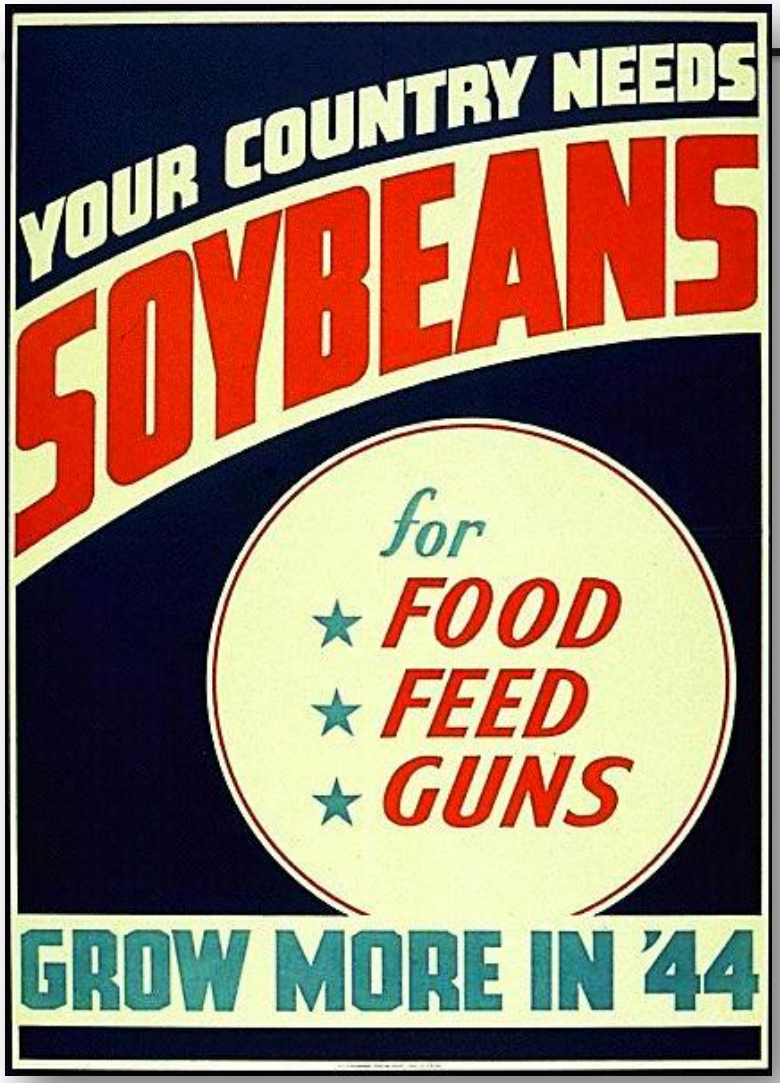
USB Update:
Center for Soybean Tissue Culture & Genetic Engineering

- Brief history and overview
- Goals and objectives
- Focus on nematode resistance

UNITED SOYBEAN BOARD
Making Your Checkoff Pay Off.

Engineering in the public domain



- Engineering technology is needed to
  - Continue finding new uses for soybean
  - Make soybean more economical to produce
  - Ensure soybean remains competitive as a crop
- Engineering also helps
  - Genomic and basic biology studies

Center members

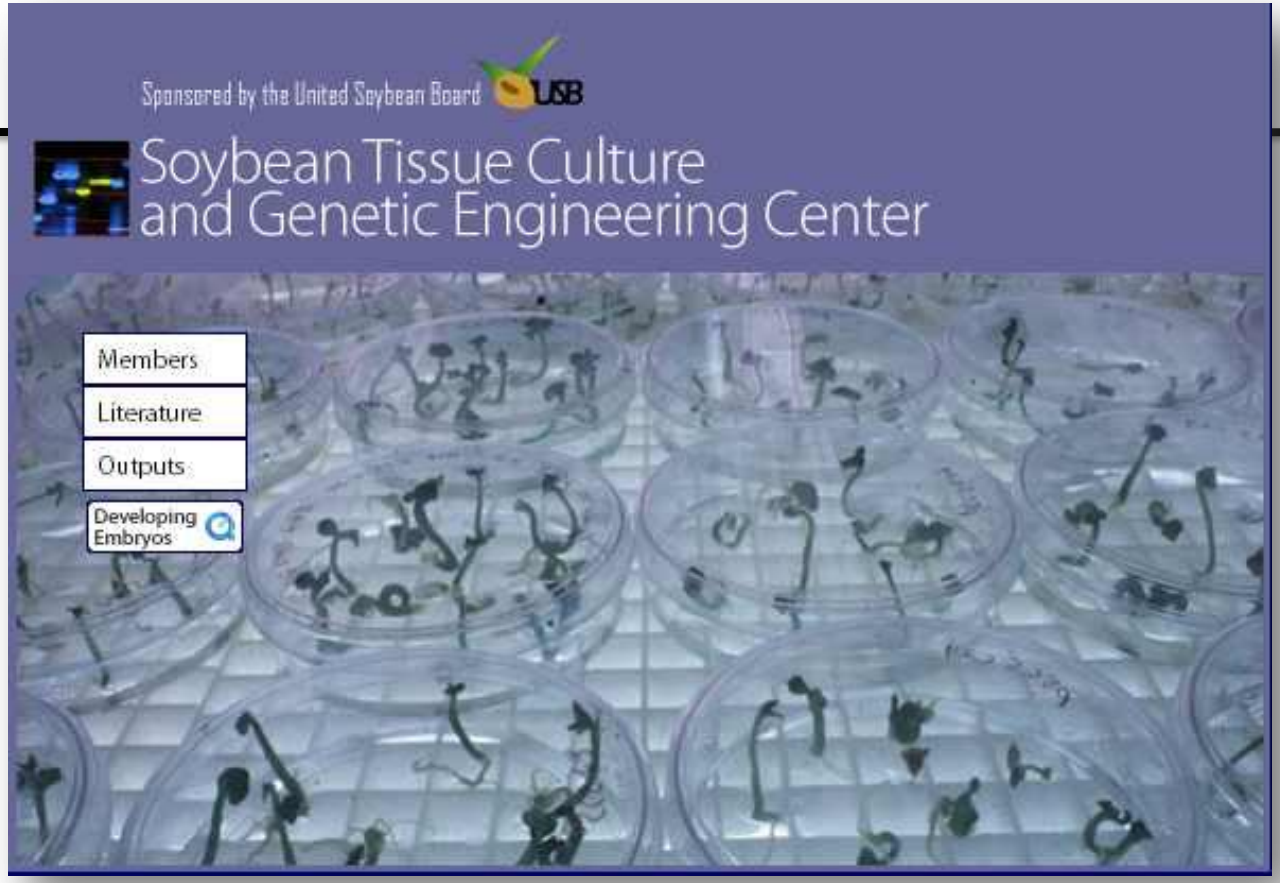
- Founding member, 1991
  - Glenn Collins
  - Retired 2007

- Joined in 1992
  - Wayne Parrott
  - John Finer

- Joined in 1998
  - Lila Vodkin
  - Jack Widholm

- Joined in 2008
  - Harold Trick

mulch.cropsoil.uga.edu/soy-engineering/



- Keeps technology in the public domain
- Viewed 40,185 times since 31 March 1998

Objective

- Develop and make available an efficient genetic engineering system for soybean
  - Freedom to operate
    - useful to anyone
- Engineer useful traits

Some engineered beans produced by Center members

Insect resistance

Bean pod mottle virus resistance

Specialty carotenoids for animal feed

SMV resistance

Additional engineered beans produced by Center members

- α-Tocopherol
  - Vitamin E
- Casein protein
  - Better quality protein
- High linolenic acid
  - For soyink
- Oxalate oxidase
  - White mold resistance
- Pinitol
  - For drought
- Phytase
  - To lessen phosphorus issues
- Zein
  - For greater methionine content

