Environmental and Regulatory Sustainability of Genetically Engineered Bioenergy Feedstocks

The case of switchgrass

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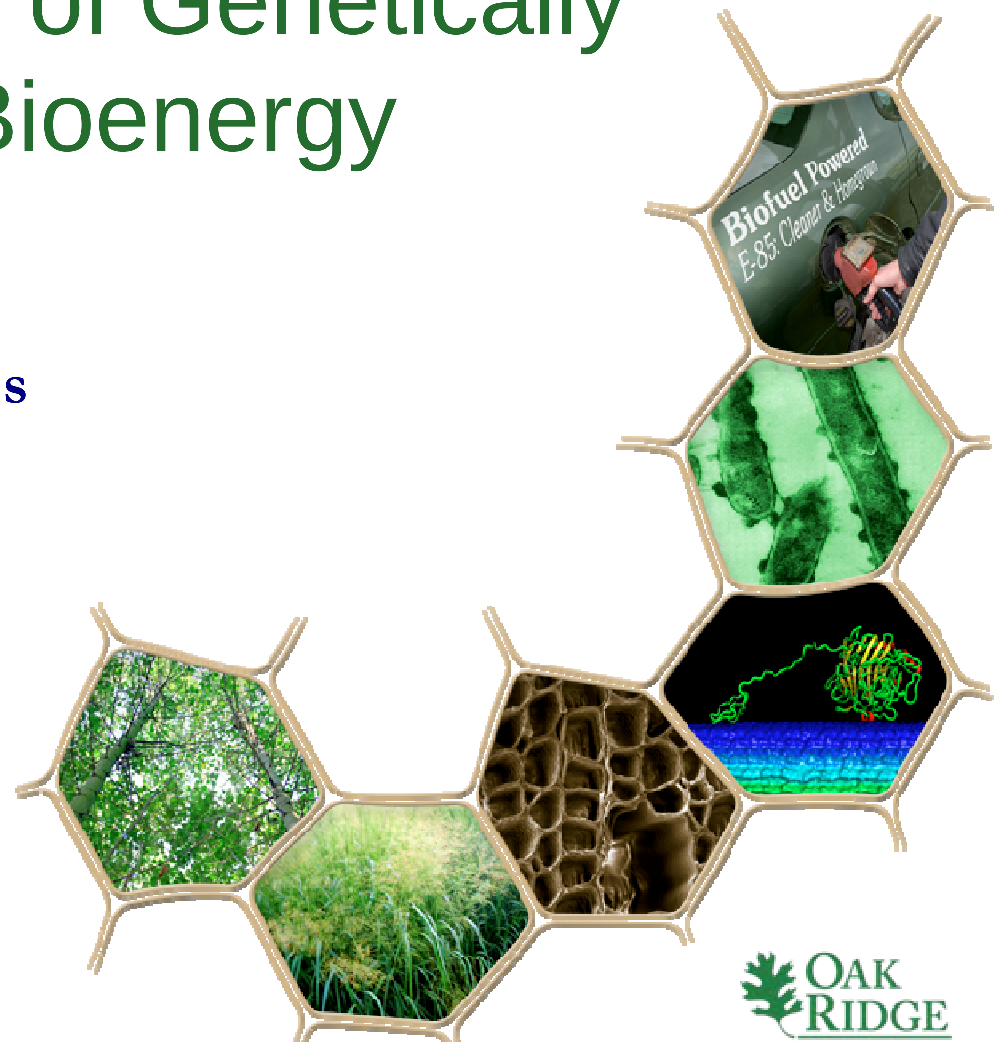
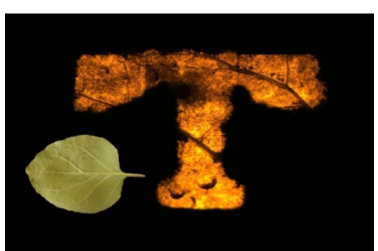
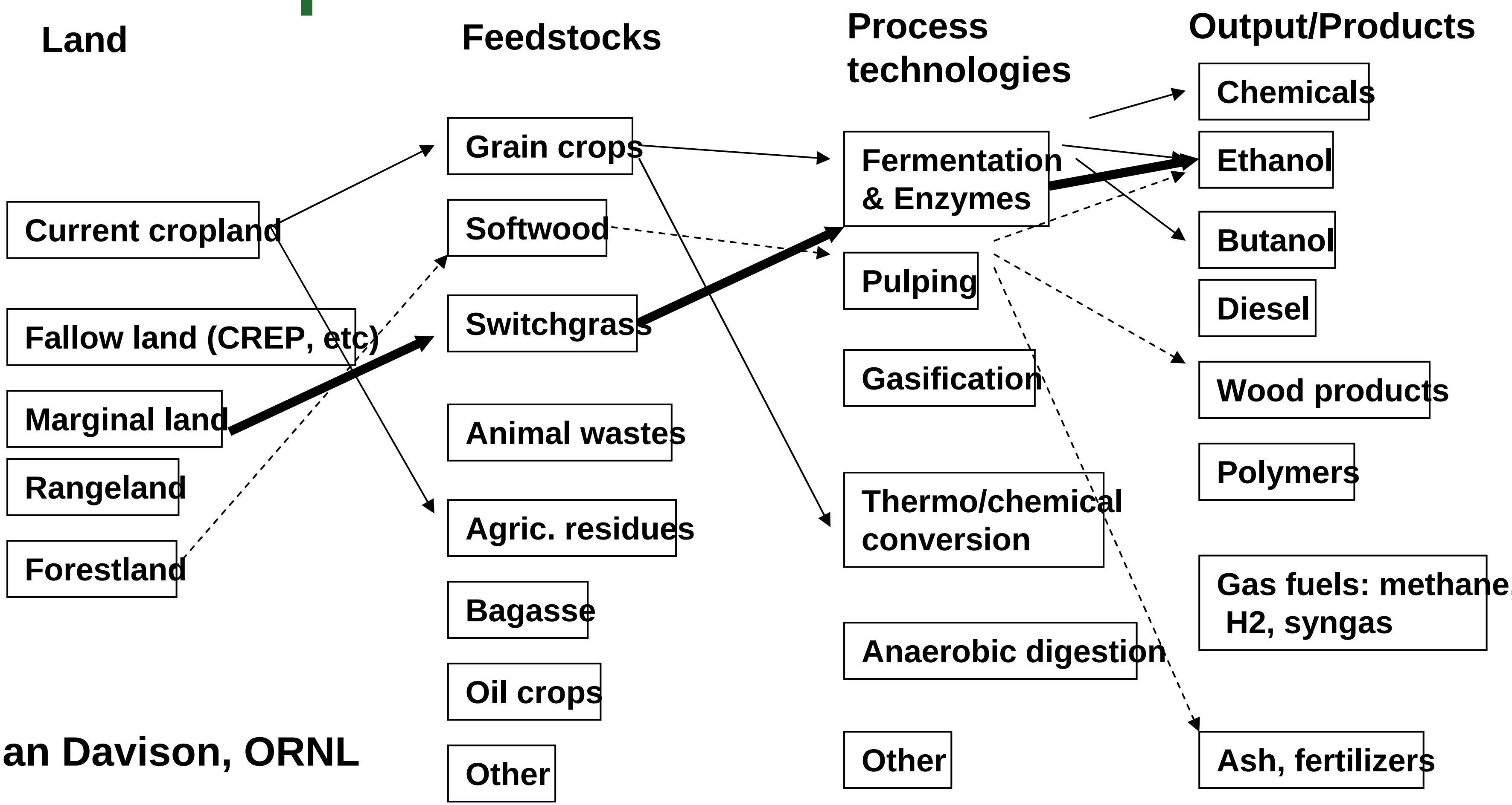


