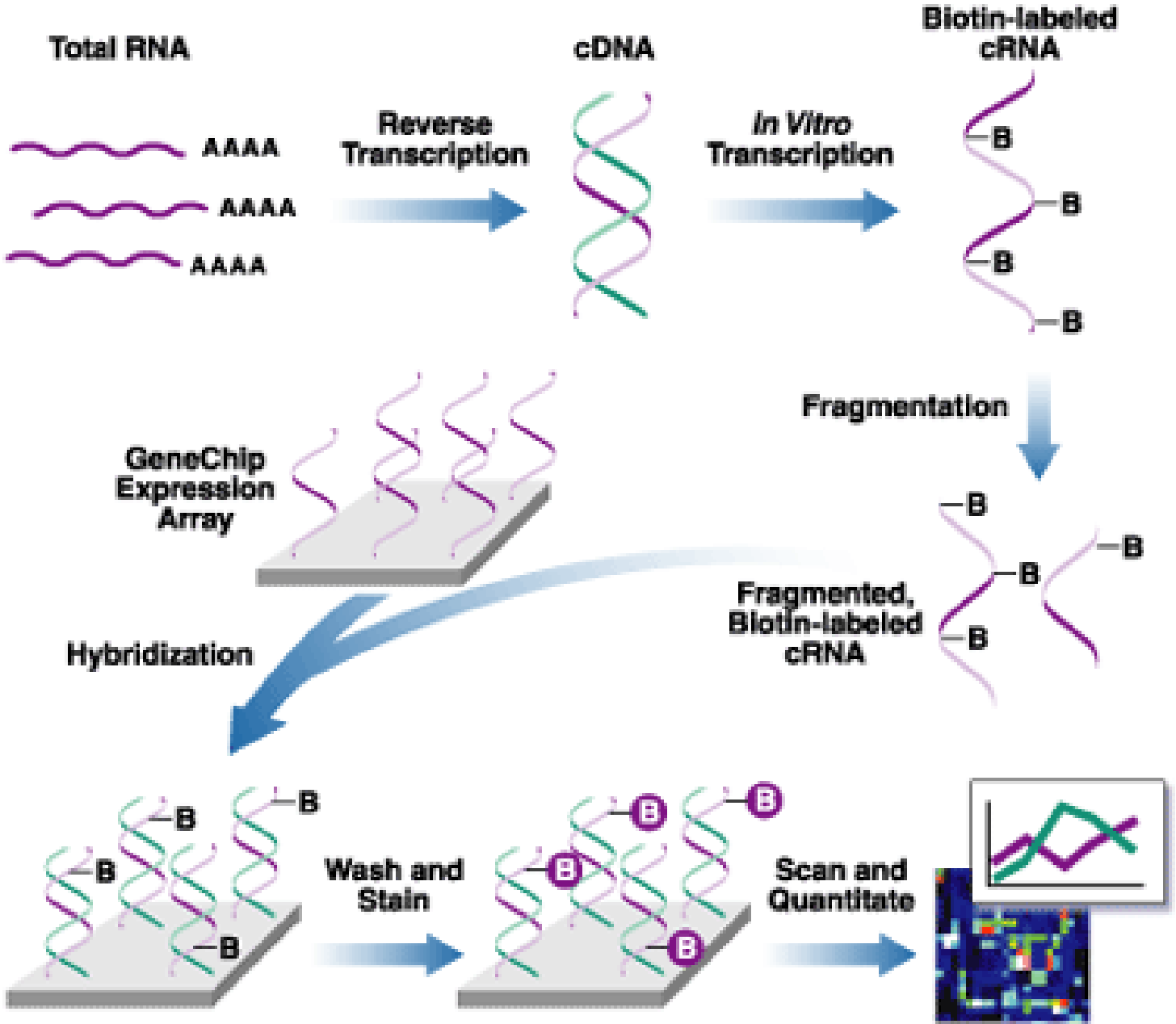
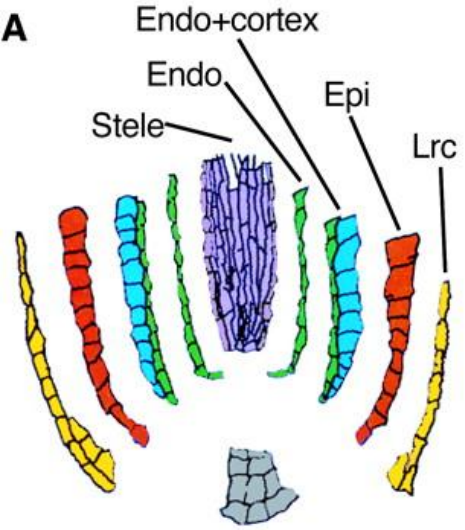
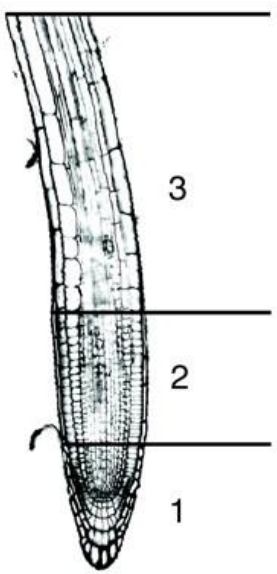
J1162
J1242
J1422
J1442
J1491
J1511
J1551
J1581
J1701
J1721
J1731
J1772
J1841
J1891
J1892
J1911
J1922
J1931
J2092
J2093
J2094
J2101

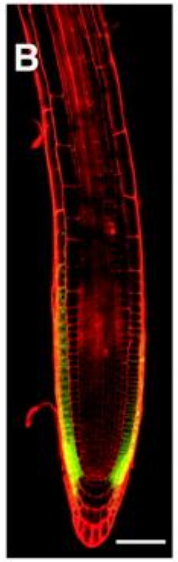
Microarray

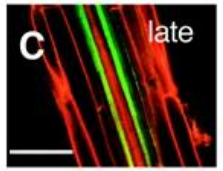

Total RNA
AAAA
AAAA
AAAA
Reverse Transcription
cDNA
In Vitro Transcription
Biotin-labeled cRNA
B
B
B
B
Fragmentation
GeneChip Expression Array
B
B
B
B
Fragmented, Biotin-labeled cRNA
Hybridization
B
B
B
B
Wash and Stain
B
B
B
B
Scan and Quantitate