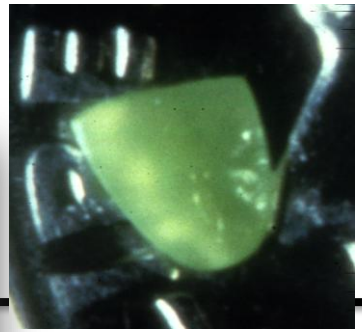
Use of Center's technology by others

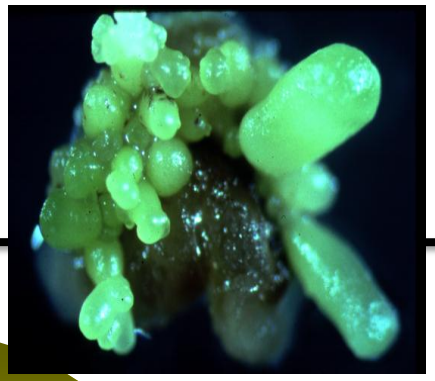
Agrius BioForms

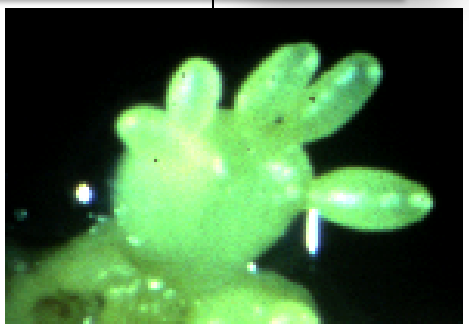
USB UNITED SOYBEAN BOARD
Making Your Checkoff Pay Off.

plenish™

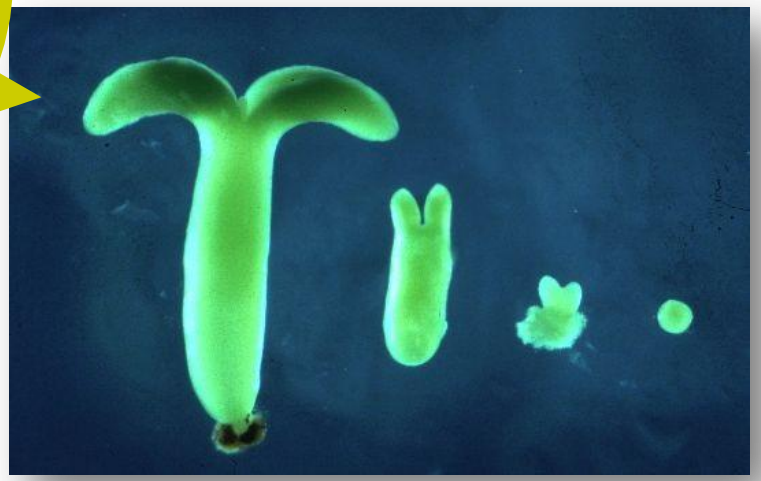
optimum® GAT®
Herbicide Tolerance

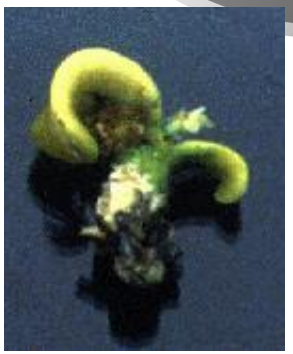






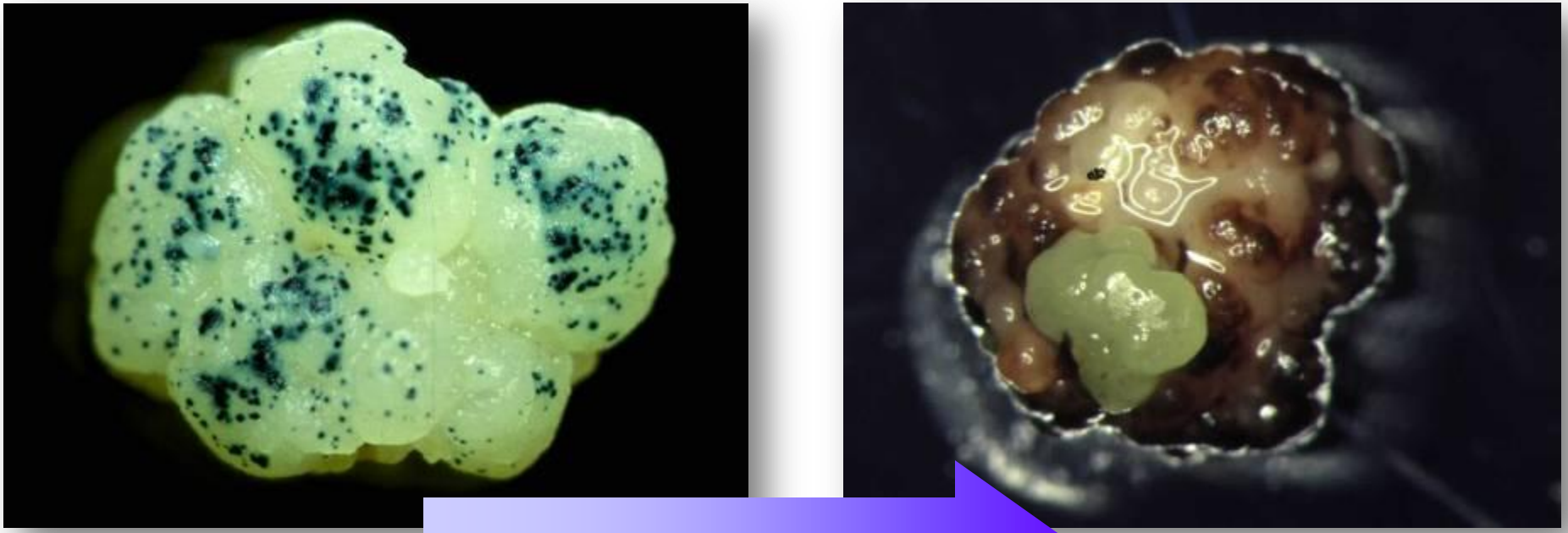
Somatic embryos





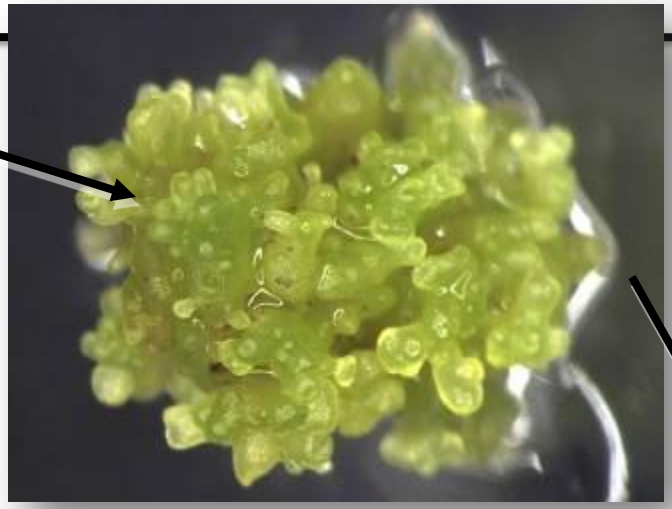
Selection for transgenic tissues

- Challenge – recover plant from engineered cell, and leave rest behind



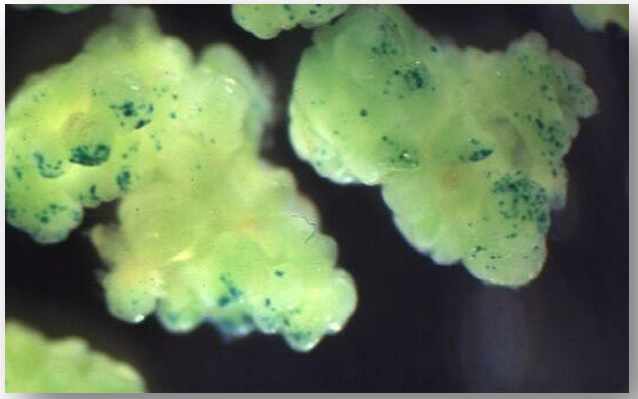
+ selective agent

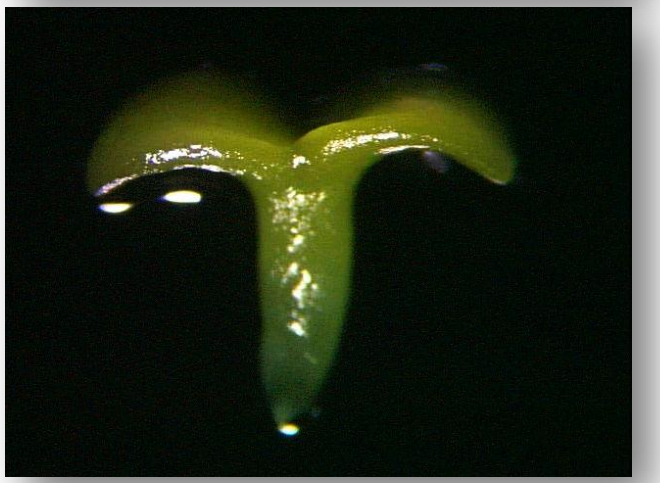
Recovery of engineered plants



The transformation system

- Best genotype = Jack
- For Jack
  - 15-80 independent events per shot
  - 6 months between shooting and taking a plant to the greenhouse
- Works with Williams 82
  - ~1/2 frequency
- Use of linear or plasmid DNA
- Great system for early analysis of seed-specific traits





