CORTEVA™
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Agriculture Division of DowDuPont™

Engineering biology applications in plant biotechnology

Lakshmi Sastry-Dent, Steve Evans
R&D External Technology, Indianapolis, IN

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CREATING GLOBAL LEADERS

In Agriculture, Materials Science and Specialty Products

We Are Bringing Together Two Complementary Portfolios to Create World-Class Independent Companies

Broad offering and robust pipeline across germplasm, biotech traits and crop protection

Low-cost integration and innovation combined with expanded customer offerings in key growth sectors

World-class innovation process and application development capabilities

CORTEVA™
agriscience

“Cor-” suggests “core” or “heart” of the earth

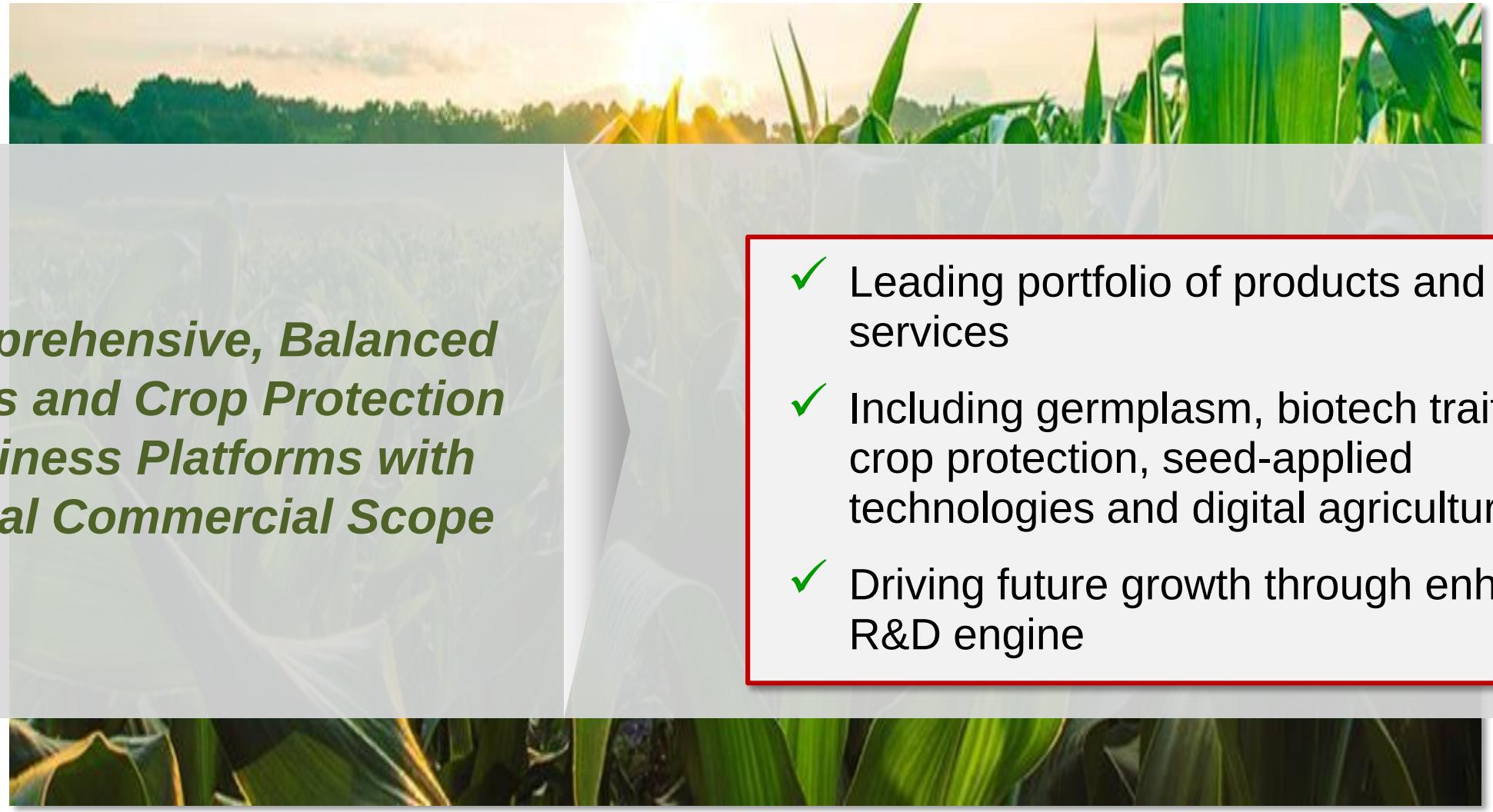
“Teva” is an ancient word which means “nature”

Pronounced: kohr-'teh-vah

Our Purpose

To enrich the lives of those who produce and those who consume, ensuring progress for generations to come.

