

Making plant transformation easier and faster

C. Neal Stewart, Jr.

Jason Burris

Muthukumar Balasubramaniam



plantsciences.utk.edu/stewart.htm

nealstewart@utk.edu

Central dogma: DNA → RNA → protein

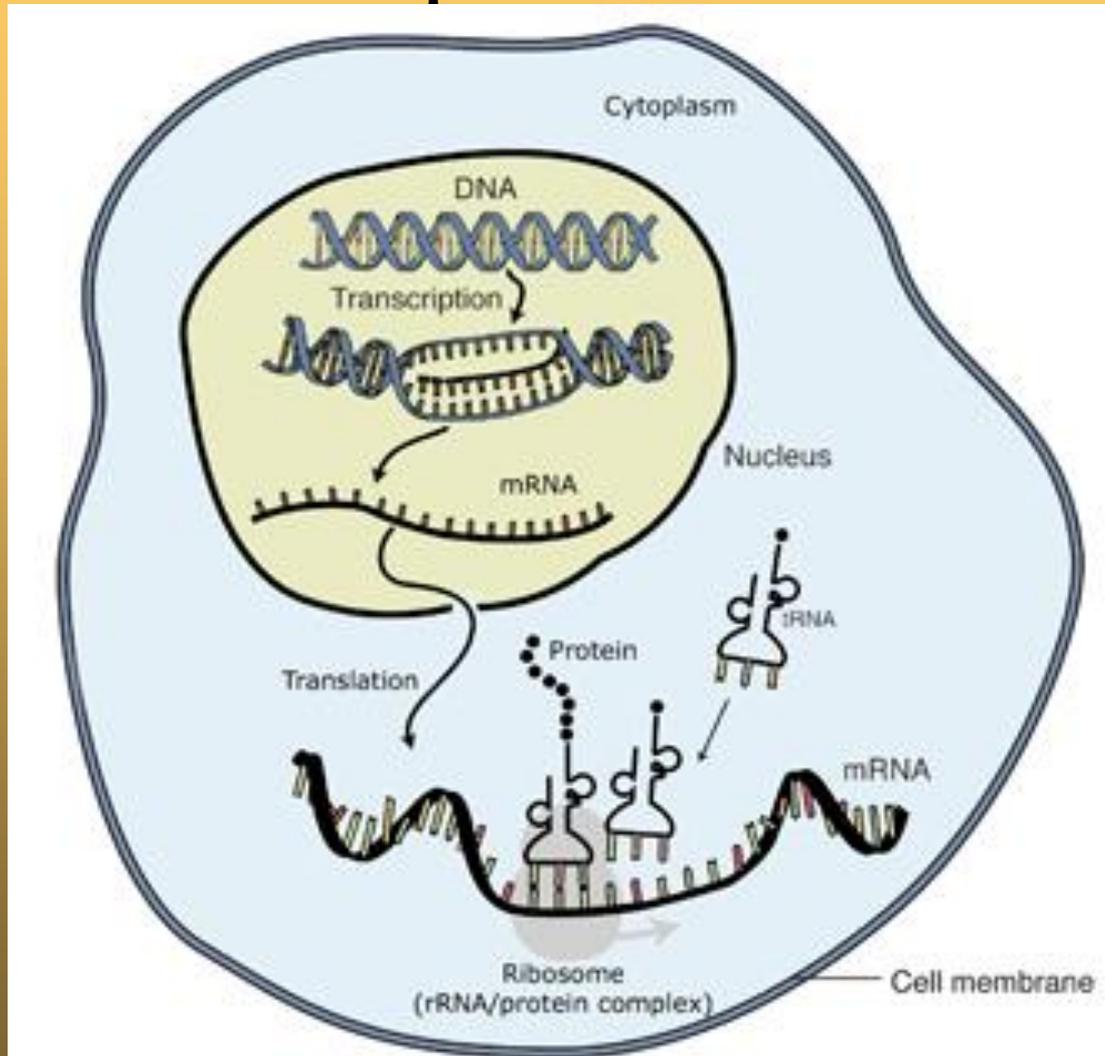
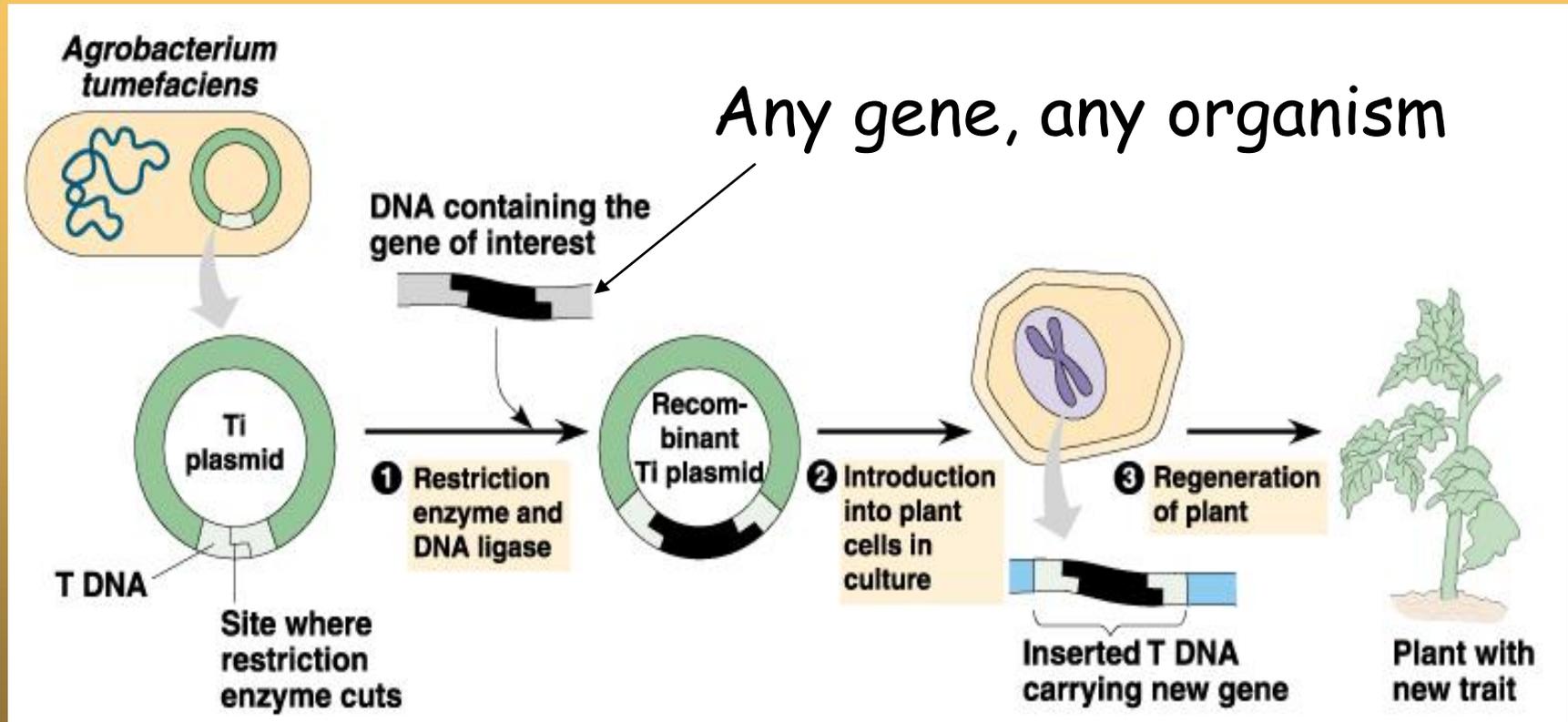