Specialty Beans ● Black Jack

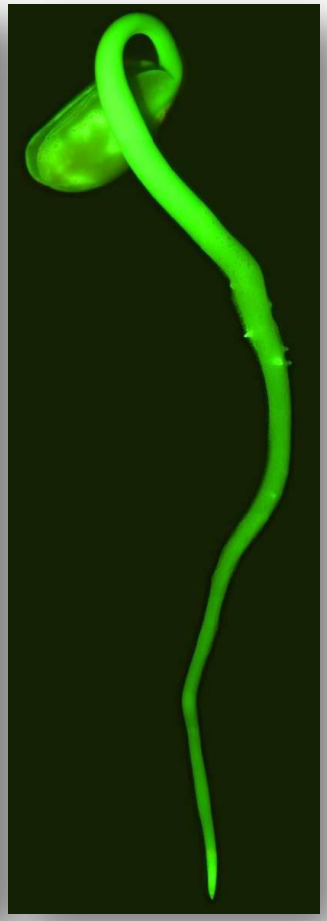
Specialty protein production

Moving forward

- Find new promoters
  - Freedom to operate
  - Tailor expression of engineered traits
- Leverage funding for genomics tools
- Nematode resistance

Green Fluorescent Protein

A way to see when and where genes are expressed

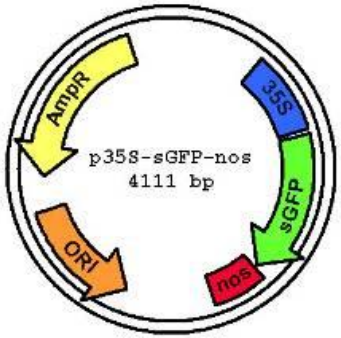


Isolation & characterization of soybean promoters

Promoter | Green Fluorescent Protein | Terminator

Robotics for automated image collection and analysis

Promoter evaluation



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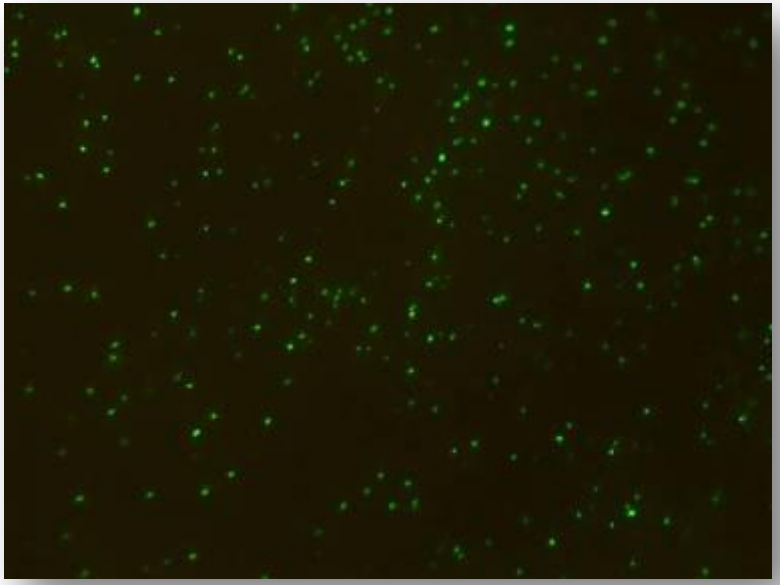
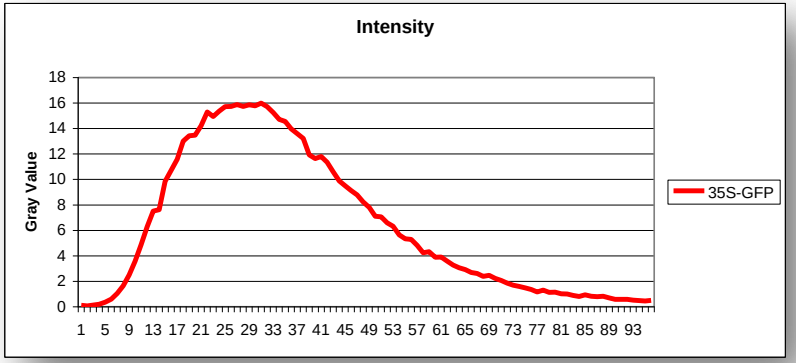
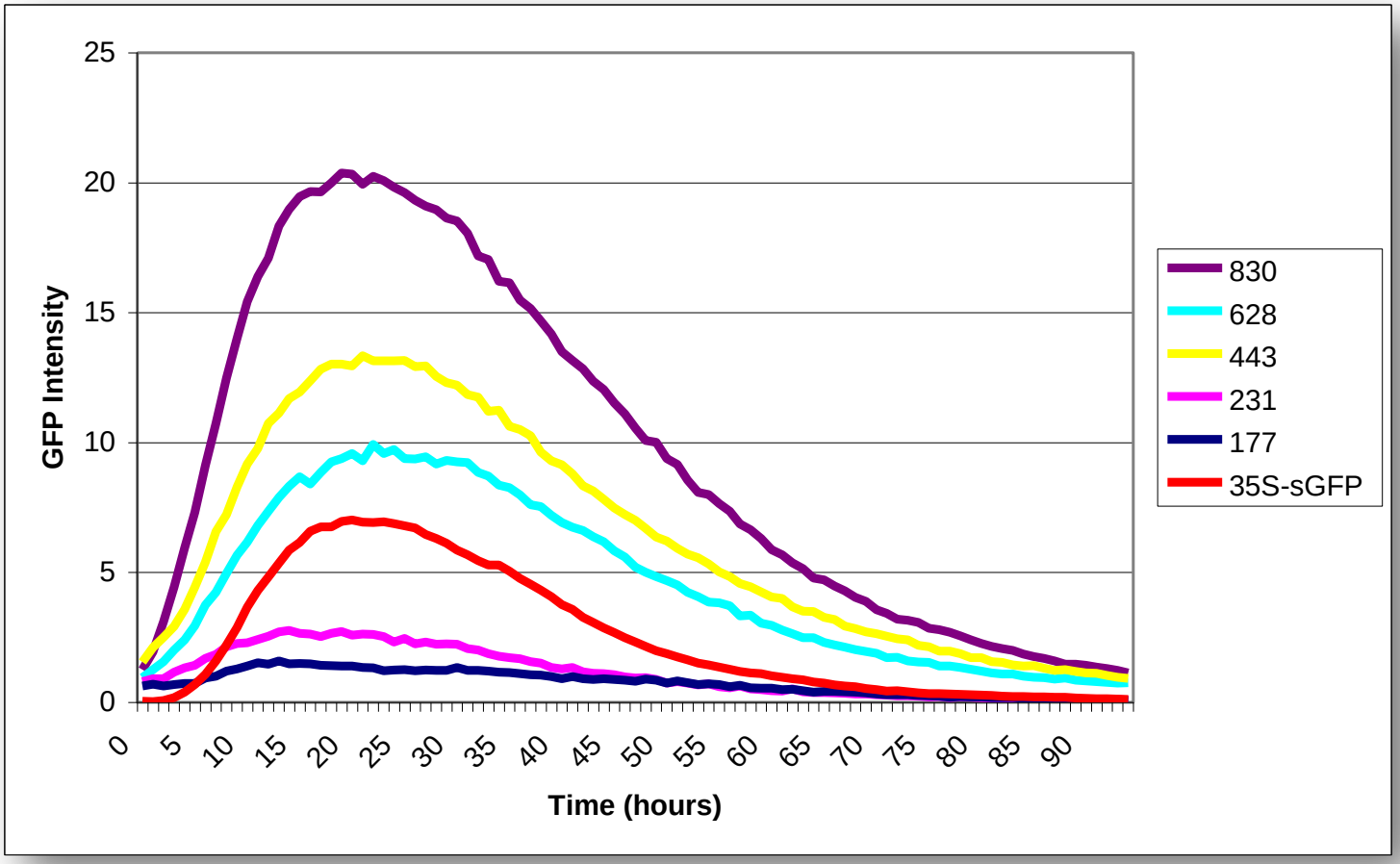
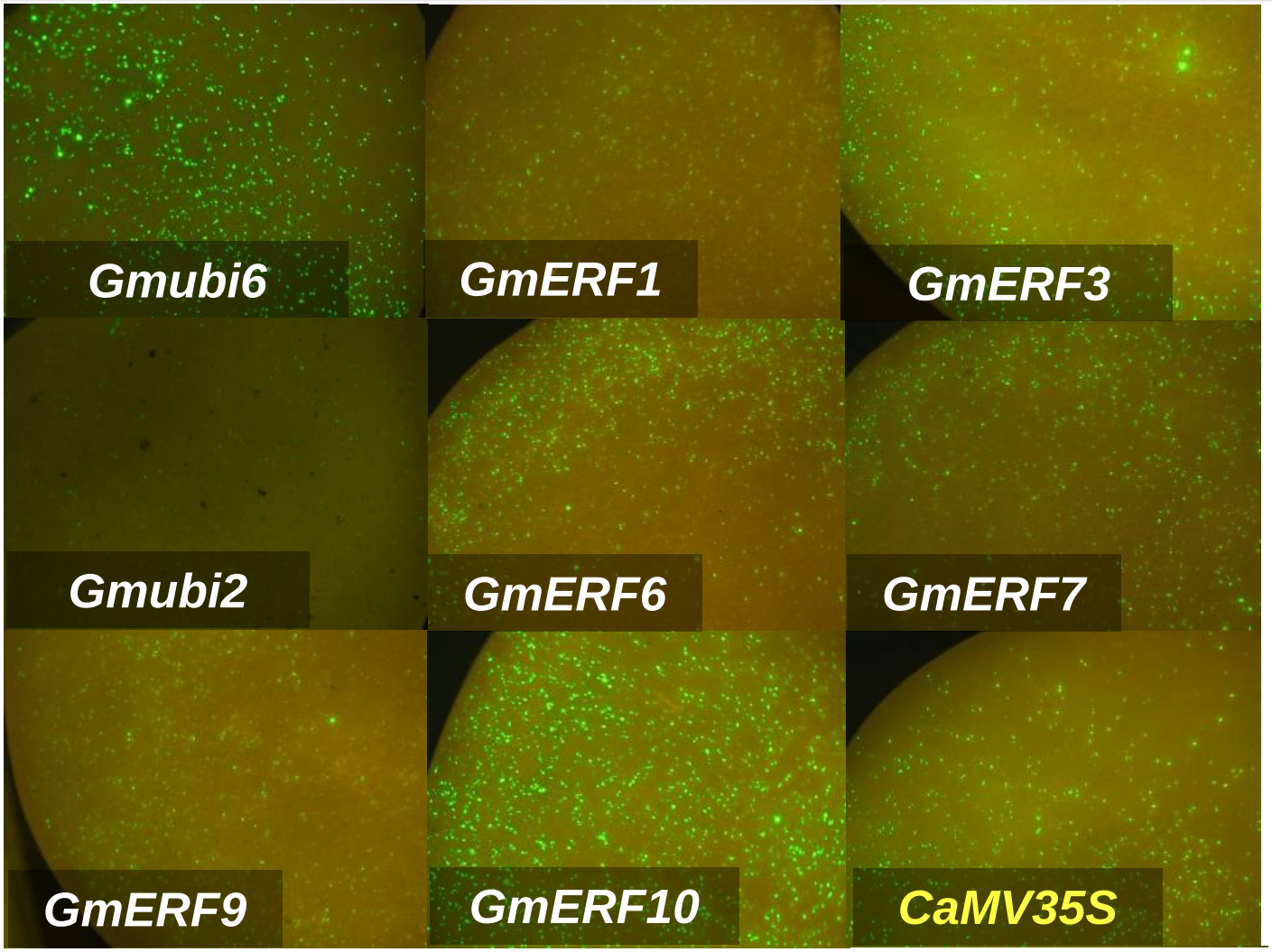