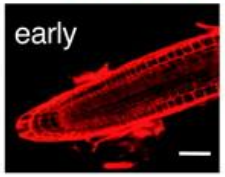
Expression map of Arabidopsis root

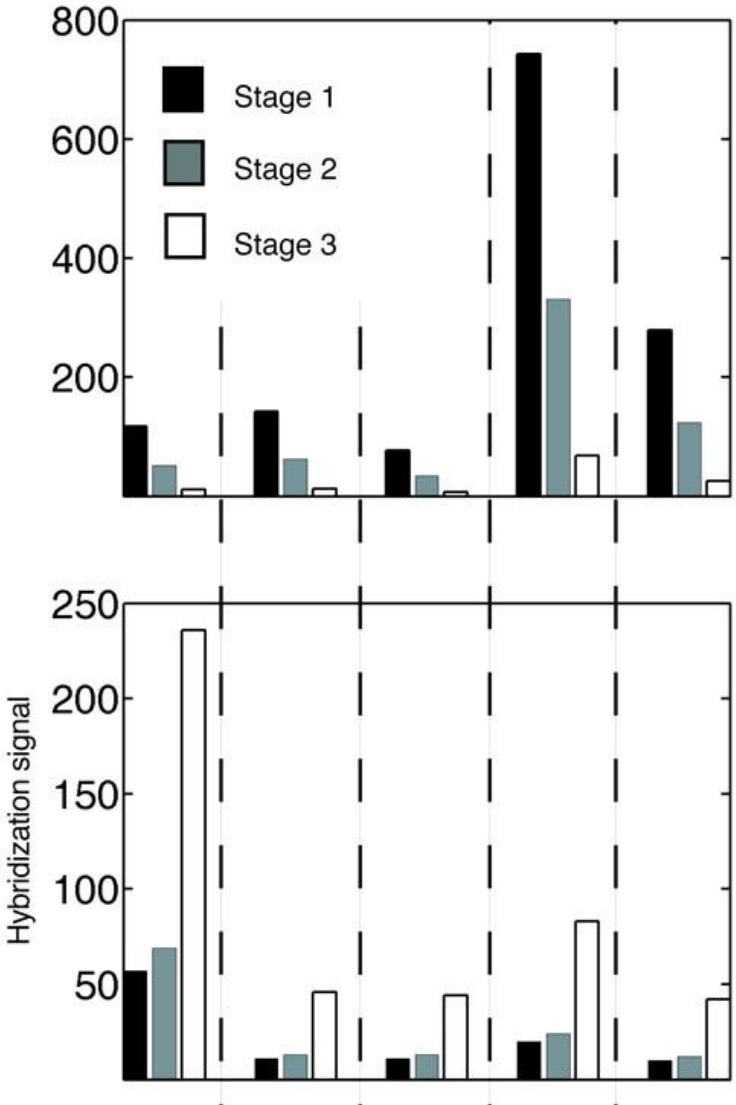




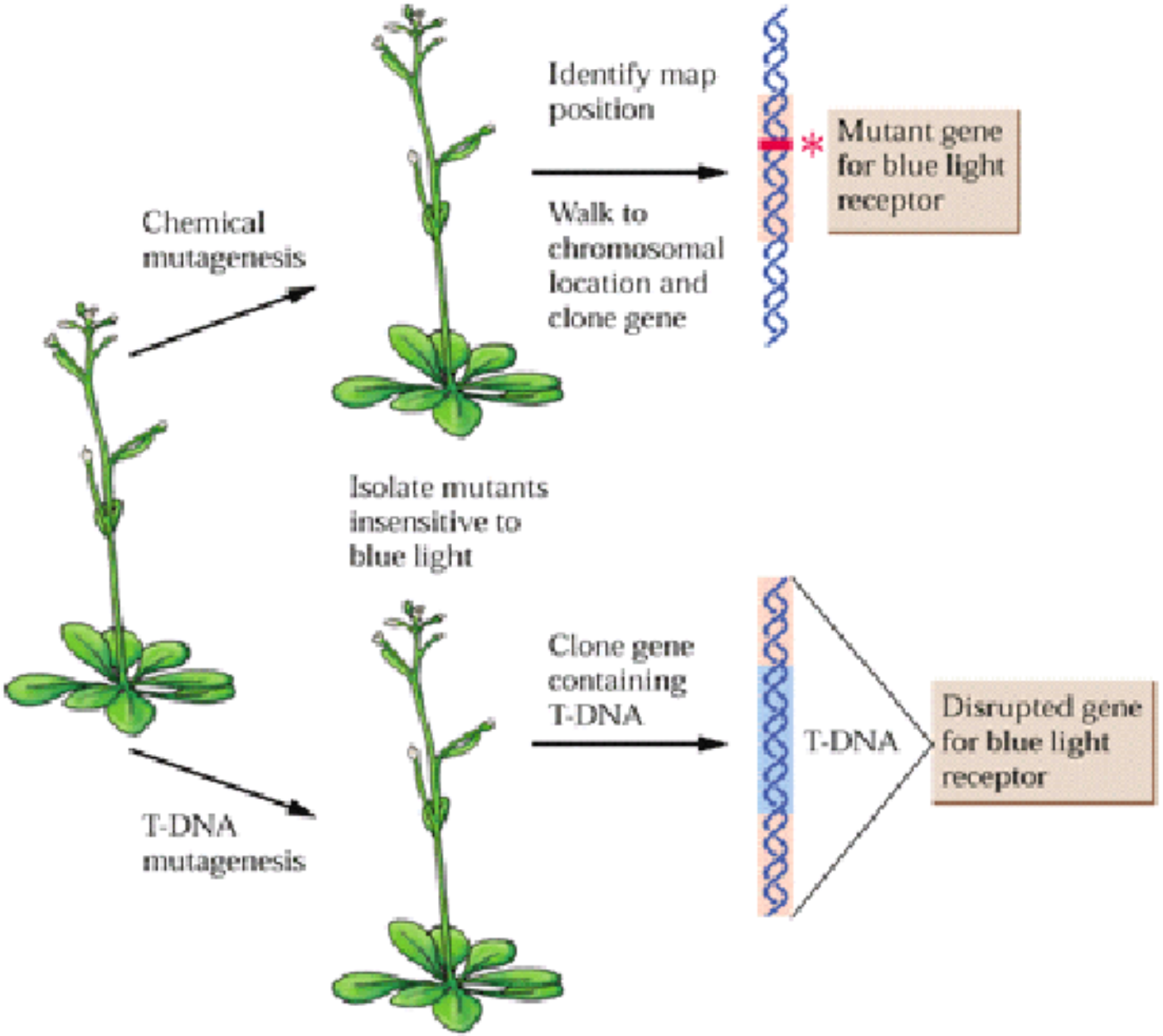




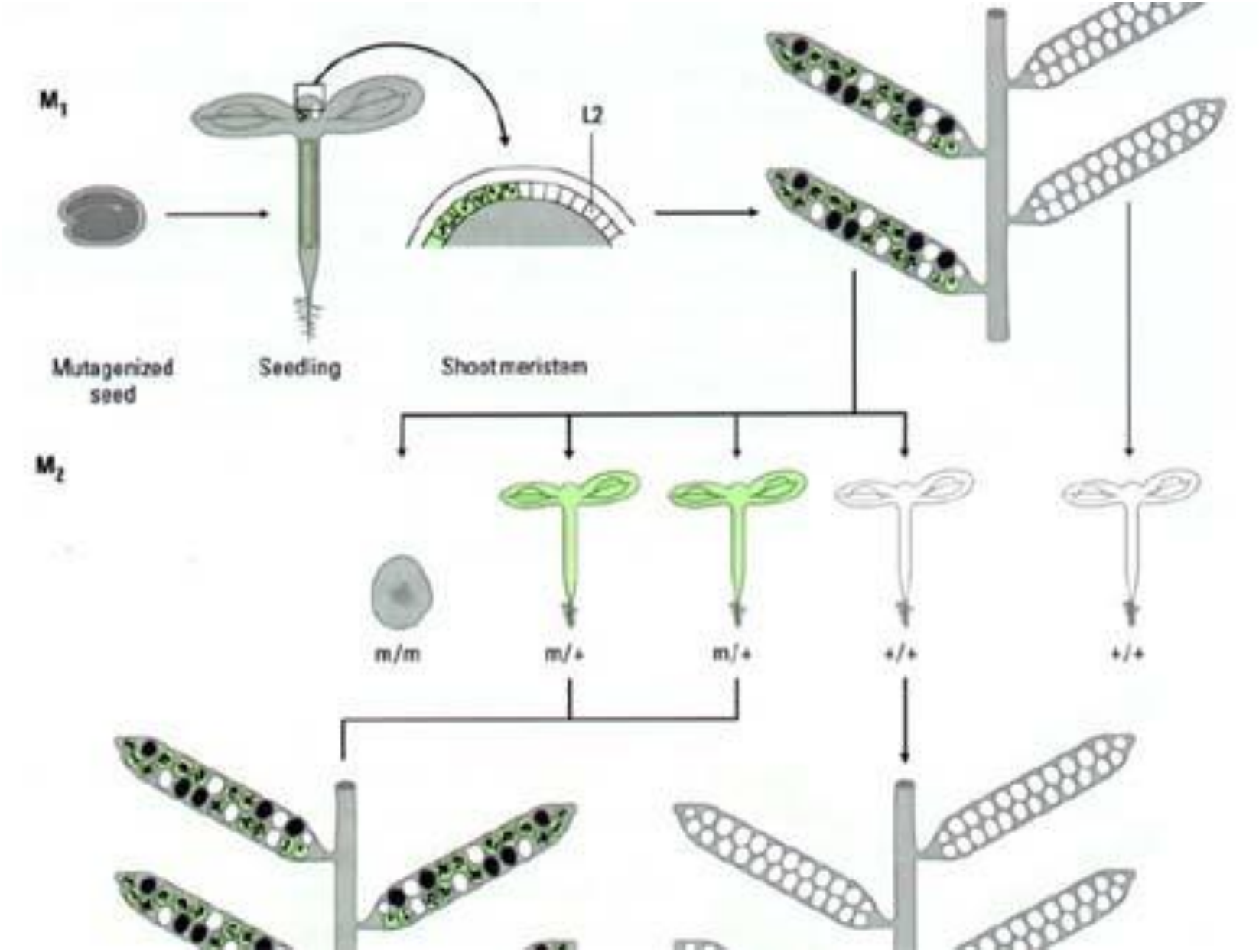




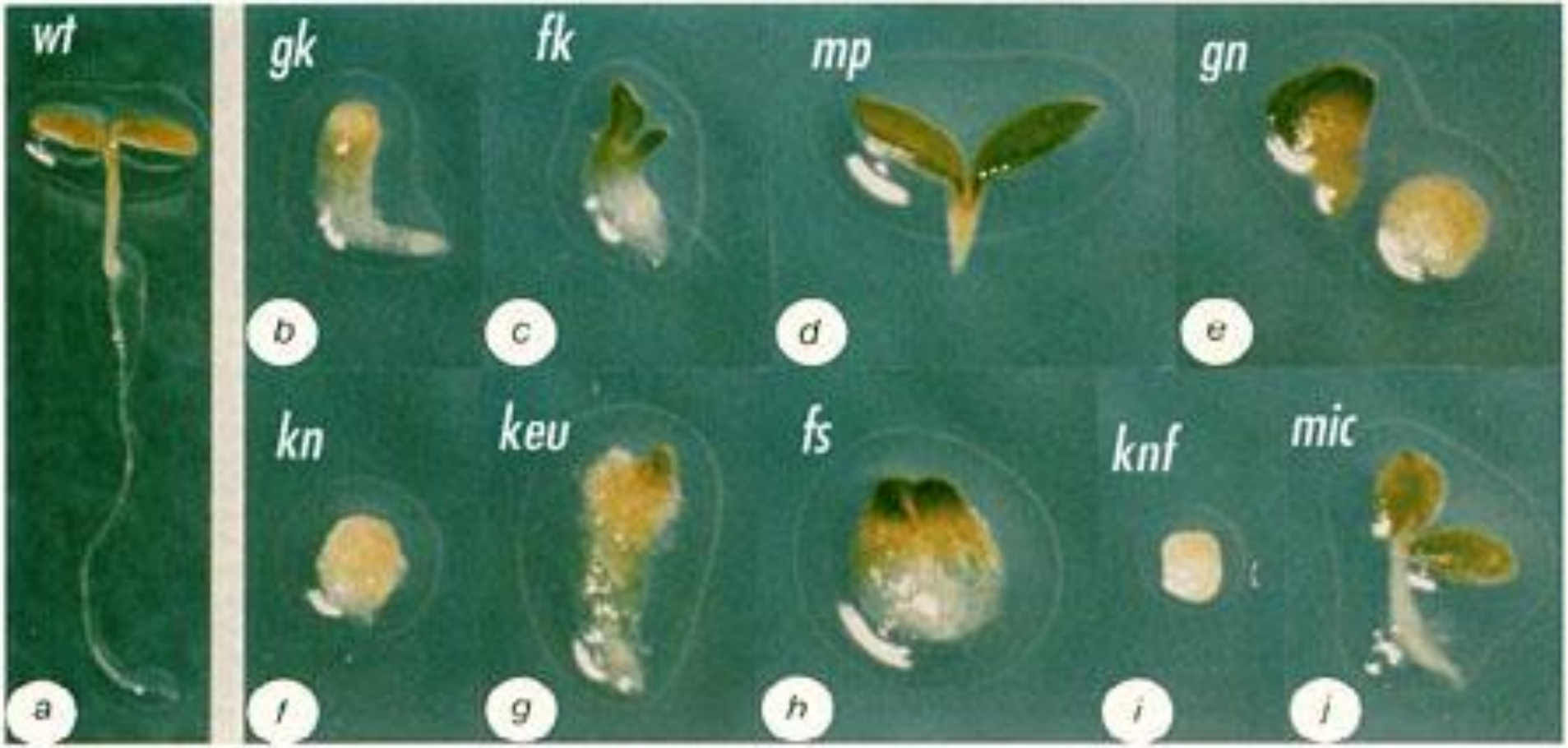
Forward genetics



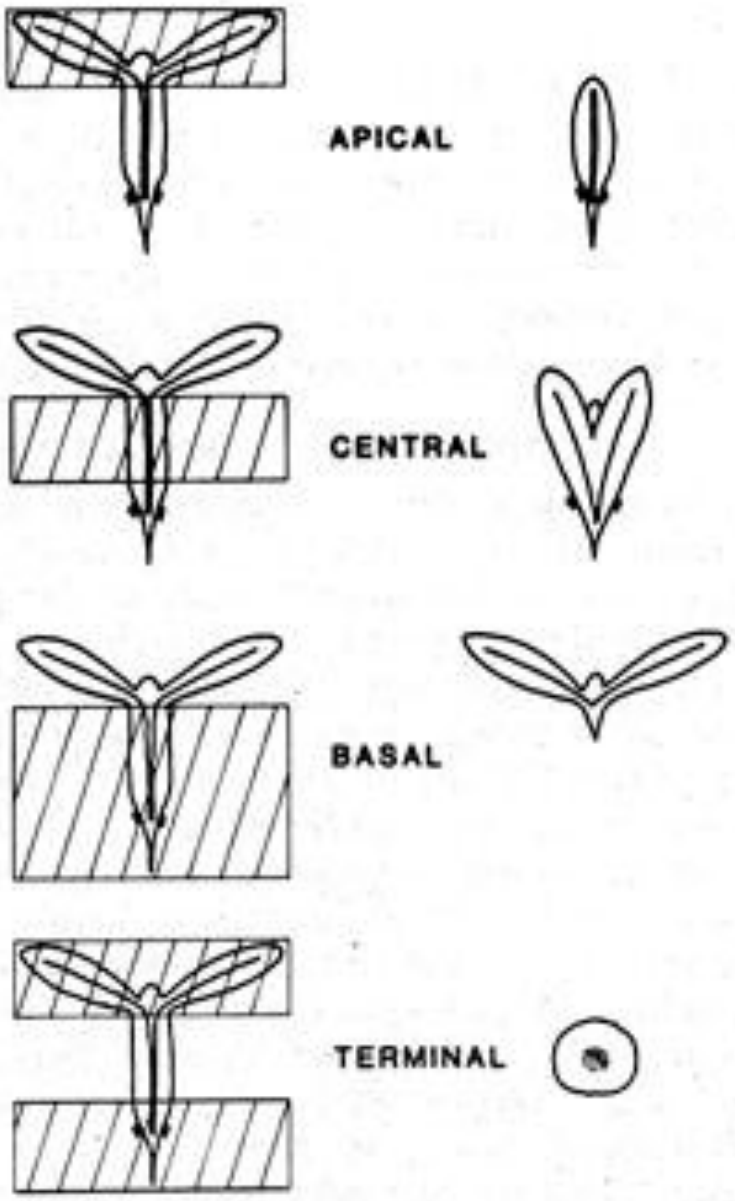
EMS mutagenesis



Mutant screen at seedling level



Patterning mutant types



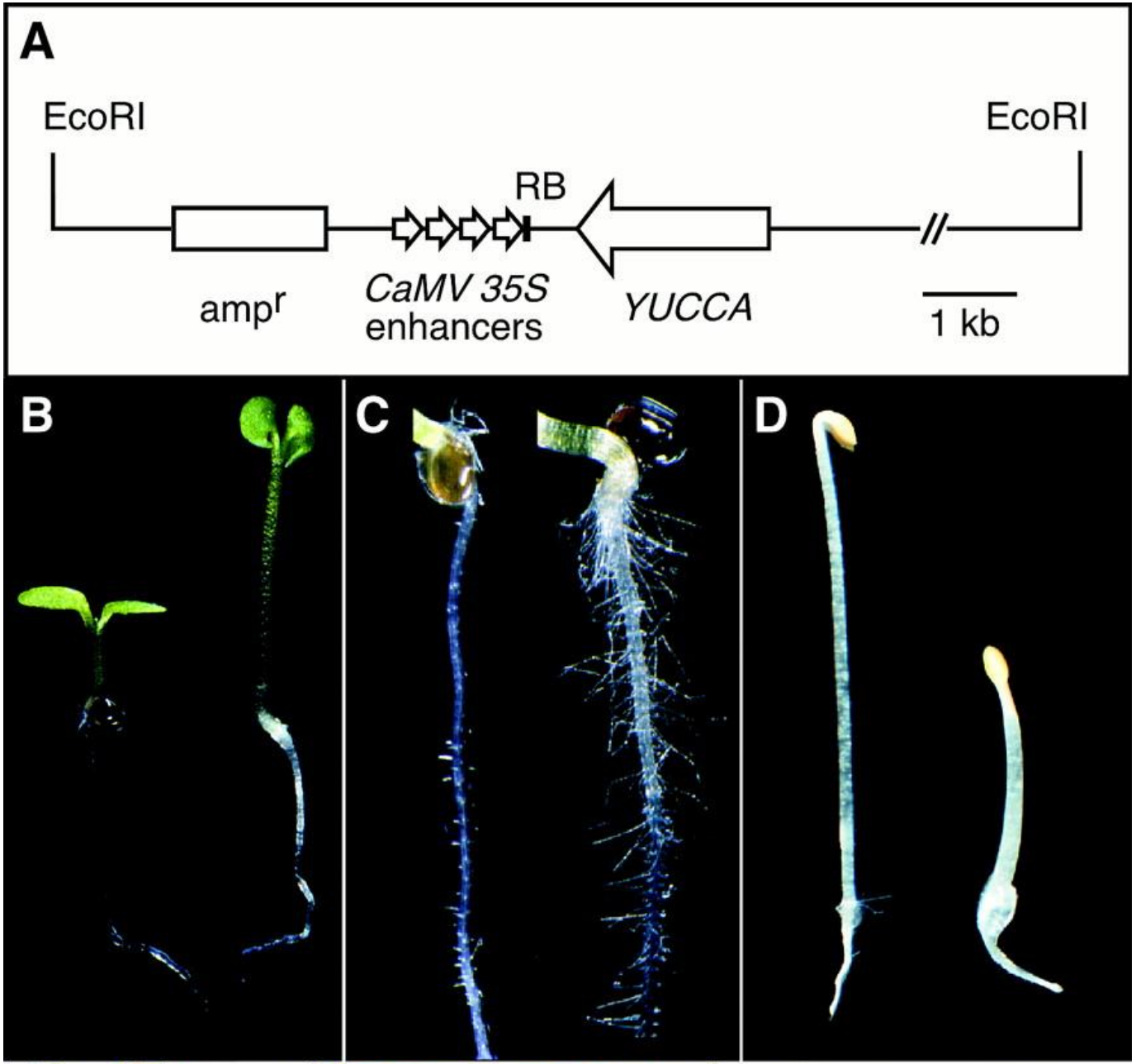
(gurke) Fatty acids

(fackel) Sterols

(monopterous) Signalling

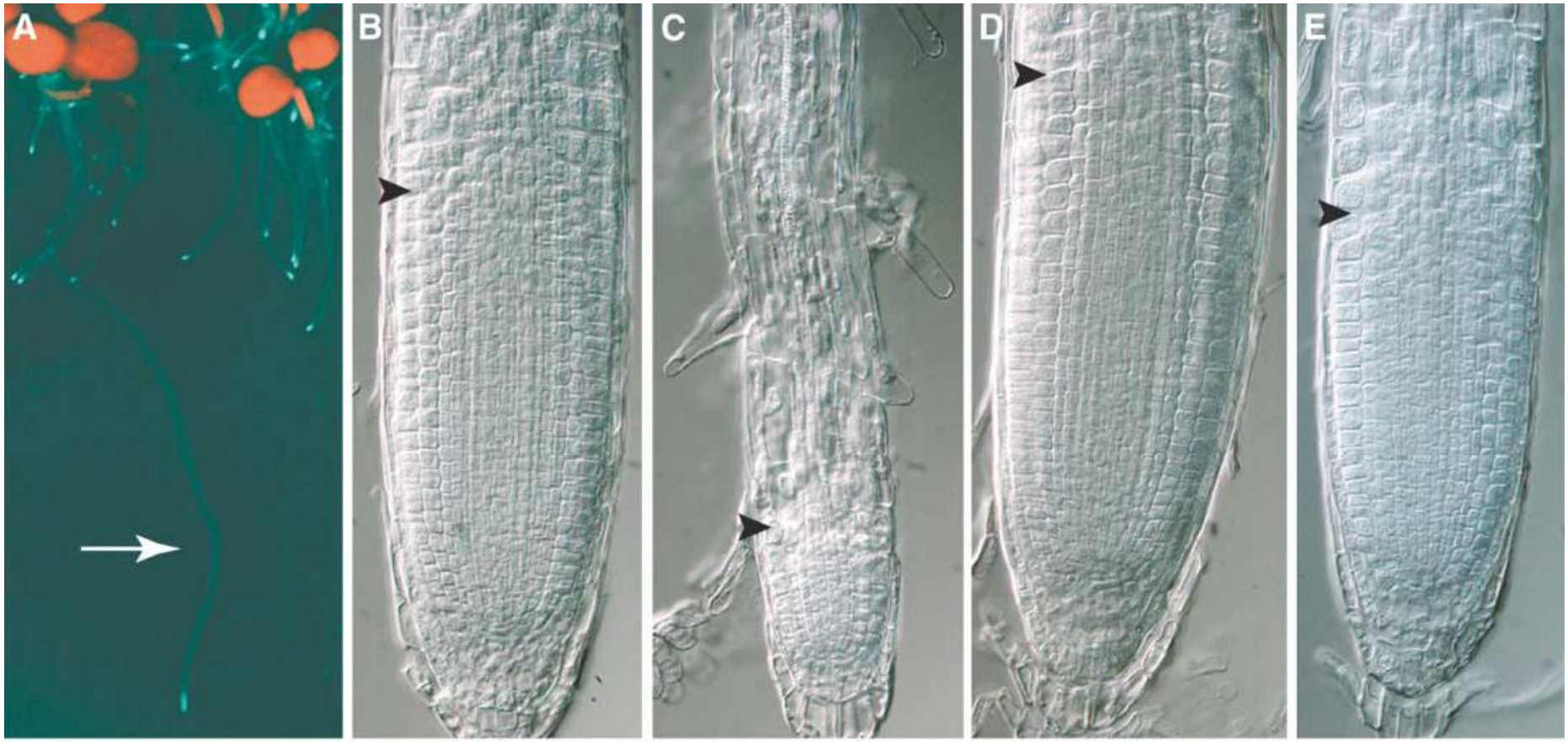
(gnom) Vesicle traffic

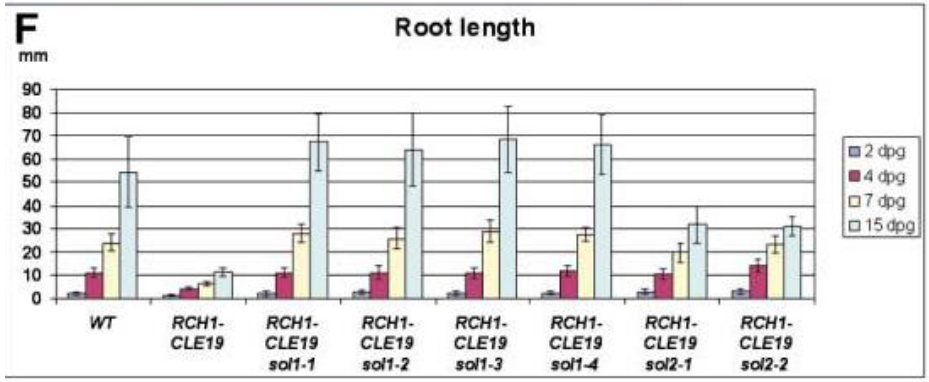
Activation tagging - YUCCA

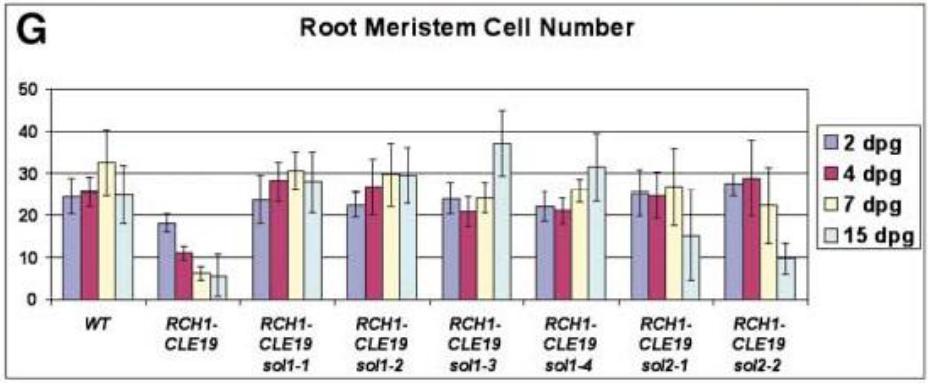


Second site mutagenesis - suppressors

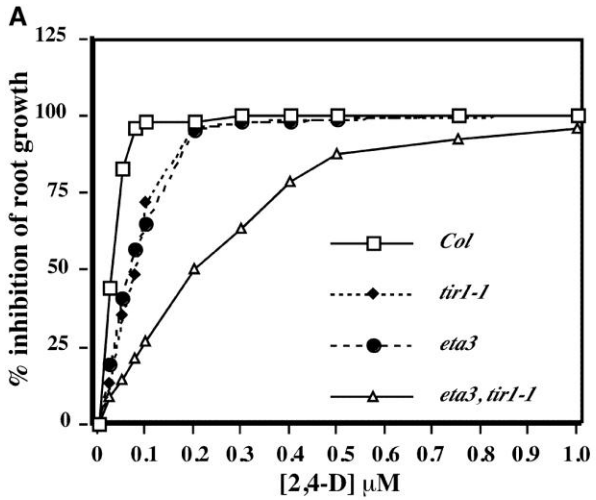
Suppressors of CLV3 overexpression

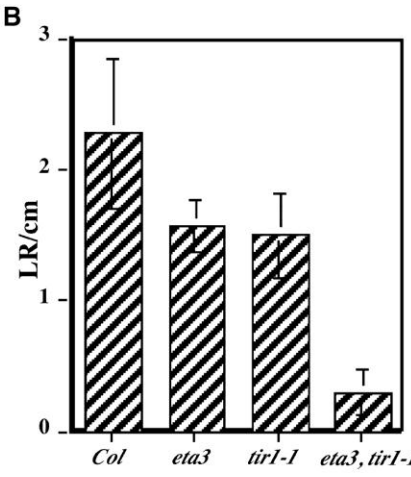


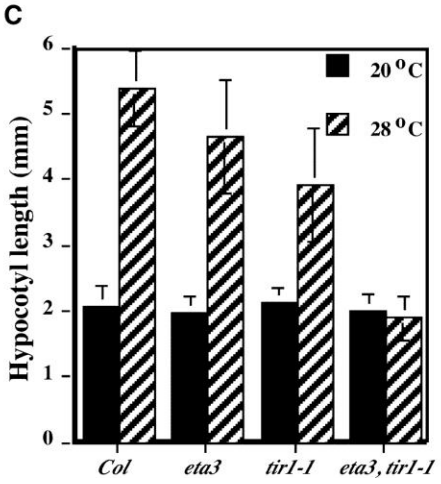




Second site mutagenesis - enhancers



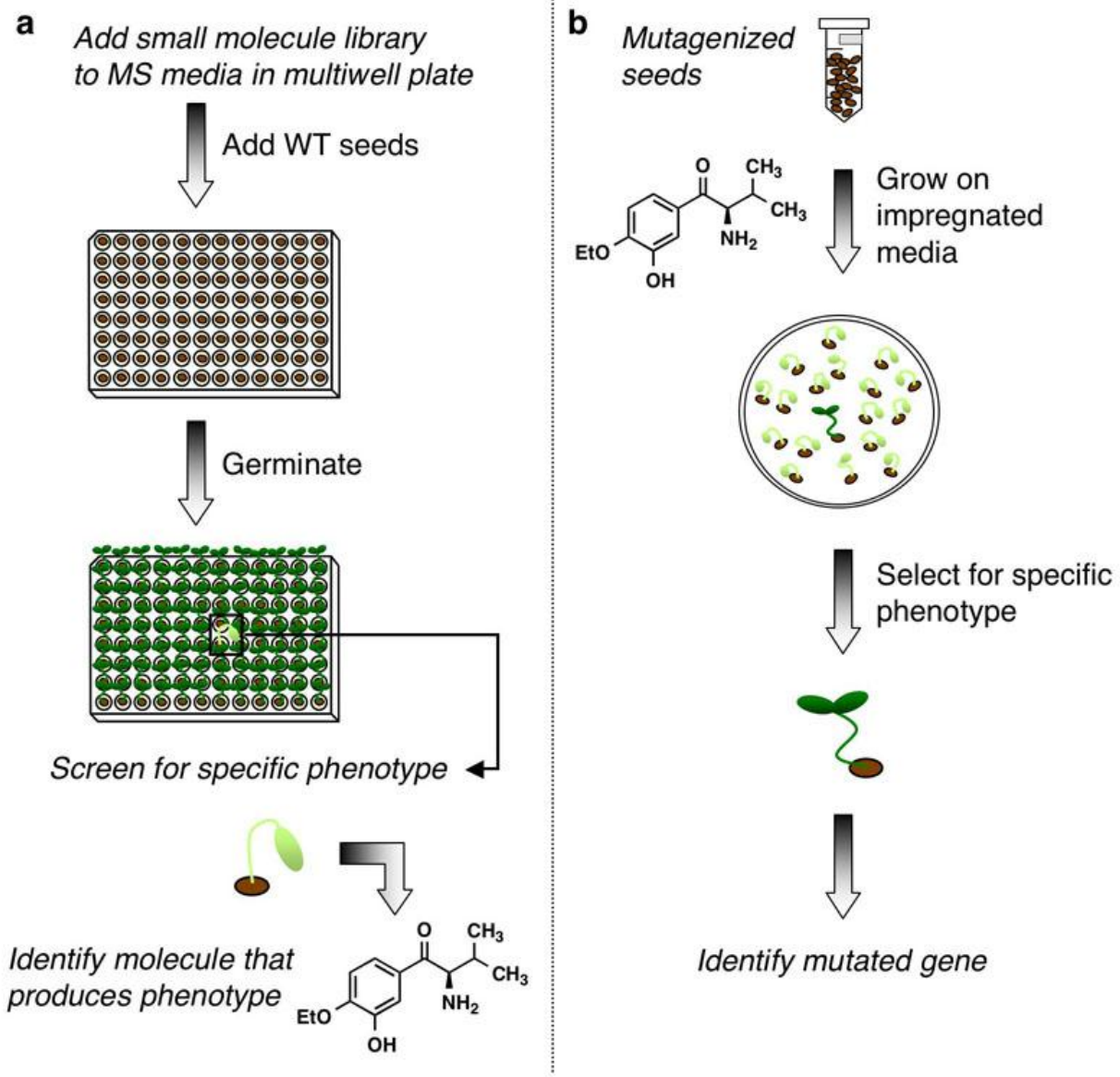




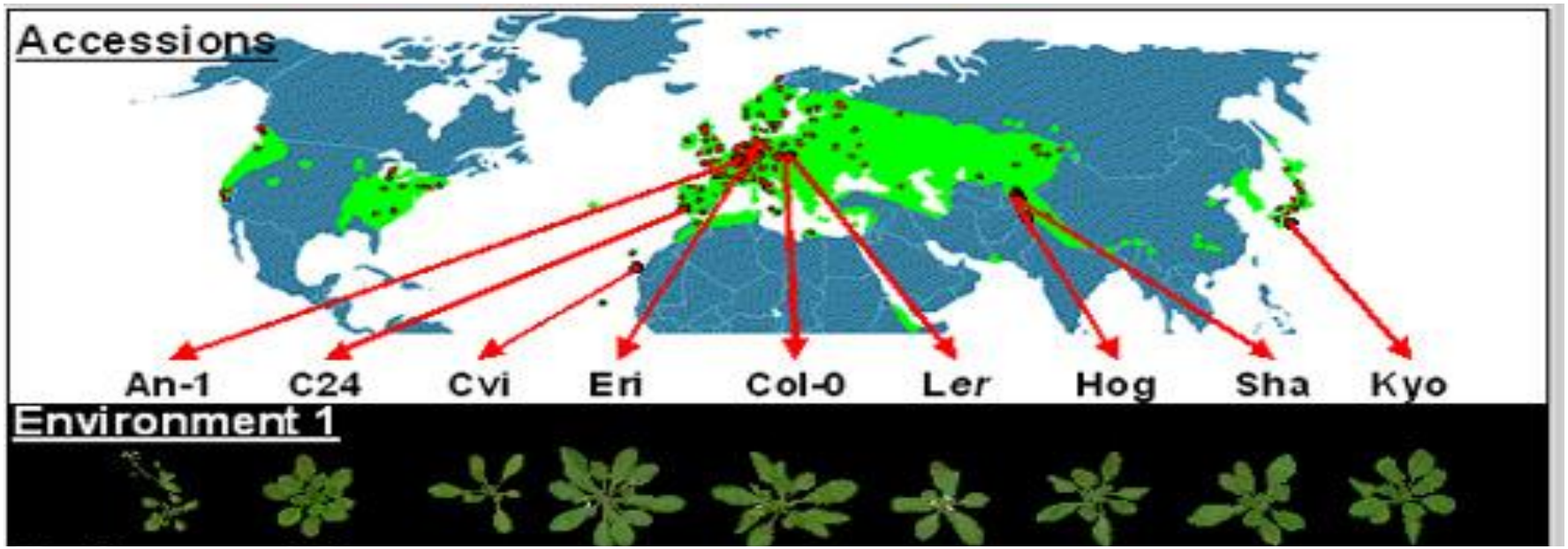
Chemical genetics

A. Screen for effective compound

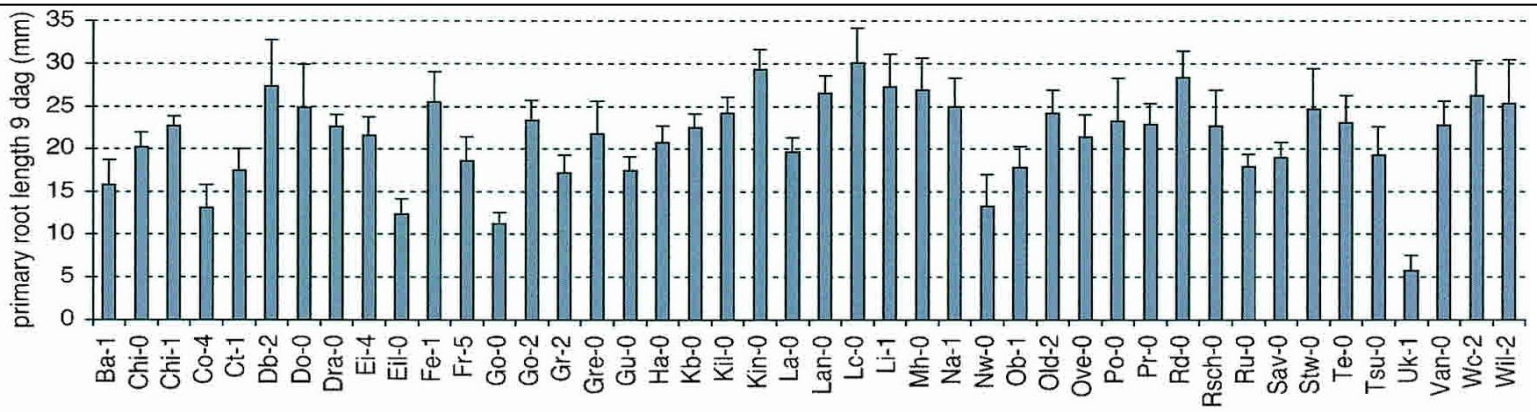
B. Screen for mutants

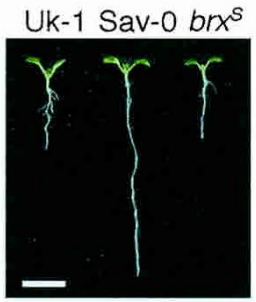


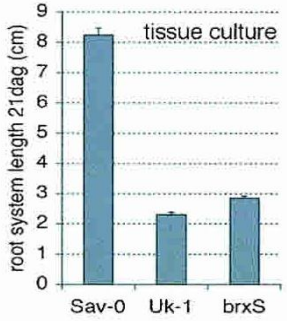
Arabidopsis natural variations = natural mutants

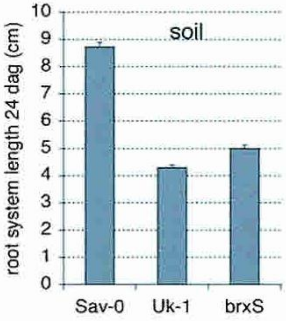


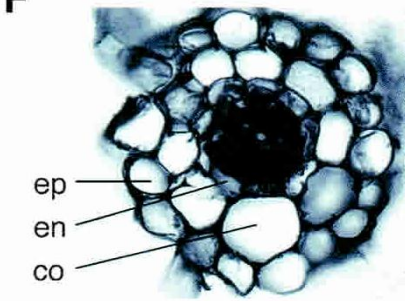
QTL –quantitative trait locus

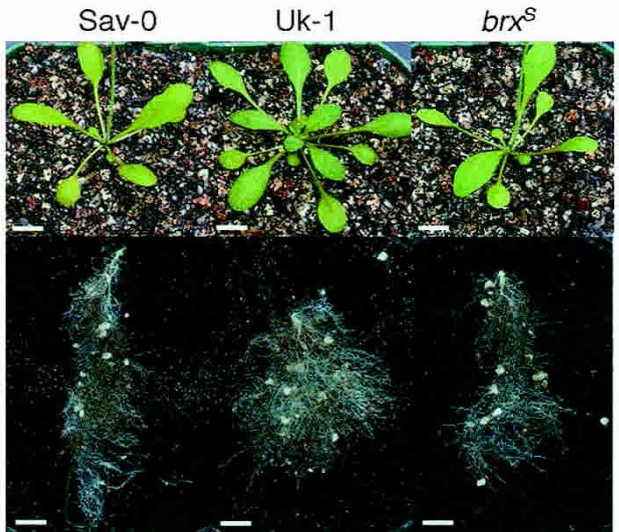


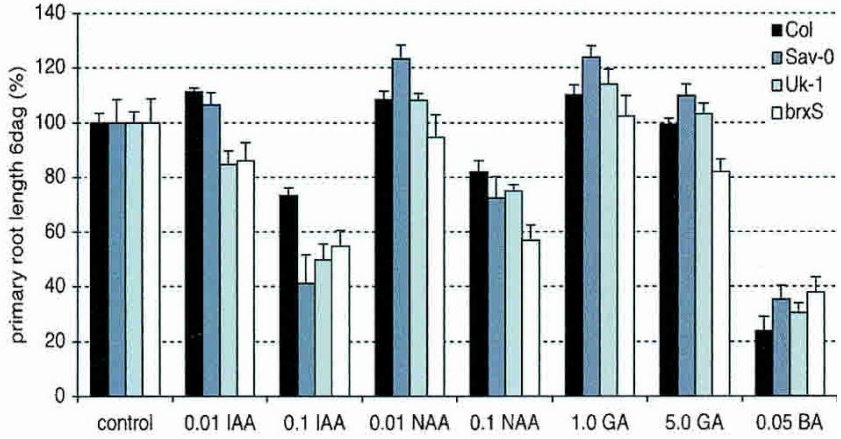












Gene verification

- Multiple alleles
- Transposone reversion
- Complementation

Towards a gene role

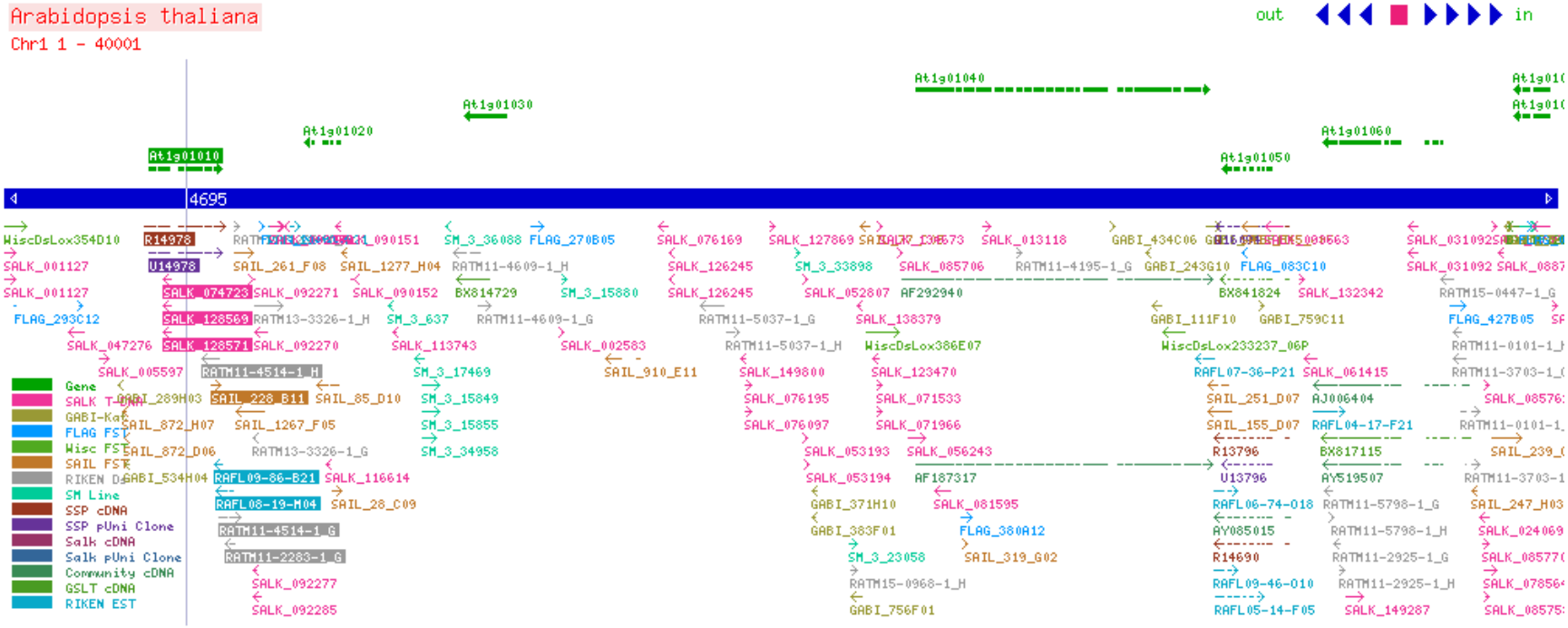
- Loss of function: Reverse genetics
- Gain of function: Ectopic expression
- Mosaics
- Sequence manipulations

- Phenotype analysis
- Biochemical function

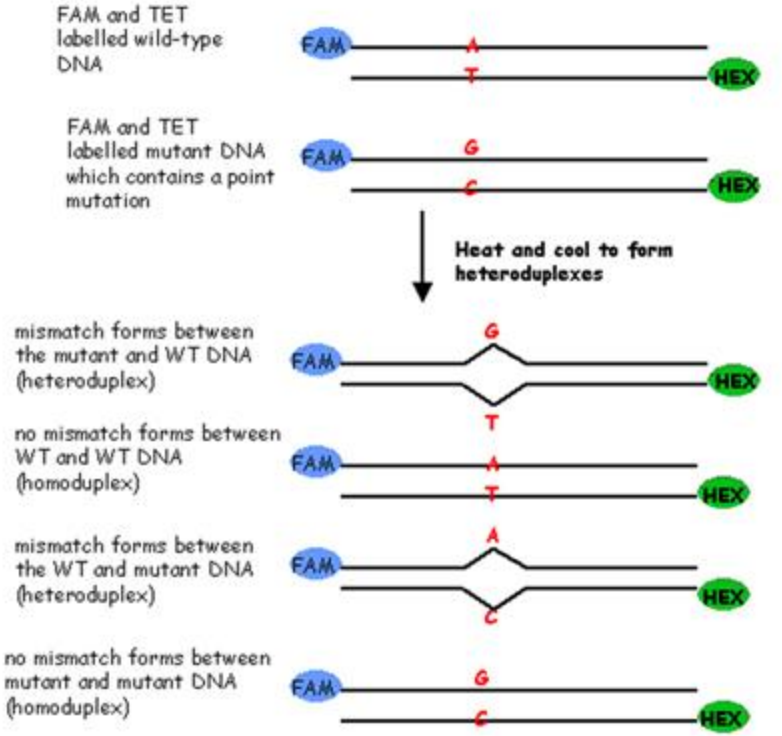
Loss of function

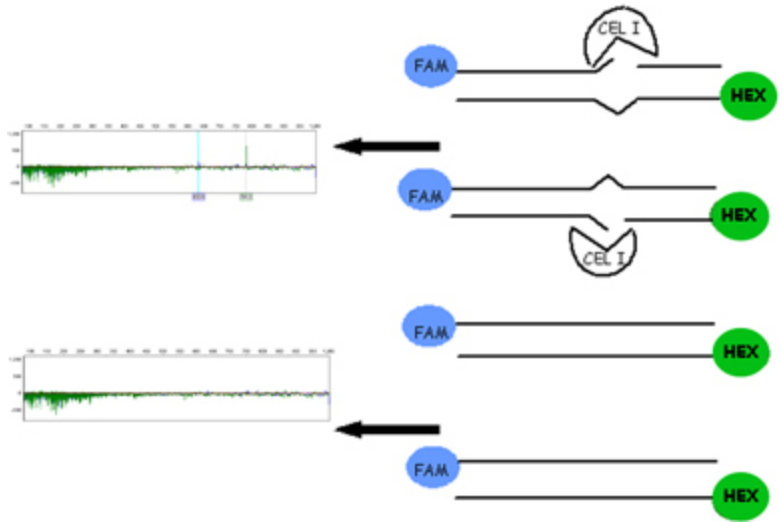
- Reverse genetics/TILLING
- Antisense and RNAi approaches
- Immunomodulation
- Repression domain
- Titration

