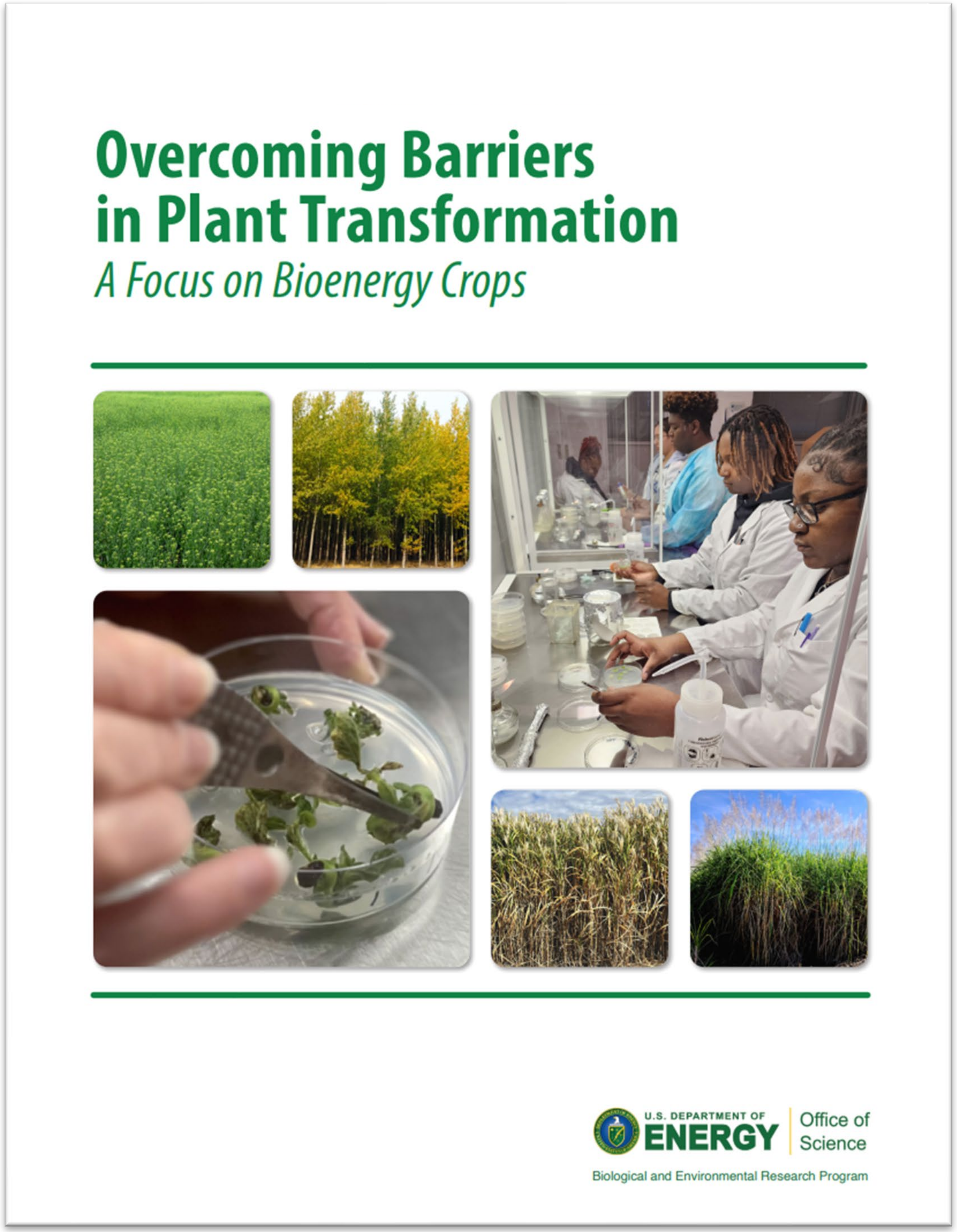
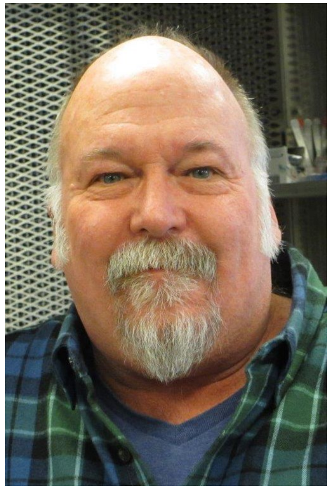