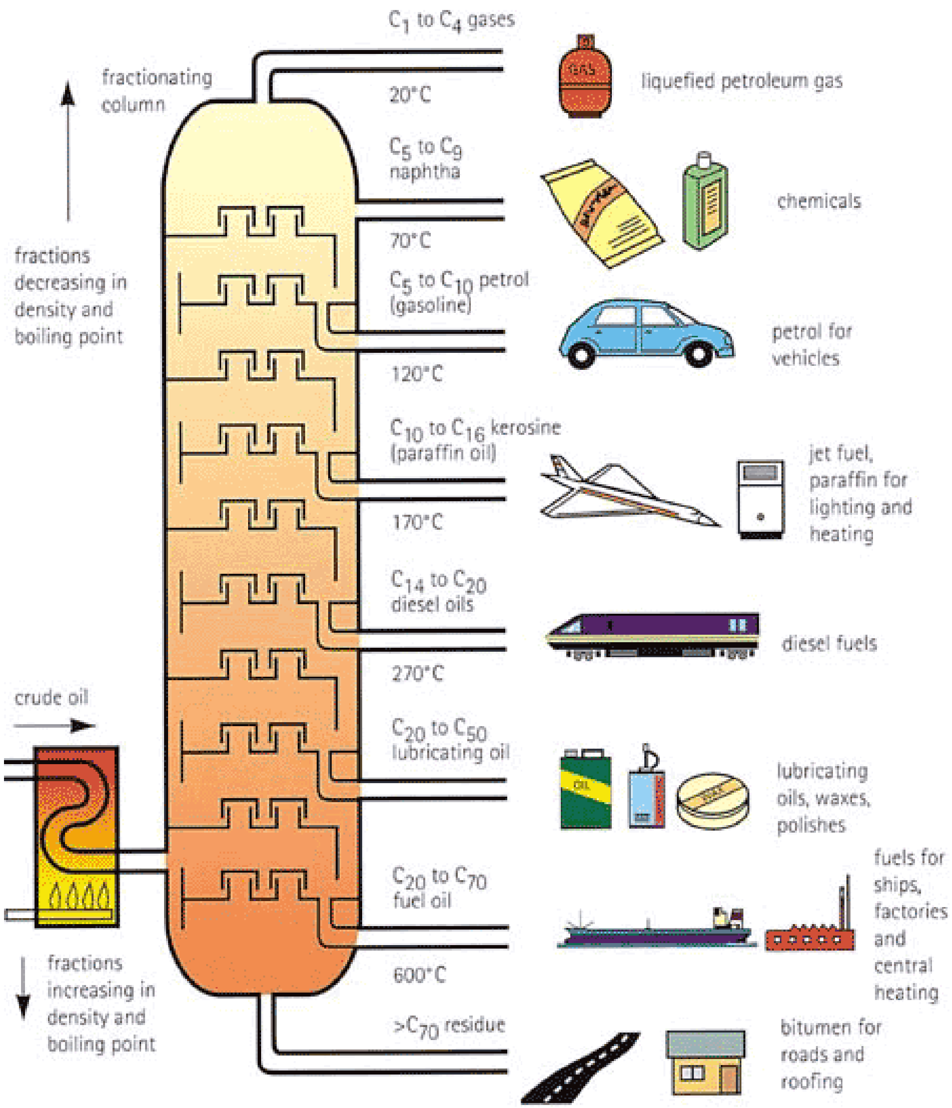


Biomass utilization is a multi-factorial problem



Brian Davison, ORNL

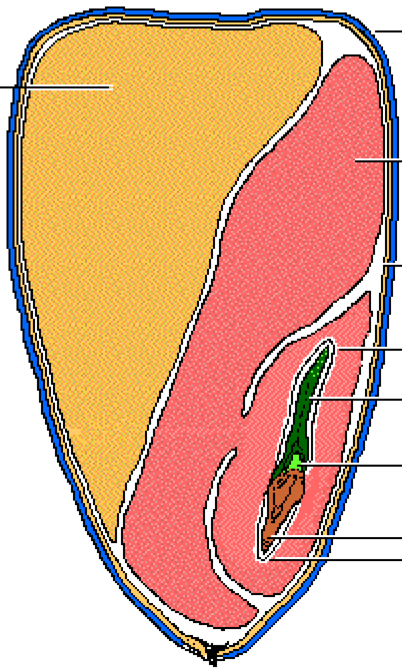
Difference between petroleum and bioenergy feedstocks