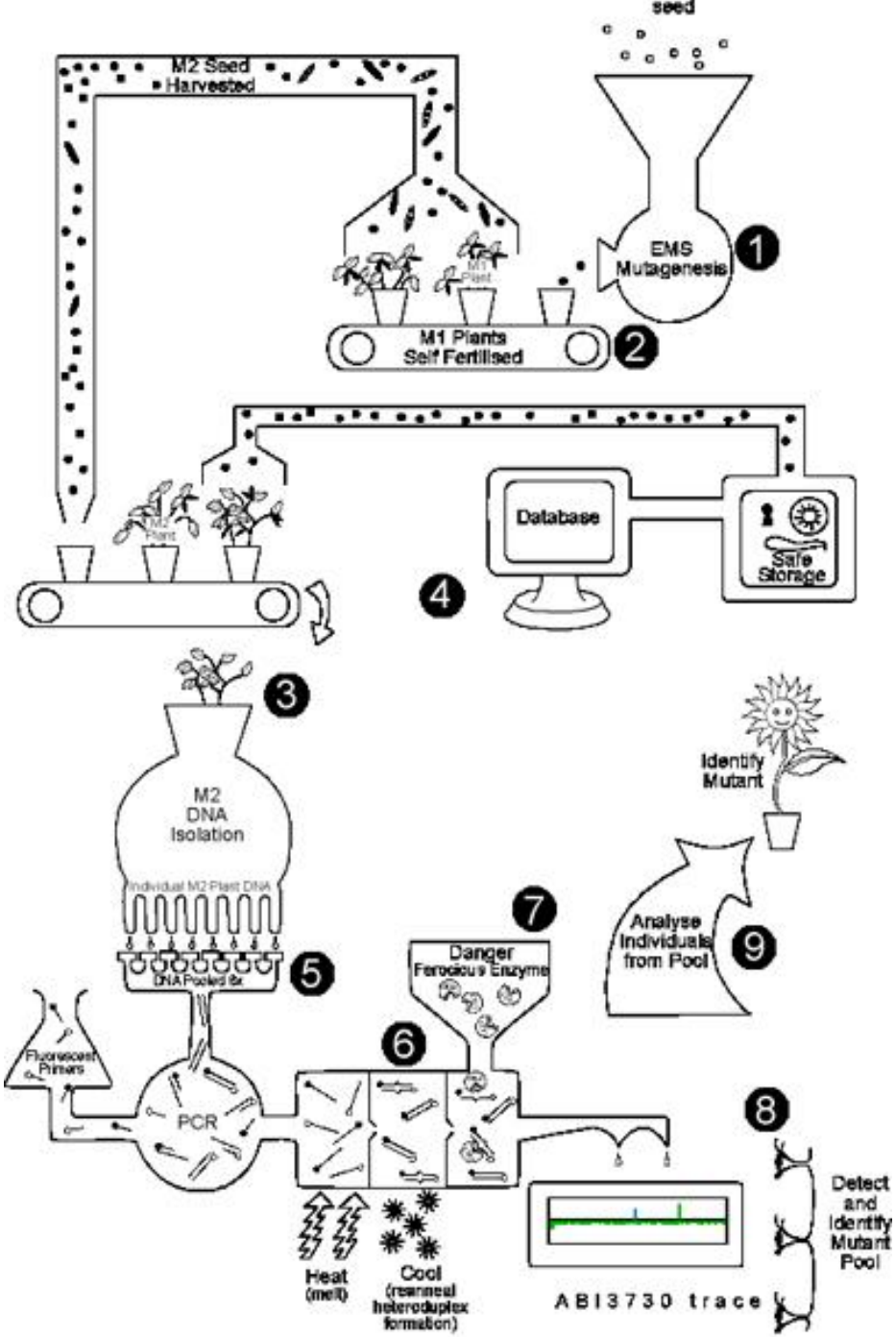
Reverse genetics – indexed mutant libraries



TILLING


FAM and TET labelled wild-type DNA
FAM
A
T
HEX
FAM and TET labelled mutant DNA which contains a point mutation
FAM
G
C
HEX
Heat and cool to form heteroduplexes
mismatch forms between the mutant and WT DNA (heteroduplex)
G
T
no mismatch forms between WT and WT DNA (homoduplex)
A
T
mismatch forms between the WT and mutant DNA (heteroduplex)
A
C
no mismatch forms between mutant and mutant DNA (homoduplex)
G
C

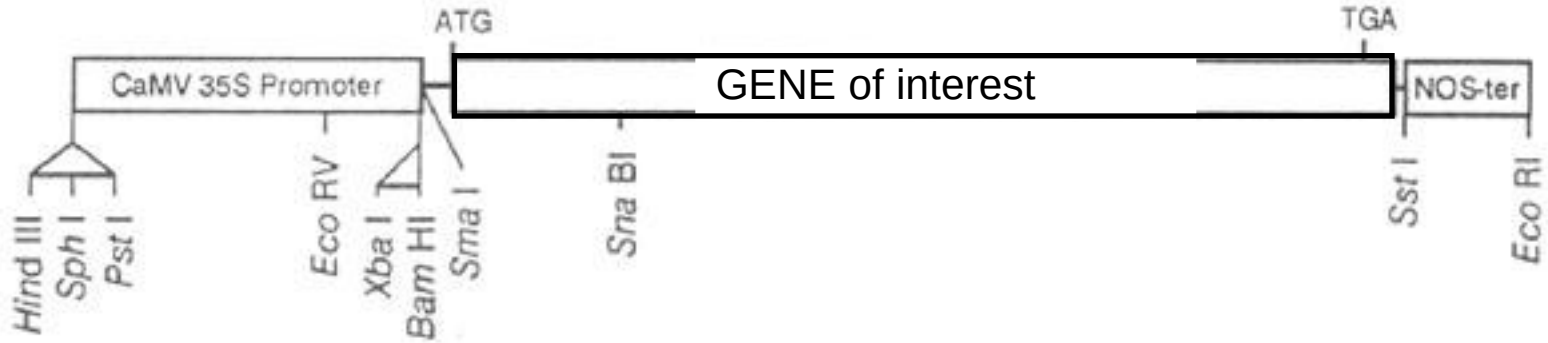

CEL I
FAM
HEX
CEL I


M2 Seed Harvested
EMS Mutagenesis
1
M1 Plants Self Fertilised
2
Database
Safe Storage
4
3
M2 DNA Isolation
Individual M2 Plant DNA
DNA Pooled 8x
5
Identify Mutant
Analyse Individuals from Pool
9
7
Danger Ferocious Enzyme
6
Fluorescent Primers
PCR
8
Heat (melt)
Cool (reanneal heteroduplex formation)
ABI3730 trace
Detect and Identify Mutant Pool

Gain of function

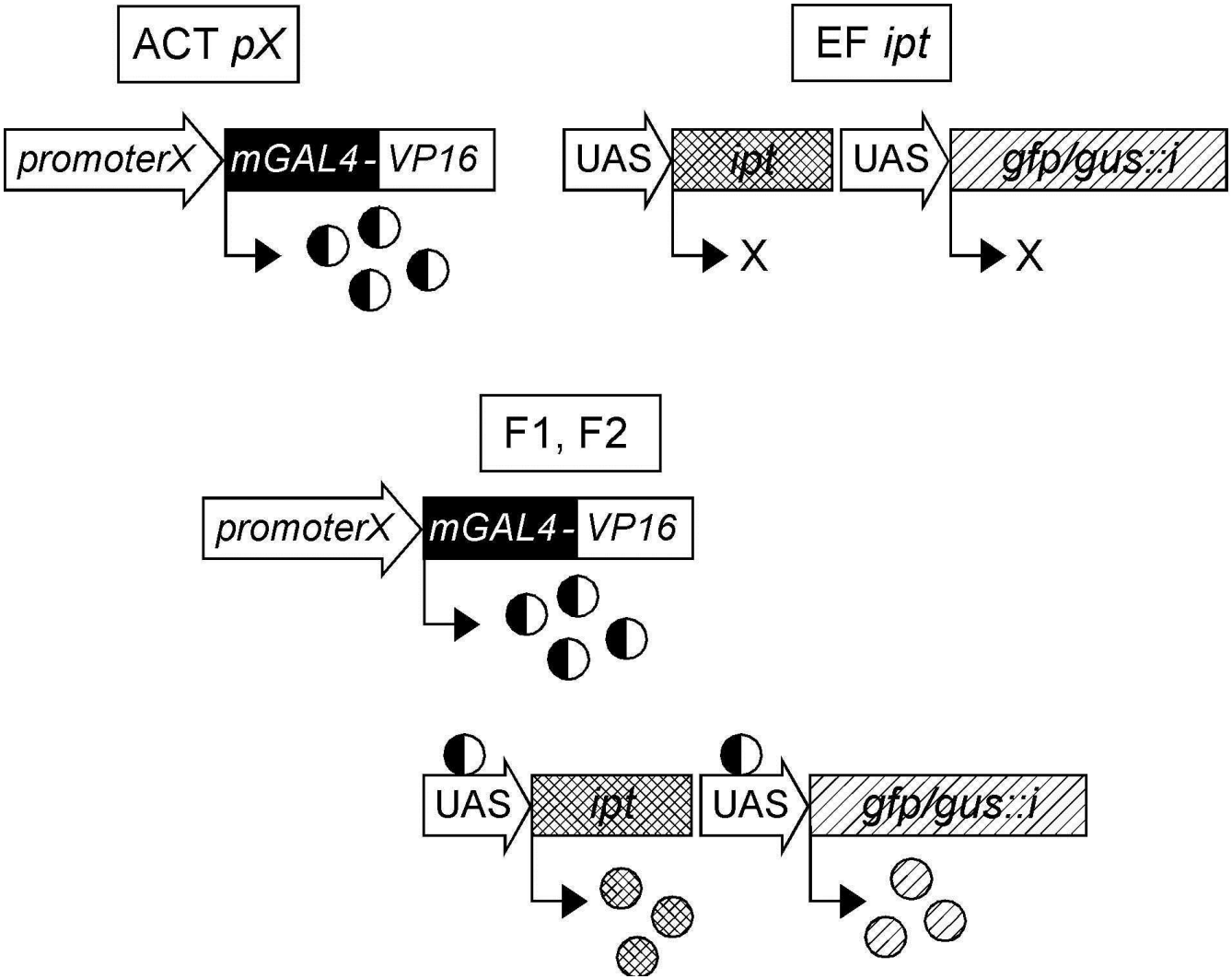
- Overexpression
- Tissue specific expression
- Conditional expression
- Protein stabilisation

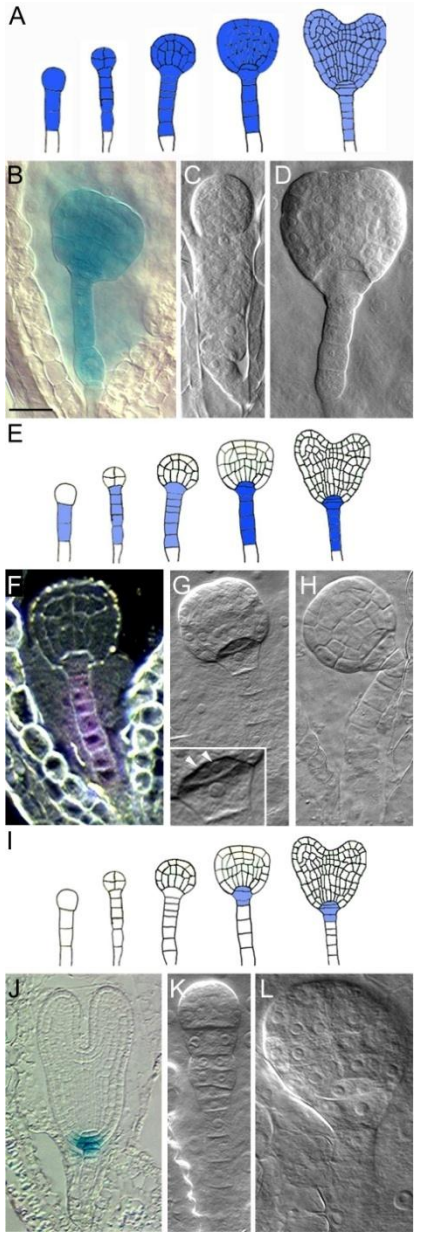
CaMV 35S Promotor

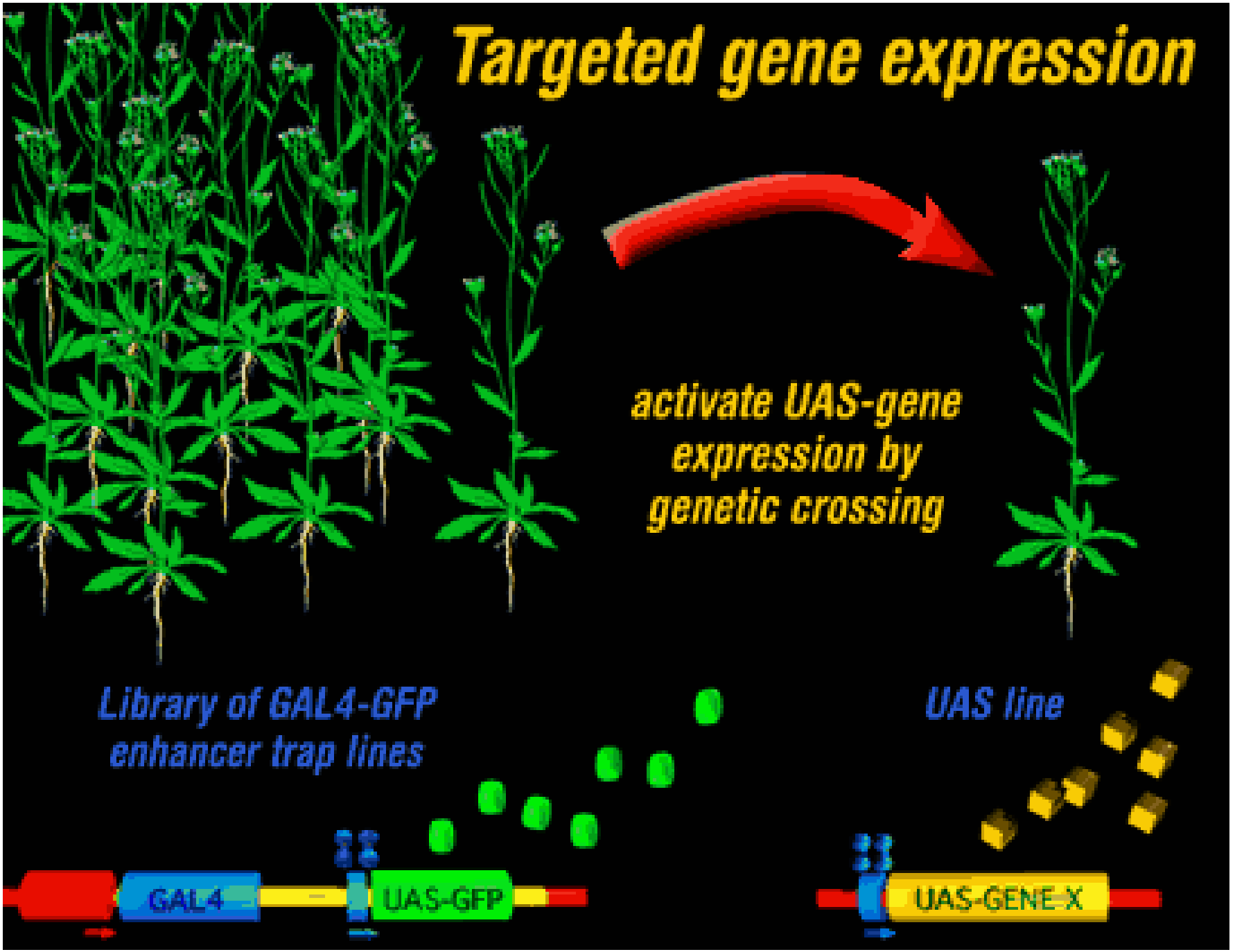


pBI221 The CaMV 35S promoter-GUS-NOS-ter portion of pBI121 was cloned into pUC19 to produce pBI221.

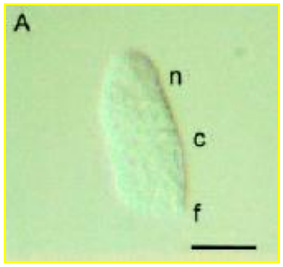
Two component system for gene expression

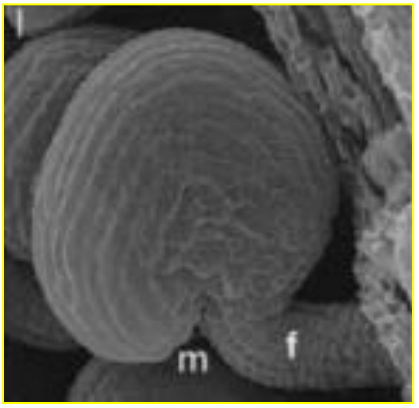


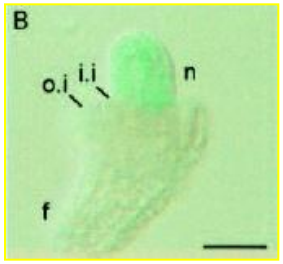


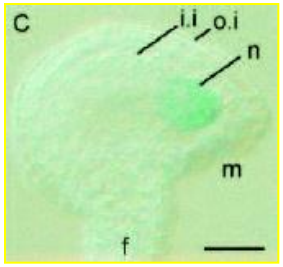

Targeted gene expression
activate UAS-gene expression by genetic crossing
Library of GAL4-GFP enhancer trap lines
UAS line
GAL4
UAS-GFP
UAS-GENE-X