Image adapted from: National Human Genome Research Institute.

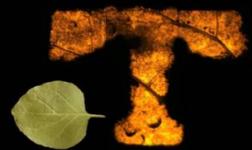


Plant transformation

Any gene, any organism

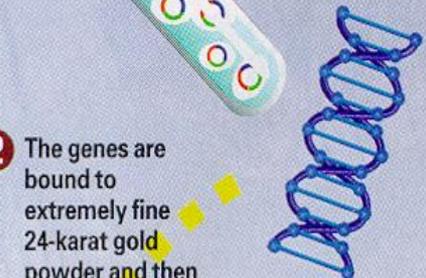


The new plant will pass the transgene to its progeny through seed.

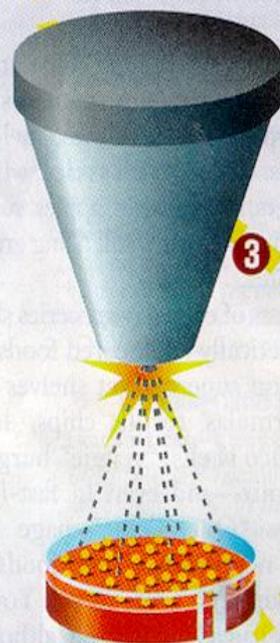


Making biotech corn

1 Scientists isolate a gene from the *Bacillus thuringiensis* bacterium that makes a protein deadly to certain insects. They modify and chemically link this gene to an antibiotic-resistance gene.



2 The genes are bound to extremely fine 24-karat gold powder and then spread on a quarter-sized plastic disc.



3 A "gene gun" slams the disc onto a mesh screen, blasting the gene-bearing gold particles onto a dish of corn cells or seed embryos.

4 The new genes are incorporated into some corn cells. To identify those, scientists add an antibiotic that kills all cells except those with the antibiotic-resistance gene.



5 The transformed cells develop into mature plants. Some, but not all, of these plants and their progeny produce the pesticidal protein.



Biolistics



Progression of transgenic plants

- 1st Generation: Input traits (herbicide tolerance, insect resistance, etc.)
- 2nd Generation: Output traits: (pharmaceuticals, enhanced nutrition, etc.)
- 3rd Generation: Non-ag— (bioenergy, biosynthesis, phytoremediation, sensors)



Limitations to development of plant transformation

- Most species have not been transformed
- Tissue culture procedure with plant regeneration from single-to few cells required
- Genotype specificity (genotype media)
- Cloning multiple genes into transformation vectors for metabolic engineering
- Targeted (time and space) transgene expression



Temptations for shortcuts

the silver bullet

- ZFNs, TALENs, or new editing technology
 - For genome editing *in situ*
 - Might not be regulated like transgenics
 - Very fast



Temptations for shortcuts *the silver bullet*

- Chloroplast transformation
 - Few events required—site directed integration
 - Very high expression
 - Multiple genes can be driven off a single promoter
- *In planta* transformation
 - Floral dipping for direct transformation of ovule
 - No tissue culture
 - Very effective for *Arabidopsis thaliana*



First steps to transforming plant X

- Select “good” genotype for culture and regeneration
- Optimize media
- Transformation system
- Promoters and vectors
- Marker genes are powerful

Case study 1: *Panicum virgatum* (switchgrass)



Switchgrass tissue culture and transformation

- Bob Conger's lab at UT: early 1990s funded by DOE
- What explant gives the right response ie. can we regenerate, transform it.
 - Mature seeds
 - Leaves
 - Inflorescences
- Surveyed a wide range of varieties/germplasm—selected Alamo clones that performed better than others
- Very low efficiency, but produced transgenic plants by both bombardment and *Agrobacterium* early 2000s—funds ended-- little progress til late 2000s-early 2010s