http://www.energyinst.org.uk/education/coryton/images/column.gif

Corn vs. cellulosics

Corn



160 bu/acre = 4.5 tons/acre

Switchgrass

12 tons/acre

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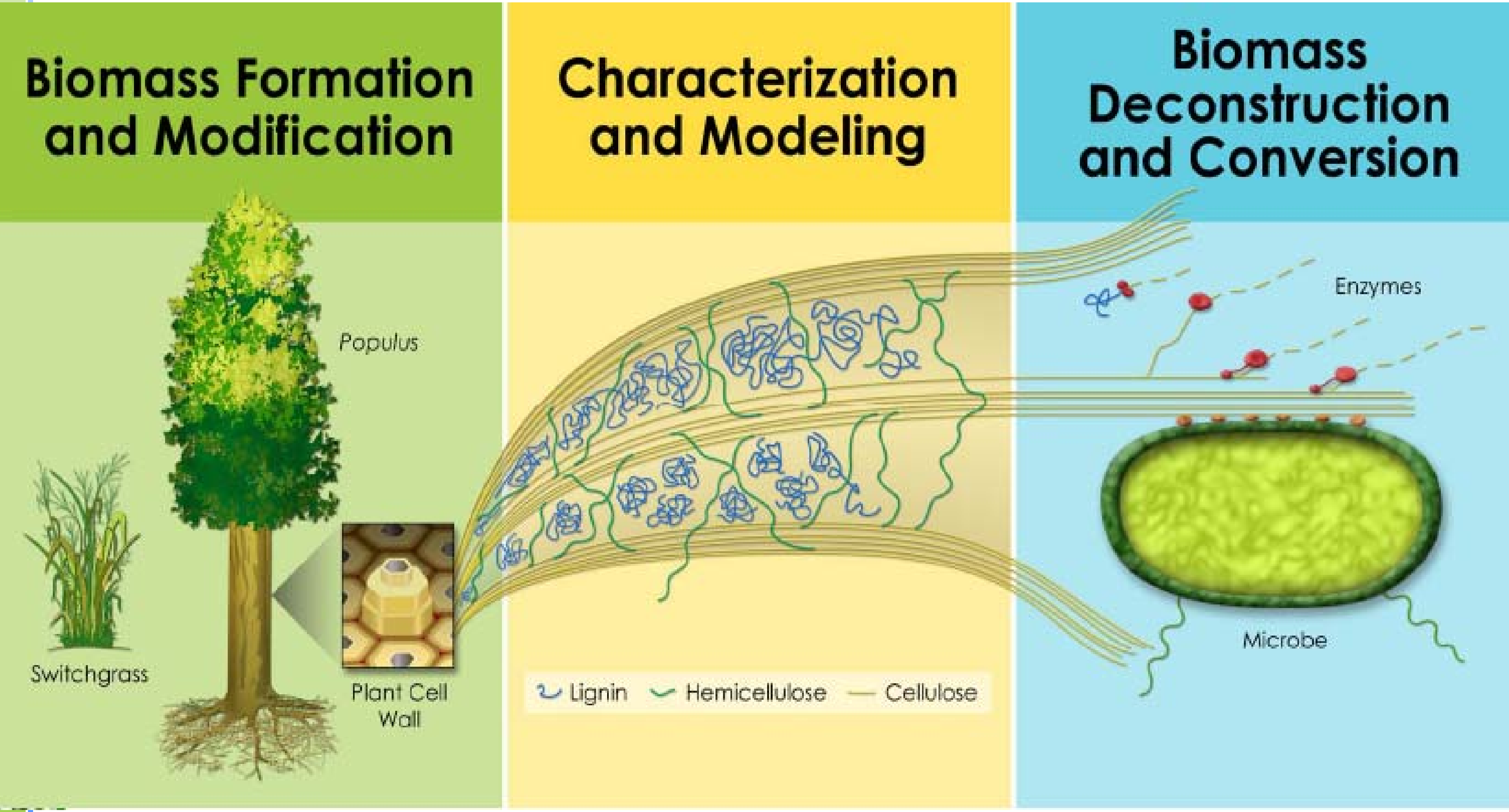
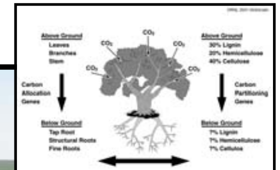
Identify, Understand and Manipulate the Plant



Bioenergy and plant genomics: Expanding the nation's renewable energy resources

Tomorrow

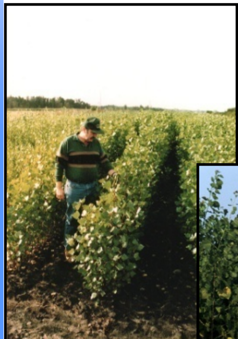
Carbon allocation



Short rotation hardwoods

Today

High yield wood crops



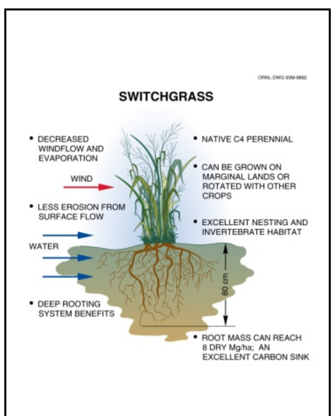
Conventional Forestry

Yesterday

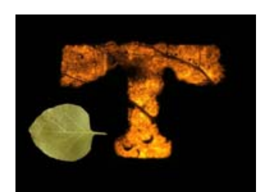
Accelerated Domestication

Metabolic Profiling

Whole Genome Microarrays



Brian Davison ORNL



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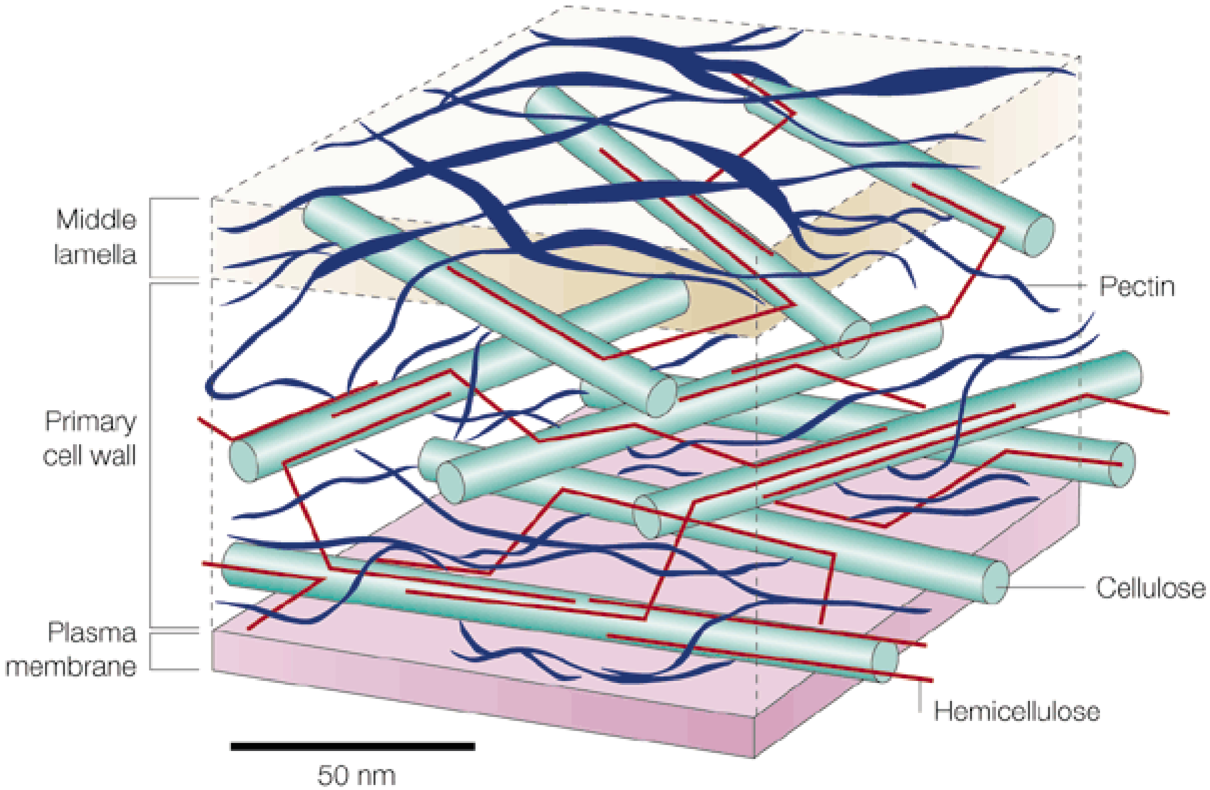
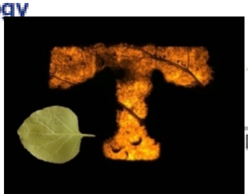
Cell wall structure