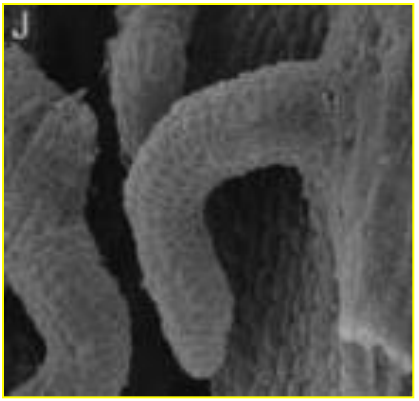
The hidden function of WUSCHEL



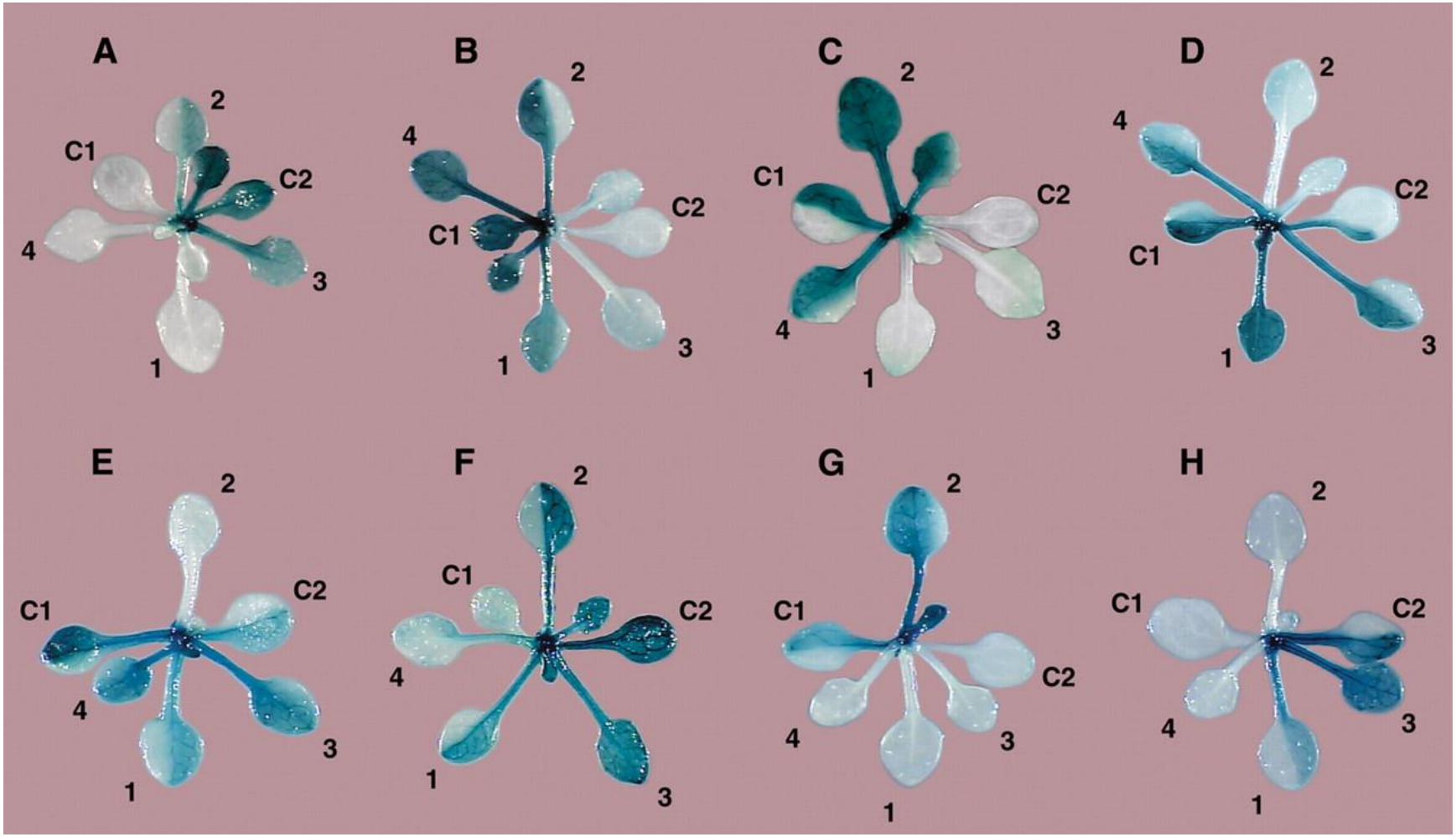








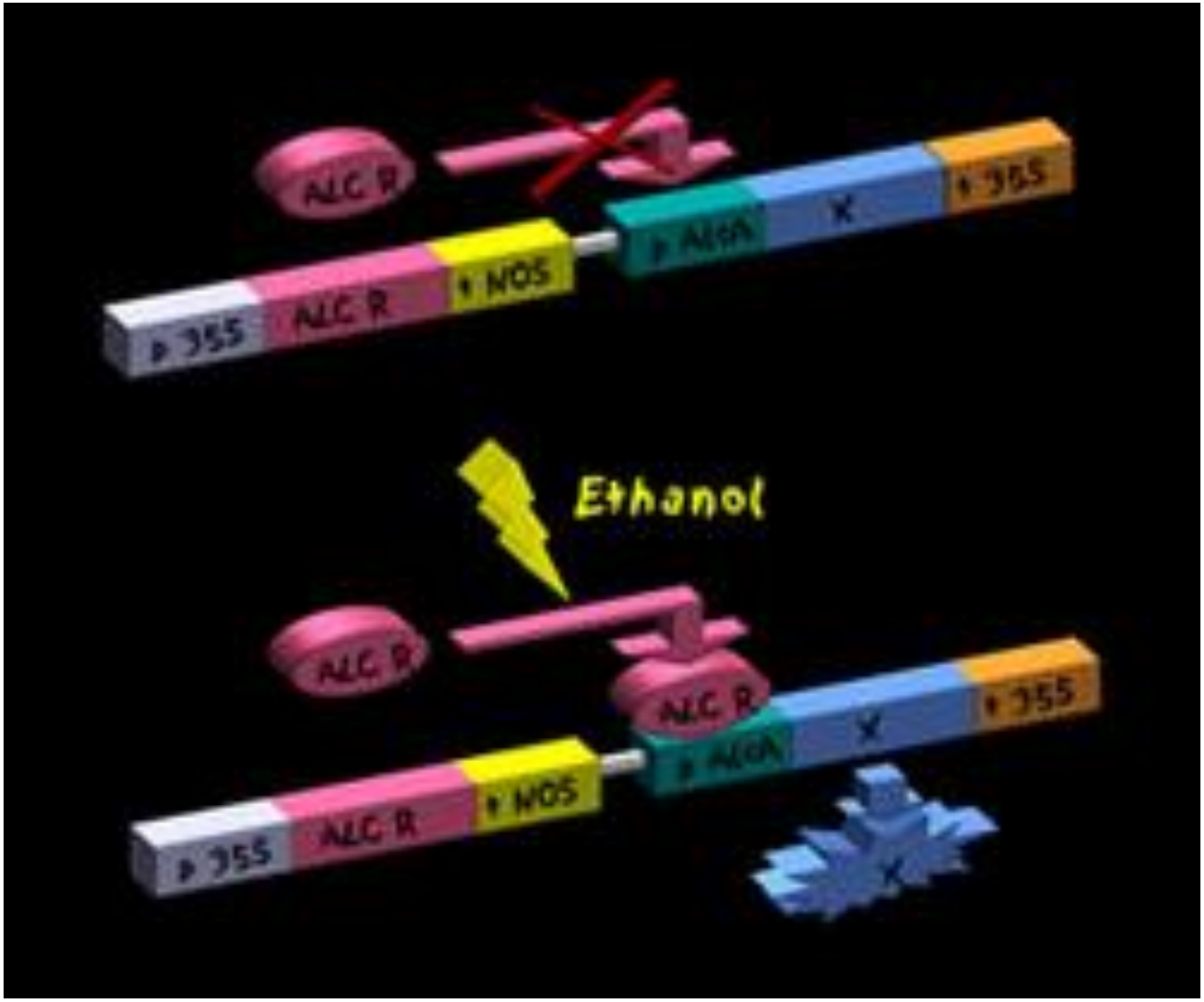
Mosaics – Cre/Lox



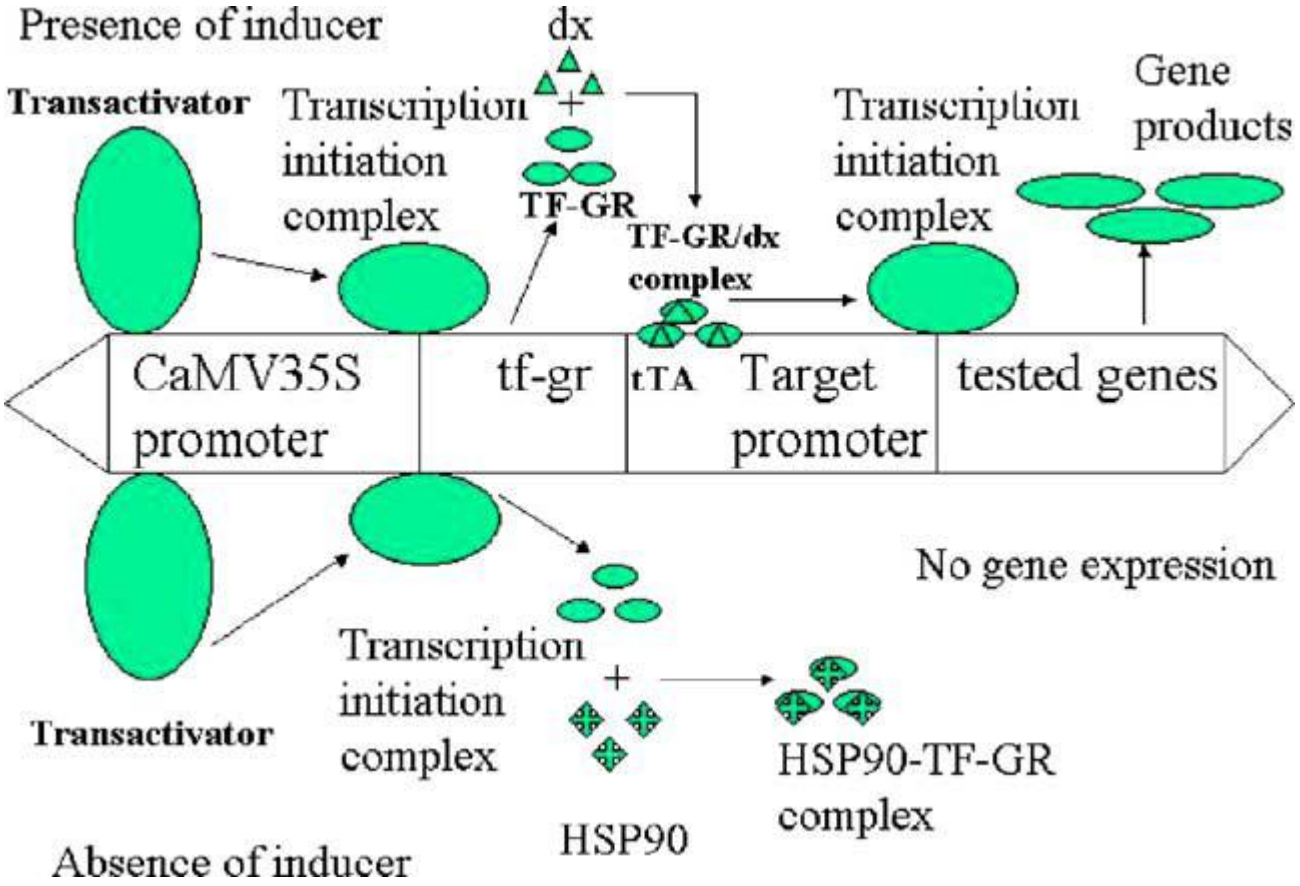
Mosaics – Cre/Lox

gene of interest

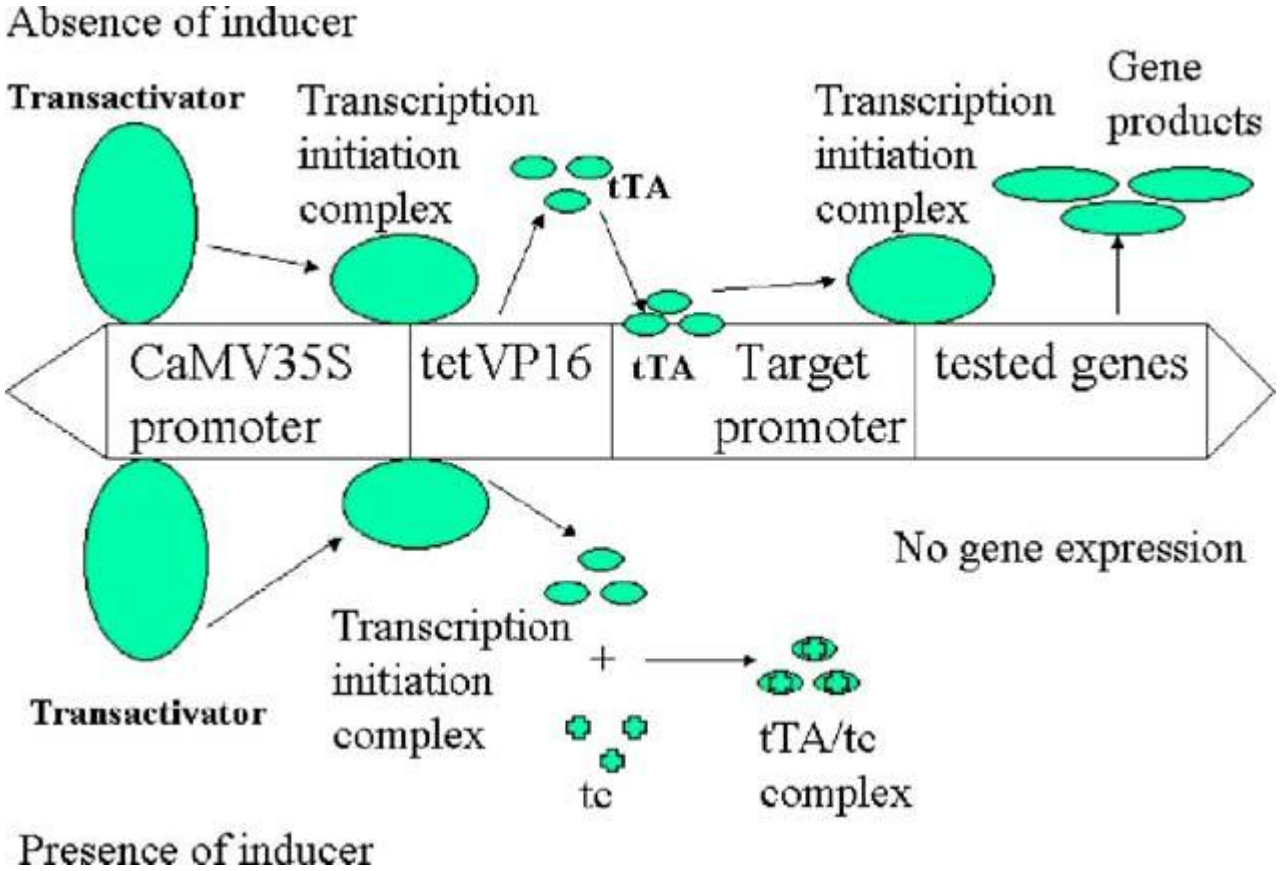
Ethanol inducible expression



The dexamethasone-inducible promoter activating system



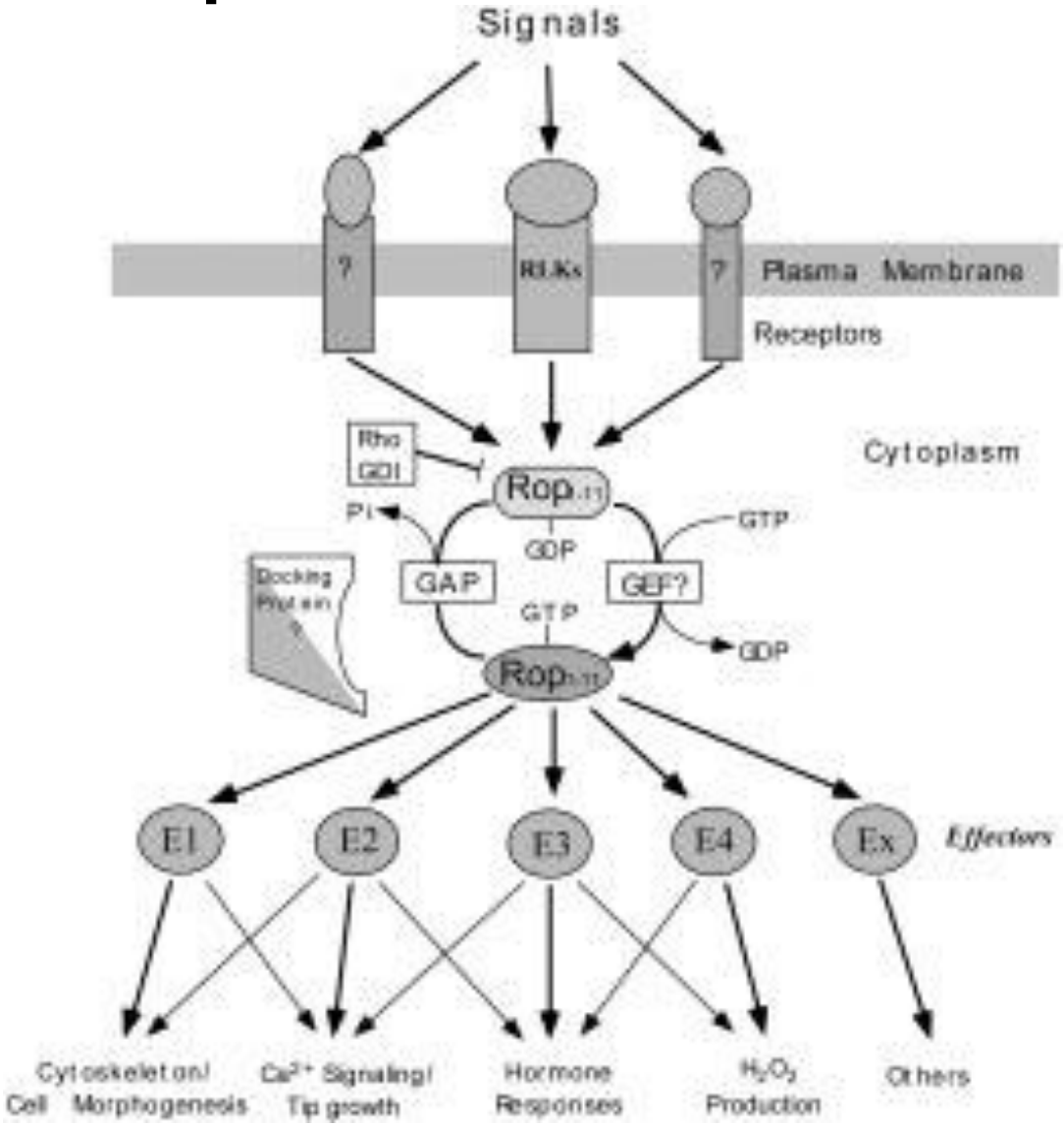
The tetracycline-inducible promoter inactivation system



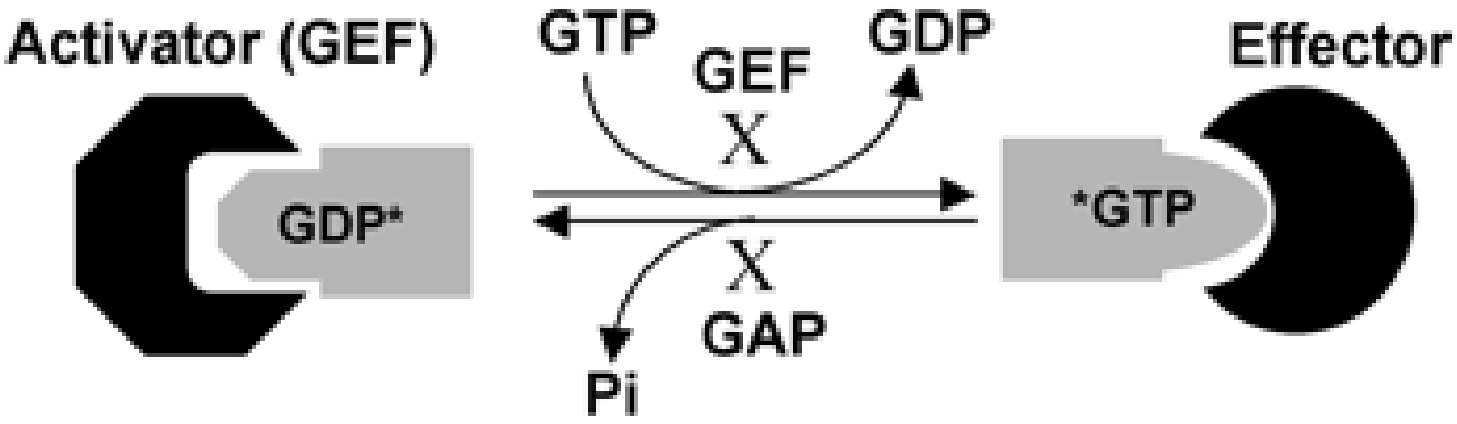
Sequence manipulation

- Site-directed mutagenesis (phosporylation sites, activity of protein domains, catalytic center)
- Domain deletions and swaps
- Chimeric proteins

rop GTPases mutants



rop GTPases mutants



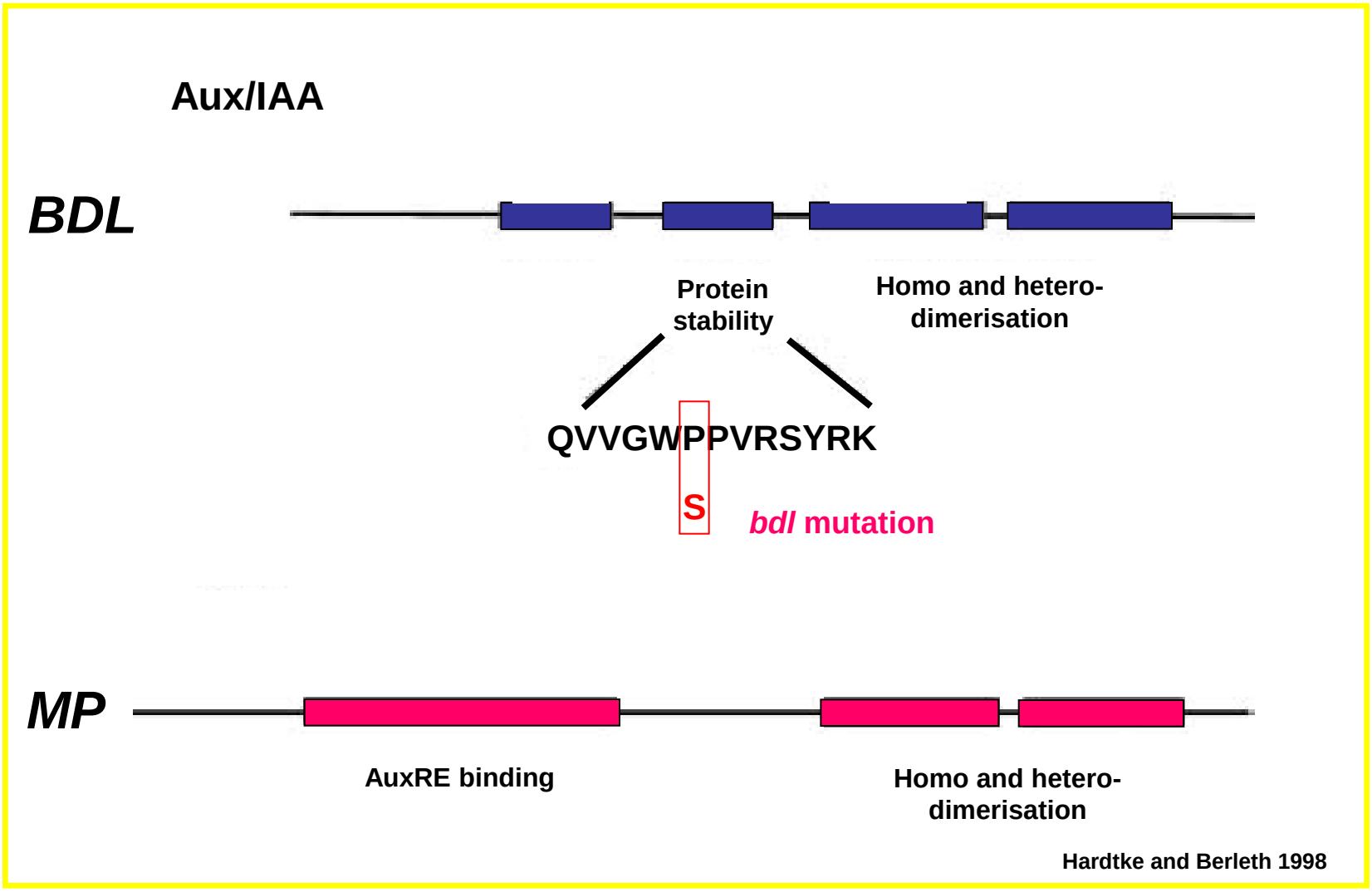
DN-rop mutants

- Permanently bind GDP or nucleotide-free
- Sequester activator (GEF) when overexpressed
- Examples:
  - ROP1/ROP2/ROP4/ROP6: T20N, A121D
  - ROP5: T20N

CA-rop mutants

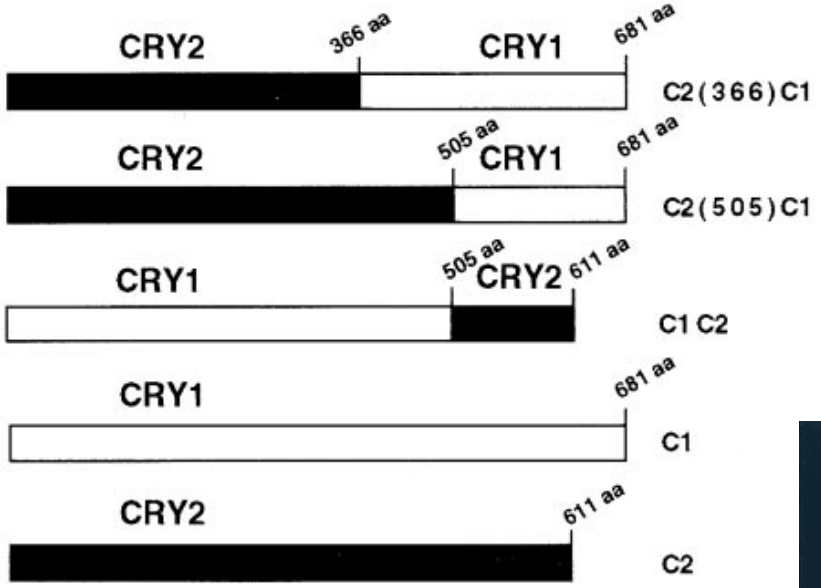
- Permanently bind GTP
- Insensitive to GAP
- Constitutively activate effectors when expressed in cells
- Examples:
  - ROP1/ROP2/ROP4/ROP6: G15V or Q64L
  - ROP5: G15V or Q64E

AUX/IAA and ARF proteins



Hardtke and Berleth 1998

Blue light photoreceptor-chimers

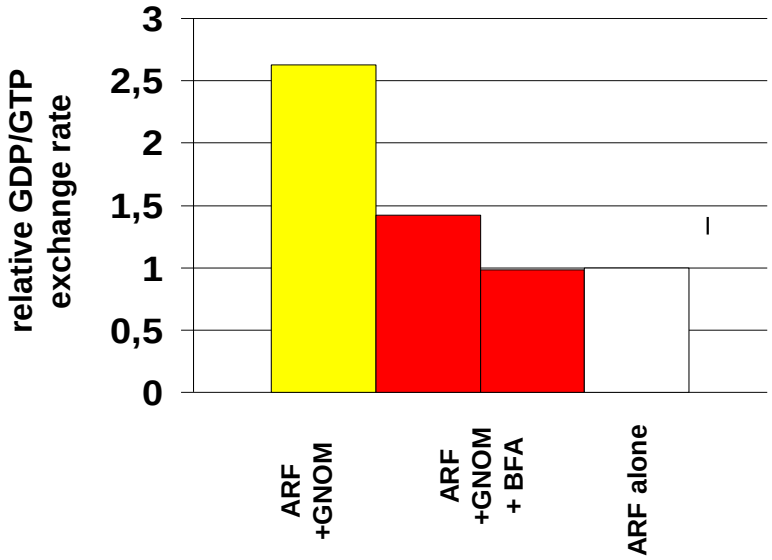


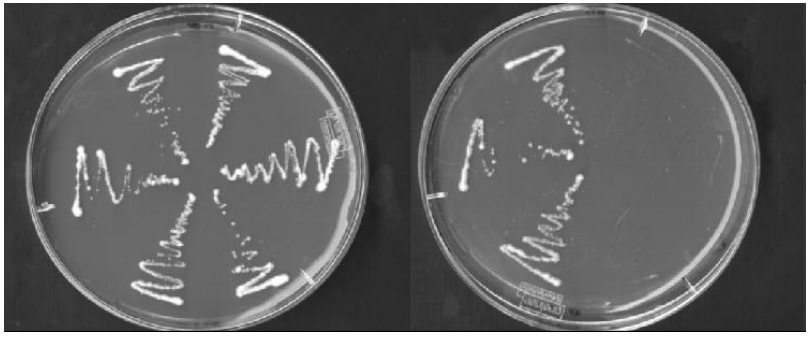
Phenotype analysis

- Visual evaluation
- Ultrastructure (EMS)
- Use of markers
- Treatments

Biochemical function – test prediction

- Protein activity
- Yeast complementation
- Xenopus oocytes





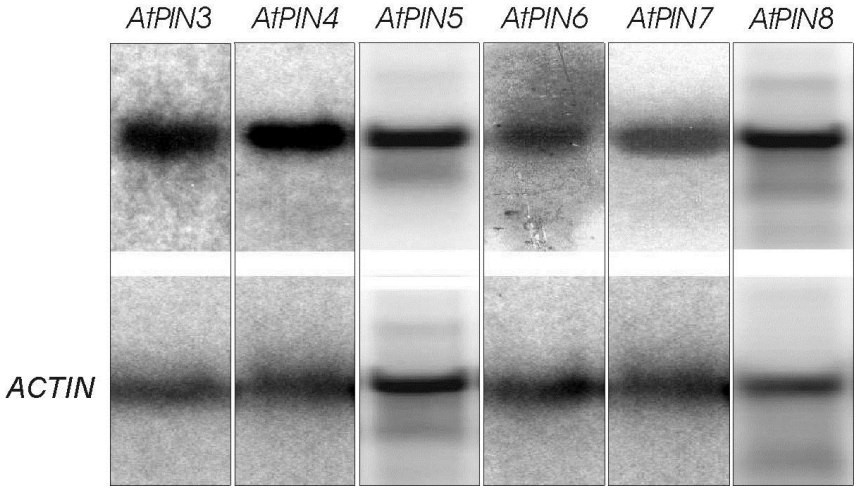
Gene Expression and Protein Localization

- Blots, RT-PCR
- Reporter genes
- In situ mRNA hybridization
- In situ protein localization
- In situ protein activity detection

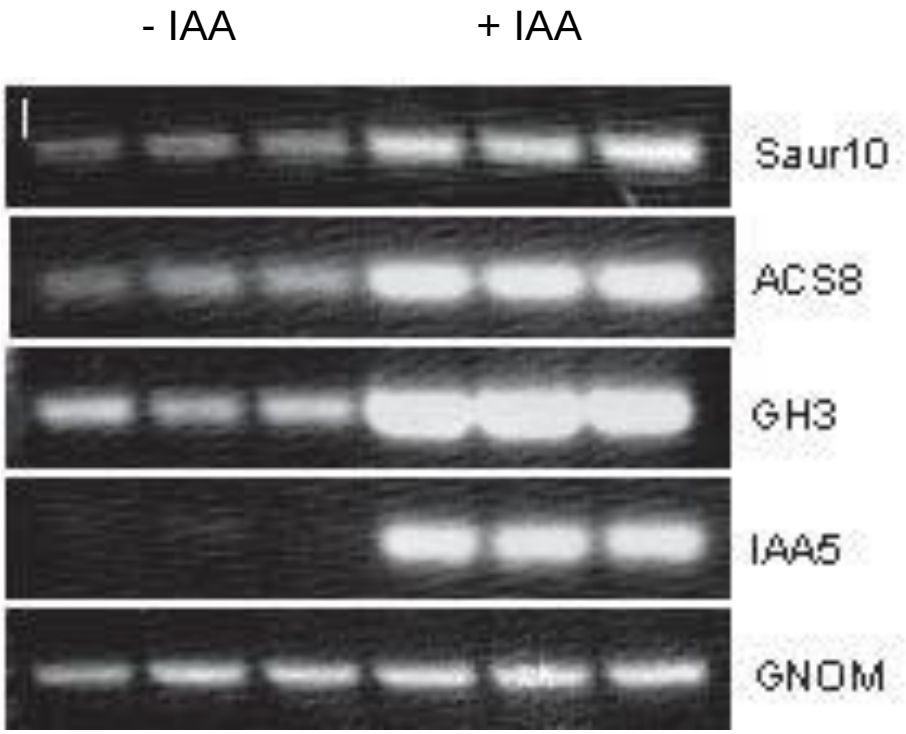
Blots and RT-PCR

Northern blots

Figure 8. Expression of *AtPIN* genes.
Northern blot and RT PCR analysis of *AtPIN3* - *AtPIN8* genes. *AtPIN3* transcript was found in stem, *AtPIN4*, *AtPIN6* and *AtPIN7* in root and *AtPIN5* and *AtPIN8* in seedling.
In the second line *ACTIN* signal is depicted.



RT-PCR

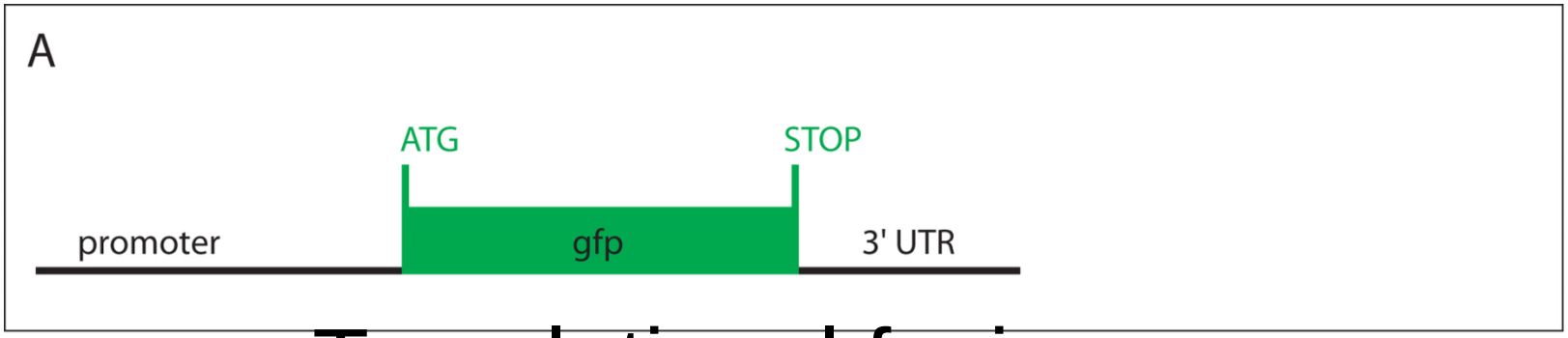


Western blots

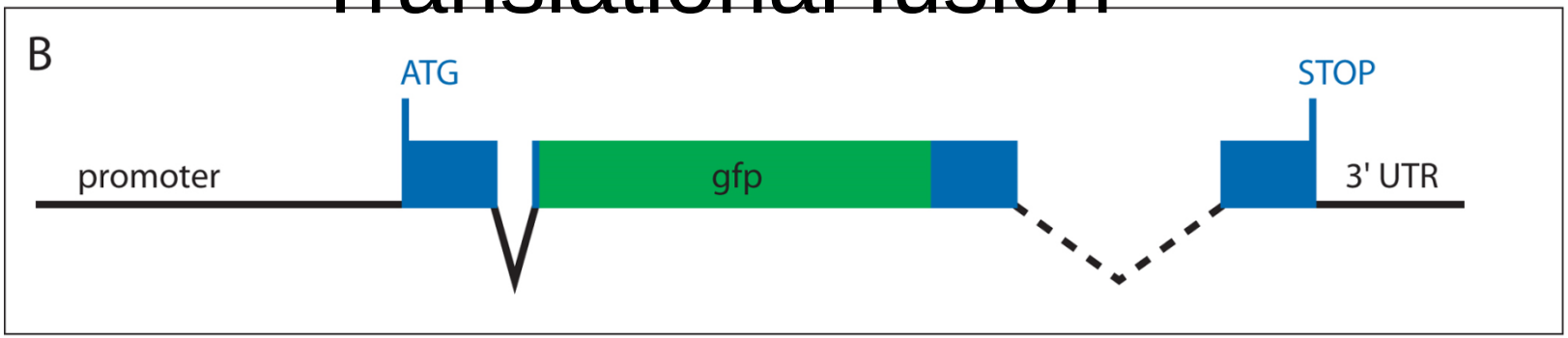
Reporter genes

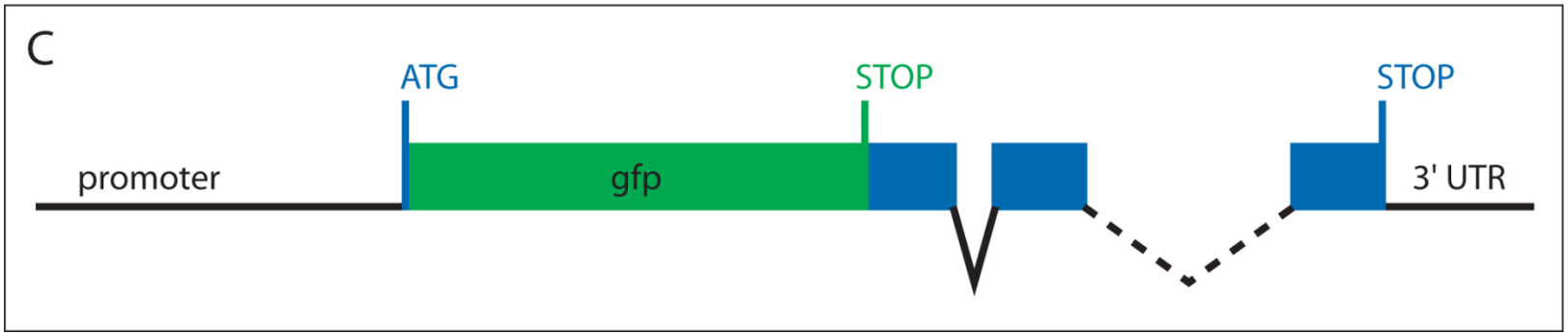
- Transcriptional fusions
- Translational fusions
- GUS, Luciferase, GFP
- Applications