Sally Assmann
PennState

Bill Gordon-Kamm
Corteva



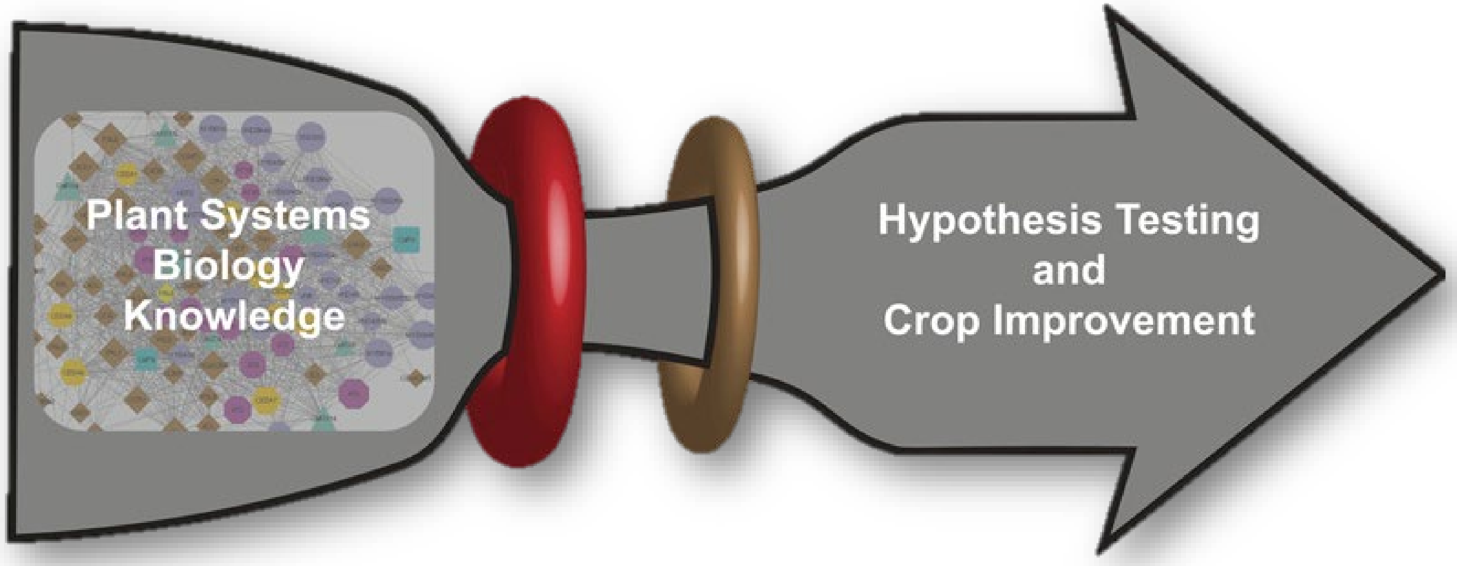
Wayne Parrott
Univ of Georgia

Jeremy Schmutz
Hudson Alpha

Veena Veena
Danforth

Margaret Young
Elizabeth College

- In the past 10 years
  - Lack of plant transformation technology & capacity identified as the major barrier to the plant sciences



[Advancing Crop Transformation in the Era of Genome Editing](https://doi.org/10.1105/tpc.16.00196)

2016. The Plant Cell, 28(7):1510-1520

https://doi.org/10.1105/tpc.16.00196

Plant Genome Research Program (PGRP)

PROGRAM SOLICITATION
NSF 16-614

Plant Transformation Challenge Grants (TRANSFORM-PGR): *Technology challenge to advance plant transformation capabilities.*

Dear Colleague Letter

Advancing Plant Transformation

October 27, 2022

Invites proposals focused on plant genetic transformation to certain existing programs at NSF and USDA. Proposals can involve basic research, long-term studies, tool development, or applications emphasizing potential outcomes with societal benefit.