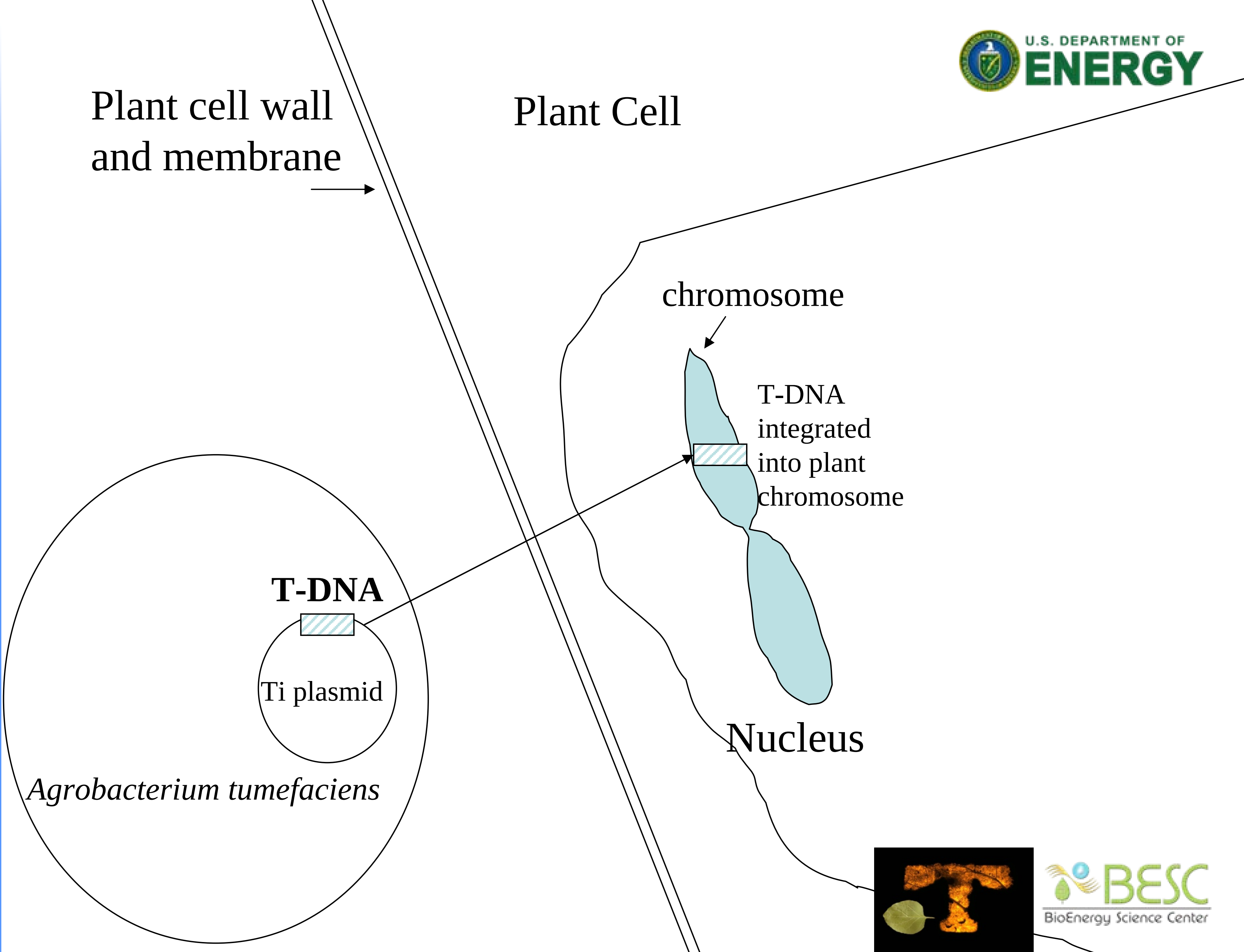
Nature Reviews | Molecular Cell Biology

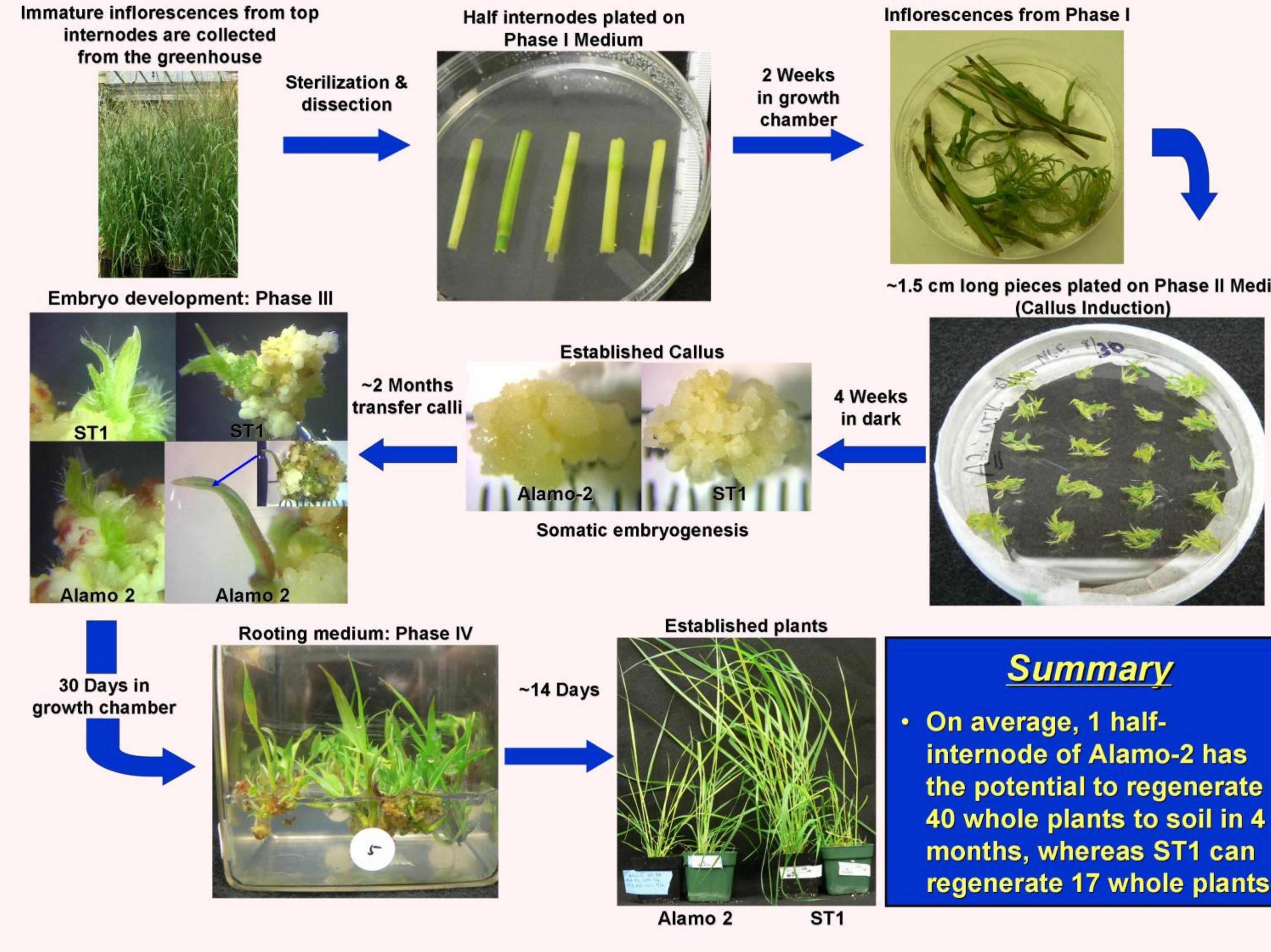
Nature Reviews Molecular Cell Biology **2**, 33-39 (2001)