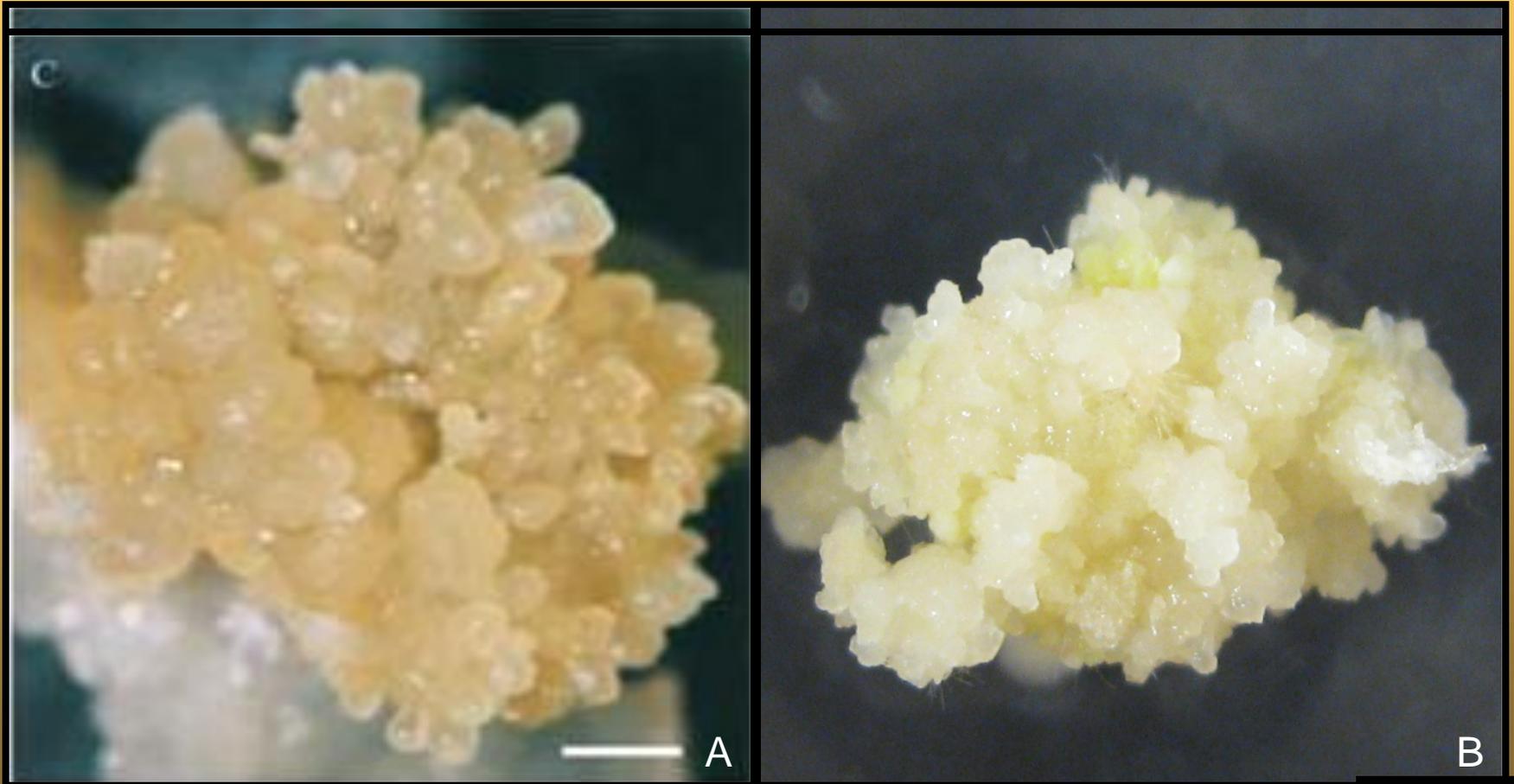


Switchgrass tissue culture and transformation

- BioEnergy Science Center funded in 2007—3 core labs at Noble, UGA and UT—decided to focus only on Alamo-types
- Much larger screen of Alamo yielded better clones for tissue culture and transformation
- Media modified for higher performance
- Transformability used as a selection metric
- Very high transformation efficiency now

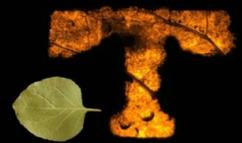


Switchgrass lessons learned: optimized media for type II callus



A. Type II callus of maize Petrillo et al. 2008.

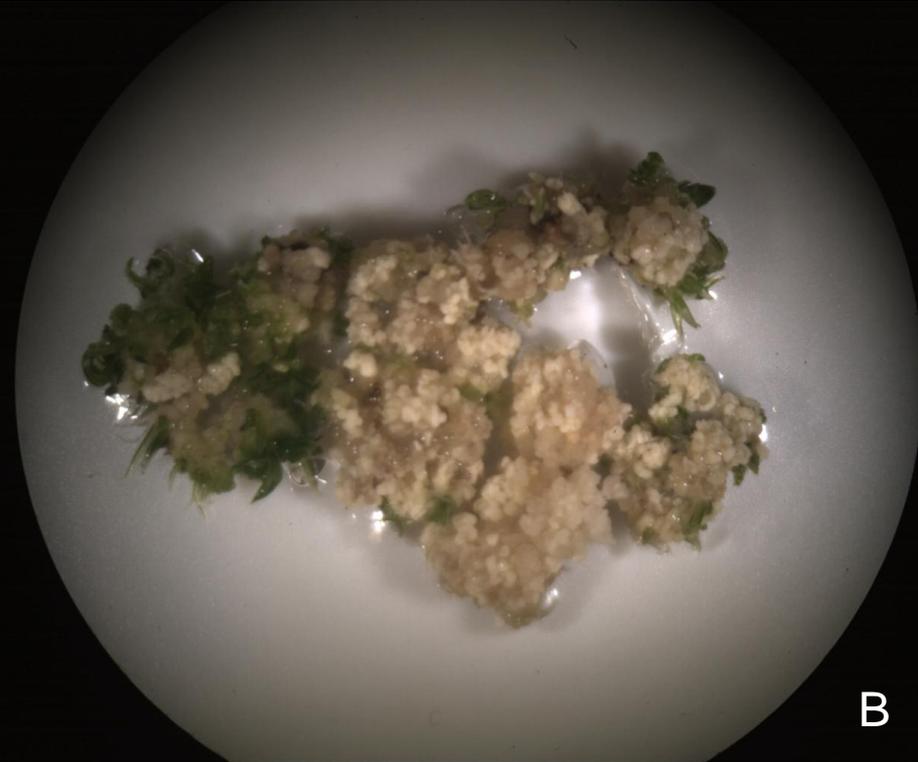
B. Type II callus of *Panicum virgatum* on LP9