Bioengineered Traits and Customizable Management Practices

Objective 1.4

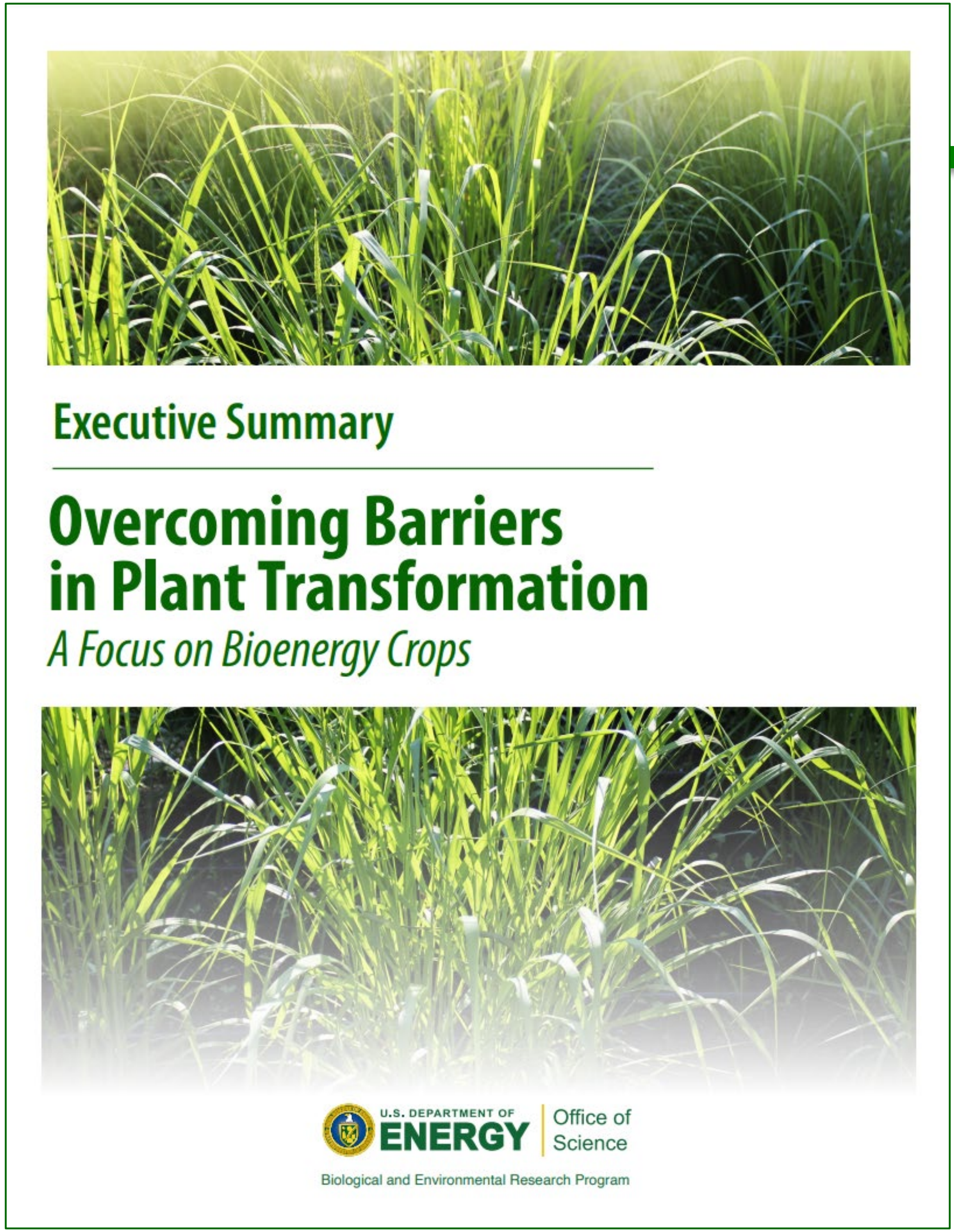
Develop genome engineering, genetic technology, and other technological tools to deliver high yield crops and forest trees for rapid adaption to extreme environmental stresses (e.g., drought) and biological threats.