Switchgrass biotechnology goals

- Enable high-throughput transformation
  - Tissue culture system
  - Transient expression tools
  - Stable transformation system
  - Vectors for genes of interest
- Altering cell wall biosynthesis/modified lignin
- Transgenic plant-expressed cellulases and ligninases
- Increased yield/domestication
- Field performance
- Biosafety/biocontainment
- SUSTAINABILITY


Plant cell wall
and membrane
Plant Cell
chromosome
T-DNA
integrated
into plant
chromosome
T-DNA
Ti plasmid
Nucleus
Agrobacterium tumefaciens

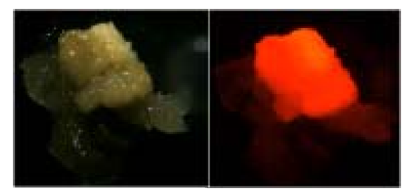


Immature inflorescences from top internodes are collected from the greenhouse
Sterilization & dissection
Half internodes plated on Phase I Medium
2 Weeks in growth chamber
Inflorescences from Phase I
~1.5 cm long pieces plated on Phase II Medi
(Callus Induction)
4 Weeks in dark
Established Callus
Alamo-2
ST1
Somatic embryogenesis
~2 Months transfer calli
Embryo development: Phase III
ST1
ST1
Alamo 2
Alamo 2
30 Days in growth chamber
Rooting medium: Phase IV
~14 Days
Established plants
Alamo 2
ST1
Summary
• On average, 1 half-internode of Alamo-2 has the potential to regenerate 40 whole plants to soil in 4 months, whereas ST1 can regenerate 17 whole plants

Improvement of tissue culture and transformation systems

Proliferating callus

Agrobacterium-mediated transformation



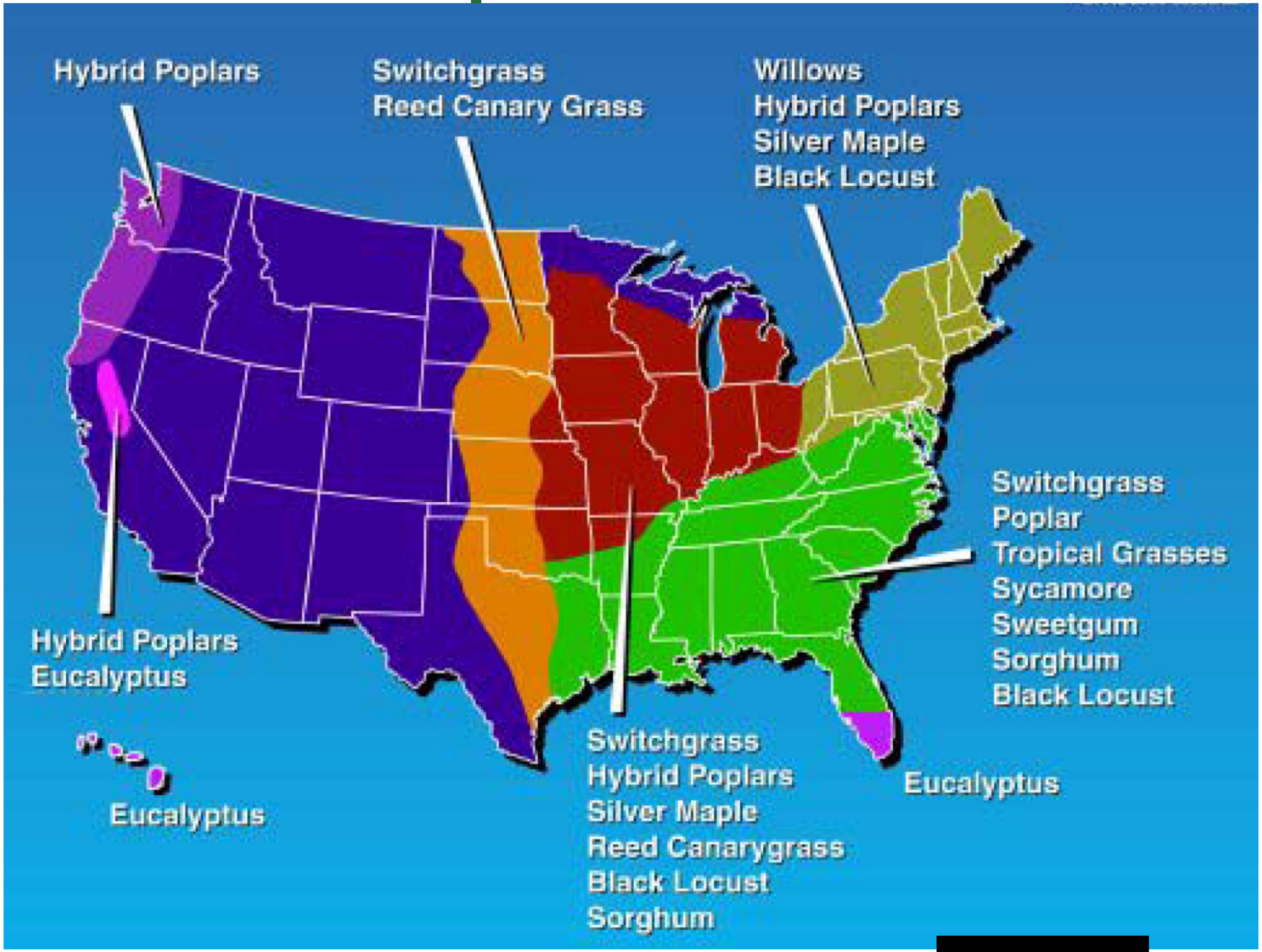
Pick transgenic callus after two months on selection.

After 2 weeks place onto regeneration to form shoots.

After 2-6 weeks place on rooting to root.

- Reduces transformation procedure by 2 months
- Higher efficiency

What biomass crops where?