Agriculture Division: A Global Innovation Leader



Comprehensive, Balanced Seeds and Crop Protection Business Platforms with Global Commercial Scope

- ✓ Leading portfolio of products and services
- ✓ Including germplasm, biotech traits, crop protection, seed-applied technologies and digital agriculture
- ✓ Driving future growth through enhanced R&D engine

To Create Comprehensive, Balanced and Diverse Seeds and Crop Protection Business Platforms with Global Commercial Scope

LEADING PORTFOLIO OF PRODUCTS AND SERVICES…

Seeds Solutions

- Alfalfa
- Canola
- Cereals
- Corn
- Cotton
- Rice
- Silage inoculants
- Sorghum
- Soybeans
- Sunflowers
- Wheat

Crop Protection Solutions

- Cereals Herbicides
- Corn and Soybean Herbicides
- Fungicides
- Insecticides
- Pasture and Land Management
- Rice, Trees and Vines Herbicides
- Seed-Applied Technologies
- Specialty Crop Herbicides
- Structural Pest Management
- Turf and Ornamental Pest Management

And We Are Building An Enhanced R&D Engine To Drive Future Growth

2017-2018	2018-2019	
CROP PROTECTION		
• Arylex™ Active • Enlist Duo® herbicide with Colex-D® • Expanded Zorvec™ launch in AP and LA • Lumisena™ seed treatment • Vessarya™ disease control in LA	• Inatreq™ • Pyraxalt™ insect control* • Rinskor™ Active • Zorvec™ expansion into EMEA*	• Fungicides 1-5(1) • Herbicides 1-2(1) • Insecticides 1-5(1) • New class of nematicides(2) • New MOA – disease control(2)
SEEDS		
• Enlist™ Corn • Enlist™ Cotton • Leptra® insect protection in Latin America • Omega-9 Reduced Saturate Sunflower • ProPound™ Advanced Canola Meal • Stewarded, limited commercial launch of Pioneer® brand Qrome™ products and Plenish™ Soybeans	• Enlist E3™ Conkesta™ Soybean • Enlist E3™ Soybean • Enlist™ Soybean • Expanded commercial launch of Qrome™ products • Herbicide Tolerant Canola with LibertyLink® trait • Pioneer® brand Optimum®	• Corn & soy insect control(2) • Herbicide Tolerance Traits 1-2(1) • Insect Traits 1-10(1) • Next-gen soybeans(2) • Optimum® GLY canola + LibertyLink® traits(2)

Engineering biology applications in plant biotechnology

Engineering Biology: Definition

What is Engineering biology?

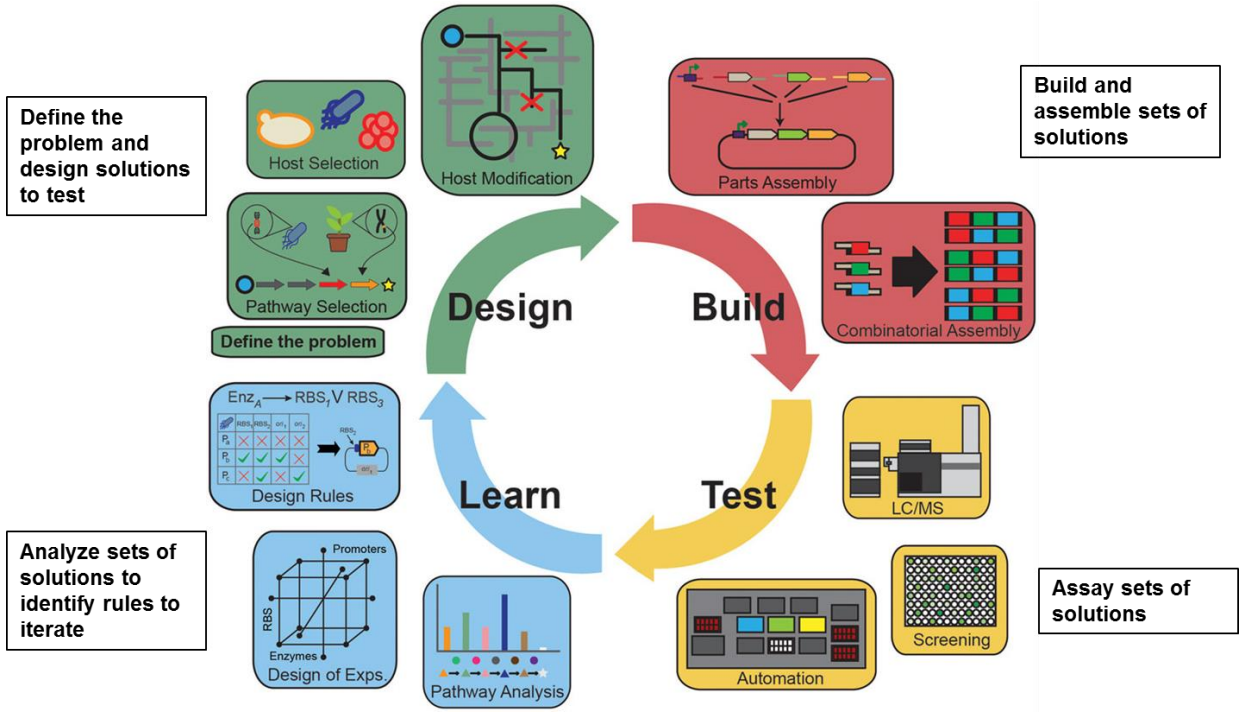
- A set of methods within biotechnology that emerged and were established as a field around the year 2000[1] . Also referred to as “synthetic biology”.
- No universal definition to date but engineering biology “***aims*** to improve the process of genetic engineering” by adopting approaches common to other engineering fields[2].
- Working definition, Corteva Agriscience:

  Engineering biology combines science, technology and engineering to accelerate the design/redesign and manufacture of genetic material, organisms and biological systems for product development.

1. Elowitz, M.B., and S. Leibler. 2000. A synthetic oscillatory network of transcriptional regulators. Nature 403(6767):335-338; ; Gardner, T.S., C.R. Cantor, and J.J. Collins. 2000. Construction of toggle a genetic switch in Escherichia coli. Nature 403(6767):339-342.

2. Voigt, C.A. 2012. Synthetic biology [editorial]. ACS Synthetic Biology 1:1-2.

Engineering Biology: DBTL Concept



- The DBT(L) Cycle is a core concept of engineering biology
- Iterative DBTL cycles are used to optimize solutions
- Enabled by computation, biology, automation and the ability to manipulate DNA (read/write/cut/paste) for rapid testing of ideas/accelerating research & product development

From Petzold, C. J. *et al* in Frontiers in Bioengineering and Biotechnology (2015)

Engineering Biology Applications

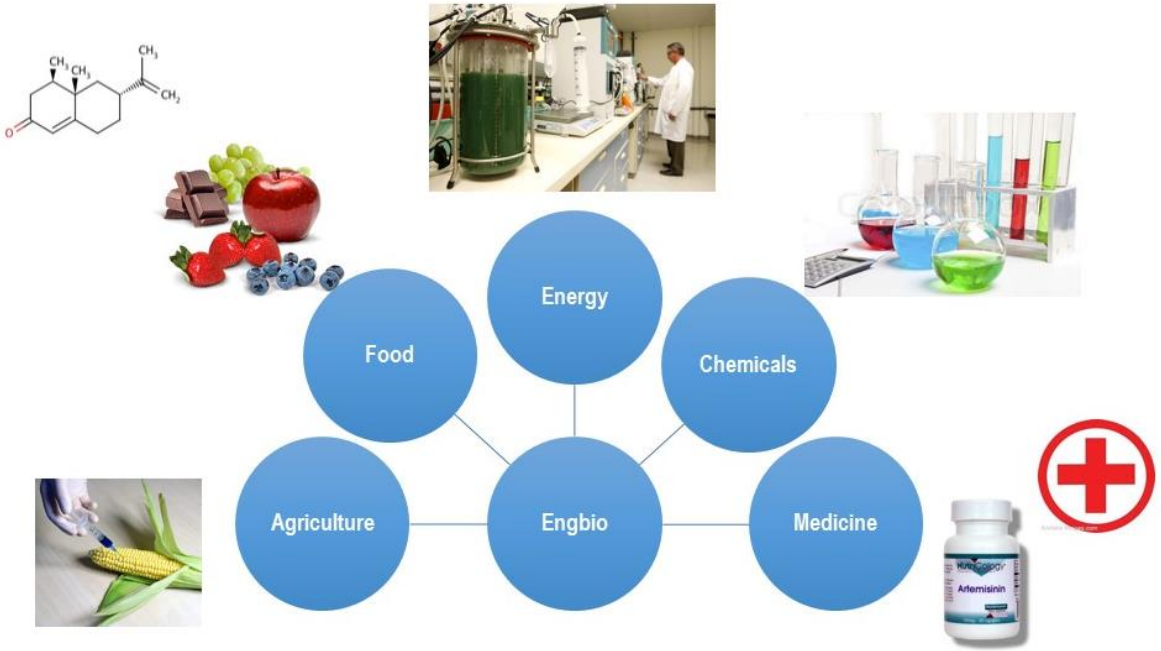


Image: Lux Research

Agriculture Specific

Microbial Engineering
- Natural products discovery & development
- Bio-pesticides