Switchgrass lessons learned: regeneration is key

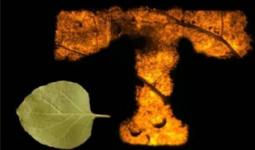


A



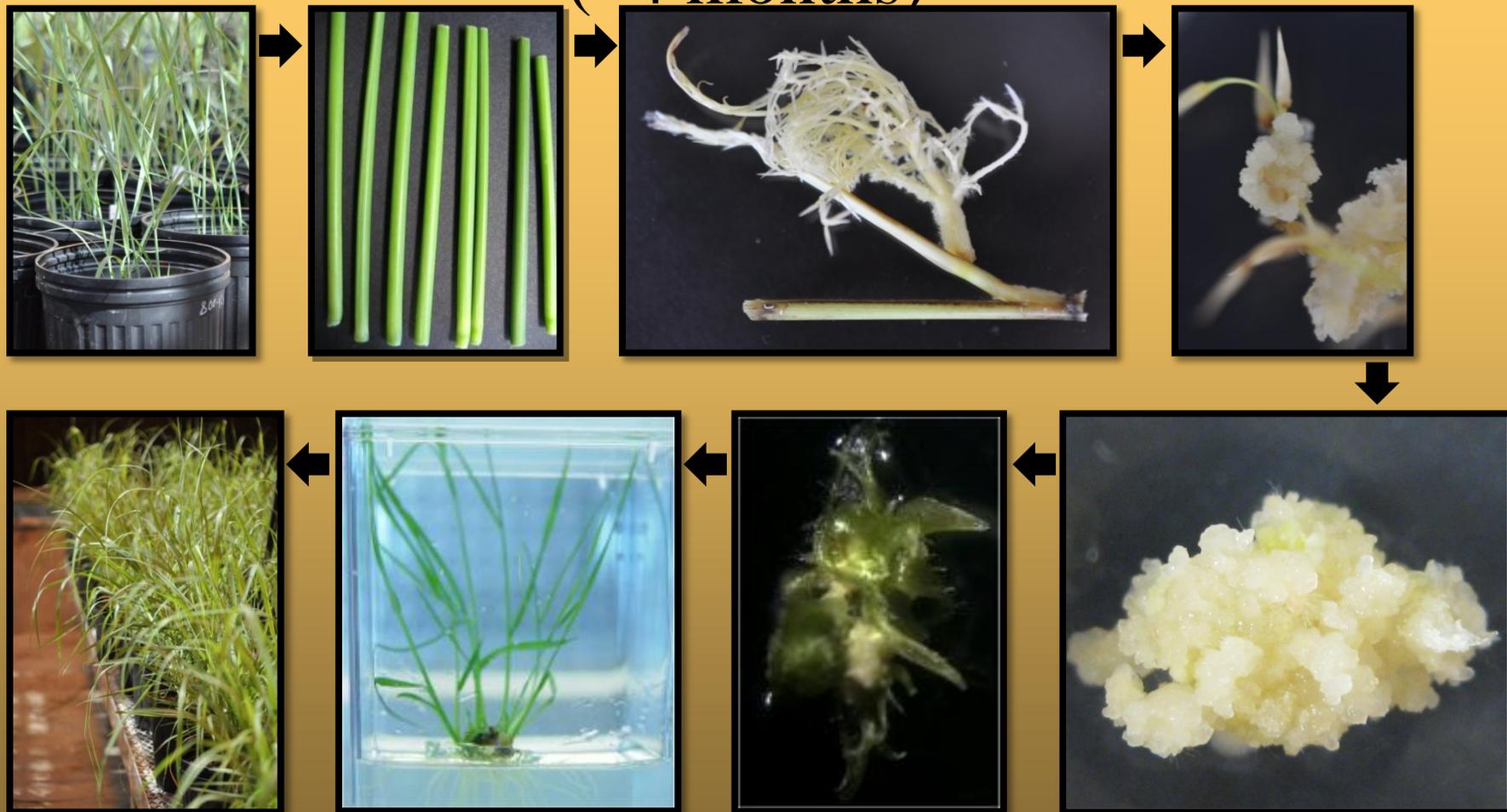
B

A. An Alamo-derivative seed clone that produces a high % of type II callus and has a high regeneration capability. B. An Alamo-derivative seed clone that produces a high % of type II callus, but has low germination capability.