Lynn Wright et al., ORNL

But switchgrass is not the perfect choice

- Tailored feedstocks for needs
- Differences in adaptation
- Resource base
- Geographic and regulatory considerations

Ceres Product Pipeline

ceres

Switchgrass	Sorghum	Miscanthus	Energycane
			
• Wide Adaptation • Low Input • Perennial • Seed Establishment	• Yield • Adaptation • Production System • Low Water Usage	• Low Input • Perennial	• Yield • Production System

the energy crop company™

Disadvantages

Switchgrass	Sorghum	Miscanthus	Energycane
•Stand establishment •Lower yields than Misc.	•Annual •Inputs •Bad candidate-biotech	•Vegetative propagation •Low genetic variation •Agronomy	•Adaptation-cold •Vegetative propagation •Inputs

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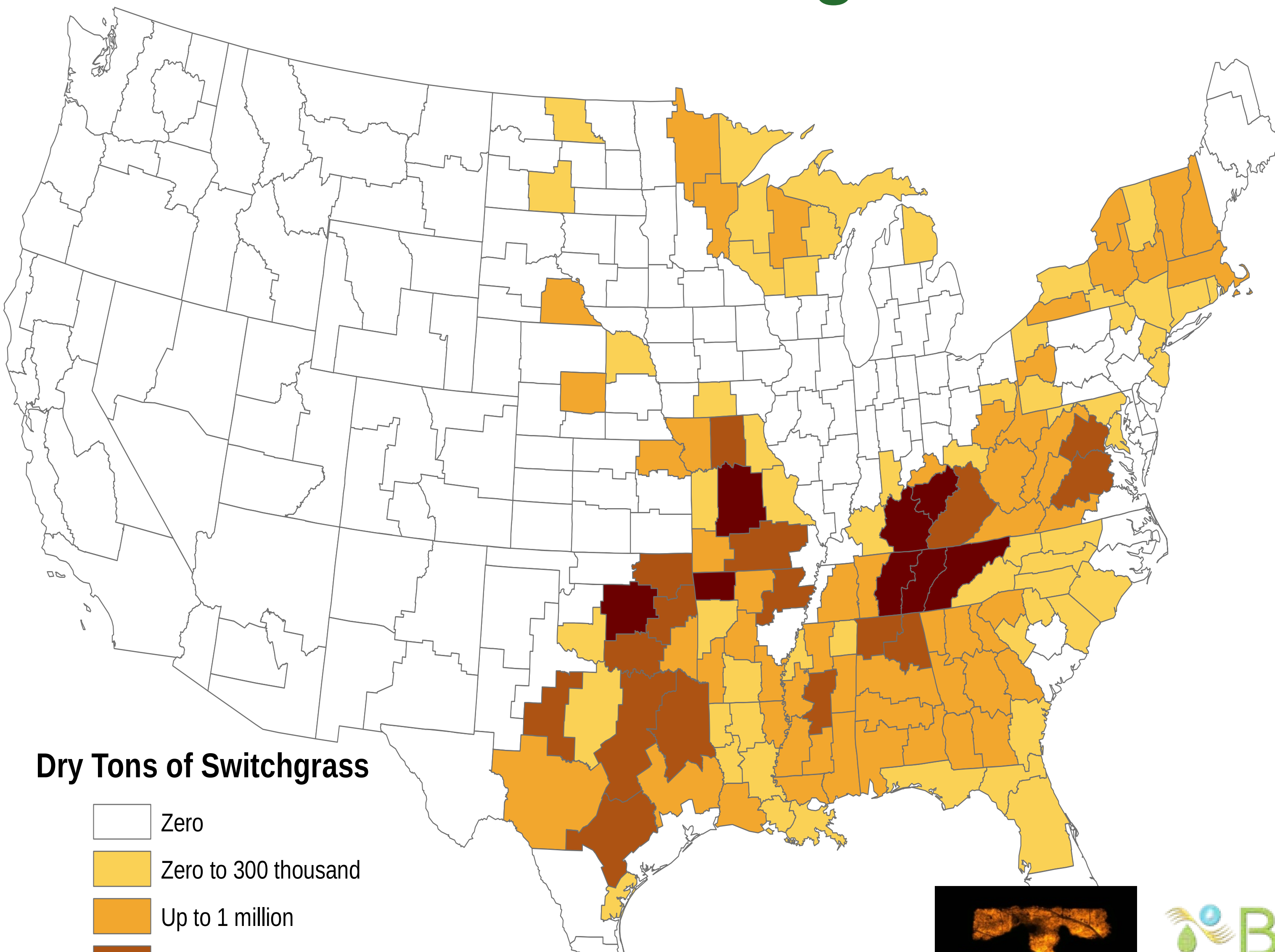
Potential biomass of switchgrass



Dry Tons of Switchgrass

- Zero
- Zero to 300 thousand
- Up to 1 million
- Up to 2 million
- Over 2 million

Daniel de la Torre Ugarte

BESC
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Ideal bioenergy feedstock?

- ✓ Widely adapted
- ✓ High yield
- Low inputs
- Not recalcitrant to digestion and processing
- Homogeneous/canalized traits
- ✓ Stress tolerant
- Farmer friendly
- Economically friendly
- Ecologically friendly

Or recipe for guaranteeing invasiveness?

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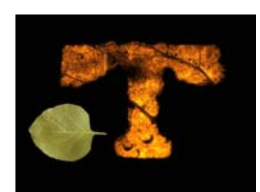
Life is full of choices

Platforms	Feedstock	NEB GJ/ha/yr	NER	CO_2 Balance	Annual	Establishment	Germplasm	Ag Practice	Ecological Benefits
Eethanol from starch or sucrose	Corn	10-80	1.5-3.0	Positive	Yes	+++	+++	+++	+
	Sugarcane	55-80	3.0-5.0	Negative	No	+++	+++	+++	++
	Sugar beet	40-100	2.5-3.5	Positive	Yes	+++	++	+++	+
	Sorghum - sweet	85-300	5-10	Positive	Yes	+++	++	++	++
Ethanol from Cellulosic feedstock	Miscanthus	250-550	15-70	Negative	Yes/No	+	+	+	+++
	Switchgrass	150-450	10-50	Negative	No	+	+	+	+++
	Poplar	150-250	10-20	Negative	No	+	++	++	+++
Biodiesel	Soybean	-20- 10	0.2-0.6	Positive	Yes	++	+++	+++	+
	Canola	-5 – 2	0.7-1.0	Positive	Yes	+++	+++	+++	+
	Sunflower	-10 – 0	03-0.9	Positive	Yes	+++	++	+++	+

Yuan et al. Plants to power:bioenergy to fuel the future, Trends in Plant Science, 2008 13:421

So, biotechnology could be a bioenergy game changer… what about regulations and public acceptance?

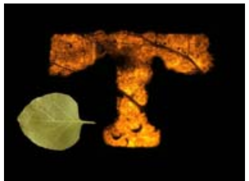
- Biotech food crops still have issues of acceptance and regulations
- But we don’t eat dedicated energy crops
- Special problems with transgenic perennials
- Special problems with transgenic plants grown in their geographic center of diversity
- Gene flow is still a regulatory train wreck



Biotech tools to mitigate transgene flow: biocontainment

- Transgenes on chloroplasts
- Transgenic mitigation: tandem constructs
- Site specific recombination or zinc finger nucleases
- Tissue specific apoptosis→male sterility