Plant Genome Engineering (via Site Directed Nucleases)
- Trait Stacking
- Accelerated trait development
- Functional genomics

Plant Sensors
- Chemical/biological environmental hazards

Engineering Biology Approaches at Corteva Agriscience™

- Crop Protection and Seeds Platforms
- Select examples (Genome Engineering):
  - Optimal Genomic Loci for Targeted Gene Addition
  - Trait Stacking

Targeted Gene Addition: Benefits for Trait Product Development

(compared to conventional transformation)

- Gene addition to a specific genetic locus
  - *Higher quality events (minimal unintended side effects)*
  - *Increased probability of success*
- Targeted analytics, efficient event sorting
  - *Reduced cycle times*
- Reuse of a genetic locus, targeting reagents, analytics for new product development
  - *Cost savings*

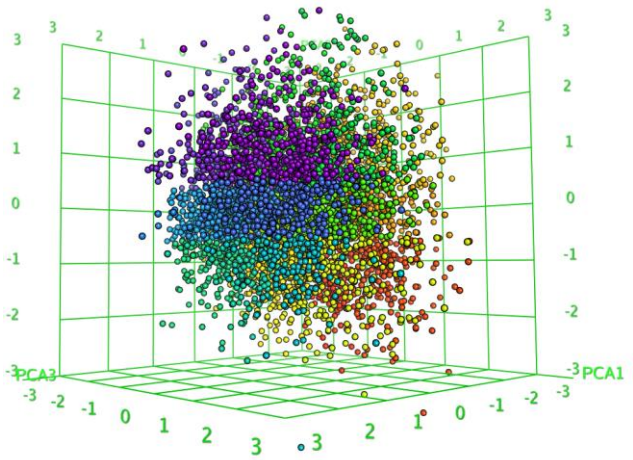
Targeted gene addition

Key question: what genomic locations are optimal for targeted gene addition?

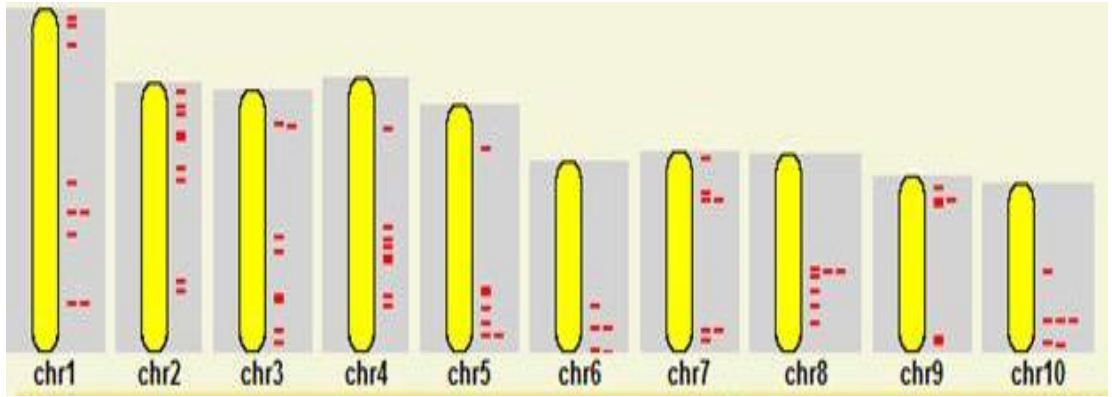
Optimal Genomic Loci for Targeted Gene Addition

- Optimal Genomic Loci (OGL): Genomic regions (sequences) that are targetable, support expression, agronomically neutral and amenable to breeding.
- Bioinformatics approach for genome wide selection of OGL

	Corn	Soybean	OGL Characteristics
Genome size	2.1Gb	1.1Gb	•*Hypomethylated* •*Proximal to genes* •*Unique, non repeat* •*Non-genic* •*Evidence of recombination*
# OGLs (>1kb)	5286	7018	
# Loci validated for targeting	76	32	



Clustered Corn OGL(32 clusters)