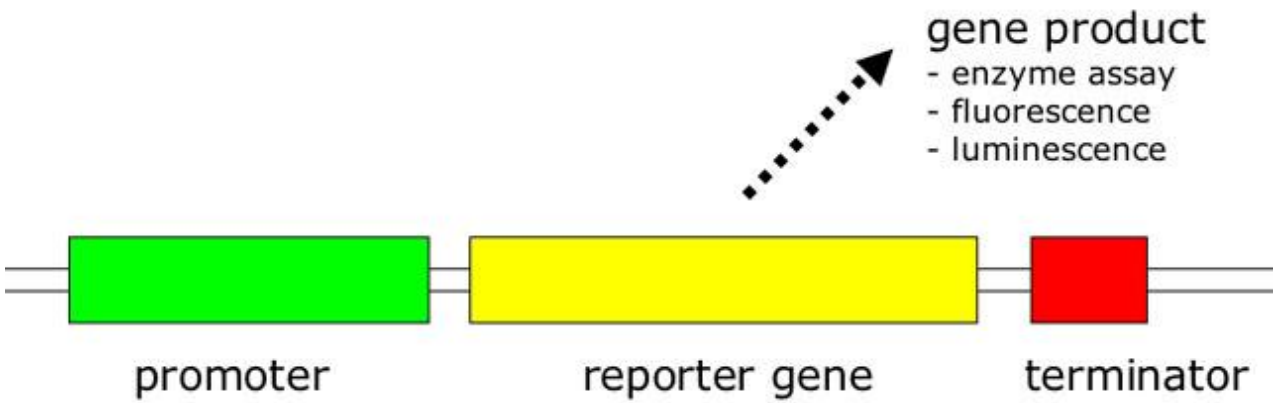
Transcriptional fusion



Translational fusion







Reporter genes: markers for gene expression

ß-glucuronidase
green fluorescent protein
luciferase

GUS – ß-Glucuronidase

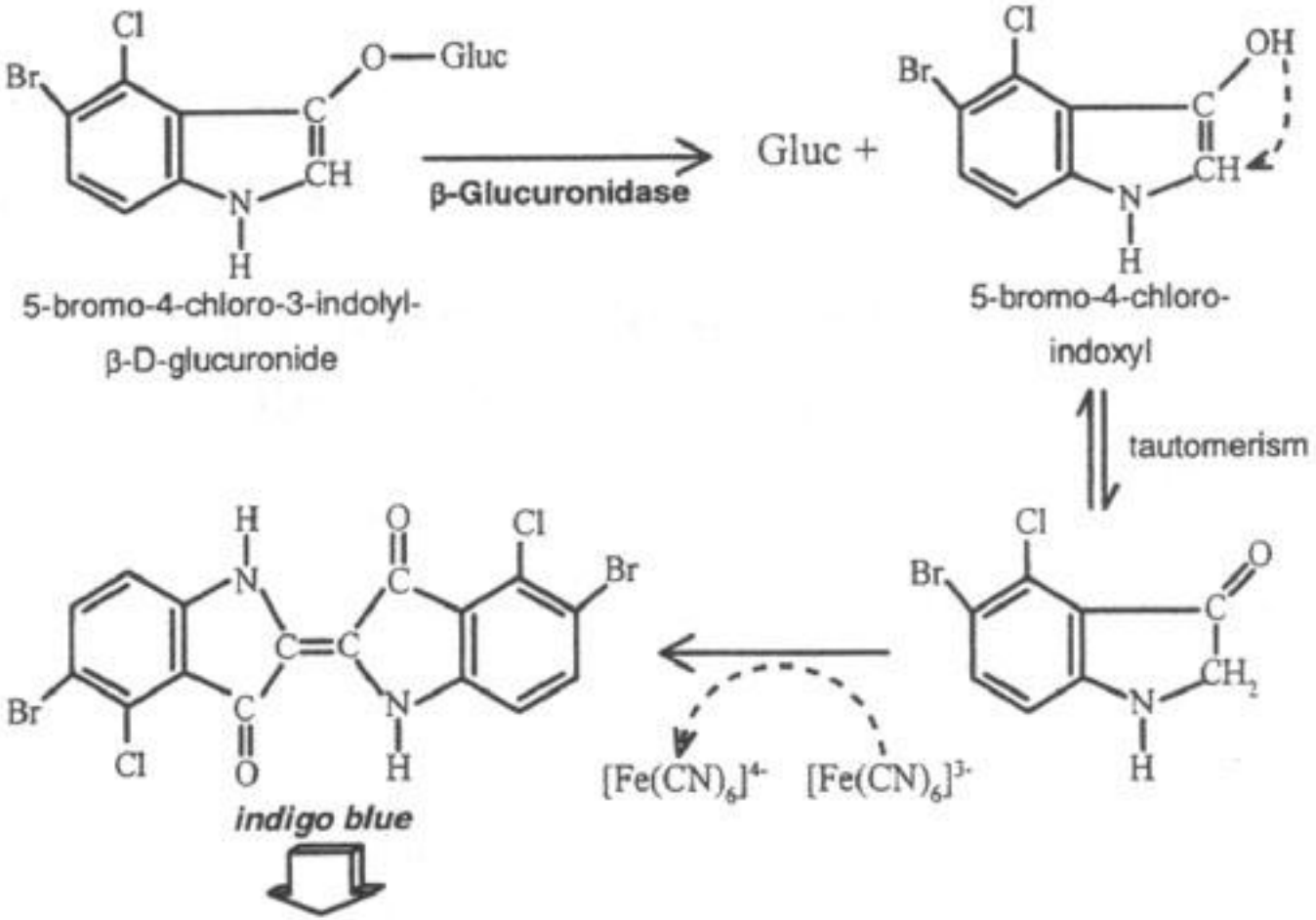
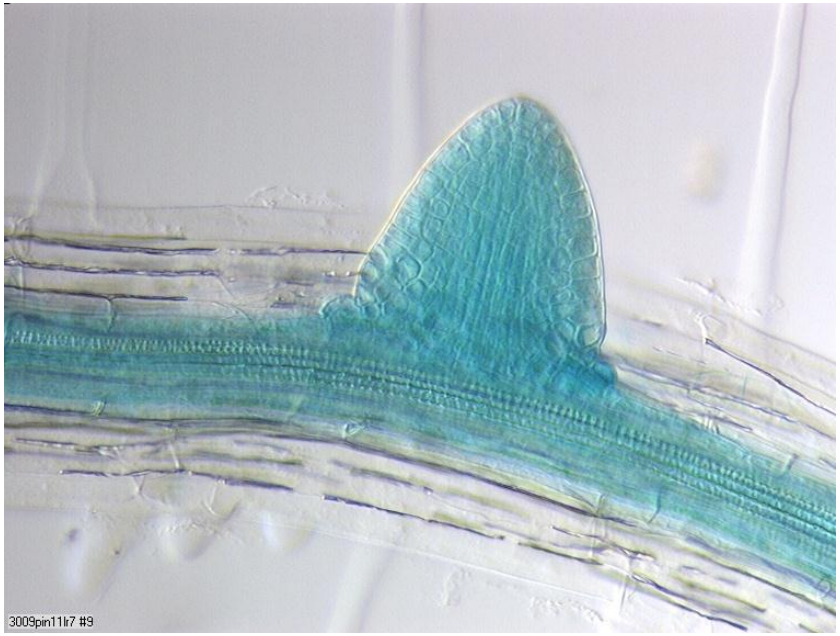
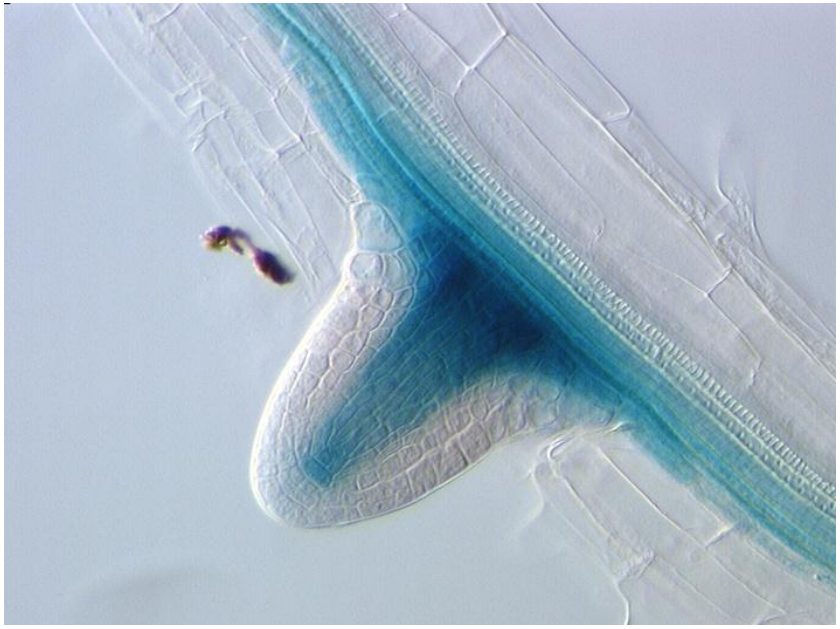
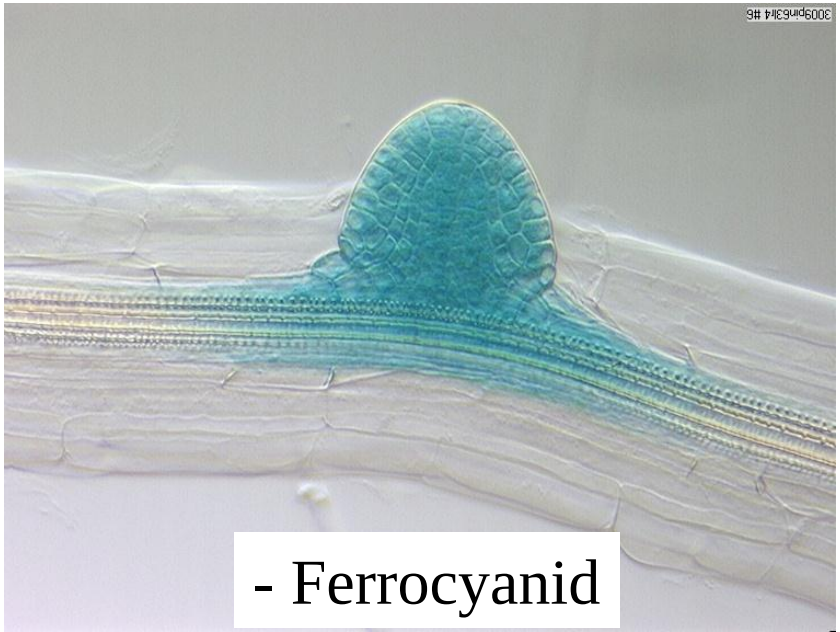


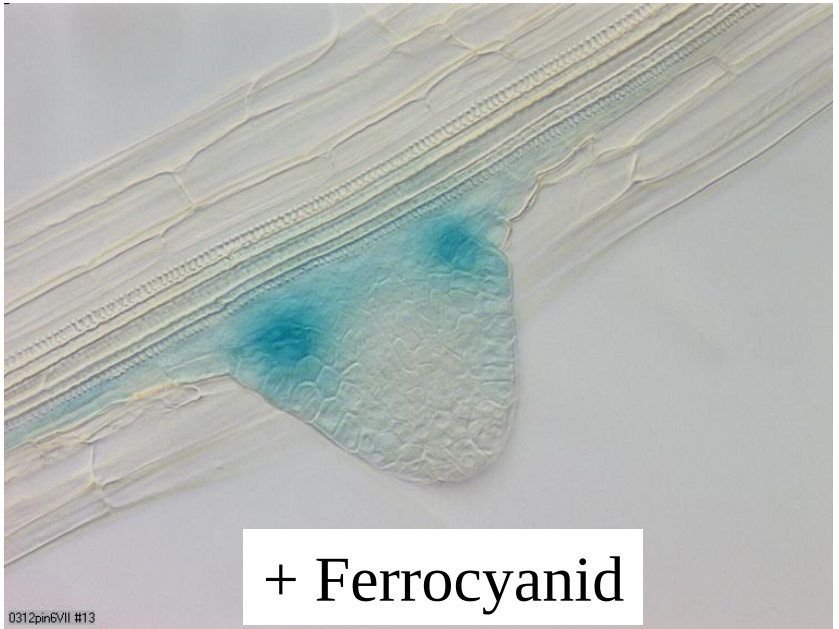
Fig. 1. Chemistry of X-Gluc reaction. Hydrolyzation of X-Gluc by the β-glucuronidase enzyme results in a reactive indoxyl molecule. Two indoxyl molecules are oxidized to indigo blue; ferri(III)cyanide enhances the dimerization.