Genomics and Genome Editing

Objective 4.1

Develop plant regeneration methods, such as recovering viable plants from single cells or plant organs; for example, for specialty crops or rare/endangered species that have the potential to benefit from genome editing tools.

Bioenergy crops remain unaddressed



Workshop on Transformation of Bioenergy Crops

Sept 18-20

https://genomicscience.energy.gov/plant-transformation/

68 participants

- Assorted academia
- Large & small companies
- Research centers

8 DOE

- Vijay Sharma, Ramana Madupu, Shing Kwok, Kari Perez, Todd Anderson, Dawn Adin, Resham Kulkarni, Pablo Rabinowicz

12 observers

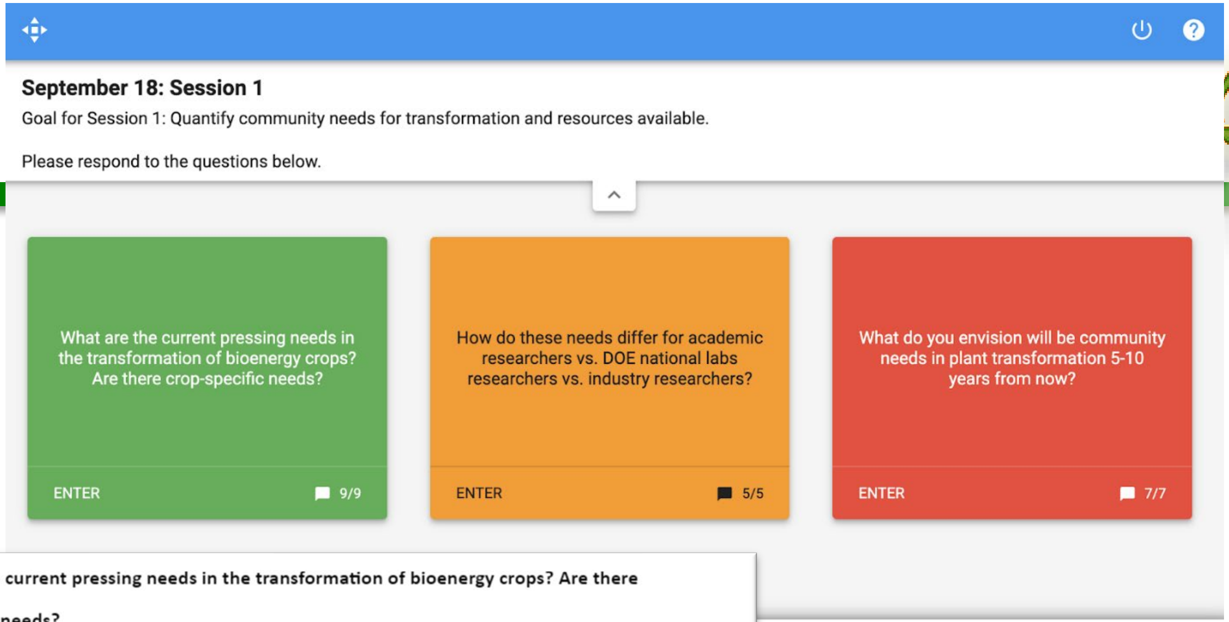
- NSF, NIFA, ARS

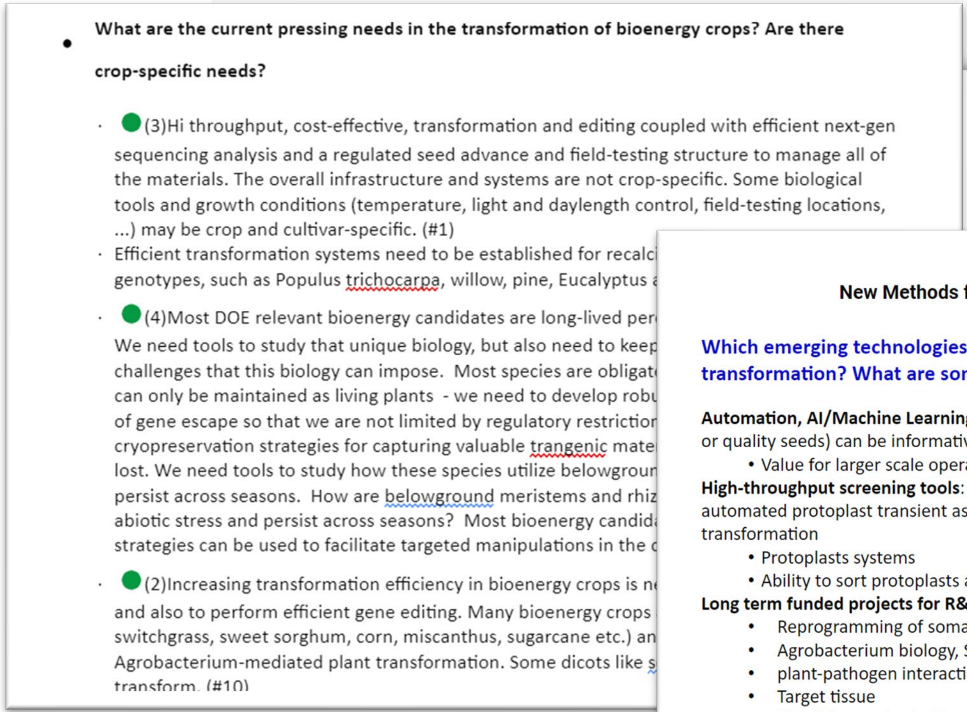
Workshop format

- Inventoried community needs for plant transformation now and in the future
- Evaluated the current state and challenges of plant transformation
- Noted promising new methods for gene delivery, transformation, and regeneration
- Looked at ways to leverage –omics approaches to develop future transformation technologies
- Identified the role of IP, regulations, and stewardship needs
- Highlighted the need to develop an inclusive community and talent pool
- Recommendations