Tissue culture - callus production and regeneration

(~4 months)



**Improvements in Alamo clones for
responsiveness,
transformability and regeneration**

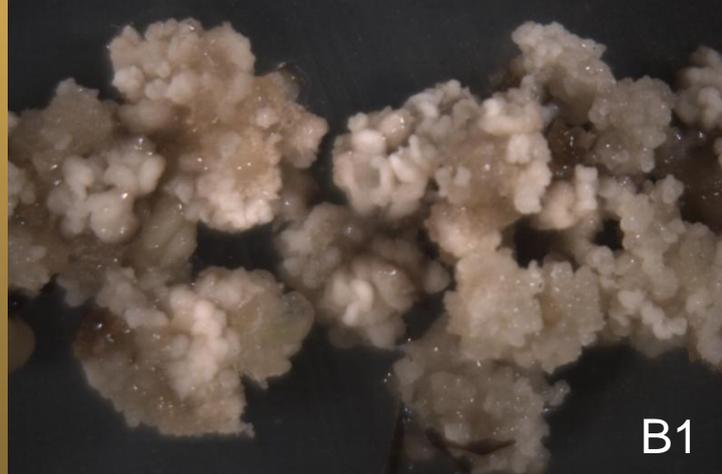
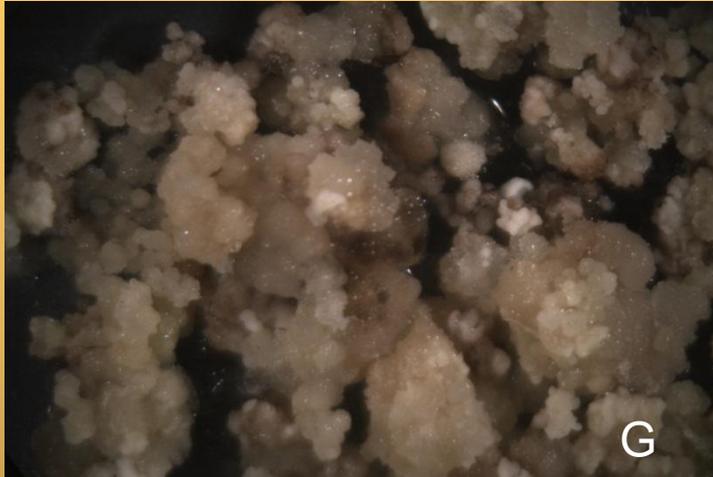


But why are some genotypes more responsive?

- No one really knows
- Transcriptomics and metabolomics could be informative
- Preliminary metabolomic screen data for switchgrass callus

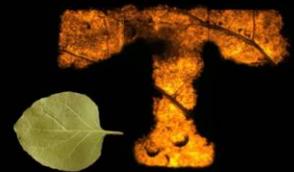


Callus produced from the three clones



Differences in metabolites among switchgrass clones

Metabolite	Ratio	
	G/B1	G/B2
lactic acid	0.42	0.42
alanine	0.43	0.36
2-pyrrolidinone	0.46	0.16
7.33 70 172 103	1.62	0.58
ethylphosphate	0.18	2.56
11.18 393 303 257	0.20	0.66
myo-inositol	0.61	0.48
linoleic acid	1.82	1.37



Metabolites

- --Alanine - major free amino acids detected in many examples of tissue culture (corn and wheat)
- --2-pyrrolidinone – Breakdown product of glutamine supplied in the culture media
- --myo-inositol – a carbohydrate commonly used in plant tissue culture media
- ++linoleic acid - common plant fatty acid



Next steps

- Combine transcriptomics with metabolomics
- Understand the genetic basis of tissue culture-ability and transformation
- And the same for regeneration



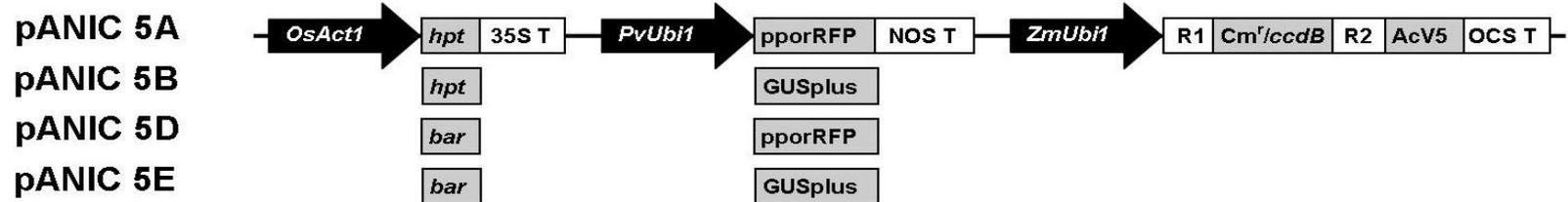
Rest of the talk

- Problem: easily subcloning genes to construct transformation vectors
- Special problem of metabolic engineering
- Case study: fern transformation
- Summary

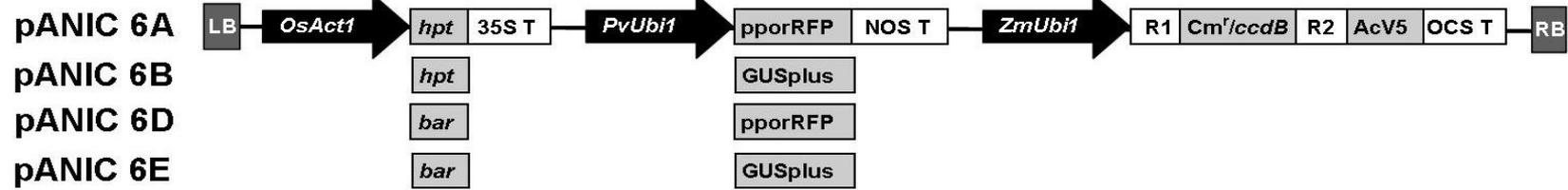


pANIC Vectors:

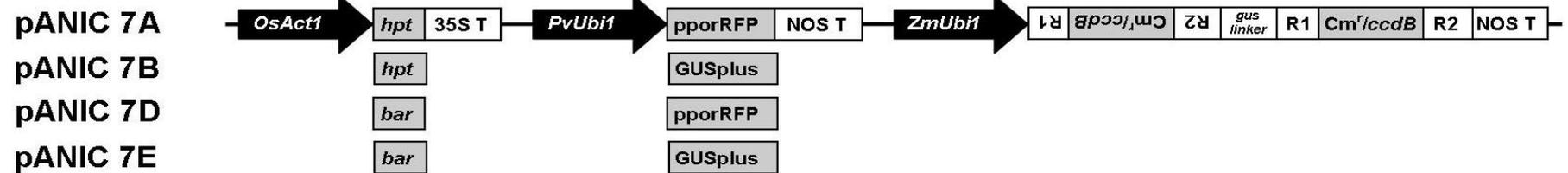
Overexpression - Biolistic backbone



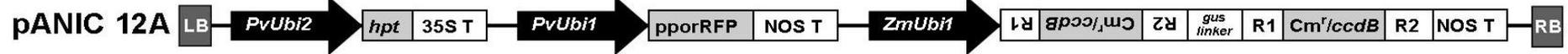
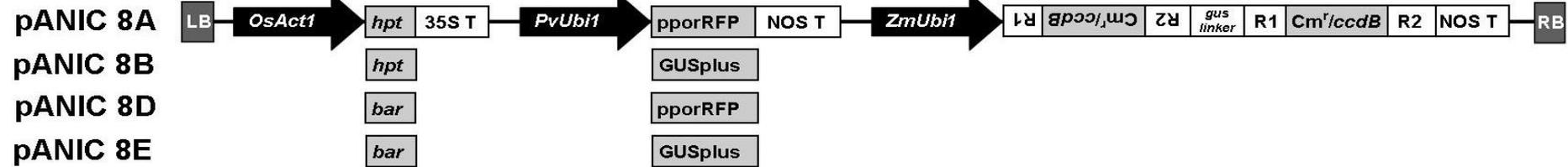
Overexpression - Binary backbone



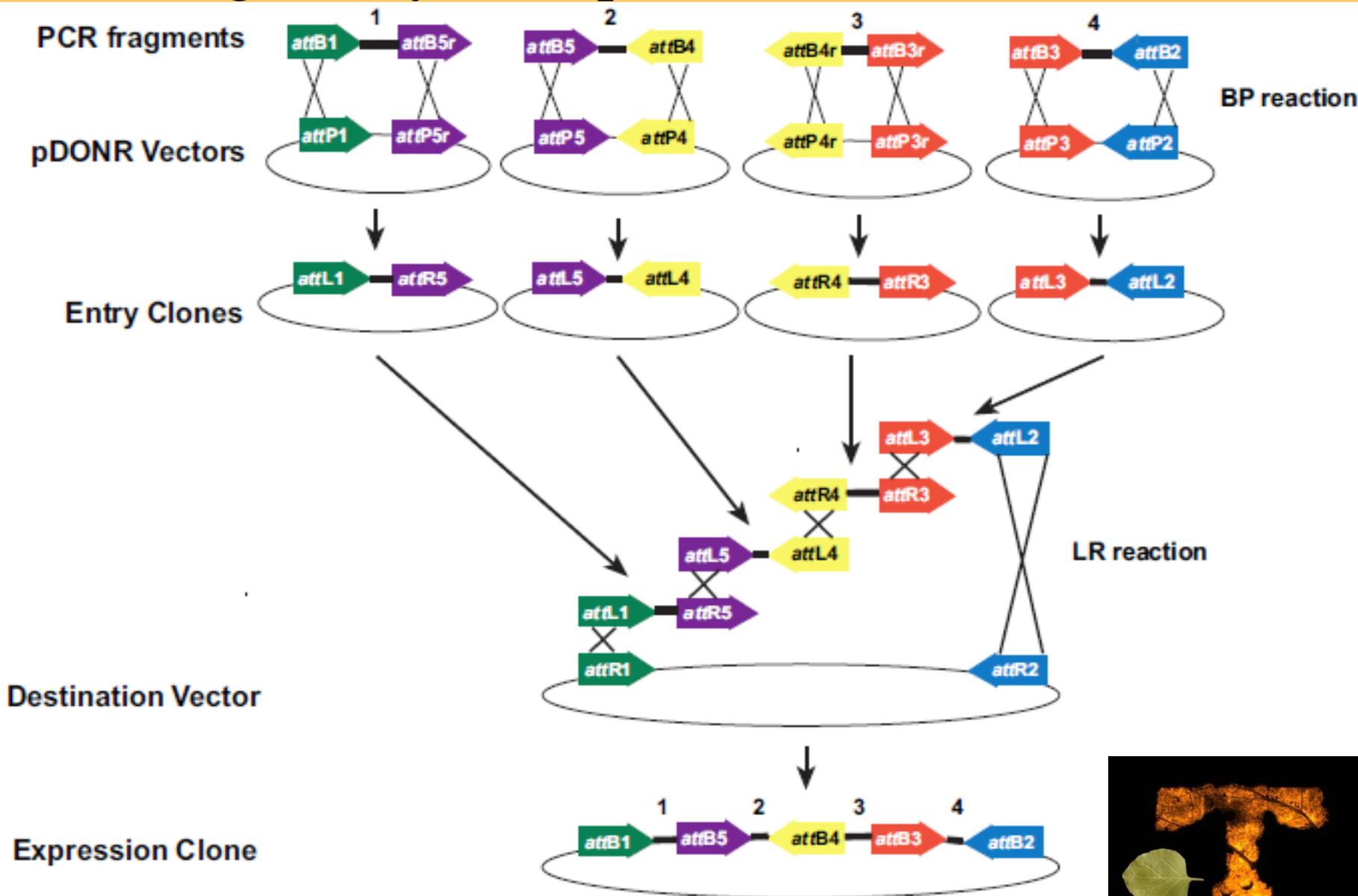
RNAi-mediated knockdown - Biolistic backbone