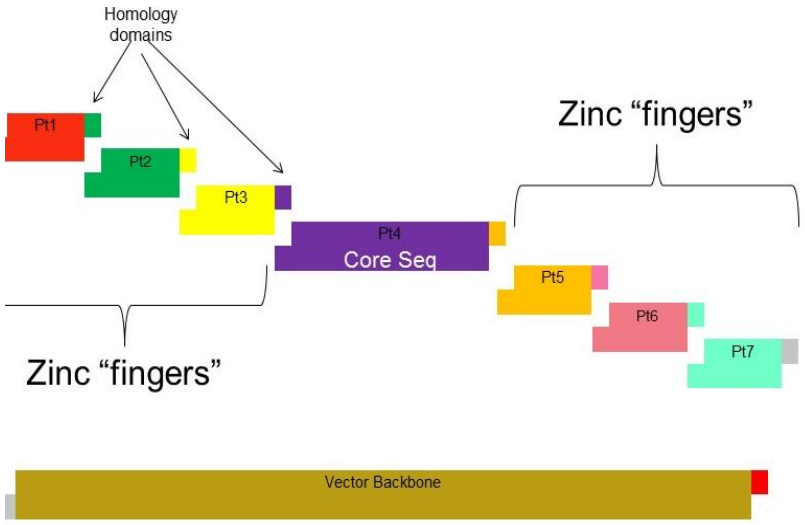
Representative Corn OGL

Targeting Validation of OGL

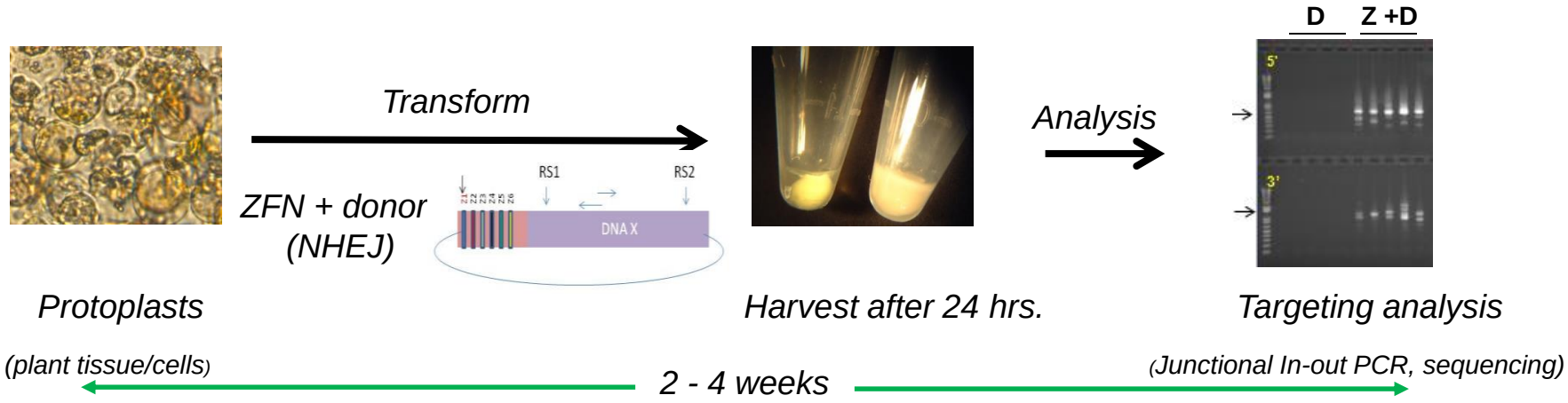
- Challenge: A large number of loci for targeting validation
  - Targeting Reagents (ZFNs): At least 4/locus [~480 ZFN constructs]
  - Develop alternative higher throughput targeting analysis methods

Design/Build of Targeting Reagents

- Automated DNA assembly used to build ~480 ZFN constructs
  - 4-6 ZFN designed per target locus
    - 6 unique “parts” per design and
    - 2 constant “parts” per design
    - ~4320 parts cataloged and used in “build” phase
- 66% 1st pass success rate
  - 480 ZFNs passed on for use in Test/Analyze phase”
  - >90% of errors were SNP’s in synthesized DNA fragments



Rapid Targeting Assay (RTA)



- RTA: Novel plant protoplast based assay for rapid targeting assessment without plant generation
- RTA established for corn, soybean, canola & wheat
- New universal donor DNA design allows evaluation of multiple loci and ZFNs
- New analytics allow targeting detection in complex heterogeneous populations (US patent 9765404)
- 6X reduction in targeting cycle time
- 50-60X increase in targeting analysis capacity

OGL Targeting Validation Outcomes

- Targeted DNA insertion demonstrated at 76 corn OGL and 39 soy OGL
- Patent grants & applications:
  - OPTIMAL SOYBEAN LOCI; Publication # 20150128307
  - OPTIMAL SOYBEAN LOCI; US patent 9909131(2015)
  - OPTIMAL MAIZE LOCI; Publication # 20150128309
  - OPTIMAL MAIZE LOCI; Publication # 20150128310