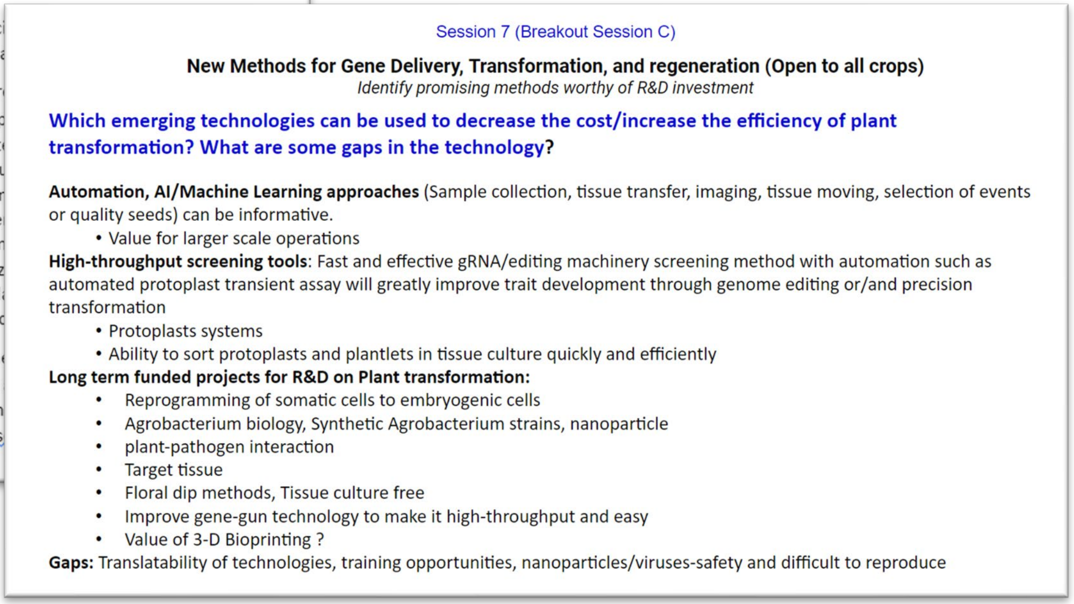
Format

- Invited talks
- Breakout sessions
  - Ranked feedback





- **What are the current pressing needs in the transformation of bioenergy crops? Are there crop-specific needs?**
  - (3)Hi throughput, cost-effective, transformation and editing coupled with efficient next-gen sequencing analysis and a regulated seed advance and field-testing structure to manage all of the materials. The overall infrastructure and systems are not crop-specific. Some biological tools and growth conditions (temperature, light and daylength control, field-testing locations, ...) may be crop and cultivar-specific. (#1)
  - Efficient transformation systems need to be established for recalci genotypes, such as Populus trichocarpa, willow, pine, Eucalyptus a
  - (4)Most DOE relevant bioenergy candidates are long-lived pere We need tools to study that unique biology, but also need to keep challenges that this biology can impose. Most species are obligat can only be maintained as living plants - we need to develop robu of gene escape so that we are not limited by regulatory restrictior cryopreservation strategies for capturing valuable trangenic mate lost. We need tools to study how these species utilize belowgroun persist across seasons. How are belowground meristems and rhiz abiotic stress and persist across seasons? Most bioenergy candid strategies can be used to facilitate targeted manipulations in the c
  - (2)Increasing transformation efficiency in bioenergy crops is ne and also to perform efficient gene editing. Many bioenergy crops switchgrass, sweet sorghum, corn, miscanthus, sugarcane etc.) an Agrobacterium-mediated plant transformation. Some dicots like s transform. (#10)



Session 7 (Breakout Session C)

New Methods for Gene Delivery, Transformation, and regeneration (Open to all crops)

Identify promising methods worthy of R&D investment

Which emerging technologies can be used to decrease the cost/increase the efficiency of plant transformation? What are some gaps in the technology?

Automation, AI/Machine Learning approaches (Sample collection, tissue transfer, imaging, tissue moving, selection of events or quality seeds) can be informative.
- Value for larger scale operations

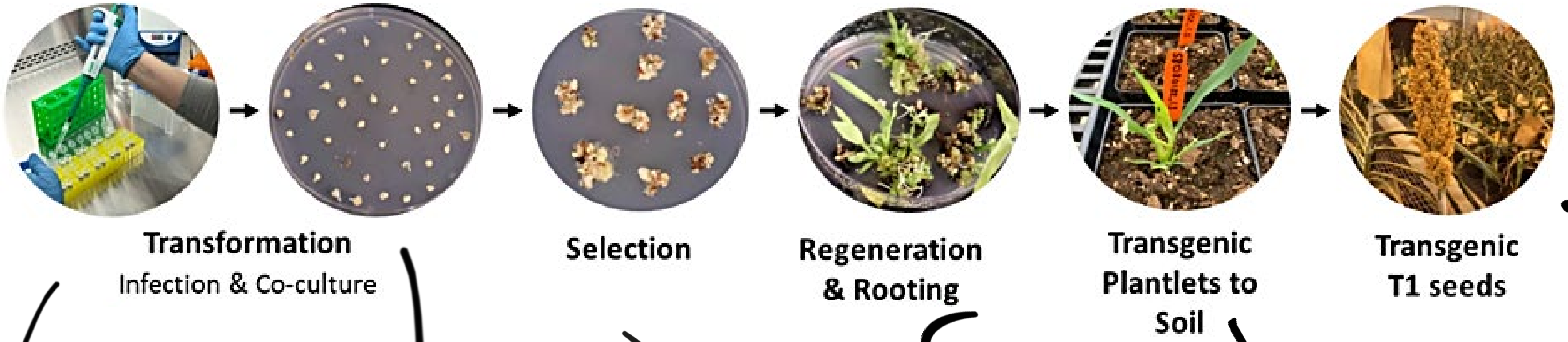
High-throughput screening tools: Fast and effective gRNA/editing machinery screening method with automation such as automated protoplast transient assay will greatly improve trait development through genome editing or/and precision transformation
- Protoplasts systems
- Ability to sort protoplasts and plantlets in tissue culture quickly and efficiently

Long term funded projects for R&D on Plant transformation:
- Reprogramming of somatic cells to embryogenic cells
- Agrobacterium biology, Synthetic Agrobacterium strains, nanoparticle
- plant-pathogen interaction
- Target tissue
- Floral dip methods, Tissue culture free
- Improve gene-gun technology to make it high-throughput and easy
- Value of 3-D Bioprinting ?