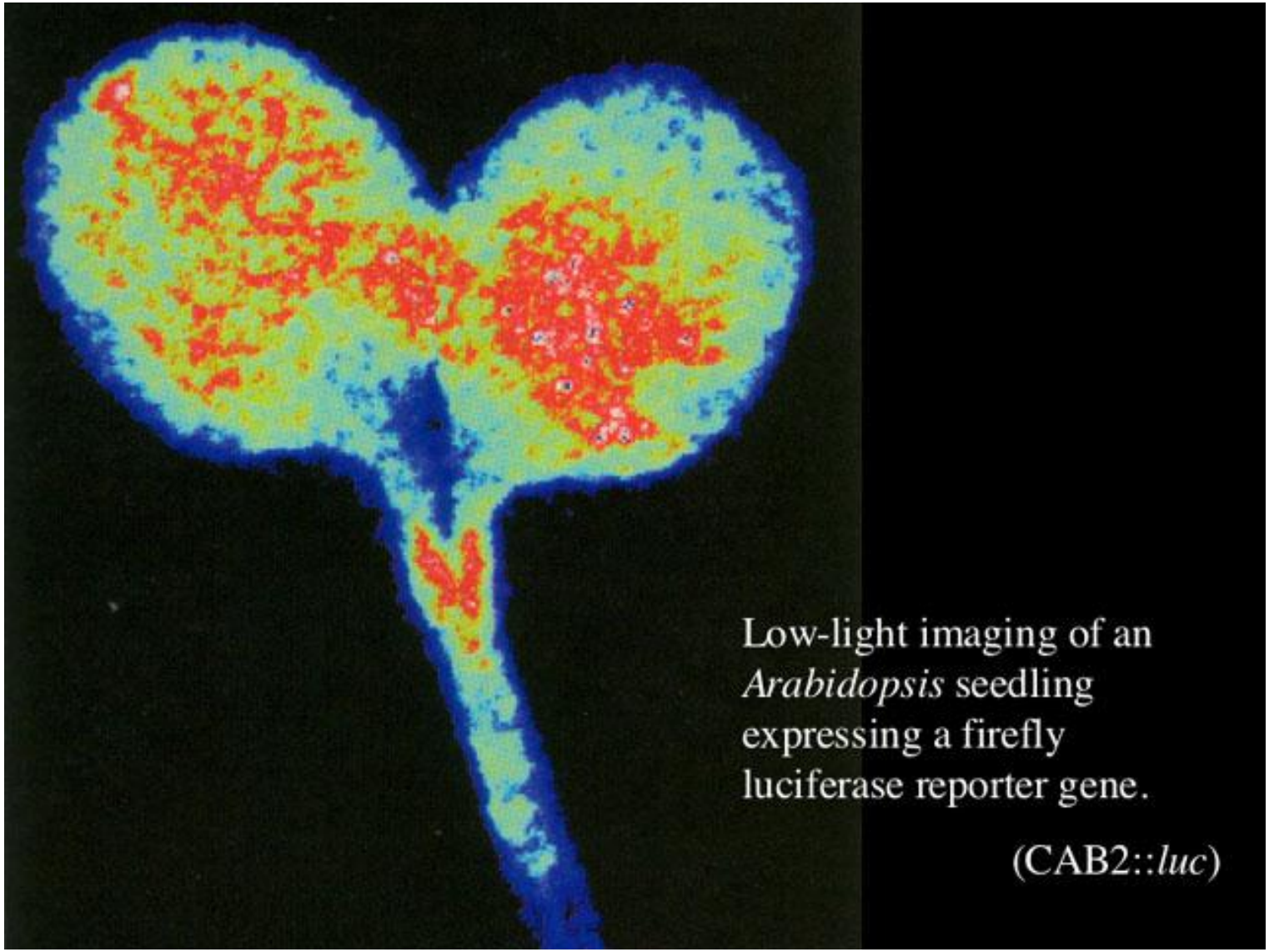
GUS – ß-Glucuronidase





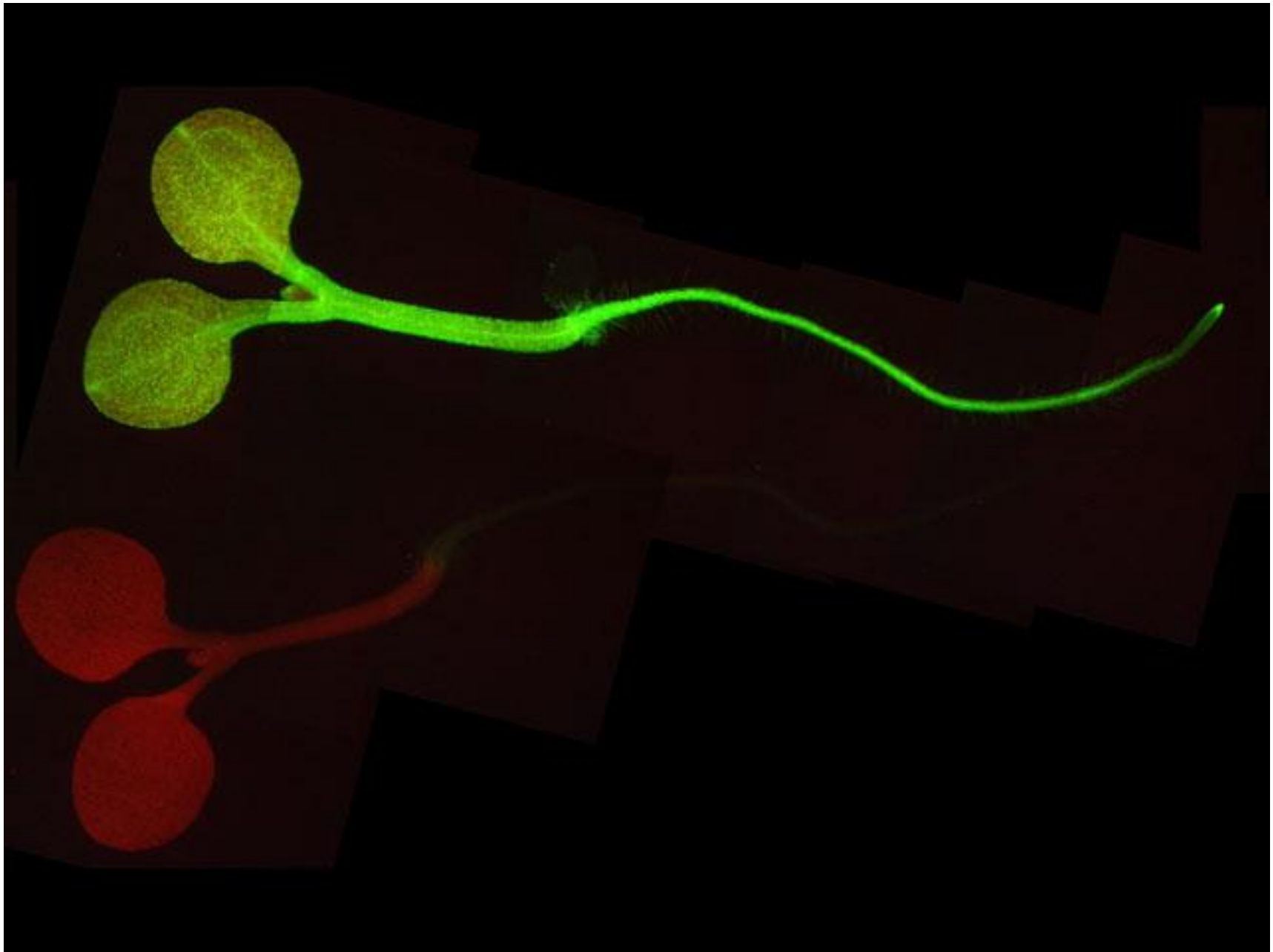


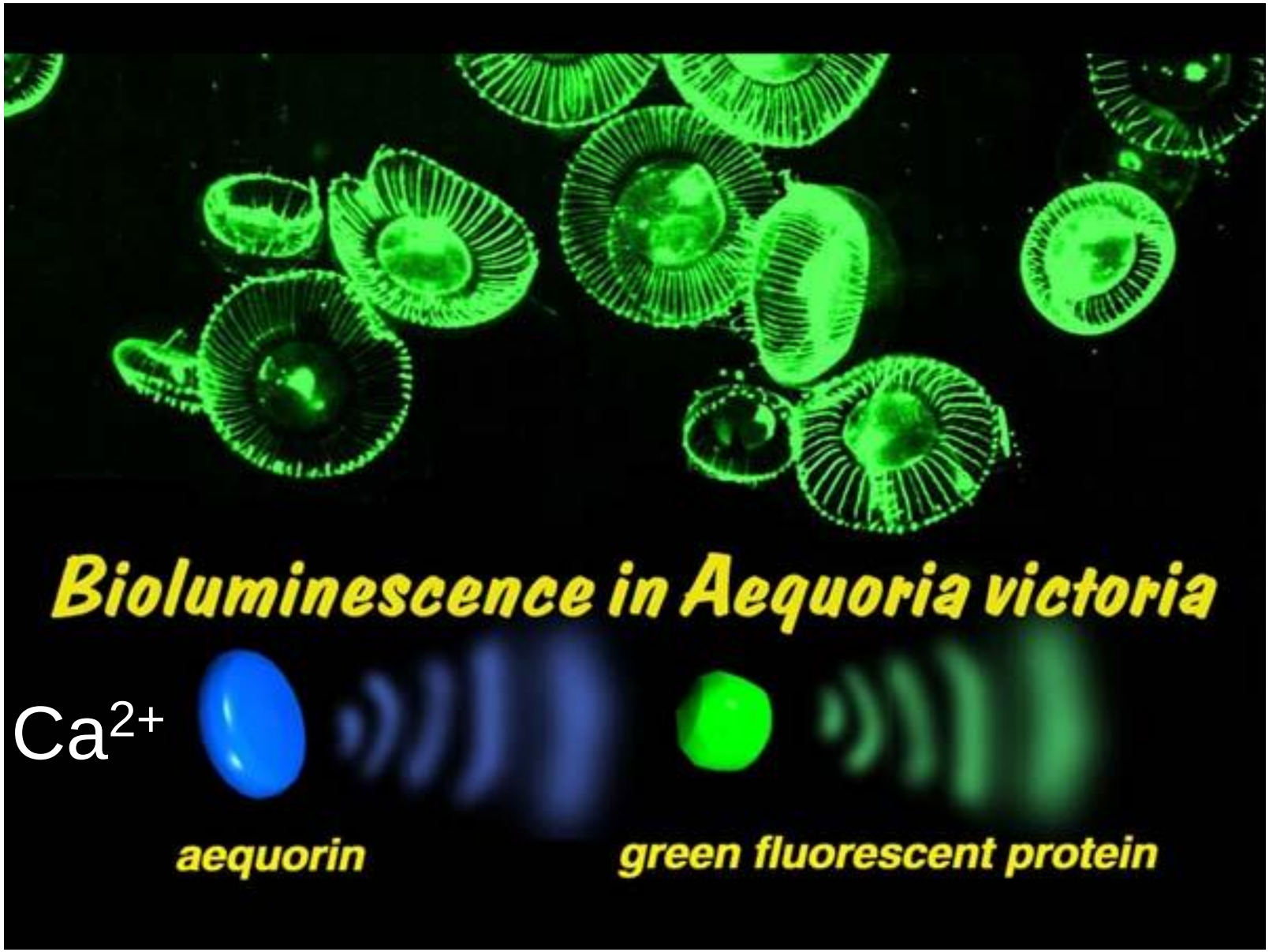


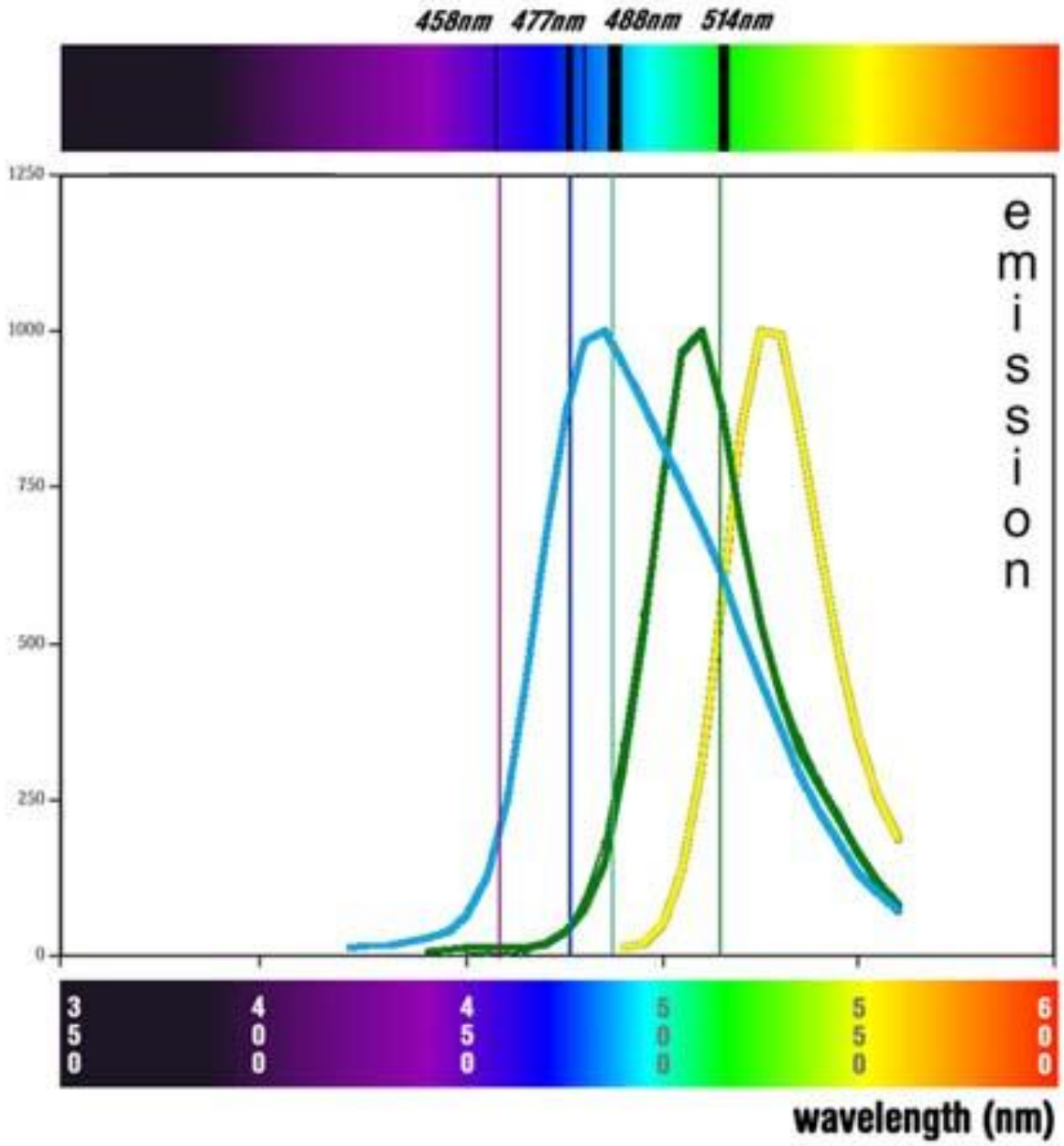


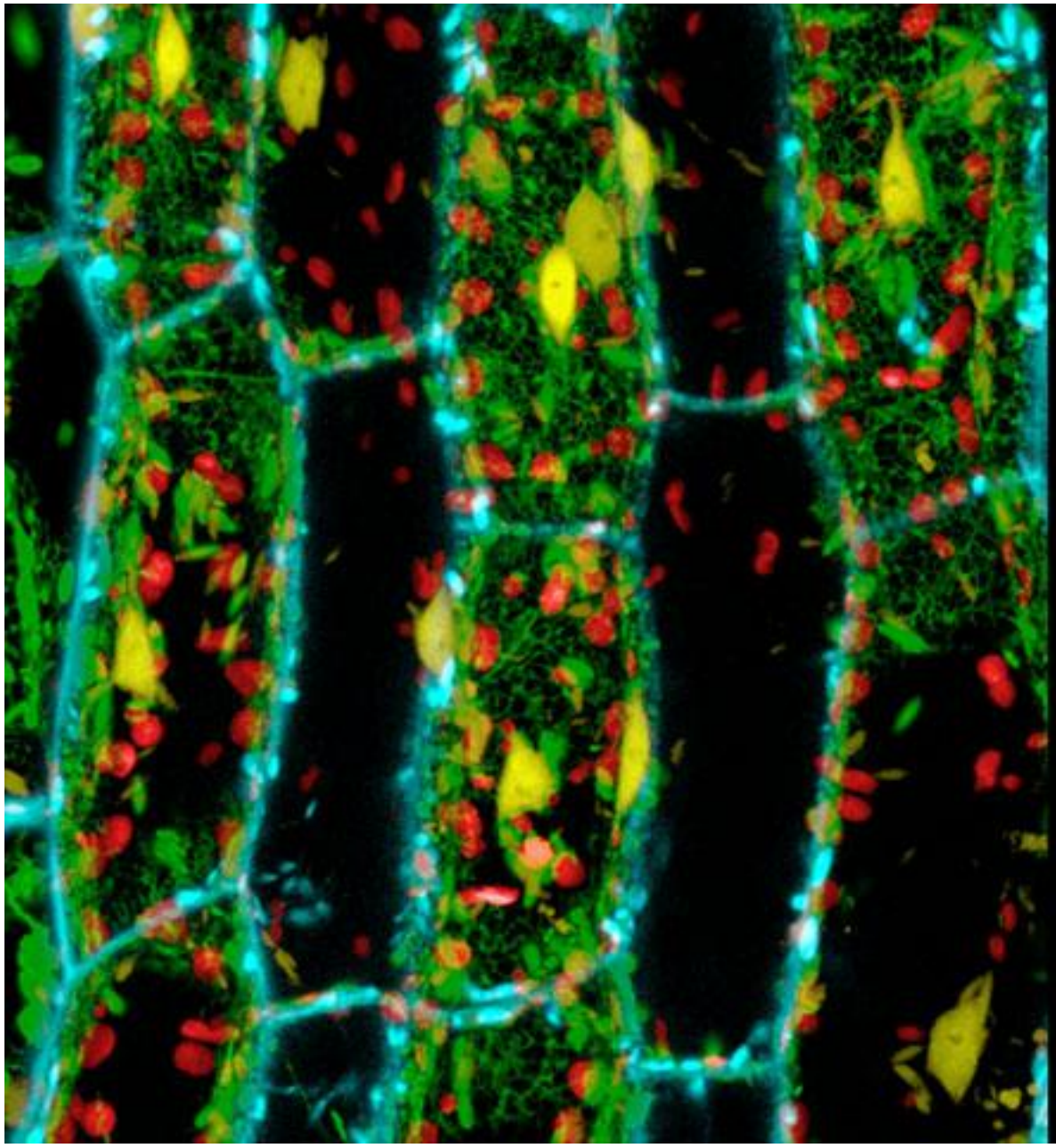
luciferin + O_2 → oxyluciferin + light

Green Fluorescence Protein




Bioluminescence in Aequoria victoria
Ca^{2+}
aequorin
green fluorescent protein


458nm
477nm
488nm
514nm
1250
1000
750
500
250
0
emission
350
400
450
500
550
600
wavelength (nm)

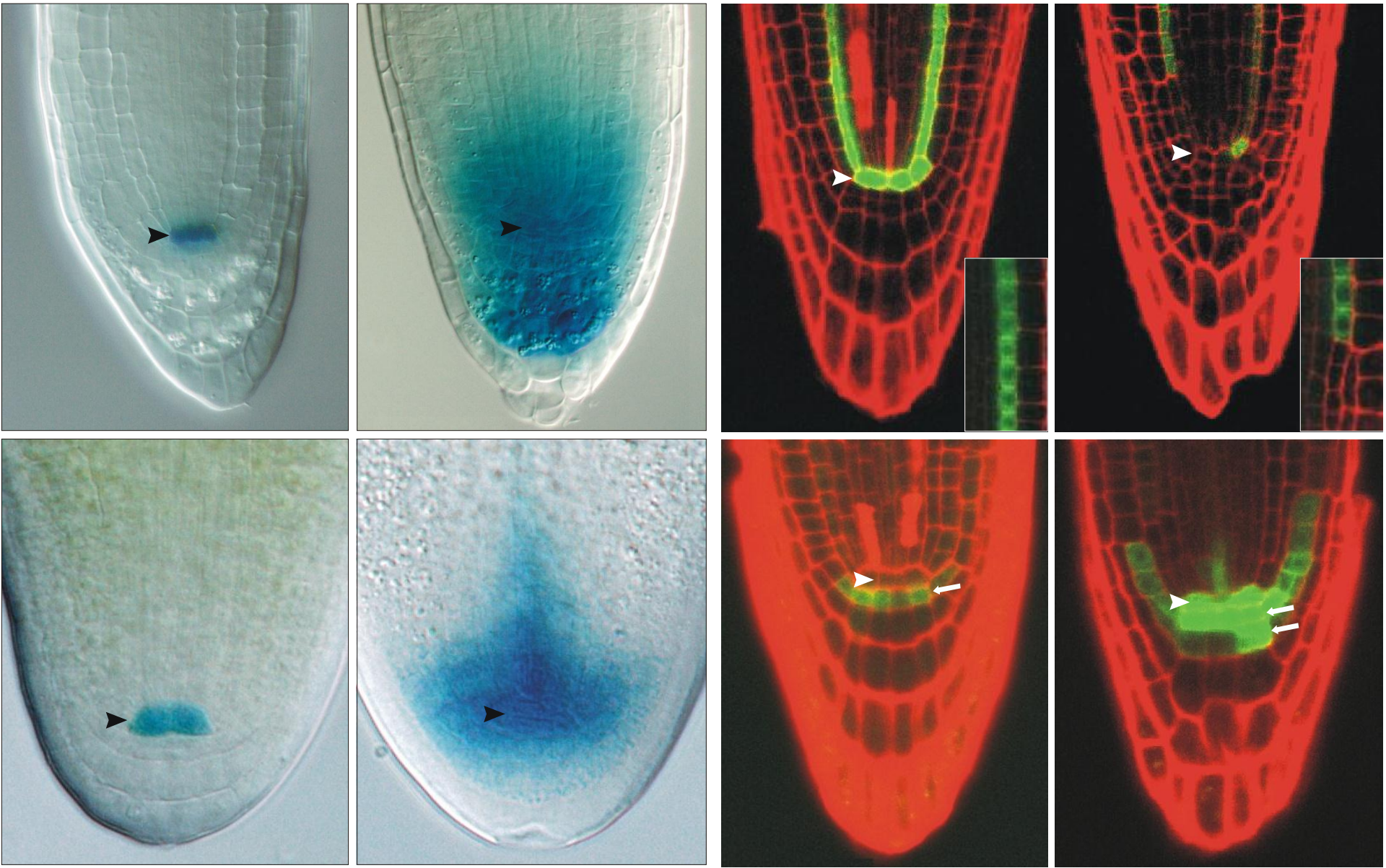


Multi-spectral Imaging with:

Extensin-CFP
GFP-ER
Histone2b-YFP
Chloroplasts

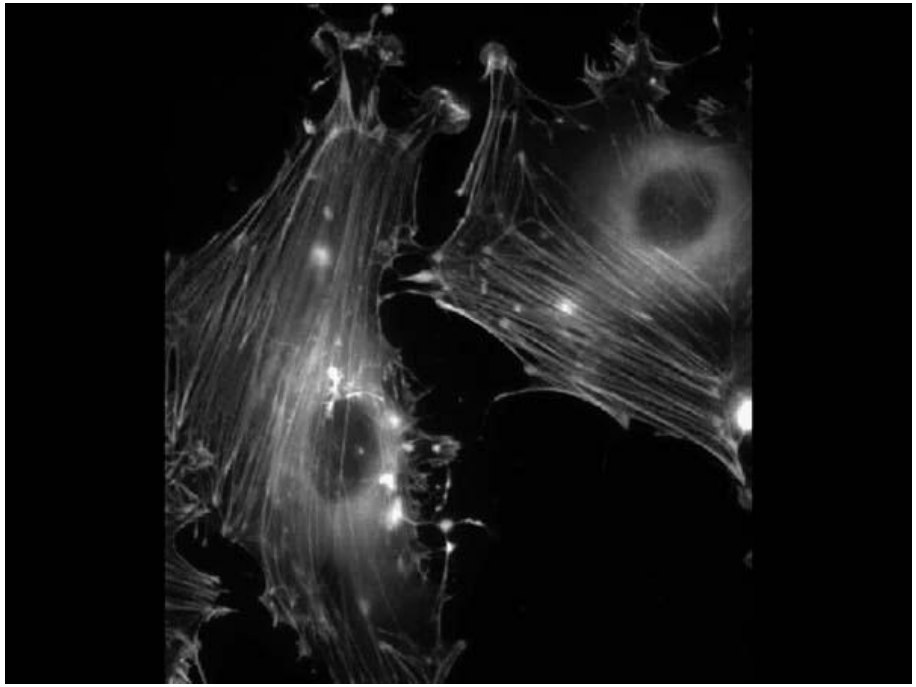
CJ Runions

Cell identity markers

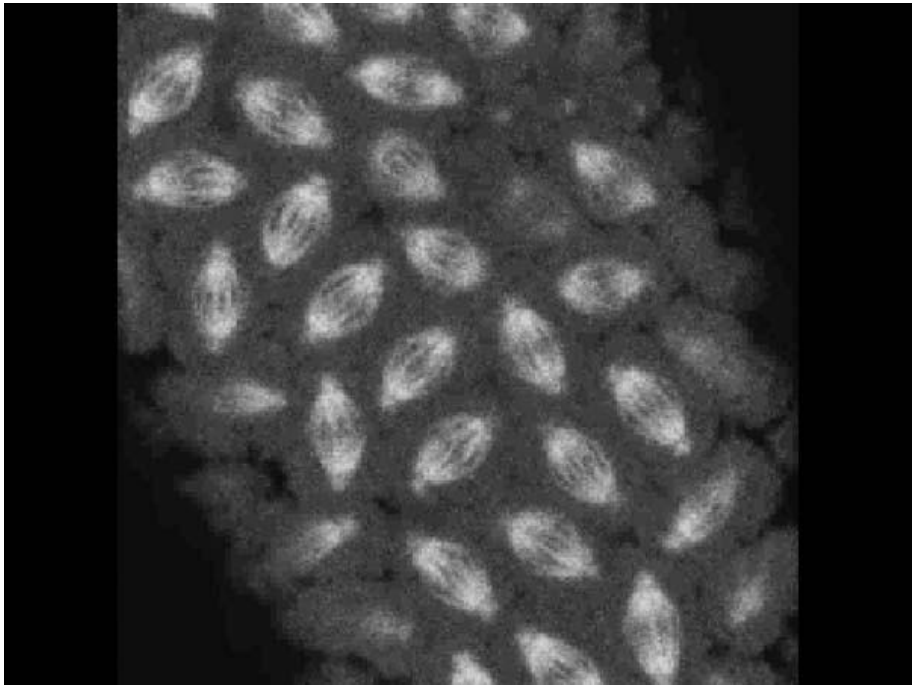


Subcellular structure markers

Actin



Tubulin

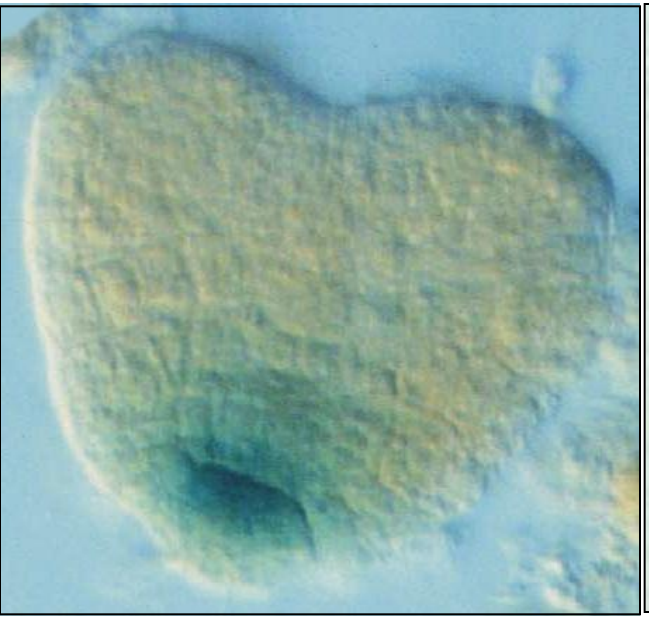


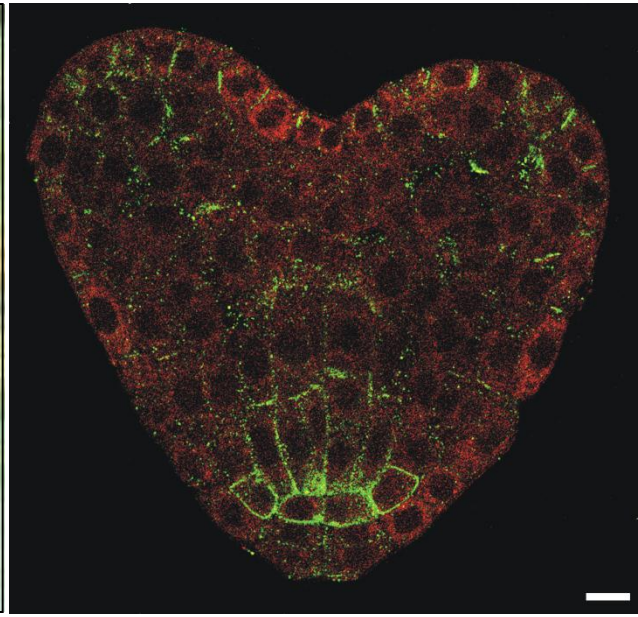
In situ mRNA/protein localisation

- Probe preparation
- Fixation
- Embedding
- Sectioning
- Deparafinization
- Treatment with probe
- Removal of unbound probe
- Signal visualization

Analysis of gene expression

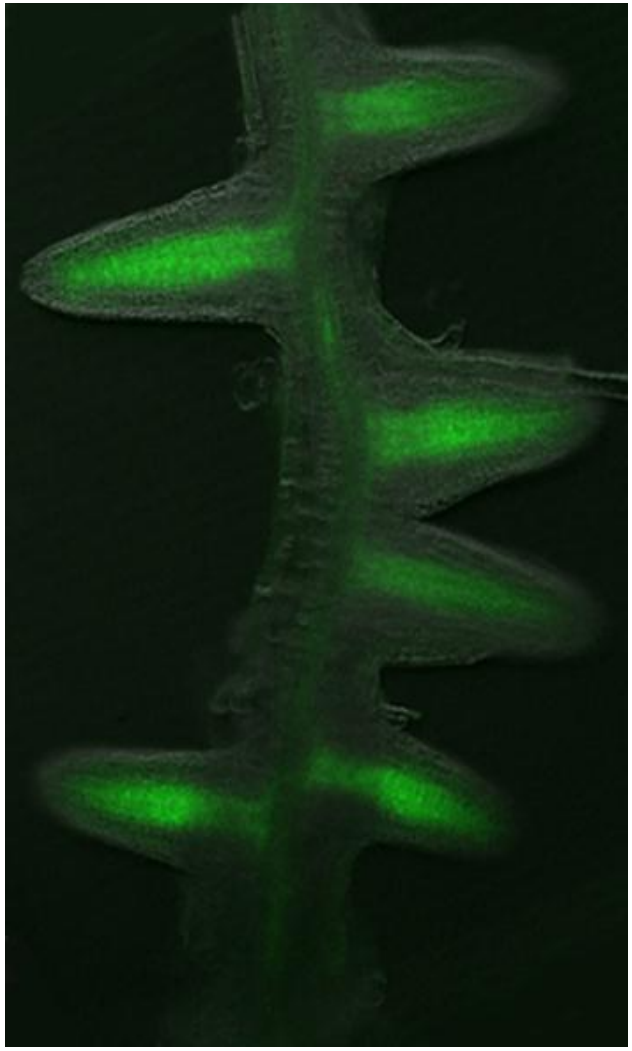
GUS **mRNA** **Protein**

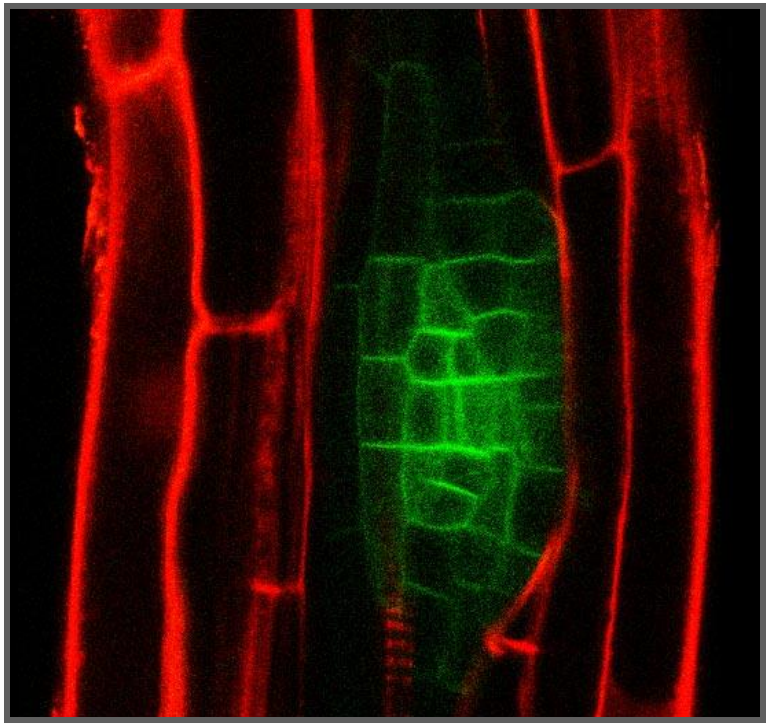


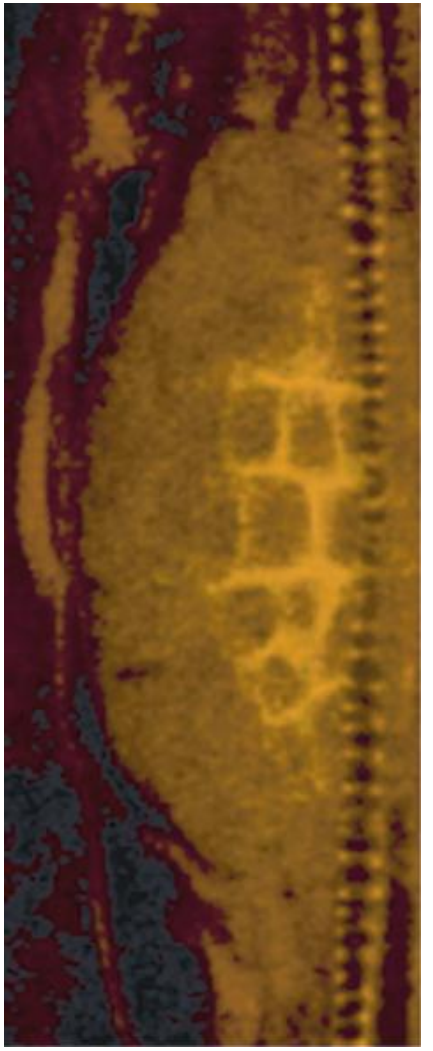


Verifications? In-situ, immunolocalisations, transcriptional fusion, translational fusions

Analysis of protein localisation







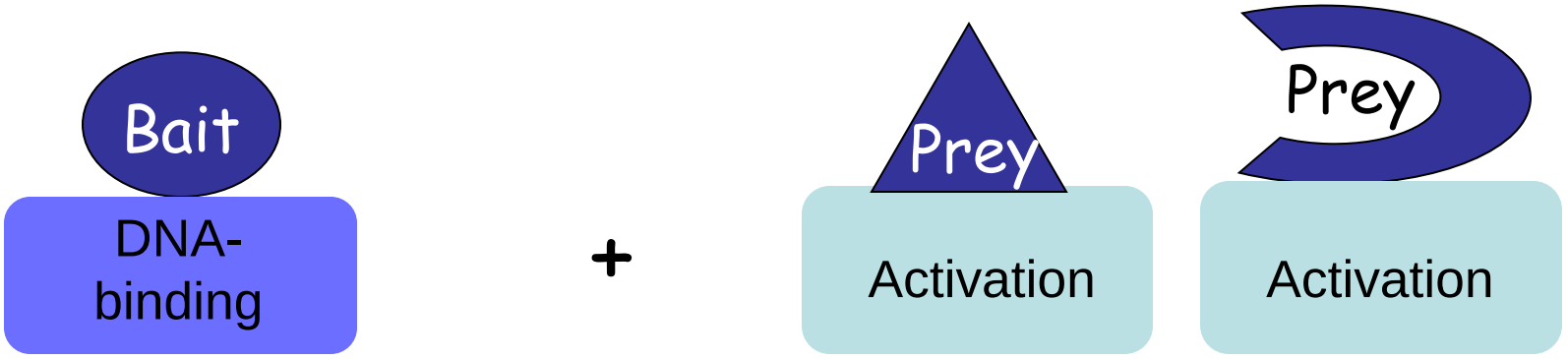
Friends and associates

- Yeast-two-hybrid
- Split ubiquitin, split YFP
- Genetic interactions
- Upstream and downstream

Yeast two hybrid

Classical transcription factor

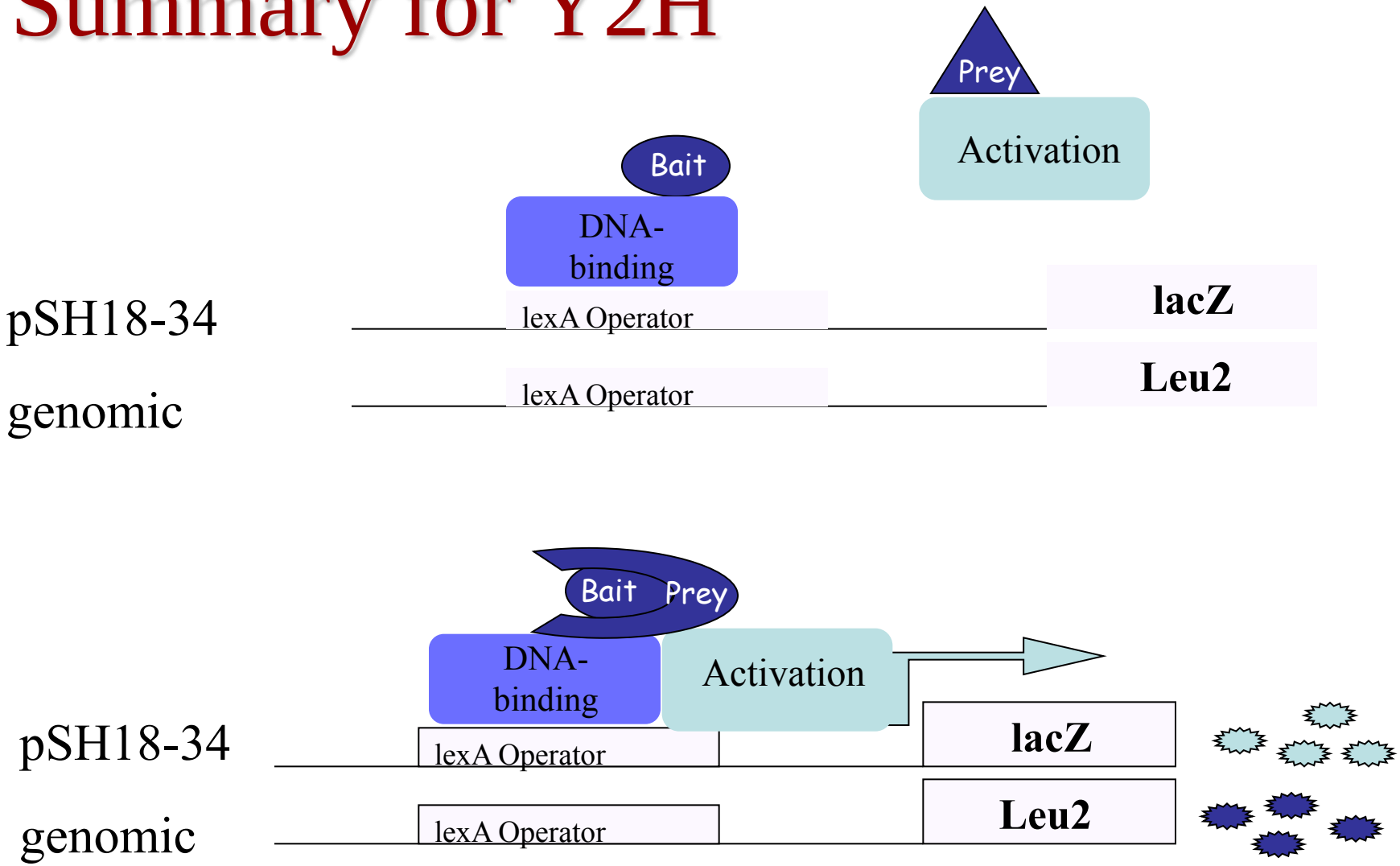
1. DNA Binding domain
2. Activation domain