Promoter comparison

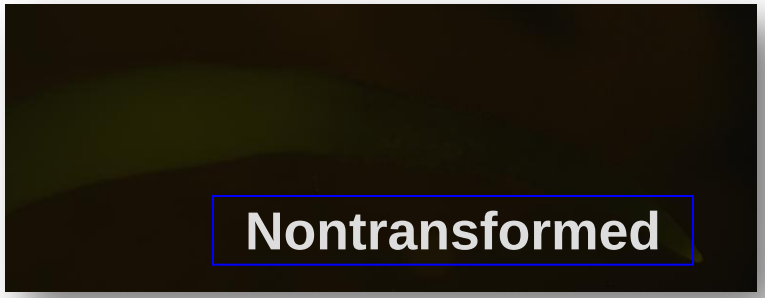


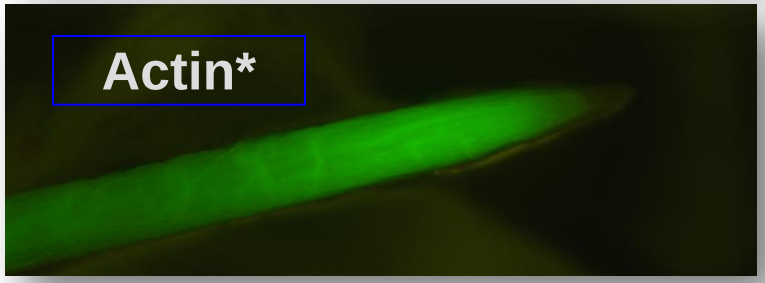
Sequence-based promoter ID

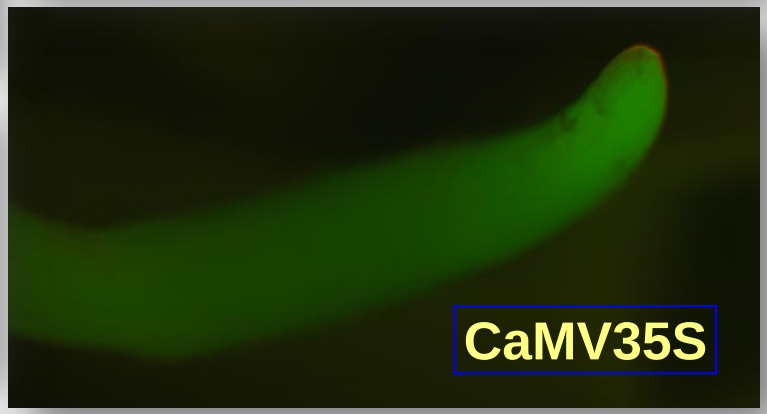


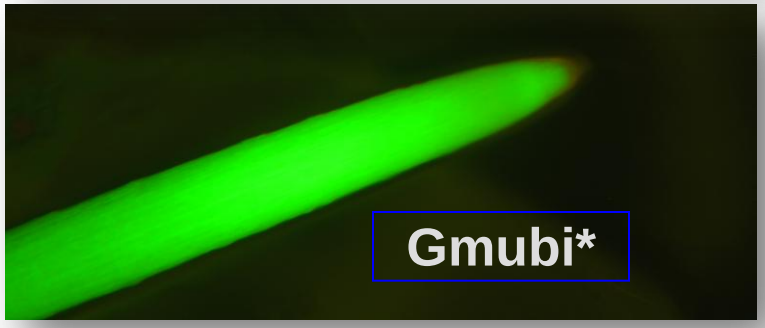
Root-specific promoters

*Cloned in the FinerLab

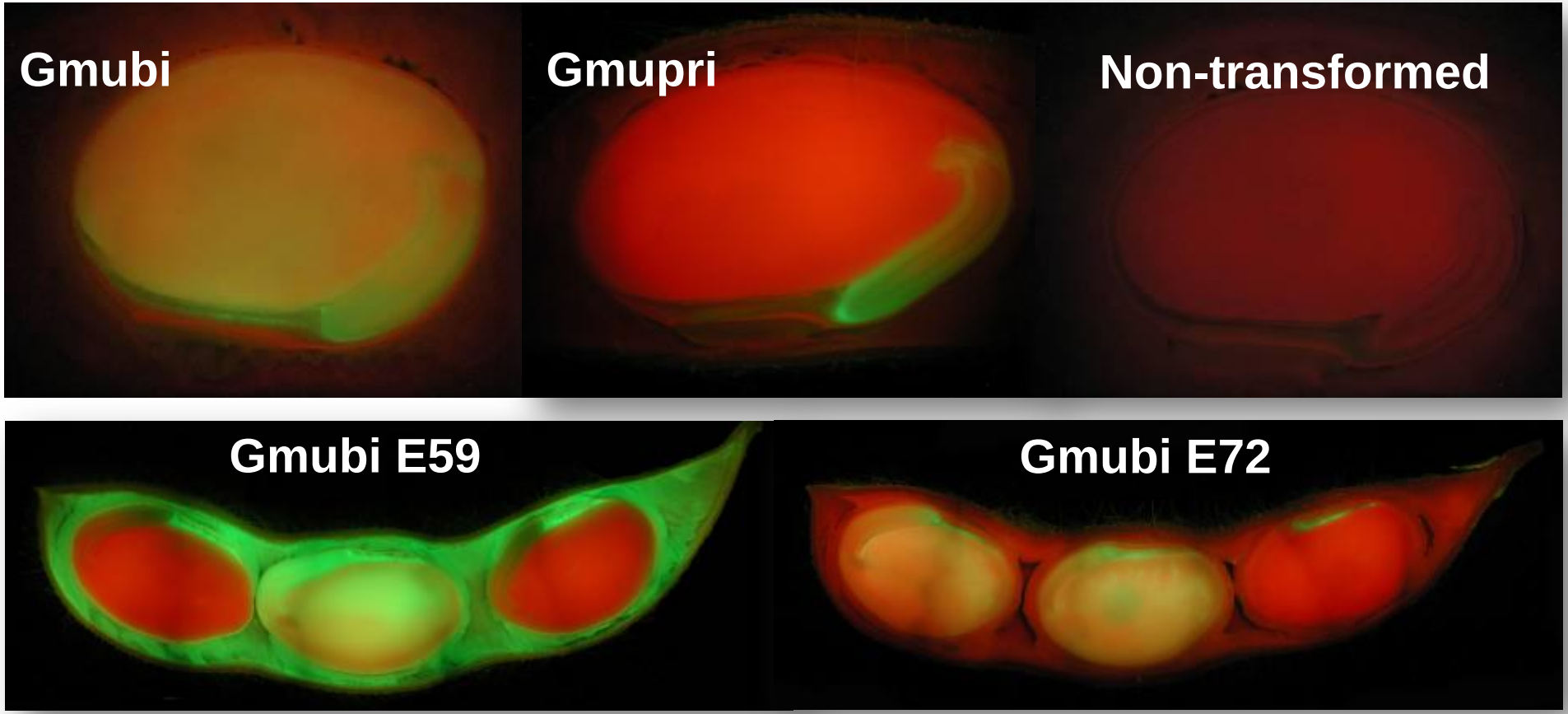






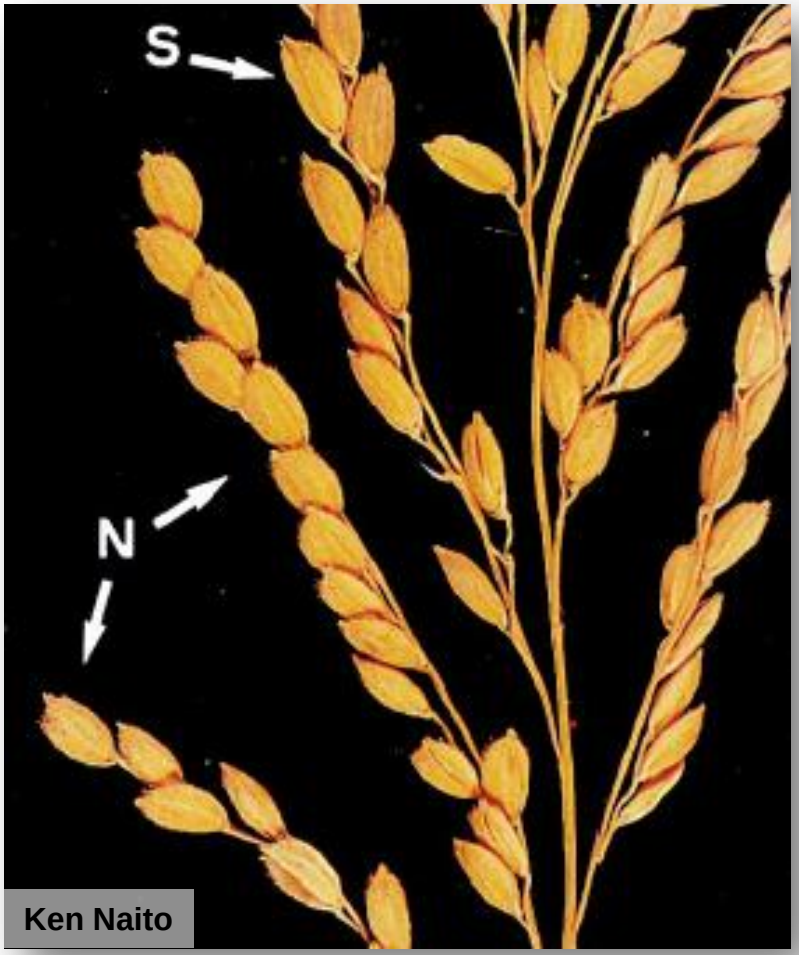