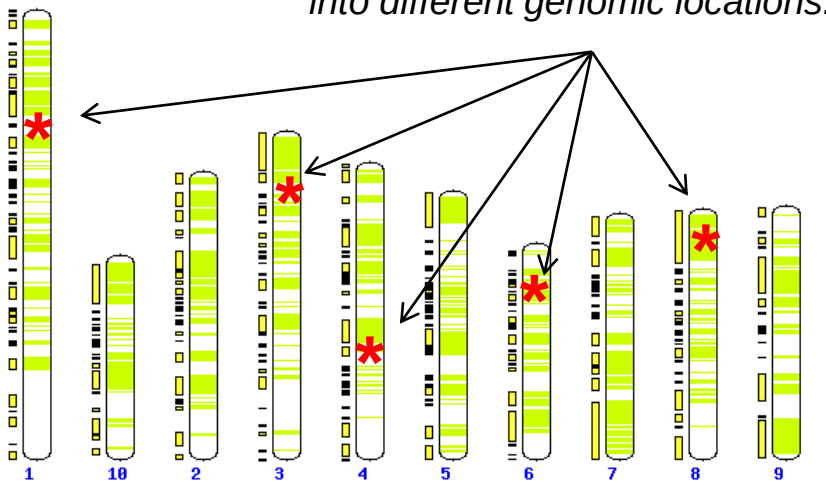
Trait Stacking

- Modern agriculture demands crops with multi-gene/multi-trait stacks
- Complex breeding process used for stacking traits

SmartStax®

Multi-trait = multi-transgene

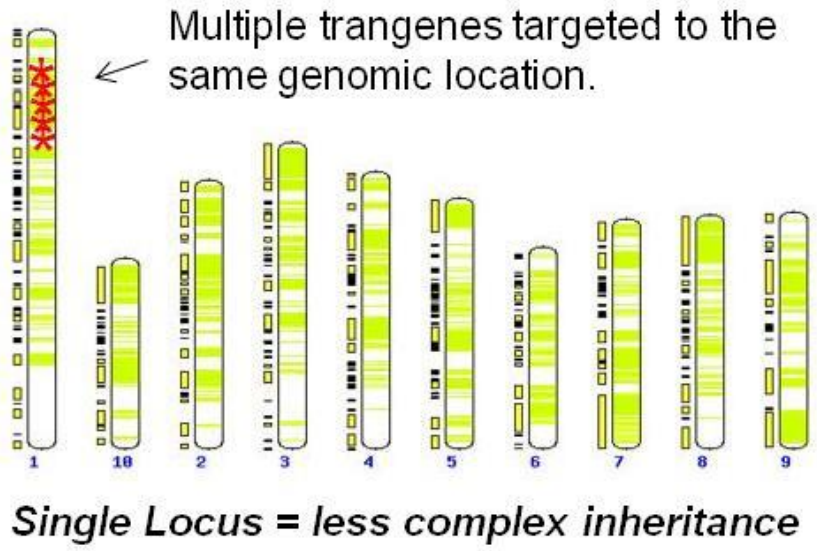
Multiple transgenes randomly integrated into different genomic locations.