Gaps: Translatability of technologies, training opportunities, nanoparticles/viruses-safety and difficult to reproduce

- X-Leap facilitator team
- BERIS

Limitations exist at every step



Ability to assemble large & complex constructs

Availability of trained personnel

Limited *Agrobacterium* strains

Need for site-specific insertion

Recalcitrance:
Few genotypes can regenerate efficiently

Need for quality events

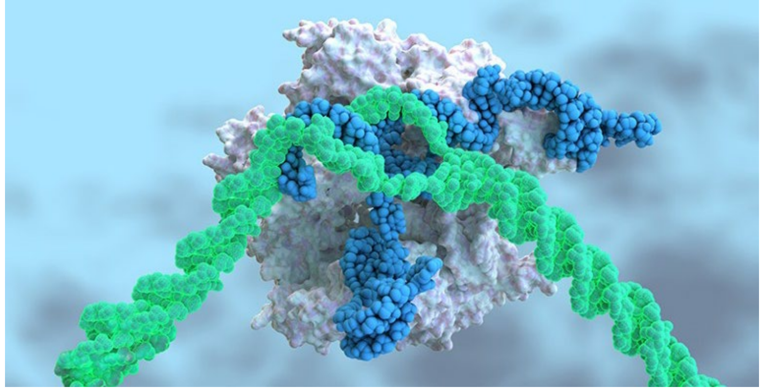
Screening & molecular characterization

The Transformation Explosion

Estimate:

- 5 -10 K transformations/year in USA
  - 6-10 events per transformation

< 10% of these involve bioenergy crops

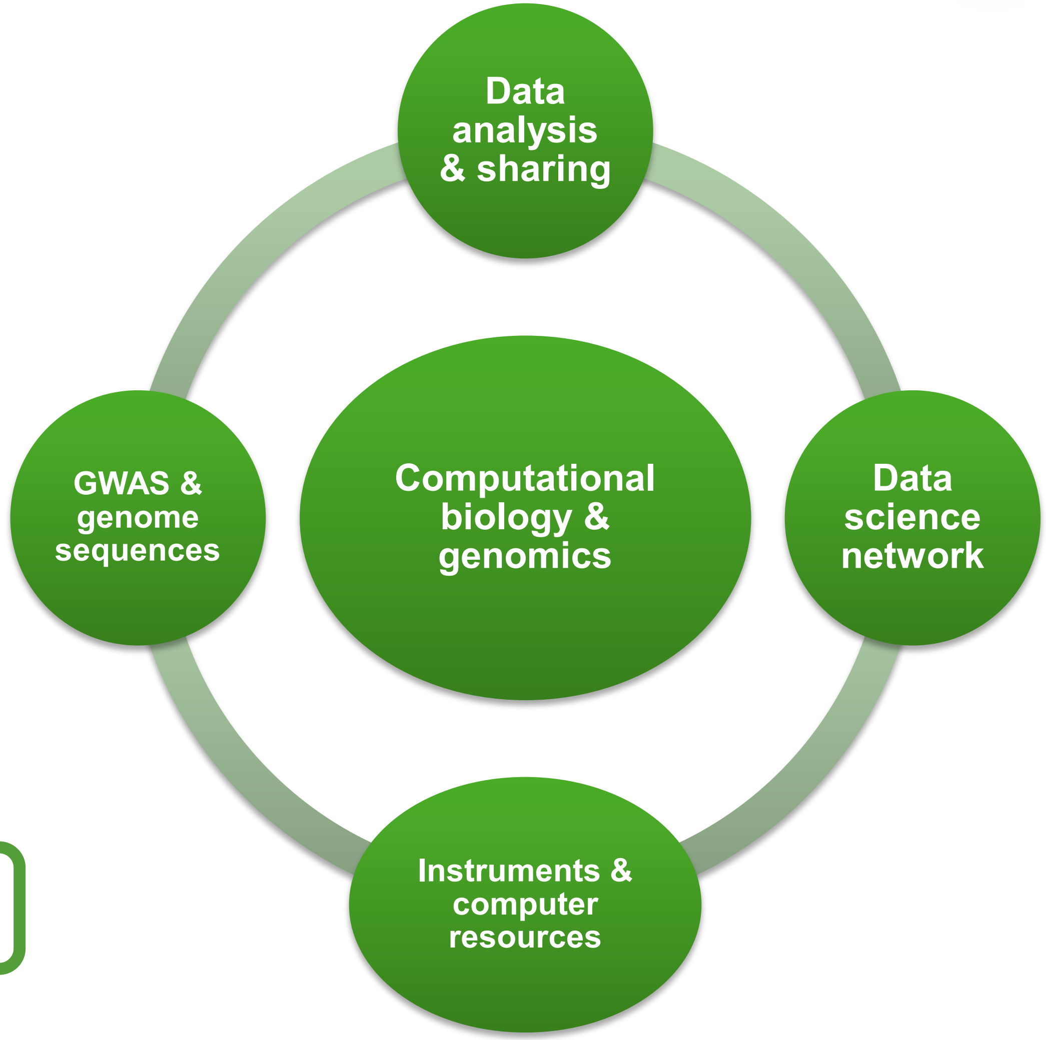


https://www.nature.com/articles/d41586-021-01776-4

Large data & computational power will drive transformation

Estimate:

Transformation needs will increase 20x in next 5 years



Efficiency will need to increase

Ability to engineer ~30 genes at once

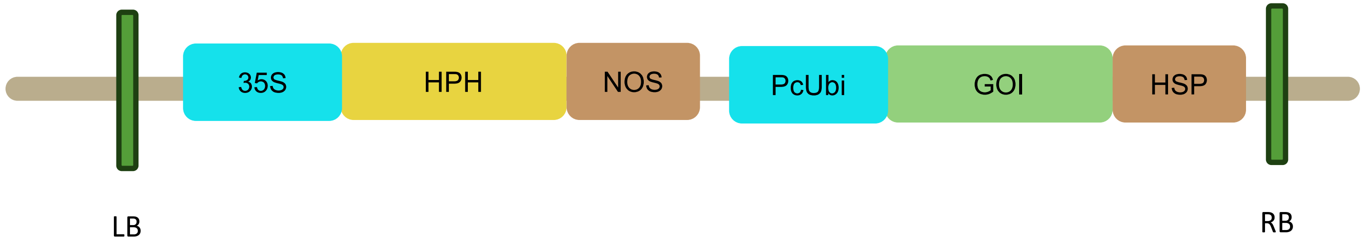
https://www.nia.nih.gov/research/labs/computational-biology-genomics-core

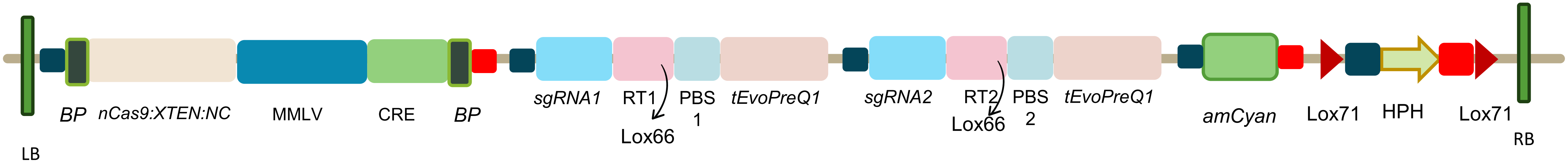
Transgenics then and now

- Restriction cloning

- Gateway assembly
- Modular cloning
- Landing pads
- Safe harbors
- Insulators
- Synthetic promoters
- Tunable expression
- Metabolic engineering

Constructs, then and now





Capacity to assemble and deliver into plants

Need advances in

- **DNA delivery methods**
  - Agrobacterium strains
  - Site-specific integration
  - Delivery of long DNA constructs
  - Viral & nanoparticle delivery
  - DNA-free editing

- **Regeneration**
  - Use of morphogenic genes
  - Site-specific integration
  - Delivery of long DNA constructs
  - Avoid tissue culture altogether

Automation
- AI & machine vision
- Robotics

Solutions and opportunities

- Basic research
  - Understand regeneration and DNA repair
- Technology development
  - Regeneration & transformation
  - Robotics
- Training opportunities
  - Knowledgeable in the science of tissue culture
  - Work force skilled in the art of tissue culture

Meeting the challenge

Thank you!