Focus on limiting gene flow via pollen



U.S. DEPARTMENT OF ENERGY

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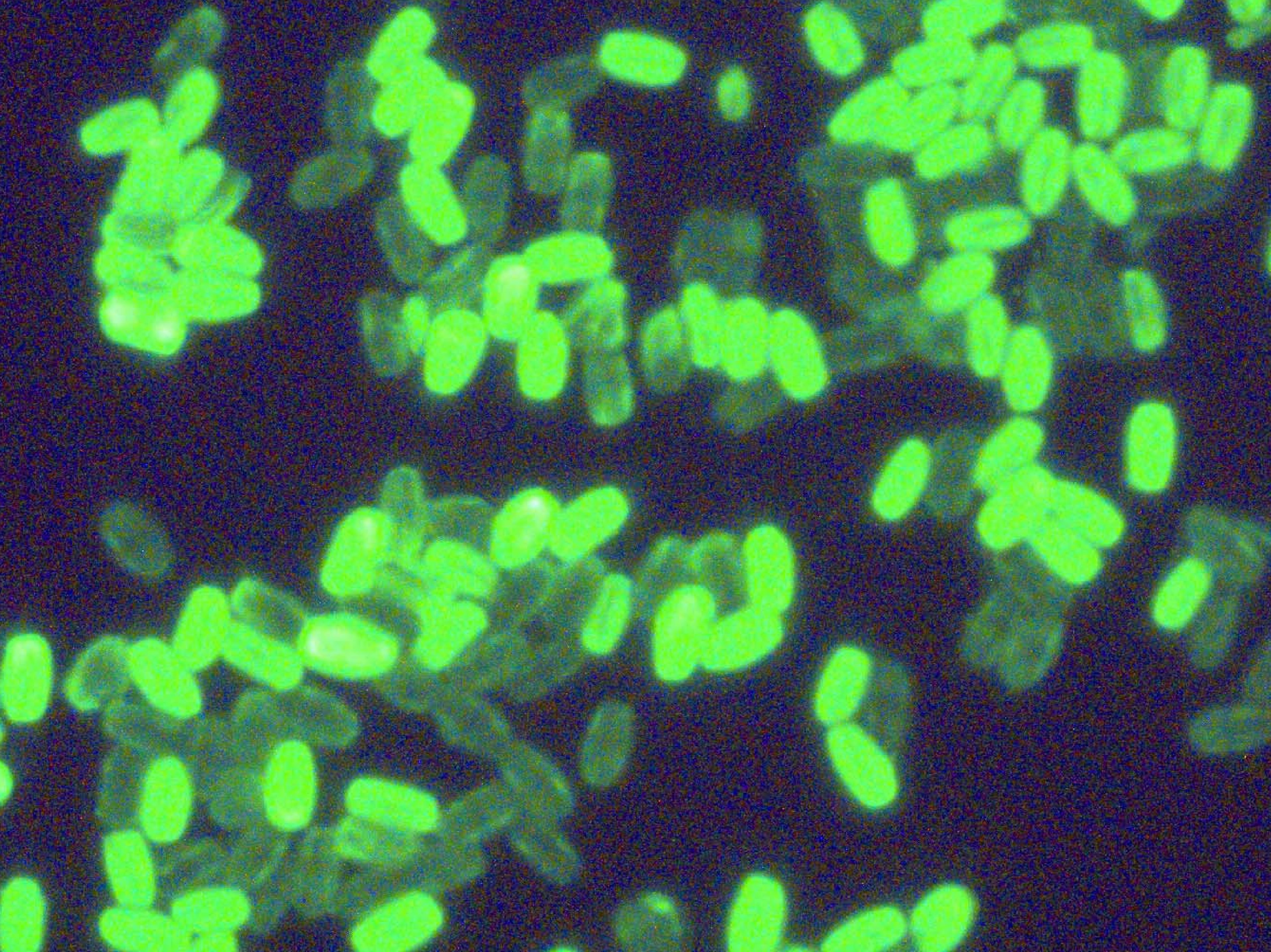
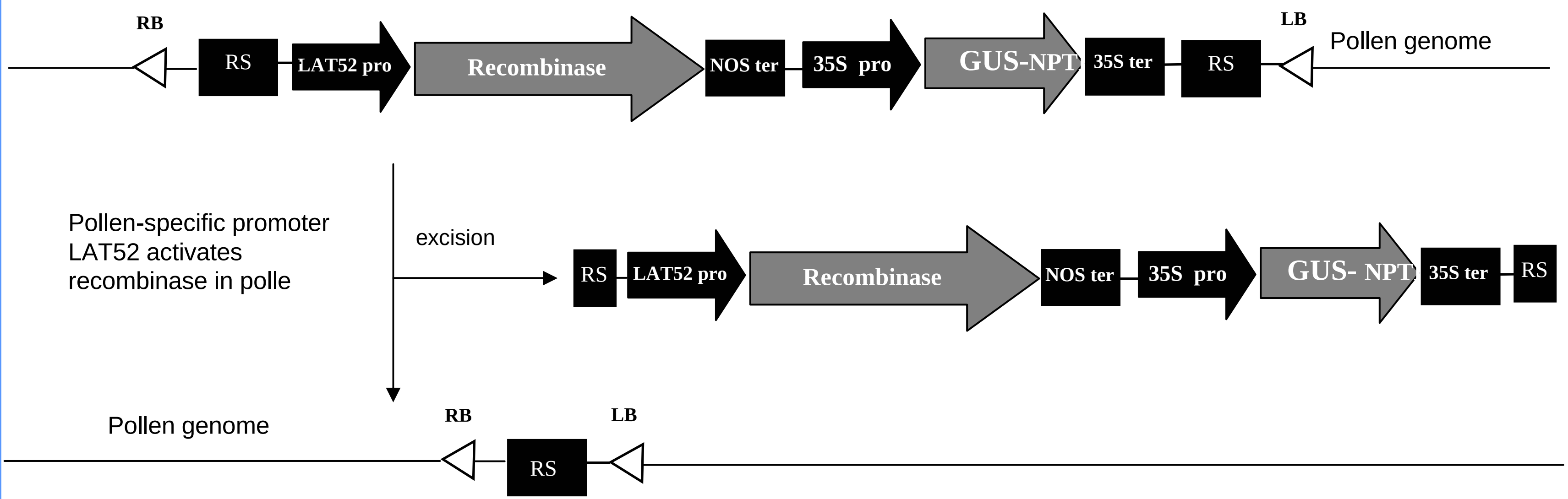
GM gene deletor

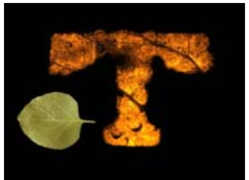
Chopping transgenes out of pollen



Gene deletor

(Luo et al. 2007 Plant Biotechnol J 5:263)



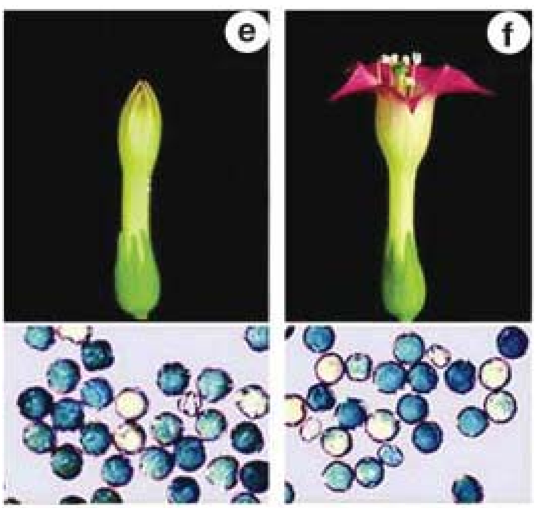


Gene deletor

(Luo et al. 2007 Plant Biotechnol J 5:263)