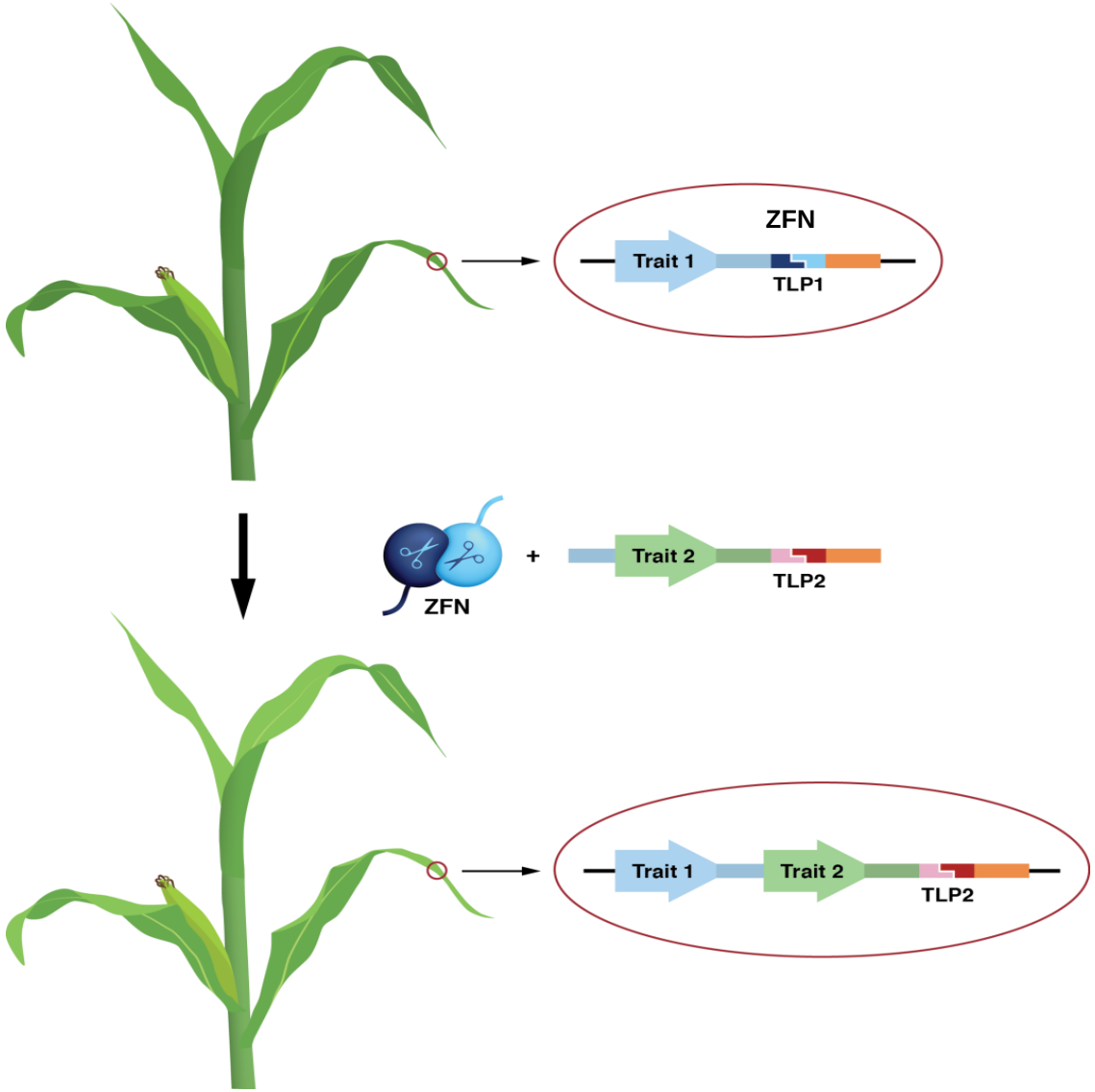


Multi-transgene = breeding complexity

Trait Stacking by Targeted Transgene integration

- Stacking Strategy: Targeted transgene integration to pre-integrated ‘Trait Landing Pads’ (TLPs) using ZFN.





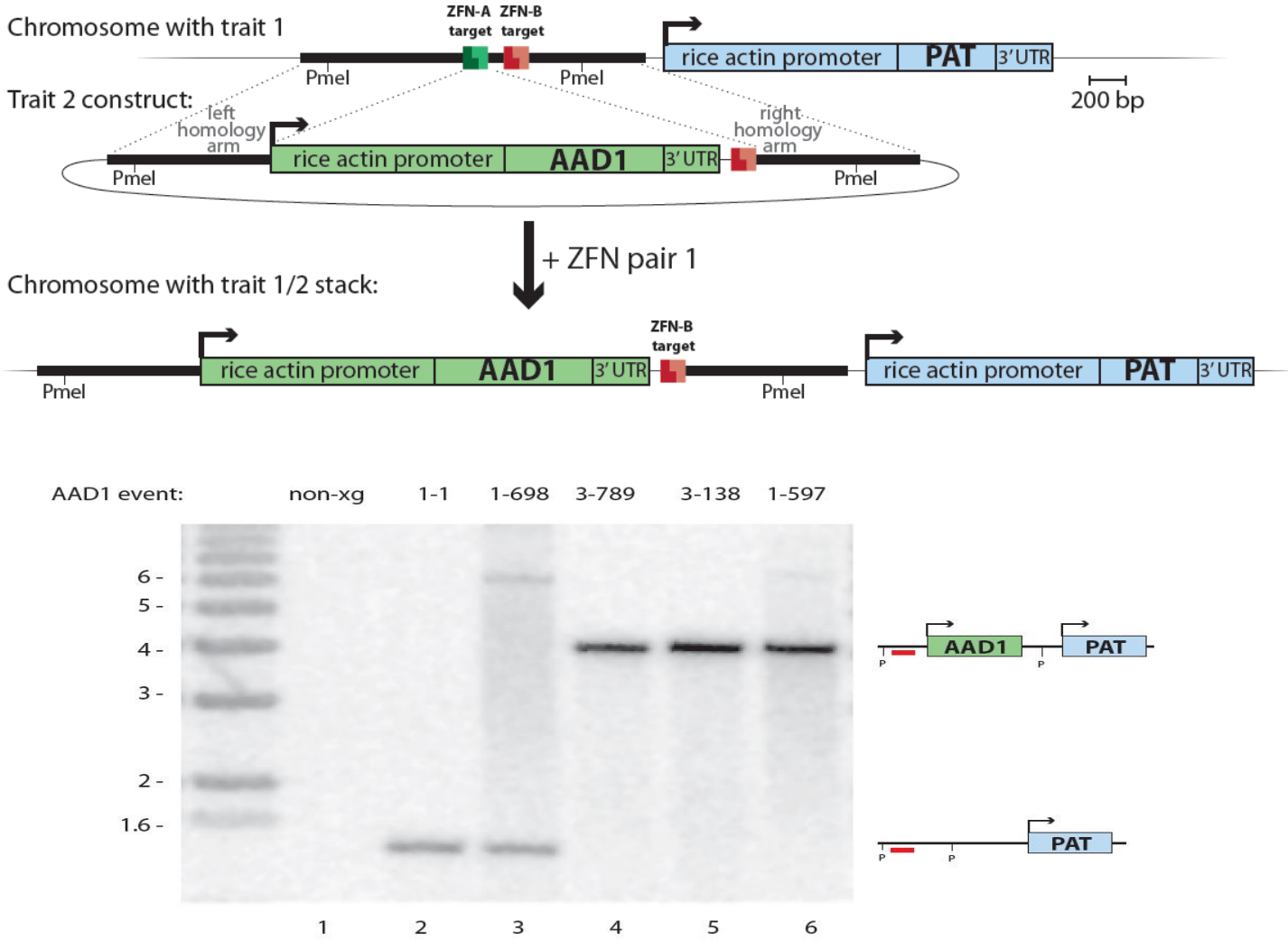
Trait Stacking by Targeted Transgene integration