RNAi-mediated knockdown - Binary backbone



Multisite gateway for rapid vector construction



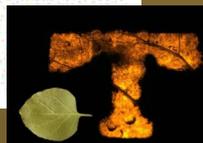
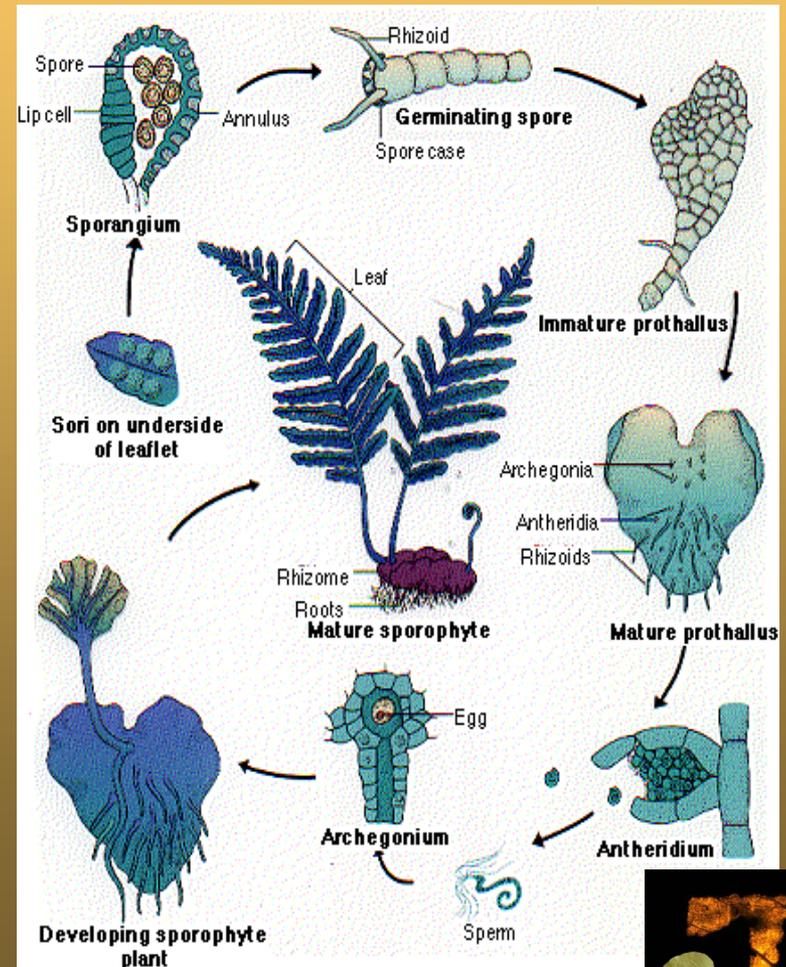
Stable transformation in ferns

- No fern transformation methods
- Few tissue culture methods
- Little genomic information
- Few-to-no fern promoters were known

Results

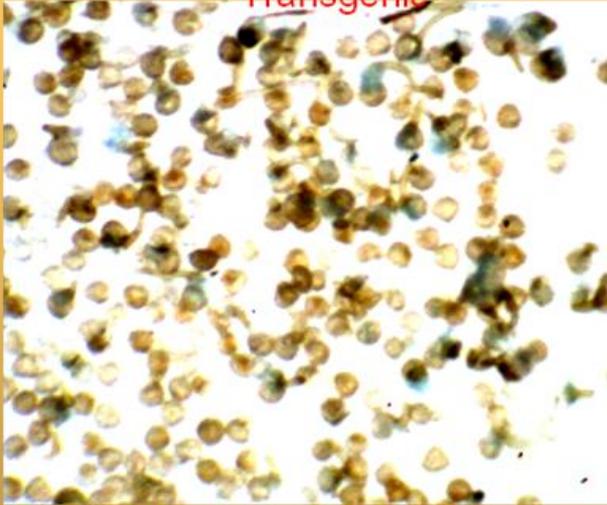
- Tissue culture was generally unsuccessful—low regeneration
- No fern promoters available for marker genes is a problem
- In desperation, we tried to use spores as a target for transformation using many different promoters

Hermaphrodite fern life cycle

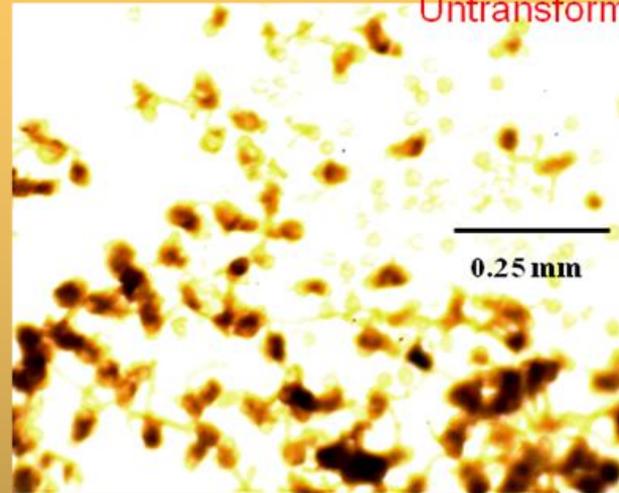


Transgene analyses in *Pteris vittata* prothalli: GUS expression

Transgenic



Untransformed control



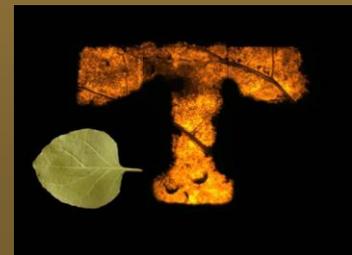
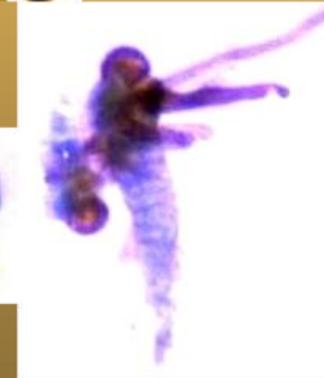
Transgenic