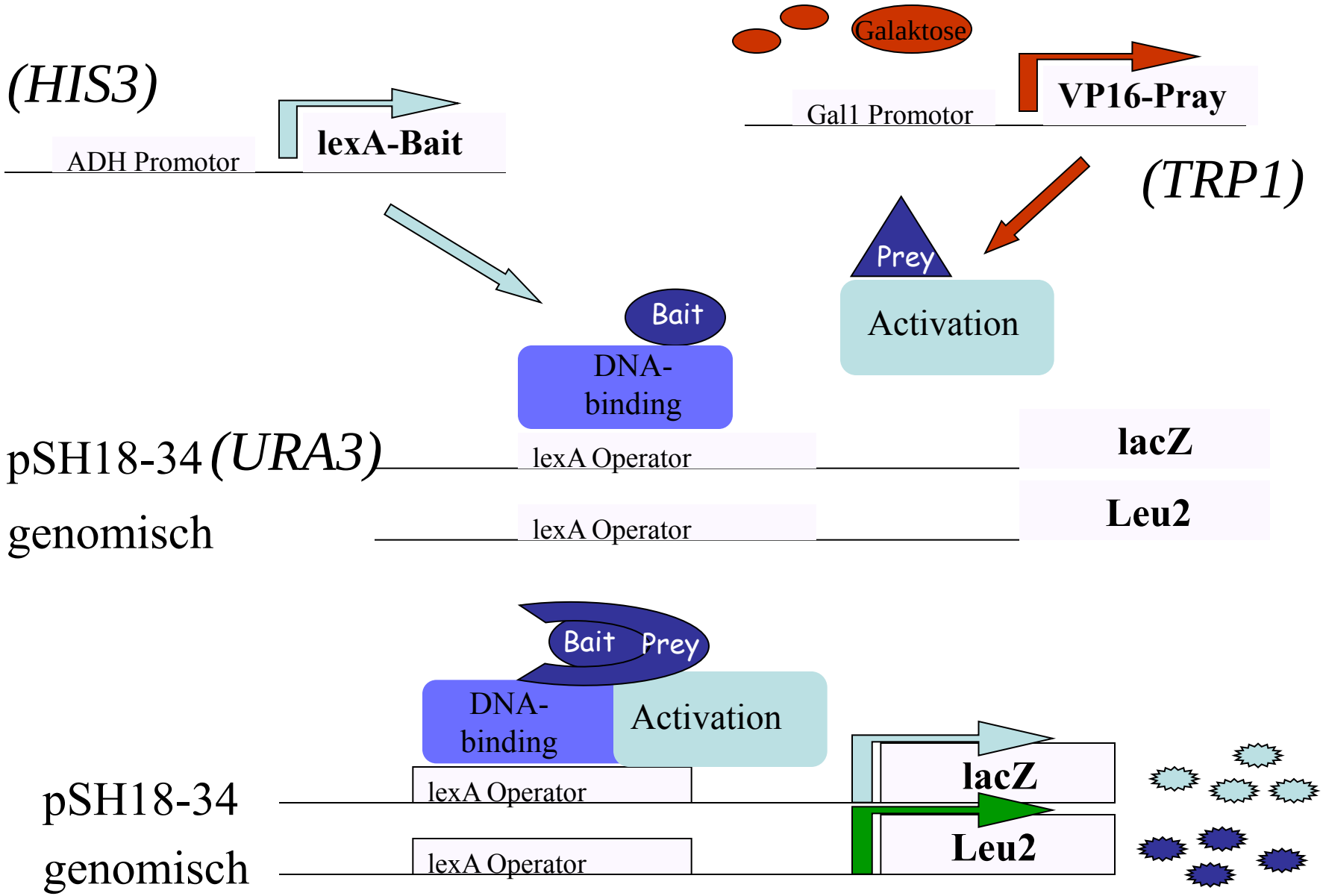


lexA Protein

VP16 Protein

Summary for Y2H





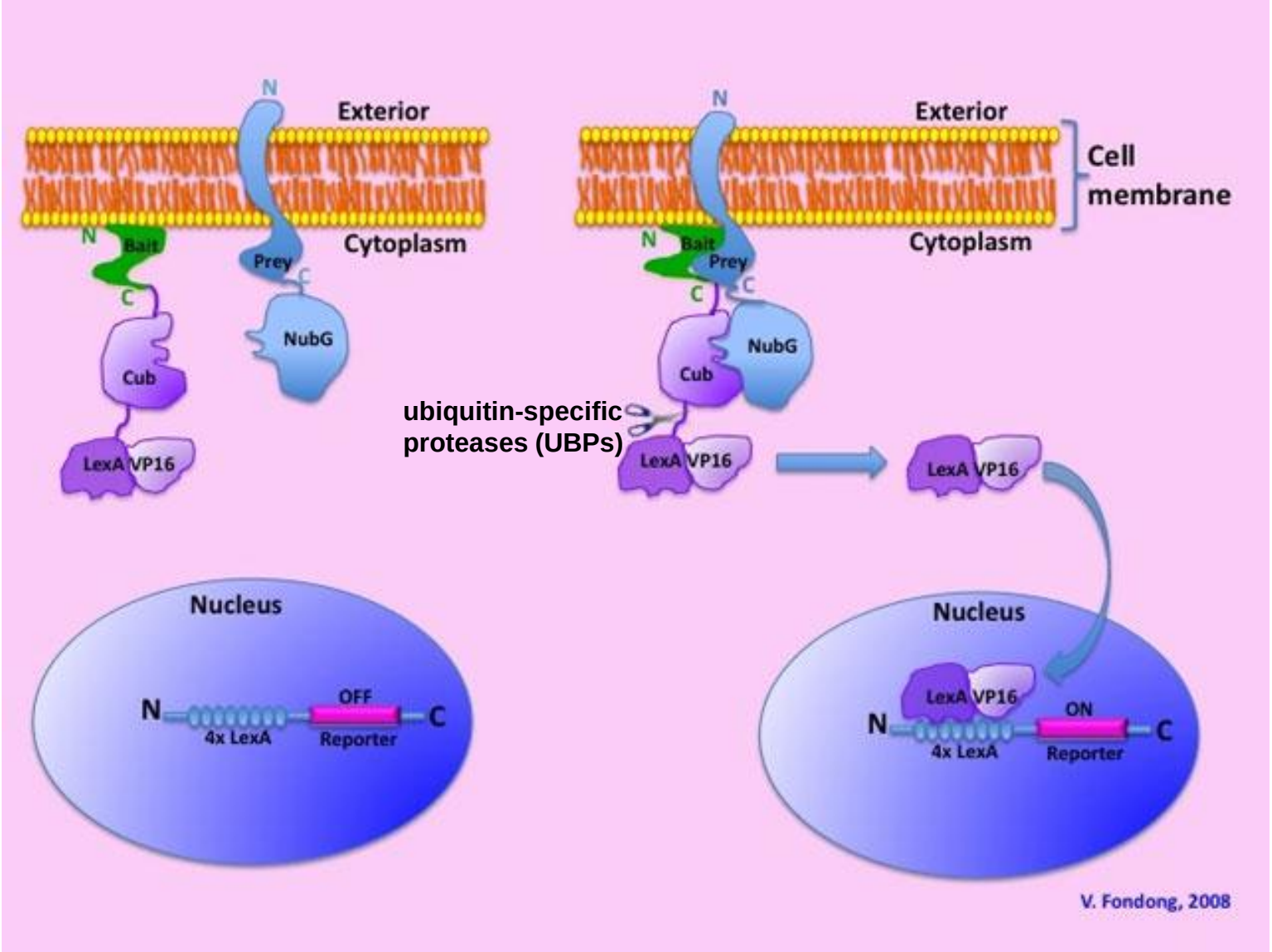
EGY48: Mutant for *HIS3, TRP1, URA3* und *LEU2*

Conditions for Y2H-System

1. Proteins must be able to localize to the nucleus

2. Bait construct must not have its own activation domain (Autoactivation)

Split-Ubiquitin



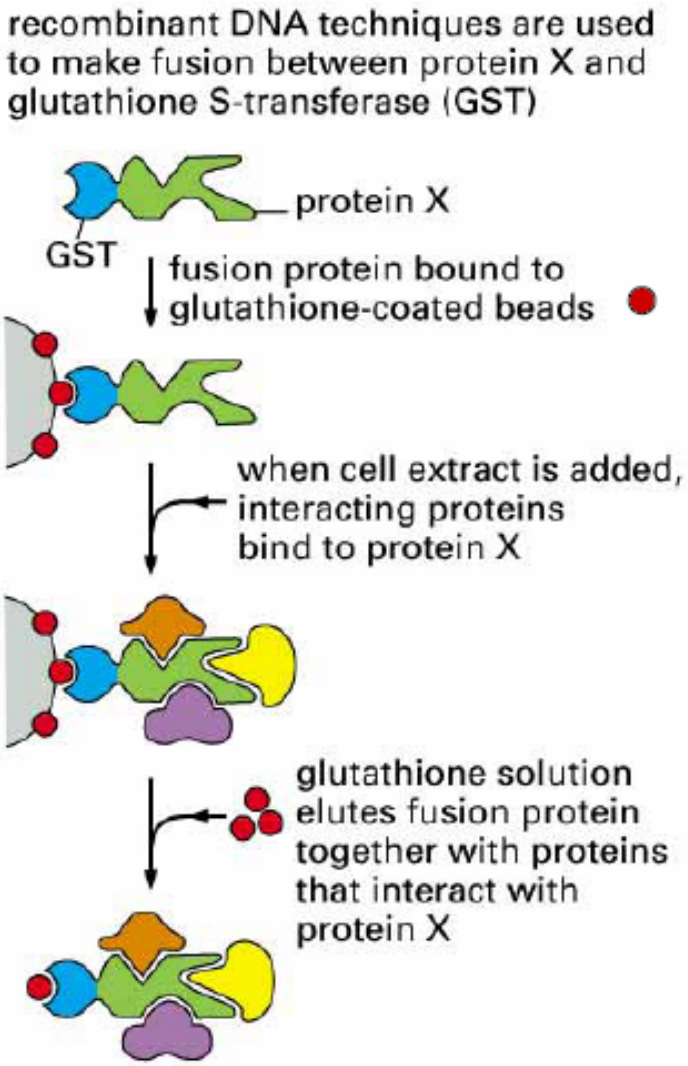
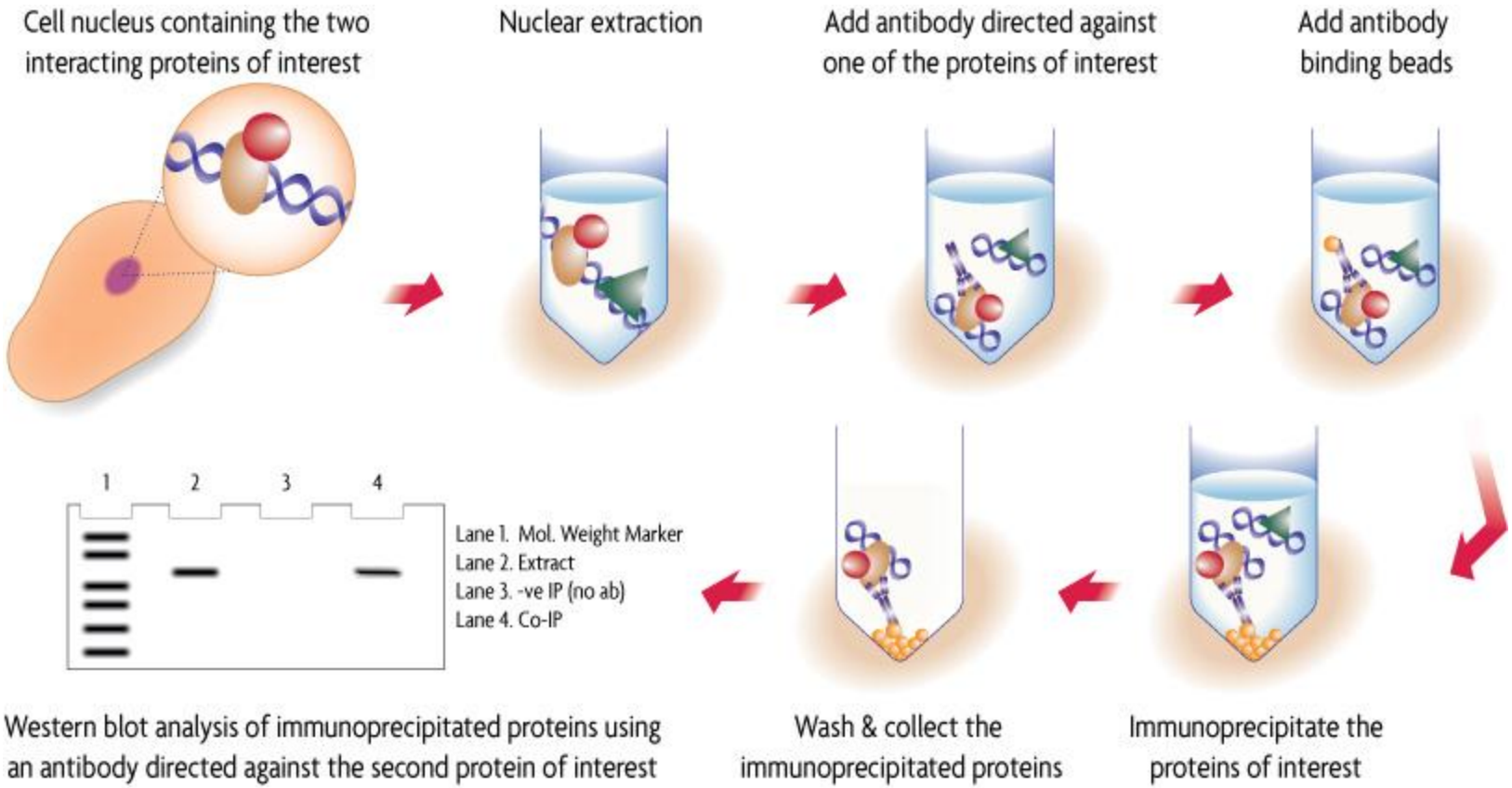


Figure 8–50. Molecular Biology of the Cell, 4th Edition.

GST "pull downs"

- GST protein is usually expressed in E. coli as microgram quantities are used in typical assays
- Detection of bound proteins are usually by western blotting, using antibody to the putative interactor
- Used extensively with GST-domain fusions in structure function studies
- New proteins can be identified if metabolically labeled cells are used

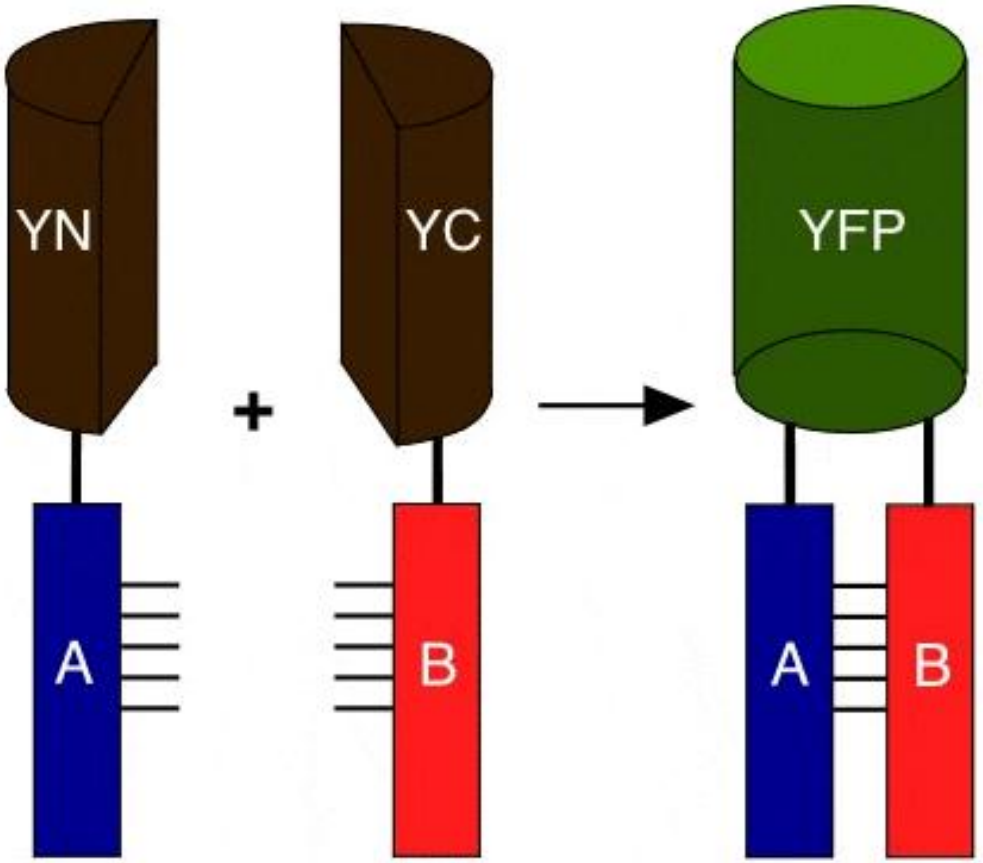
Co-immunoprecipitation



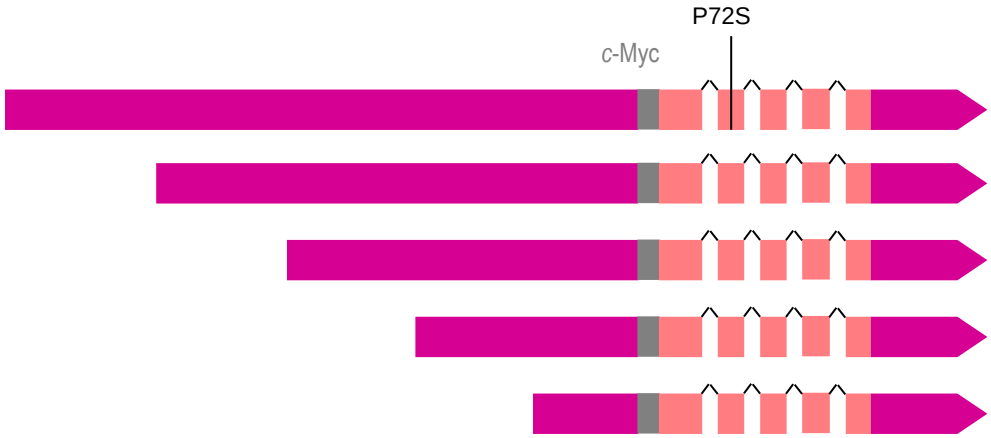
Flow chart of the Co-Immunoprecipitation procedure used in the Nuclear Complex Co-IP Kit.
Nuclear extract is prepared using a combination of low-salt buffers and enzymatic shearing, which helps protect nuclear protein complexes as they are released from the DNA. Immunoprecipitation is then carried out and the protein complex is washed using buffers that can be adjusted by addition of salt and detergent to optimize the stringency required to maintain the complex while eliminating non-specific proteins. Western blot is then performed using a 2nd antibody directed against a 2nd protein of interest.

Split-YFP

- Protoplast transfection



Upstream - Promotor analysis (yeast one hybrid)

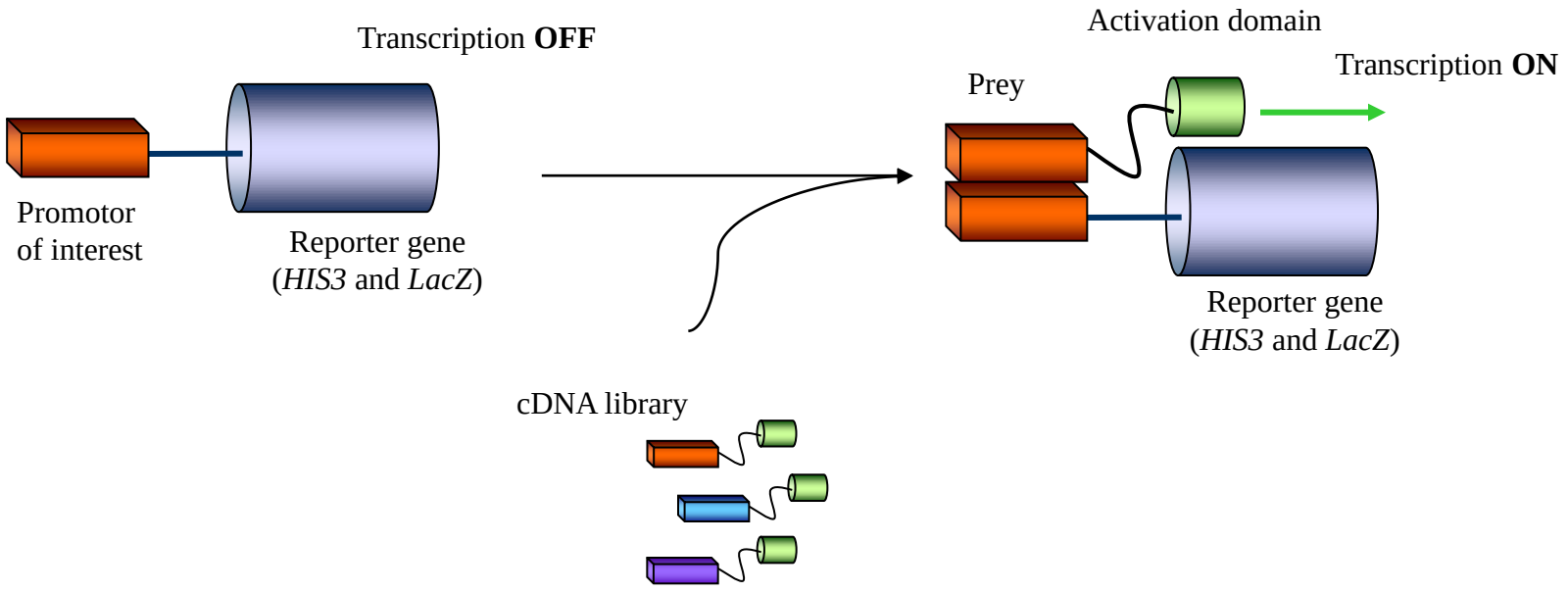


Promoter (bp)	Activity
2,400	Yes
1,500	Yes
1,000	Yes
500	No
150	No

promoter::GUS

Yeast One-hybrid

- Identification of protein-promoter interactions



Transcription Factors – Targets verification

Chromatin immunoprecipitation (ChIP)

Electrophoretic mobility shift assay (EMSA)

Co-expression

Downstream targets

- expression profiling
- proteomics
- second site mutagenesis
- educated guess

Special methods and tools

- DR5 auxin response reporter
- Transient transfection
- Laser ablations and laser capture

DR5 (Auxin) Response Reporter

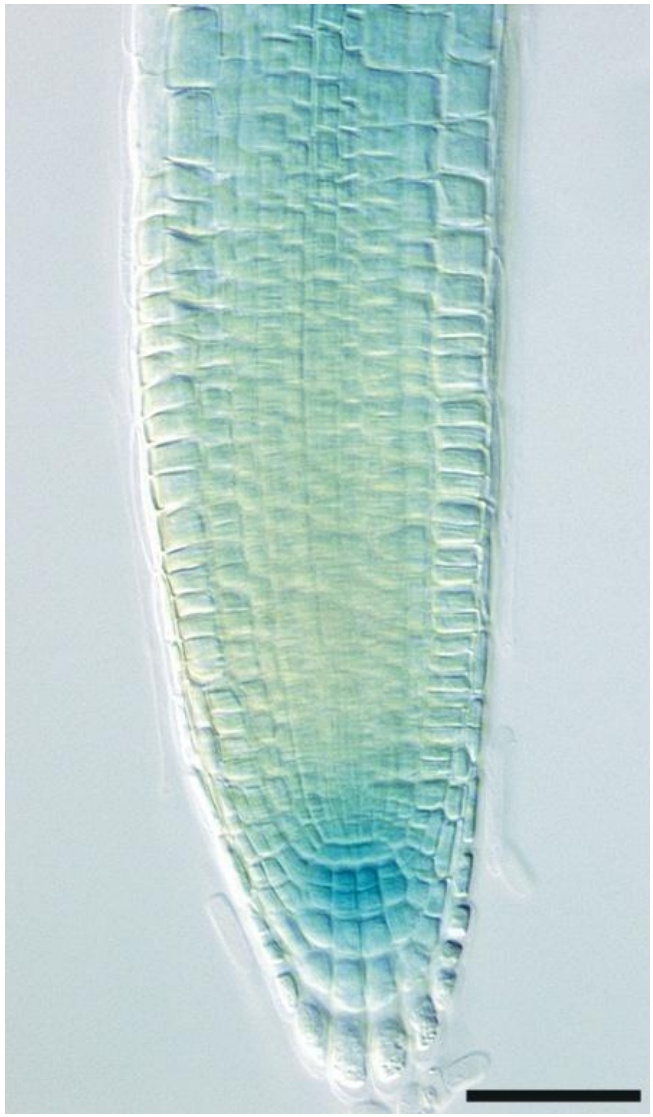
5´ CCTTT TGTCTC *3´*

9x inv.