Herbicide resistance genes can be stacked using targeted transgene integration in corn*

- A TLP flanking the *pat* herbicide resistance gene was targeted by a second herbicide resistance gene, *aad1*, using ZFN-mediated transgene addition.

Immature embryos bombarded	8,558
Transgenic events produced	1,738
Confirmed targeting to the TLP Locus	54
Targeted integration frequency	3.1%

- Targeting/stacking frequency: 3%
- Both transgenes co-segregated in progenies (physically linked) and were functional (positive phenotype).



Ainley et al., 2013. Plant Biotechnol. J. 11:1126-1134

Engineering Biology: Future Applications

- Plant engineering biology is an emerging field, adoption of a standard definition and distinction from existing fields will further accelerate growth.

- Technical challenges such as long/repetitive DNA assembly, efficient delivery of DNA into plant cells impact implementation of the DBT concept in plants.

- Emerging Trends

Funders get plants

OpenPlant
Sharing tools for a sustainable future

NSF Grand Challenges: Rules of Life

https://www.openplant.org/

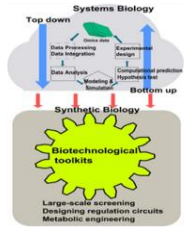
http://www.sciencemag.org/sites/default/files/documents/Big%20Ideas%20compiled.pdf

http://www.frontiersin.org/files/Articles/249725/fpls-08-00786-HTML/image_m/fpls-08-00786-g001.jpg

Hybrid Bio-Nano

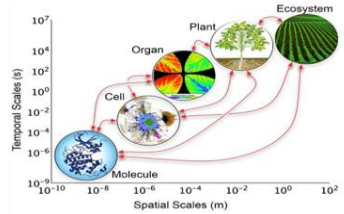
http://www.draper.com/news/equipping-insects-special-service

Integrate systems and engineering biology

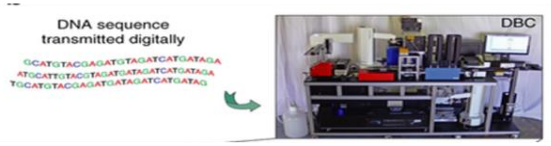


http://onlinelibrary.wiley.com/doi/10.1111/pbi.12283/full

http://www.nature.com/nbt/journal/vaop/ncurrent/fig_tab/nbt.3859_F1.html



Multi-scale modeling



Computers from sequence to product

Trends identified based on funder initiatives (US & Global)

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Thank You!

The Global Challenge

By 2050, the world must feed 9 billion people. The demand for food will be 60% greater than it is today.

https://www.weforum.org/agenda/2016/01/food-security-and-why-it-matters/

Impact of New Technologies on Food Production

- New product offerings
- Higher quality products
- Faster product development
- Products with reduced environmental footprint
- Efficient farm management

Engineering Biology has the potential to accelerate new product development and impact global food security needs.