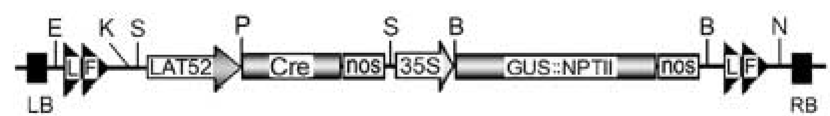
No recombinase vector

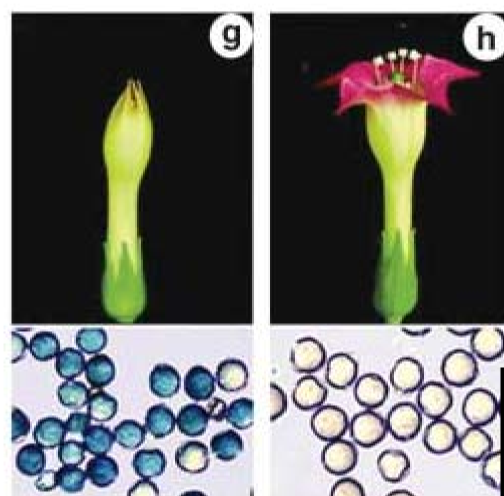
pLF



Cre-*loxP/FRT* vector

pLF_polL-Cre





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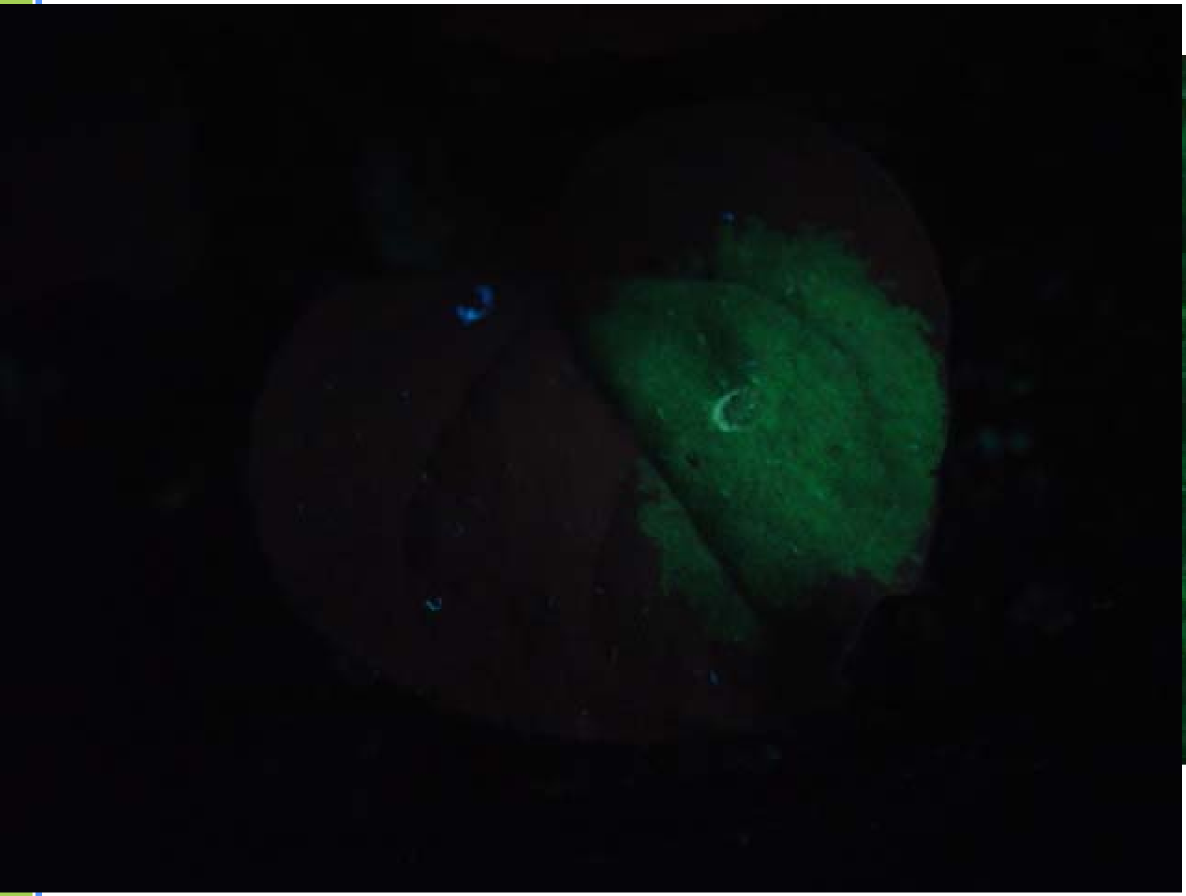
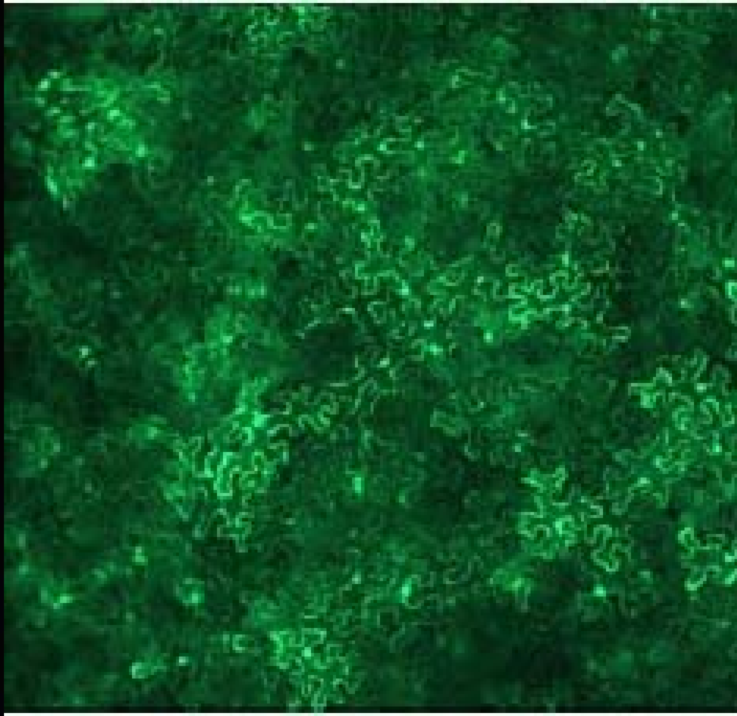
Tissue-specific apoptosis

Killing pollen cells before they can pollinate

Agroinfiltration—a means of rapid assessment of gene expression

Agroinfiltration—marker gene





Tissue specific apoptosis

Conclusions

- The choice of feedstock is critical—no clear perfect choice, but lots of ways to go wrong
- Switchgrass will benefit from biotechnology
- Switchgrass tissue culture system and transformation tools are available
- Regulatory concerns: gene flow and controlling gene flow are both important
- Transgenic switchgrass will require biocontainment for deregulation
- Several biocontainment tools are available
- We must learn from our past mistakes

Stewart Lab

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