Promoter evaluation in transgenic plants

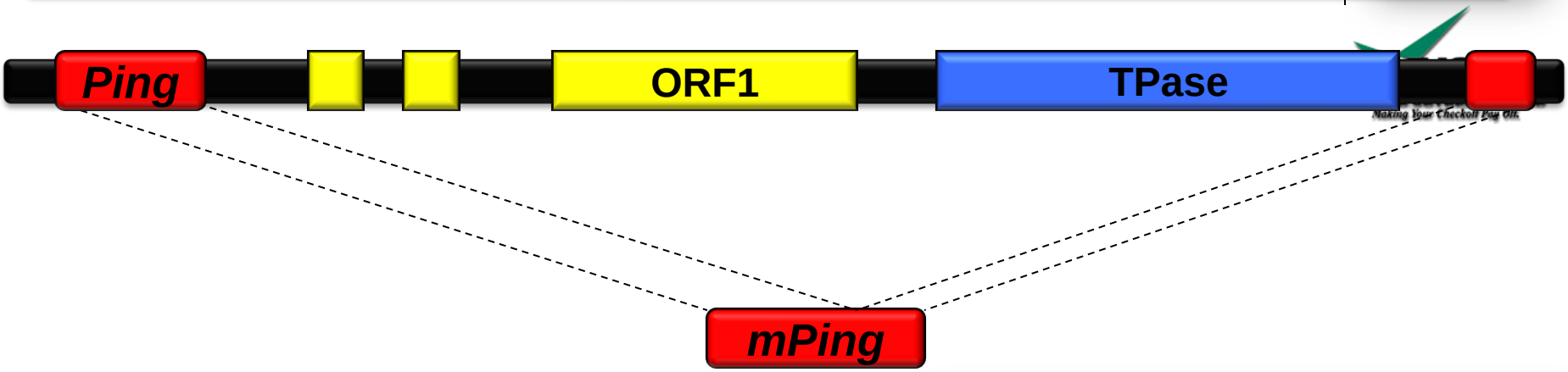


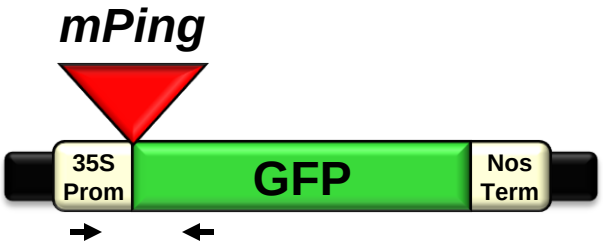
Transposon mutagenesis

for gene discovery & function



Ping & mPing

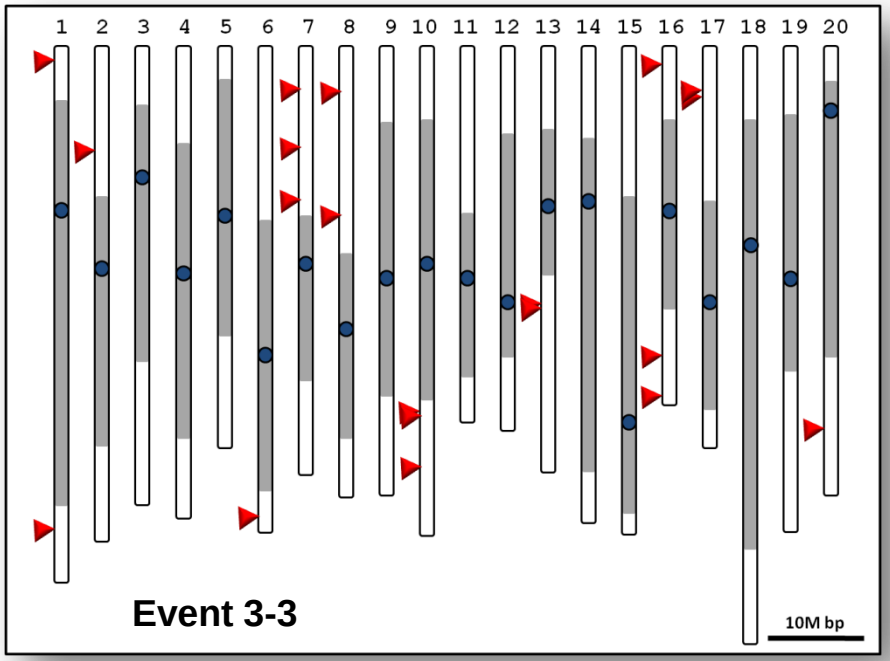


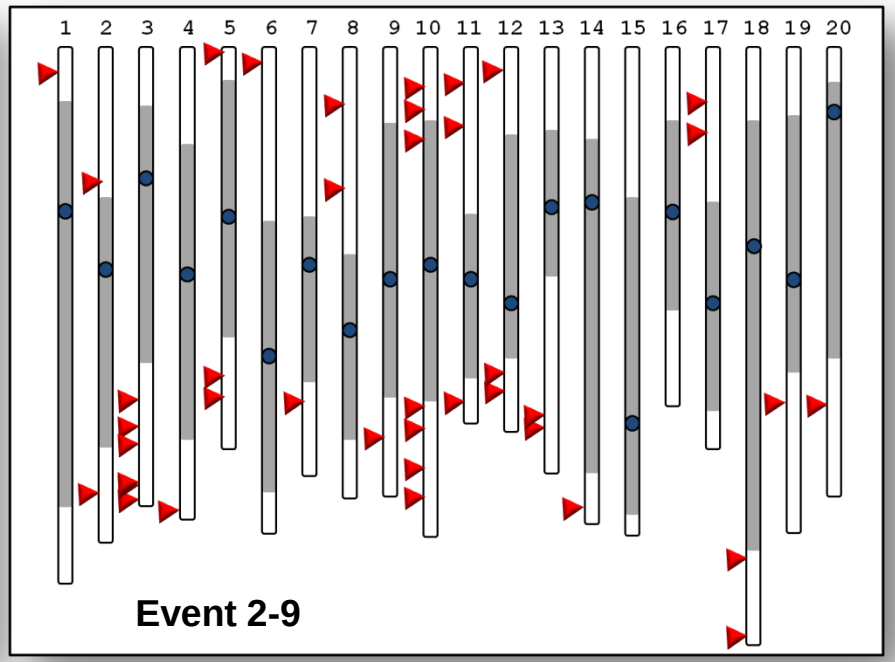


Yang et. al. (2007) PNAS

mPing in soybean

- Preference for gene-rich regions
- Transposes to unlinked sites