Transgenic

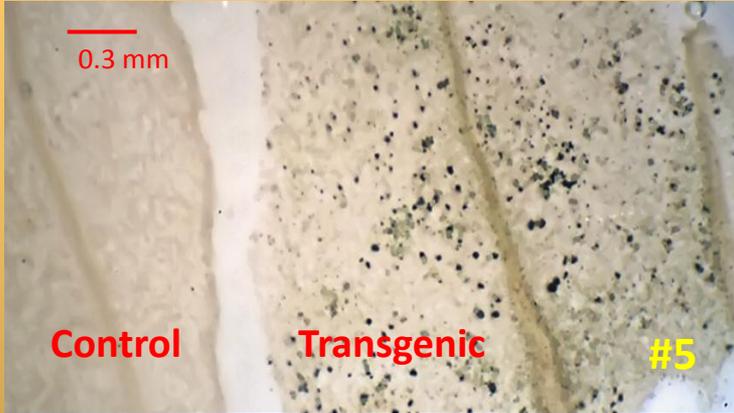


0.25 mm

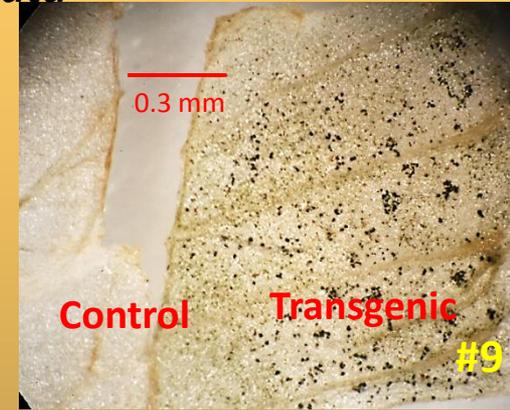
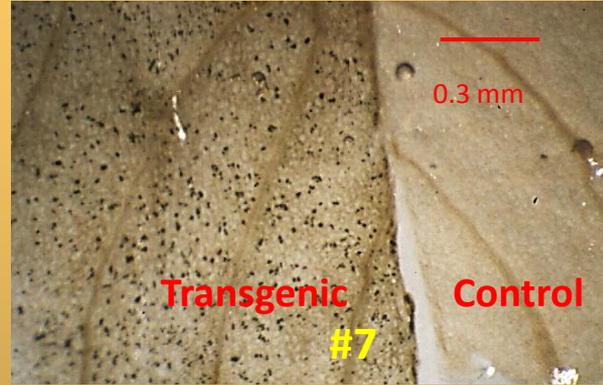


Transgenic "regenerated" *P. vittata* and C-fern sporophytes

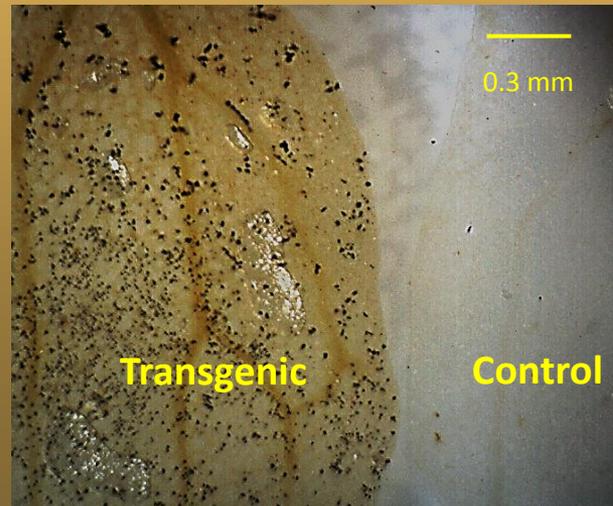
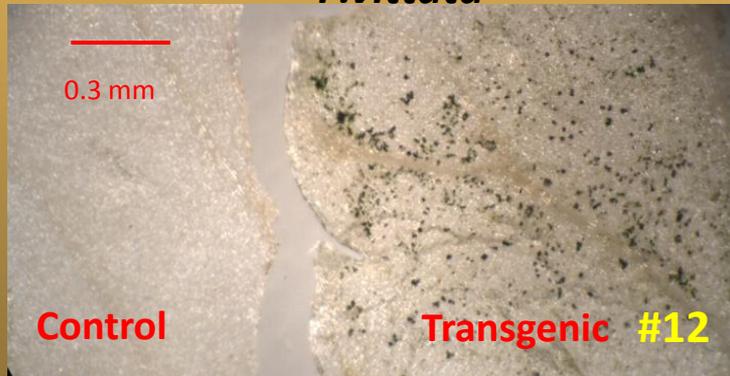
P.vittata



P.vittata



P.vittata



C-fern



Take home message: making a “new” species transgenic by an undergraduate

- No silver bullet
- Tissue culture and nuclear genomic transformation still best bet for genomic additions and knockdown for most species
- Genotype media
- Omic tools
- Promoters needed
- Vectors needed: Gateway technology
- Can compact 20 years into 5 years of research





Zeng-Yu Wang
Wayne Parrott
Tim Tschaplinski