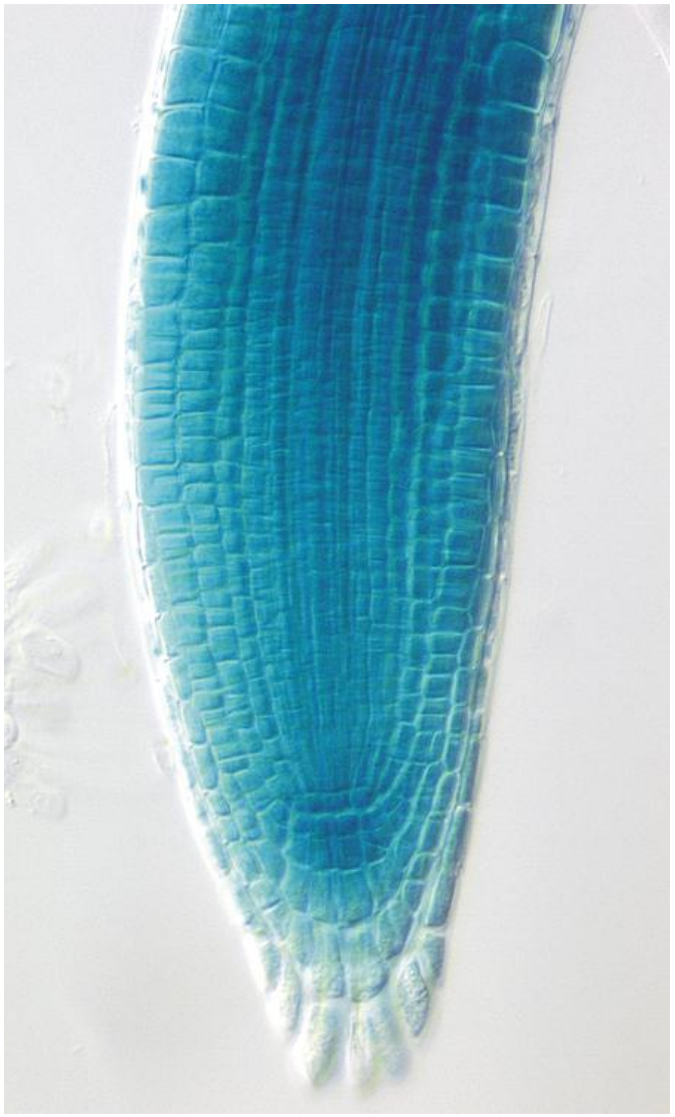
DR5: Ulmasov *et al.*, 1997

DR5::GUS

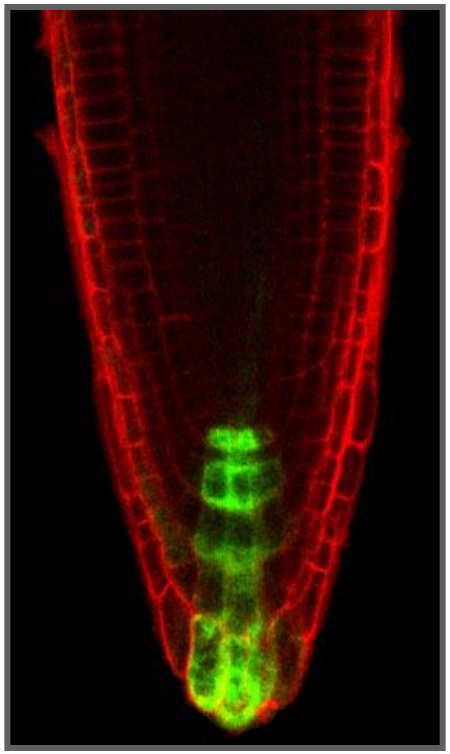
- Auxin



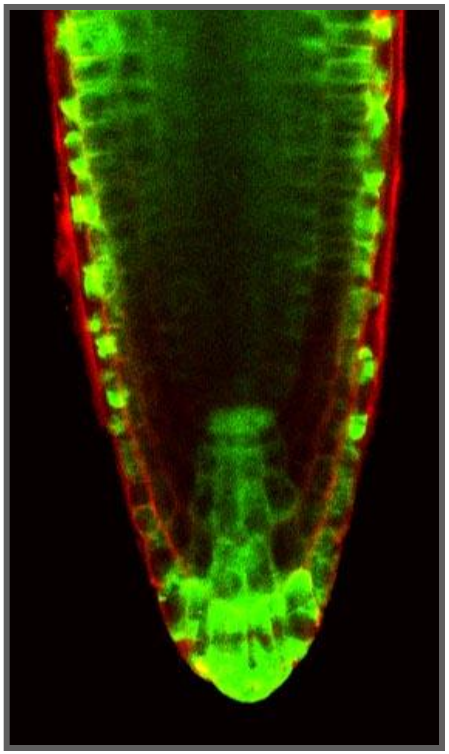
+ Auxin



DR5::GFP Auxin Reporter

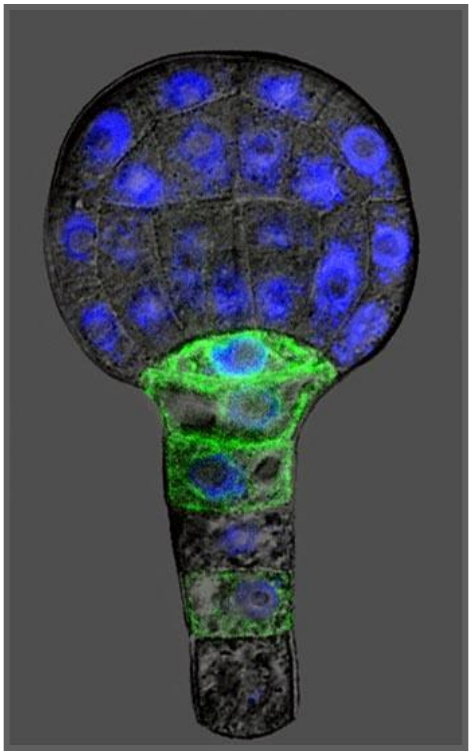


Root



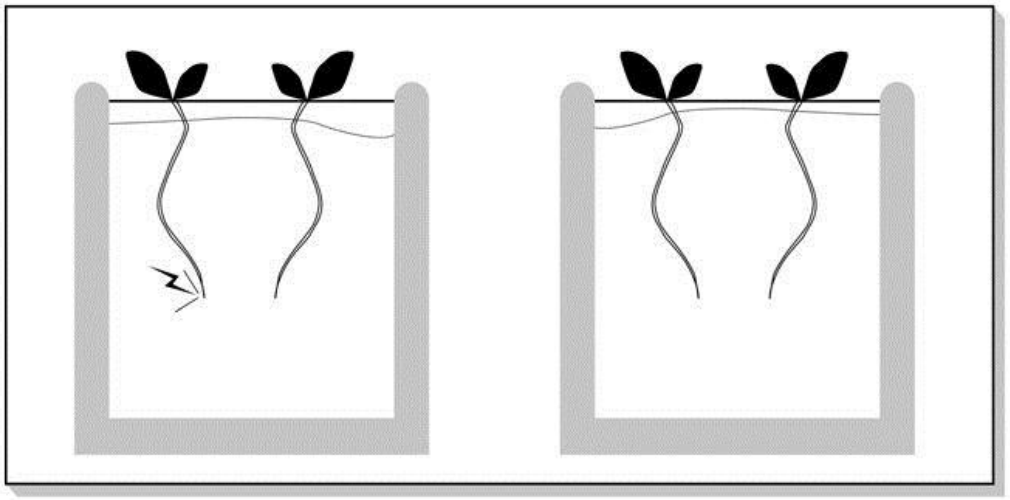
Root + Auxin

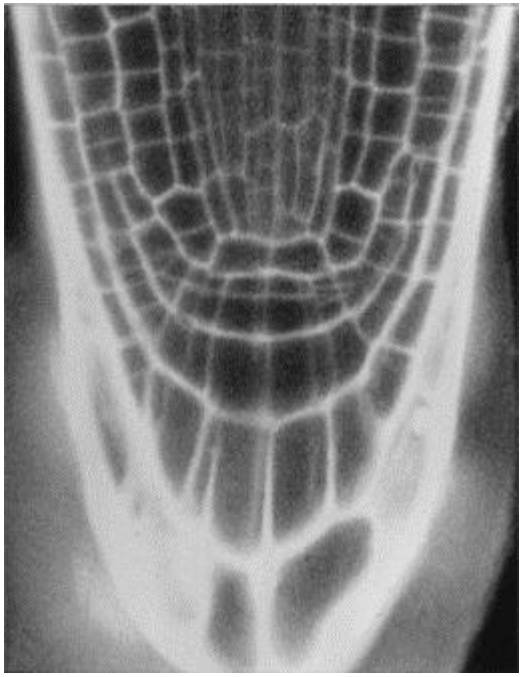
anti-IAA AB

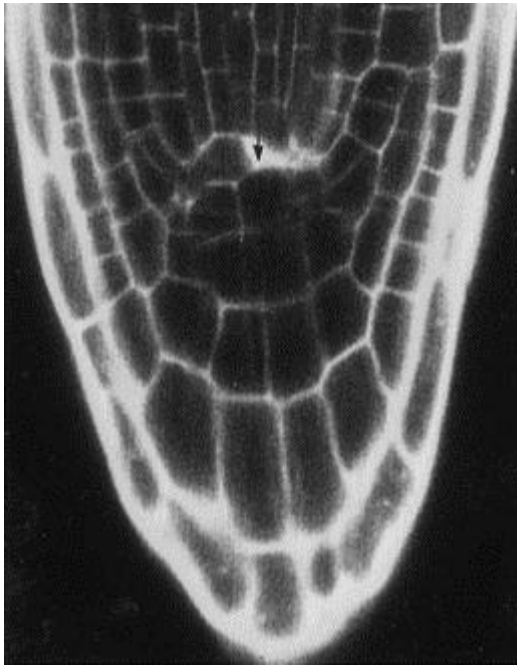


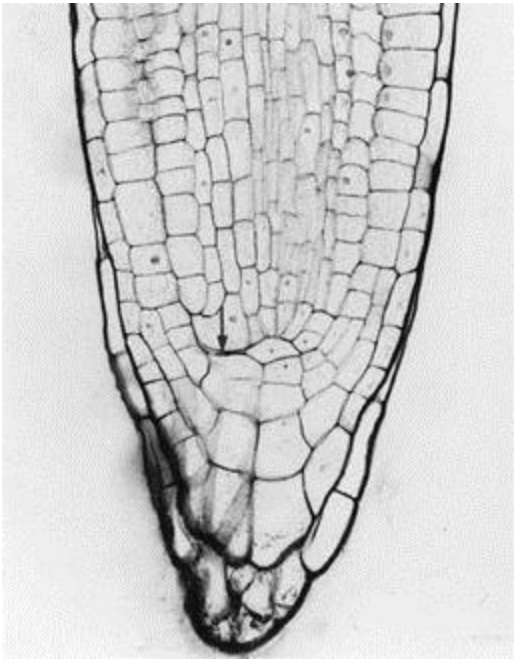
Embryos

Laser ablations

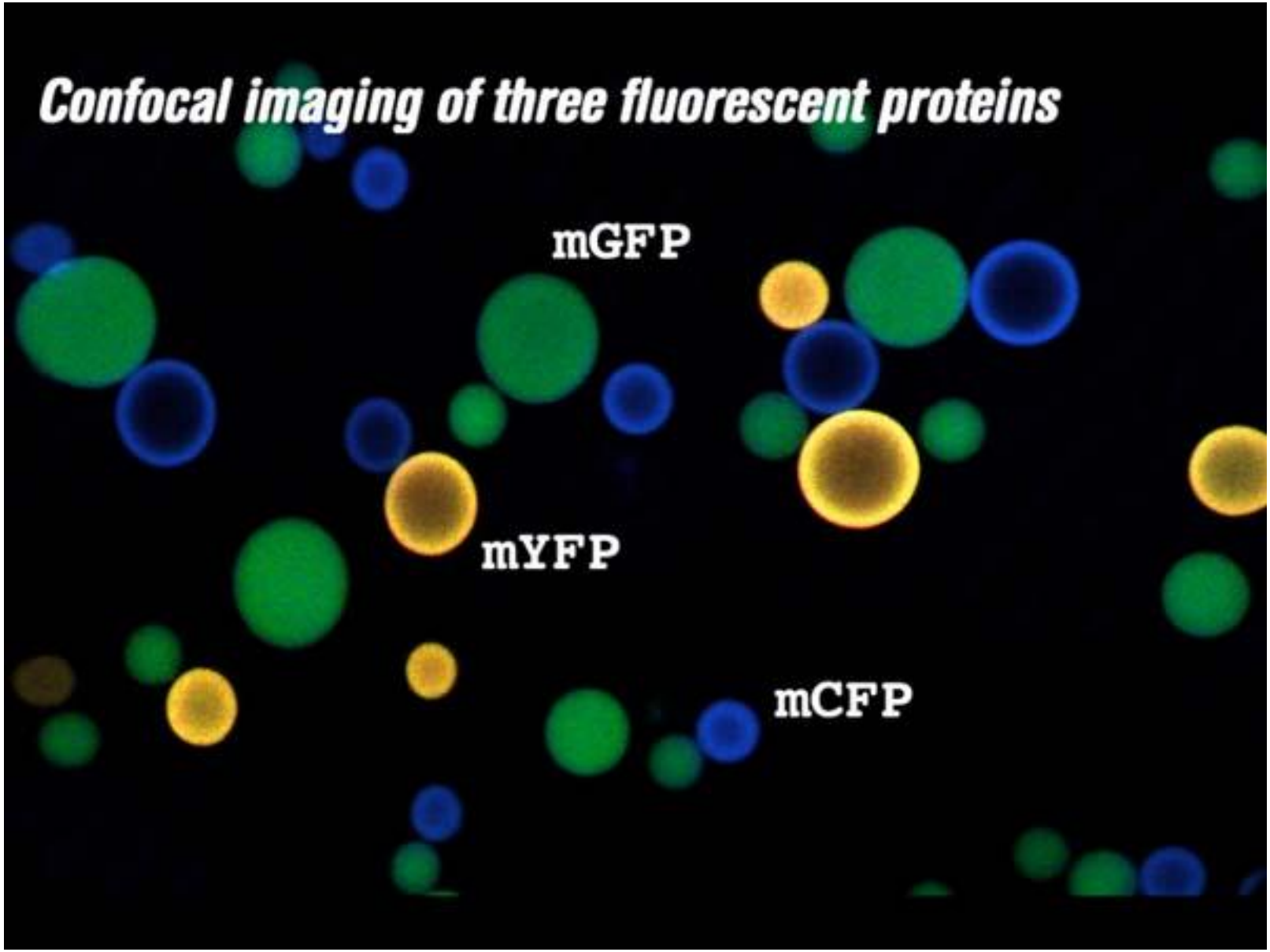






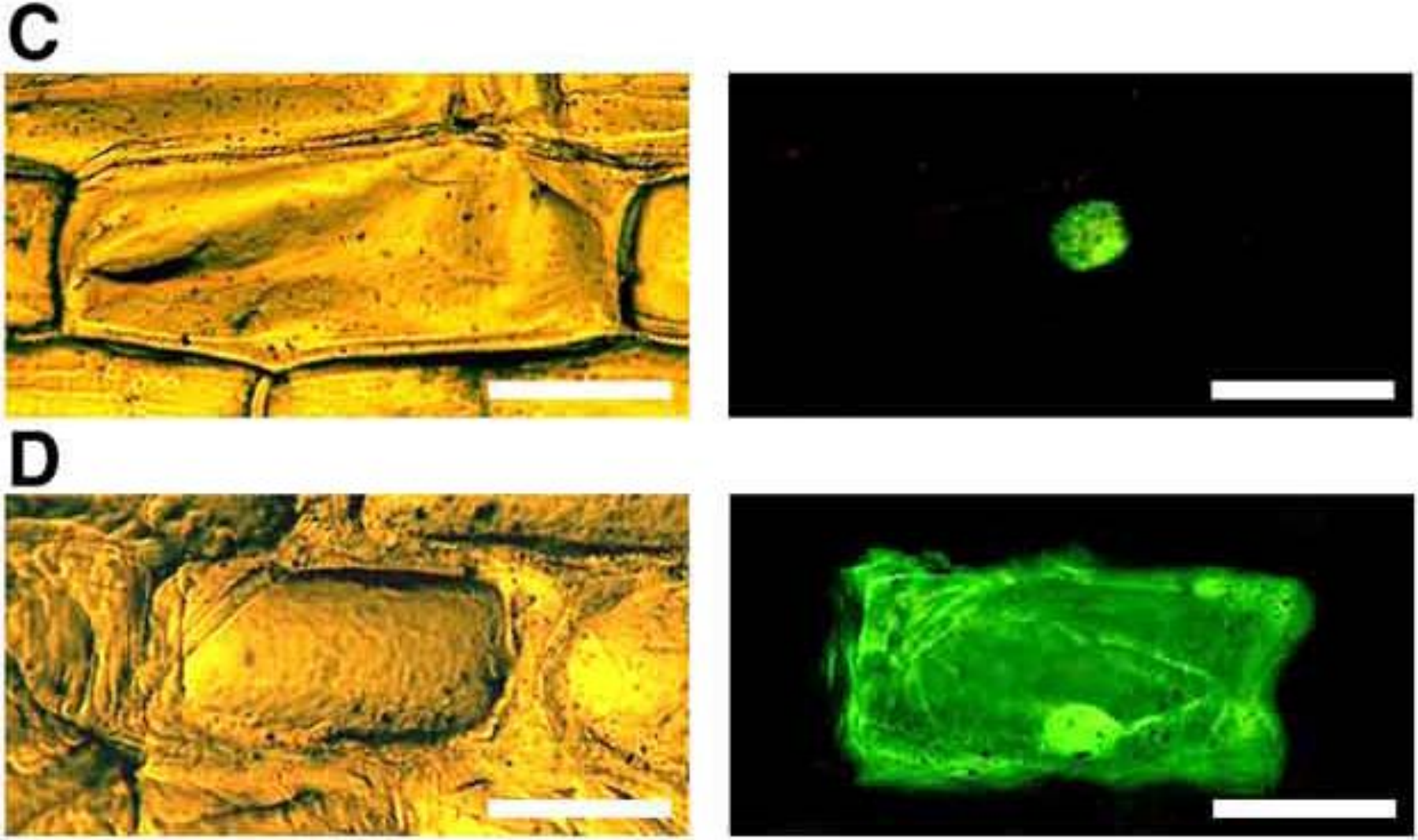


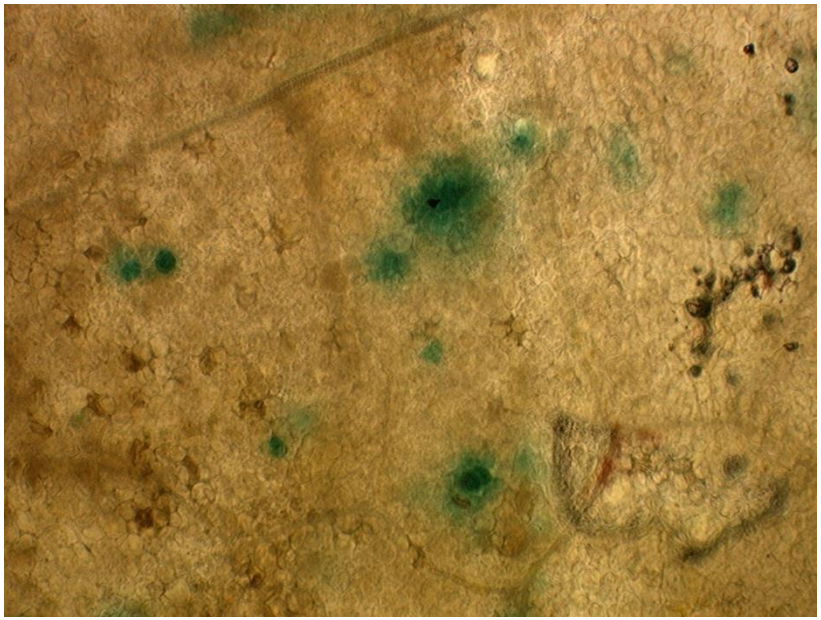
Transient transfection



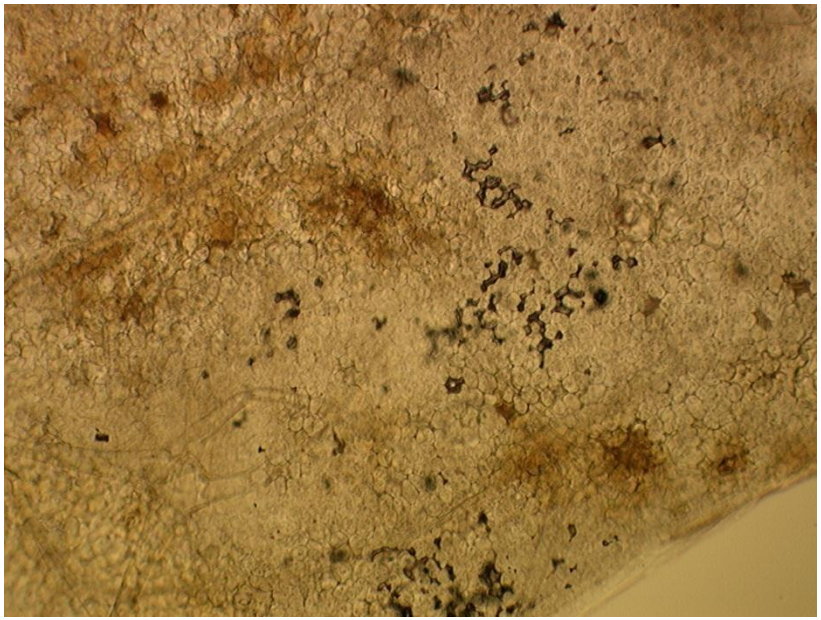
Protoplasts

Onion epidermis cells

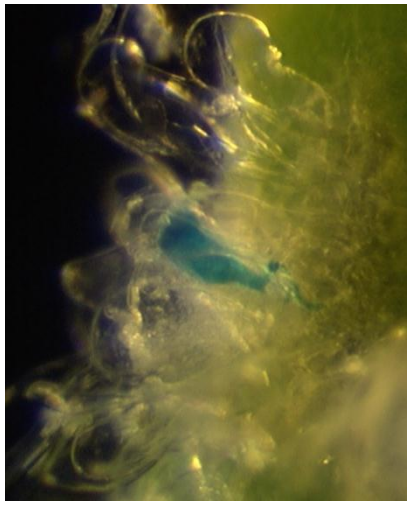




GUS

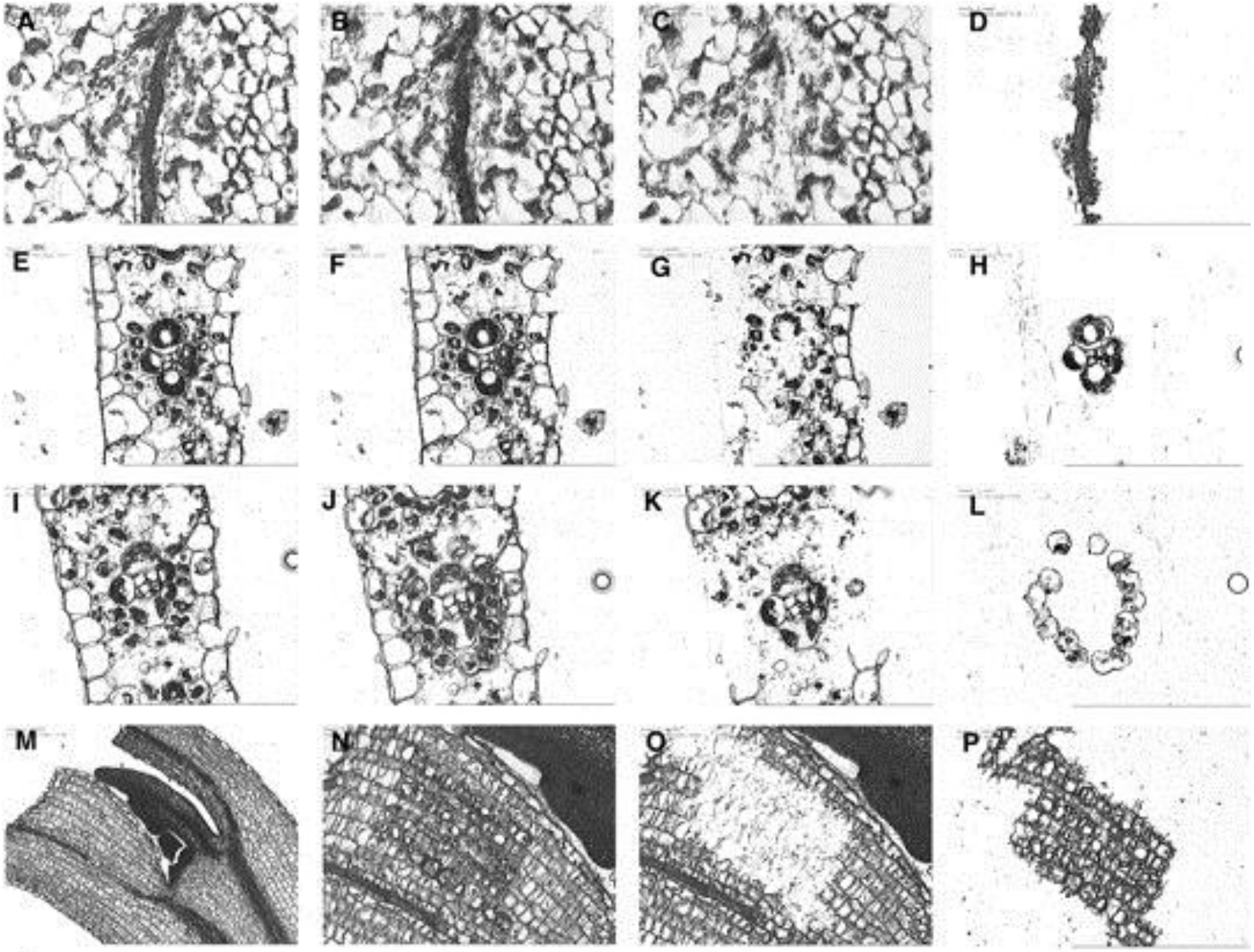


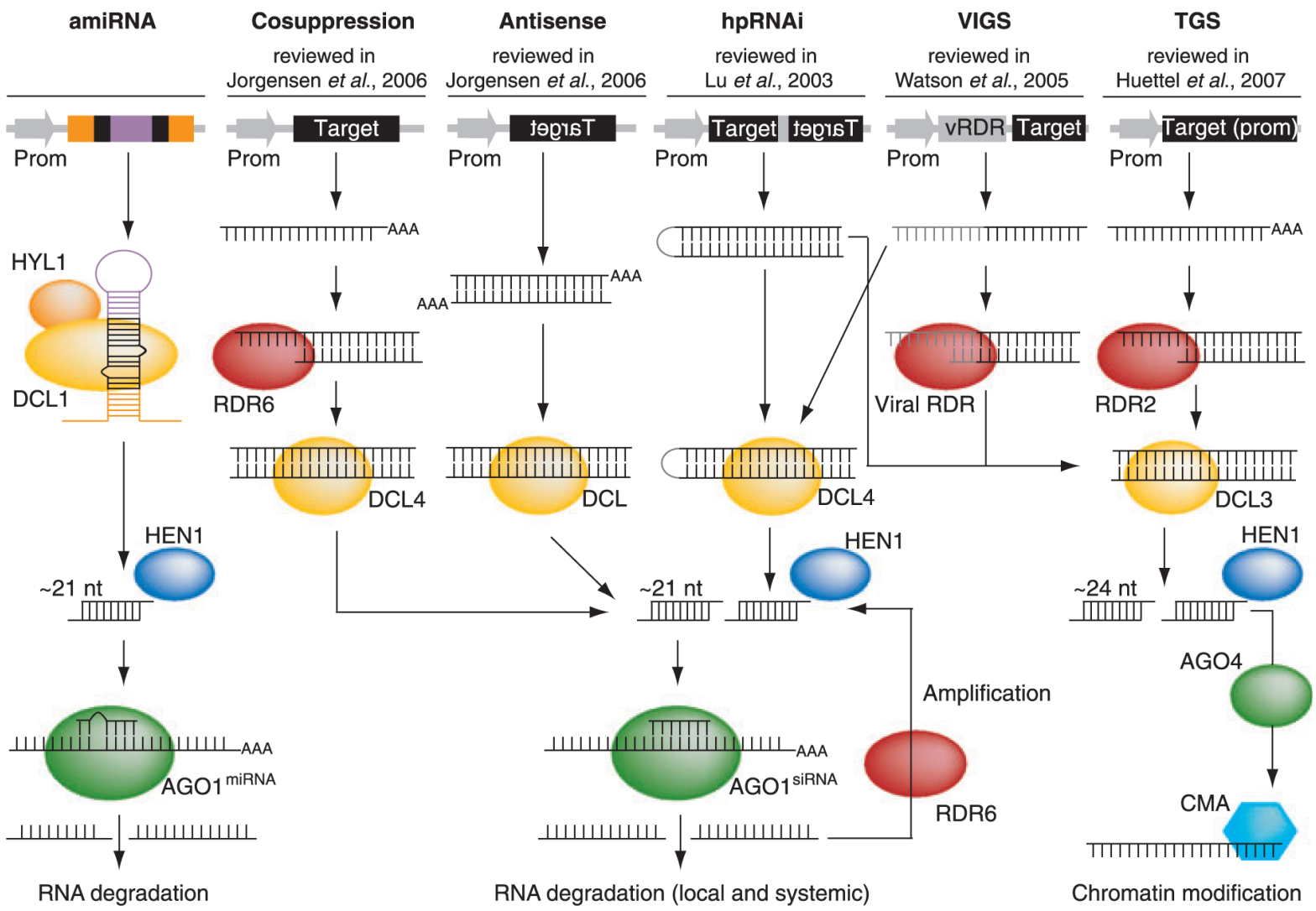
GUS + Diphteria Toxin



GUS + IPT (cytokinin biosynthesis)

Laser capture




amiRNA
Cosuppression
reviewed in Jorgensen et al., 2006
Antisense
reviewed in Jorgensen et al., 2006
hpRNAi
reviewed in Lu et al., 2003
VIGS
reviewed in Watson et al., 2005
TGS
reviewed in Huettel et al., 2007
Prom
Target
Target
Target
vRDR
Target
Target (prom)
AAA
HYL1
DCL1
RDR6
DCL4
DCL
DCL4
Viral RDR
RDR2
DCL3
HEN1
~21 nt
~21 nt
~24 nt
AGO4
Amplification
AGO1 miRNA
AGO1 siRNA
RDR6
CMA
RNA degradation
RNA degradation (local and systemic)
Chromatin modification