- Adaptable for trapping/discovery





Nematode resistance

- Shifted focus away from seed traits
- Goal: Strain-independent resistance to cyst and root-knot nematodes

- Thomas Baum
- Rick Davis
- Dick Hussey
- Melissa Mitchum

RNAinterference

A totally natural phenomenon to turn genes off

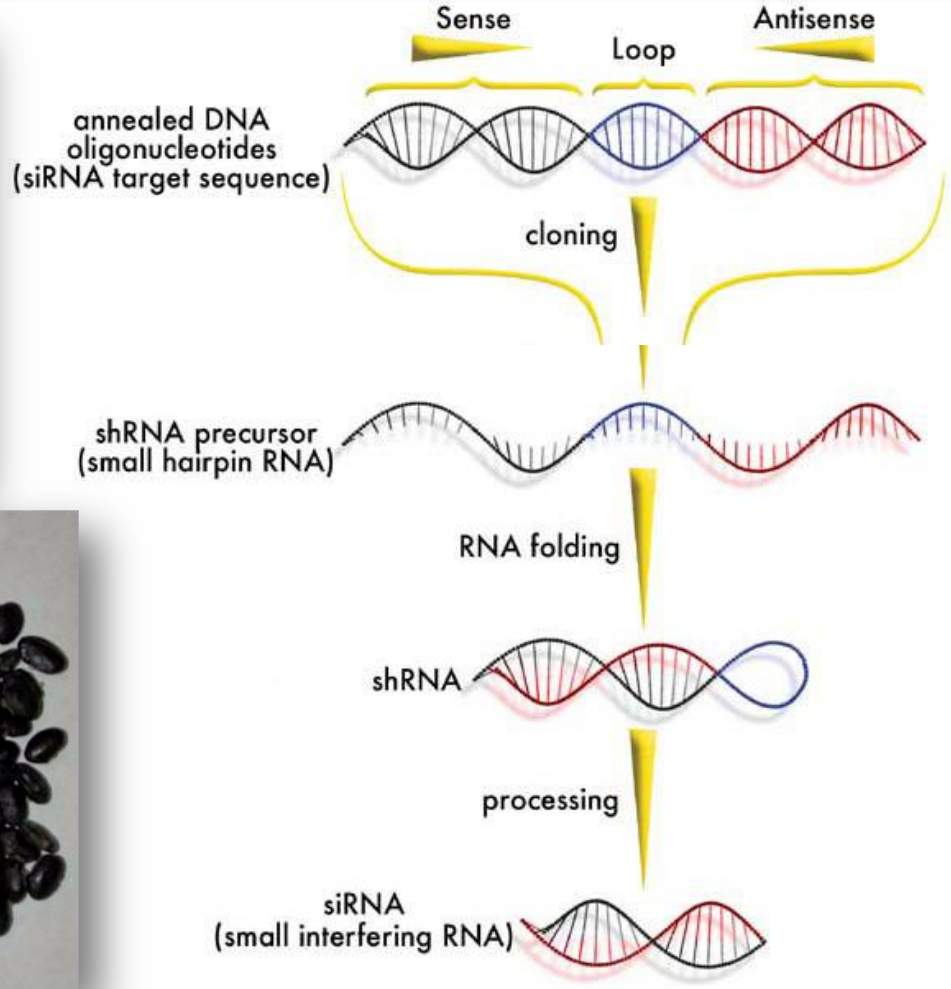
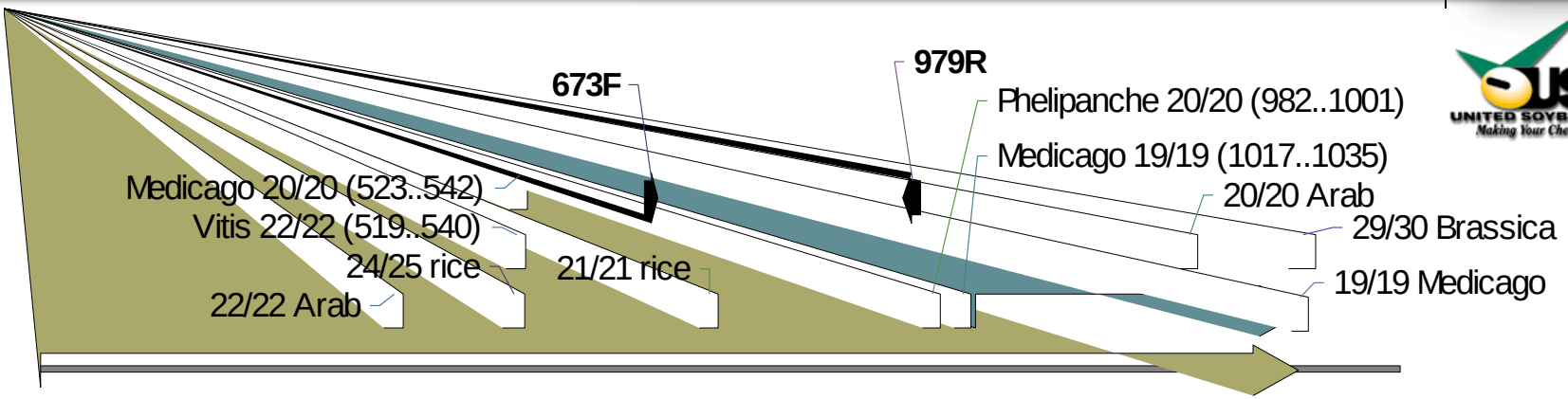


Diagram by Millipore

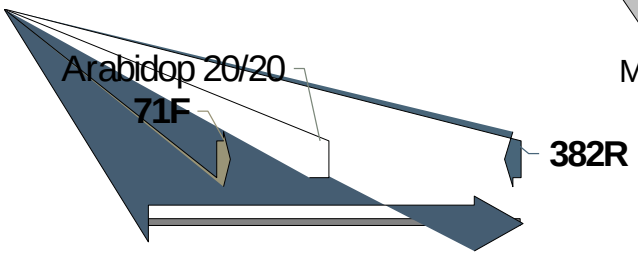
Gene target analysis

Find SCN/RKN parasitism gene regions that do not show homology to plant genomic DNA



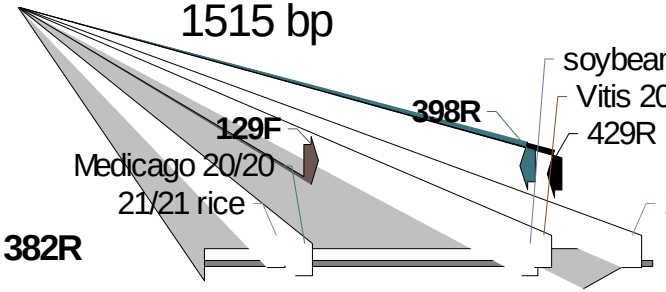
28B03

1515 bp



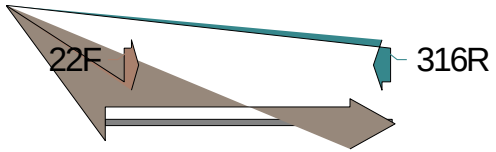
30D08

384 bp



30C02

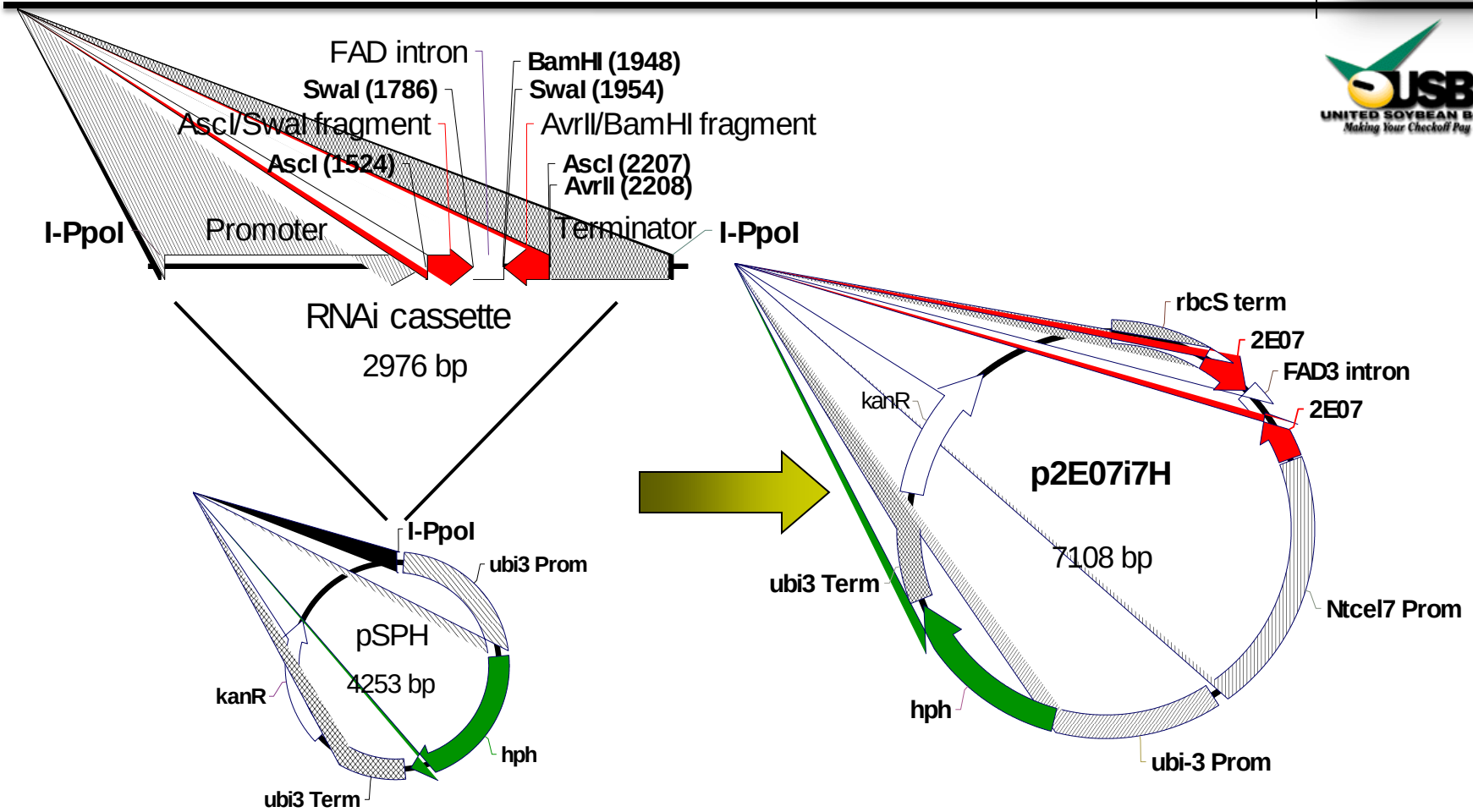
538 bp



23G12

321 bp

Silencing vectors



Plants engineered with nematode RNAi genes

Nematode objectives

Cyst & RKN

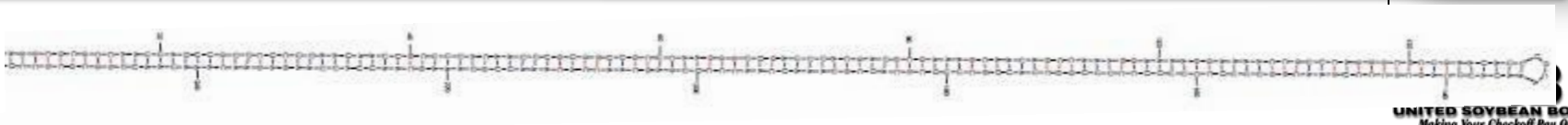
- Identify best gene(s) for resistance
  - Evaluate gene combinations
  - Evaluate different promoters

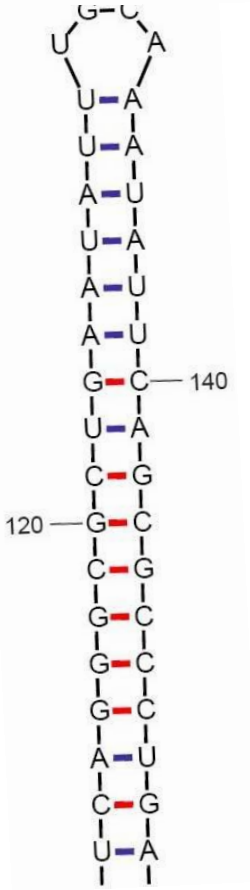
Proof of concept

Jack soybean infected with root-knot nematodes

Jack soybean engineered for resistance

Results to date

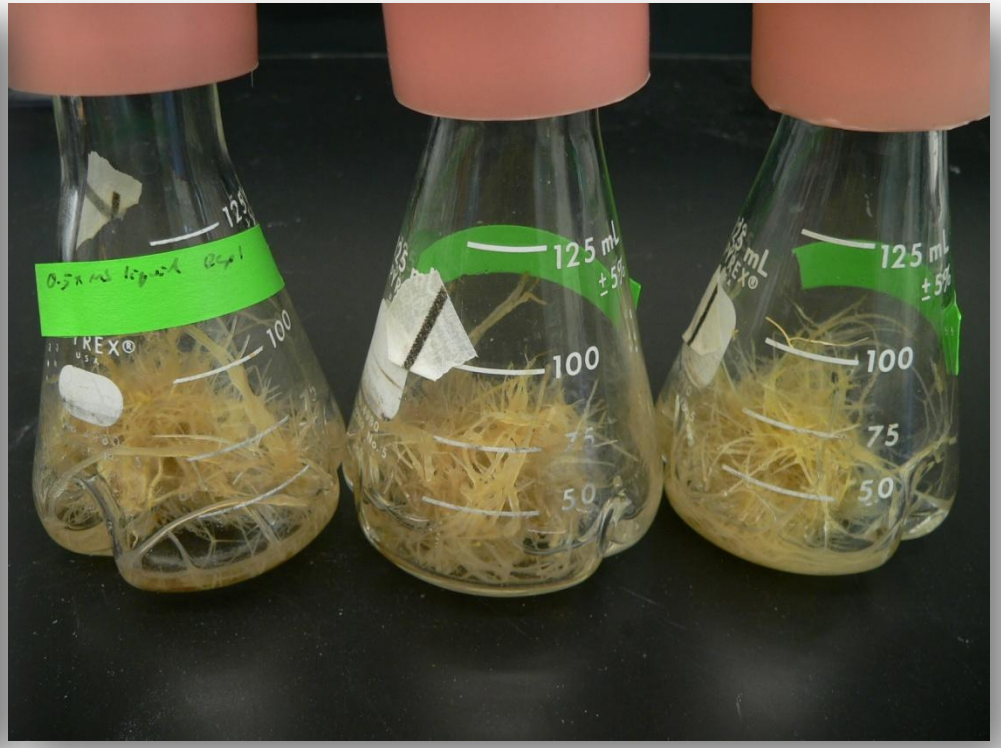




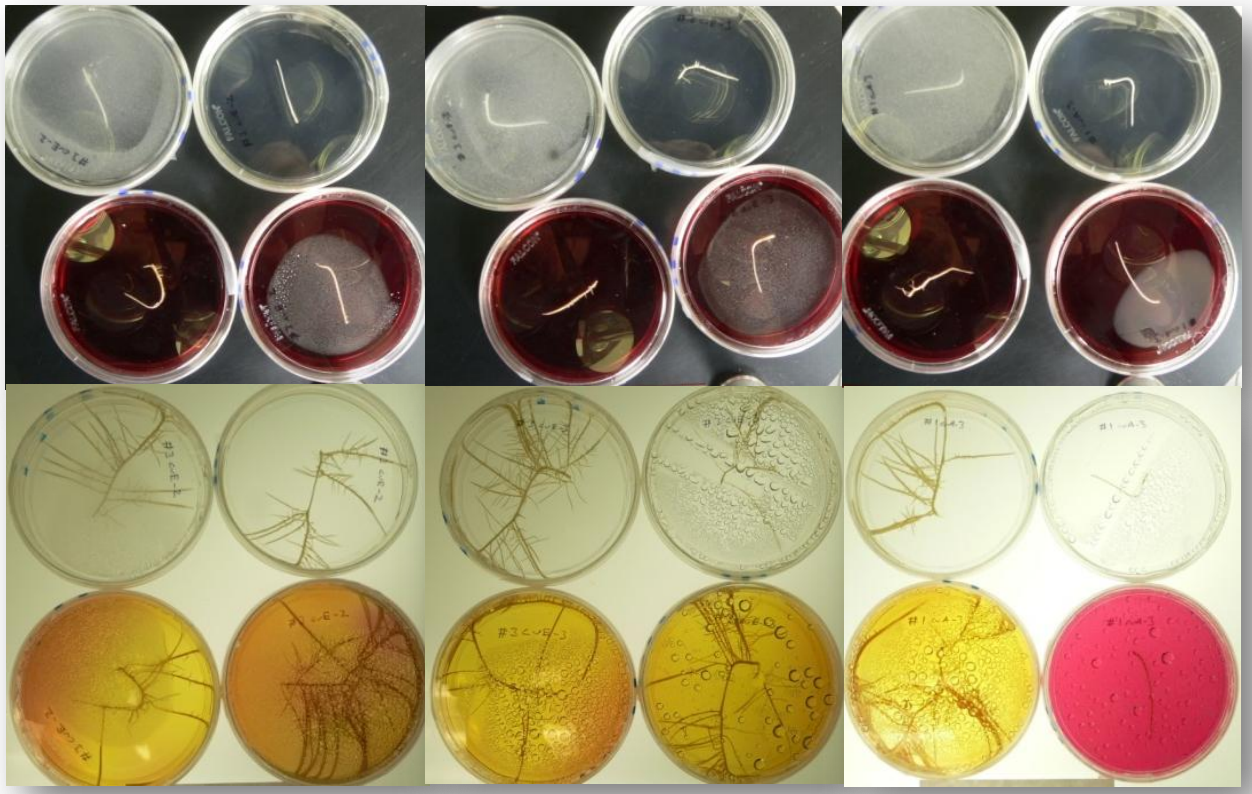
- Resistance is highly variable
  - Need ways to stabilize high levels of RNAi production
- Many unanswered questions on RNAi
  - Random?
  - Sense or anti-sense strand preference?
  - GC-rich vs AT-rich?
- Different sites are preferred
  - Can predict?

Hairy roots

The need for a high- throughput system



Hi-throughput screens



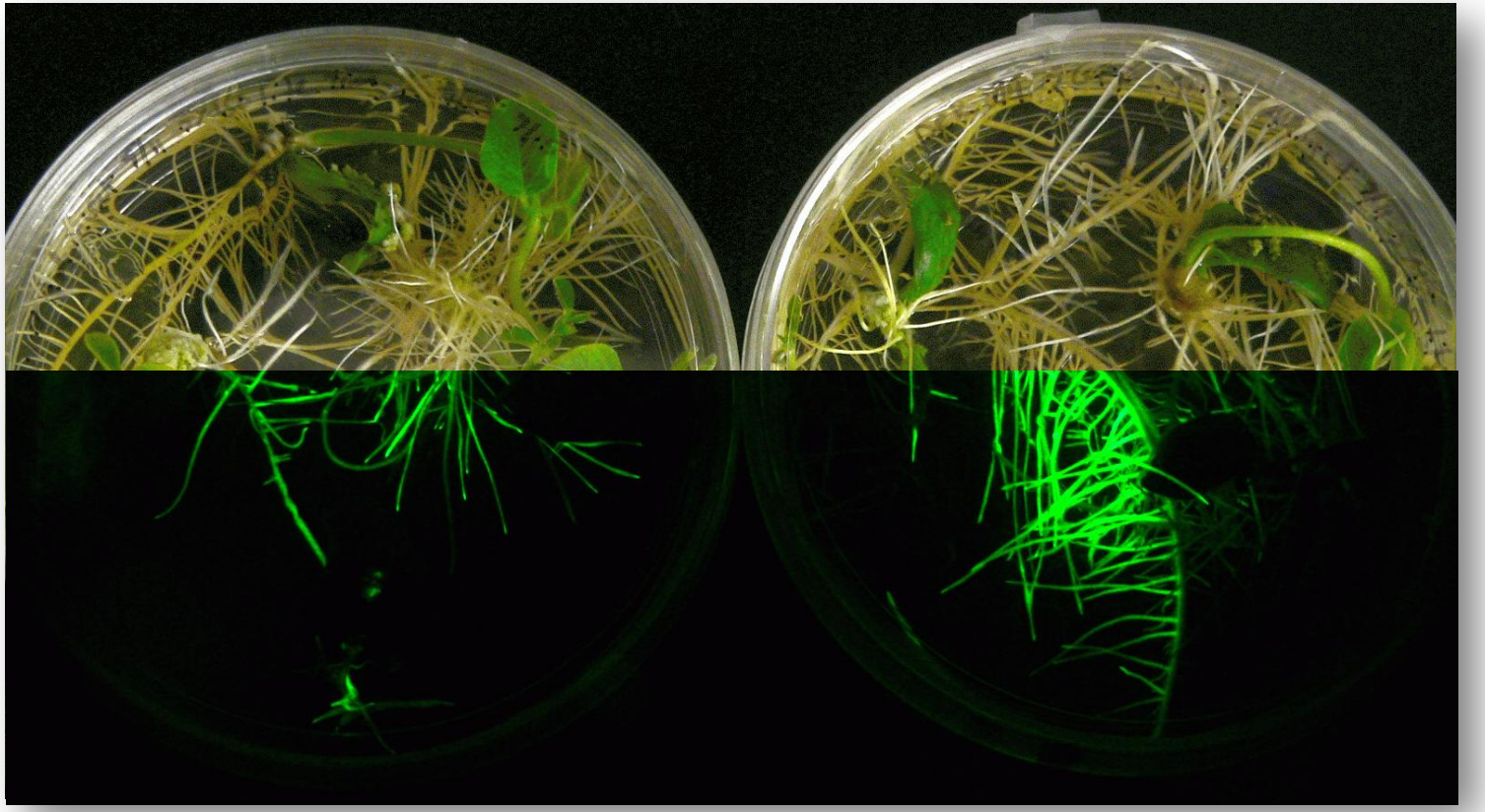
p201GBR6 cv E #2 p201GBR6 cv E #3 p201GR6TA cv A #3

1/26/10

- ½ MS
- ½ MS + BAR
- ½ MS + CRR
- ½ MS + Bar + CRR

2/7/10

Hi-throughput screens



Thanks