Zinc Finger Nucleases

ILSI Jaipur October 9 2014

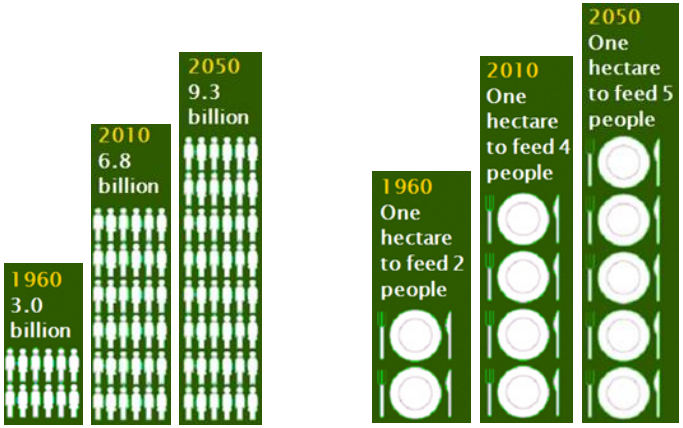
Lakshmi Sastry Dent, Gary Rudgers, Steve Webb, **Matthew Cahill**

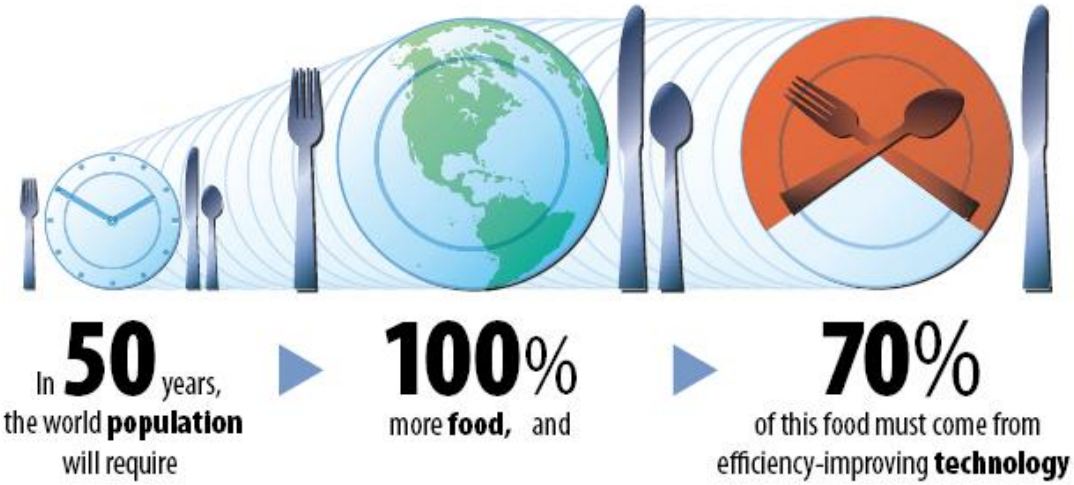
Dow AgroSciences

Solutions for the Growing World

The Global Challenge

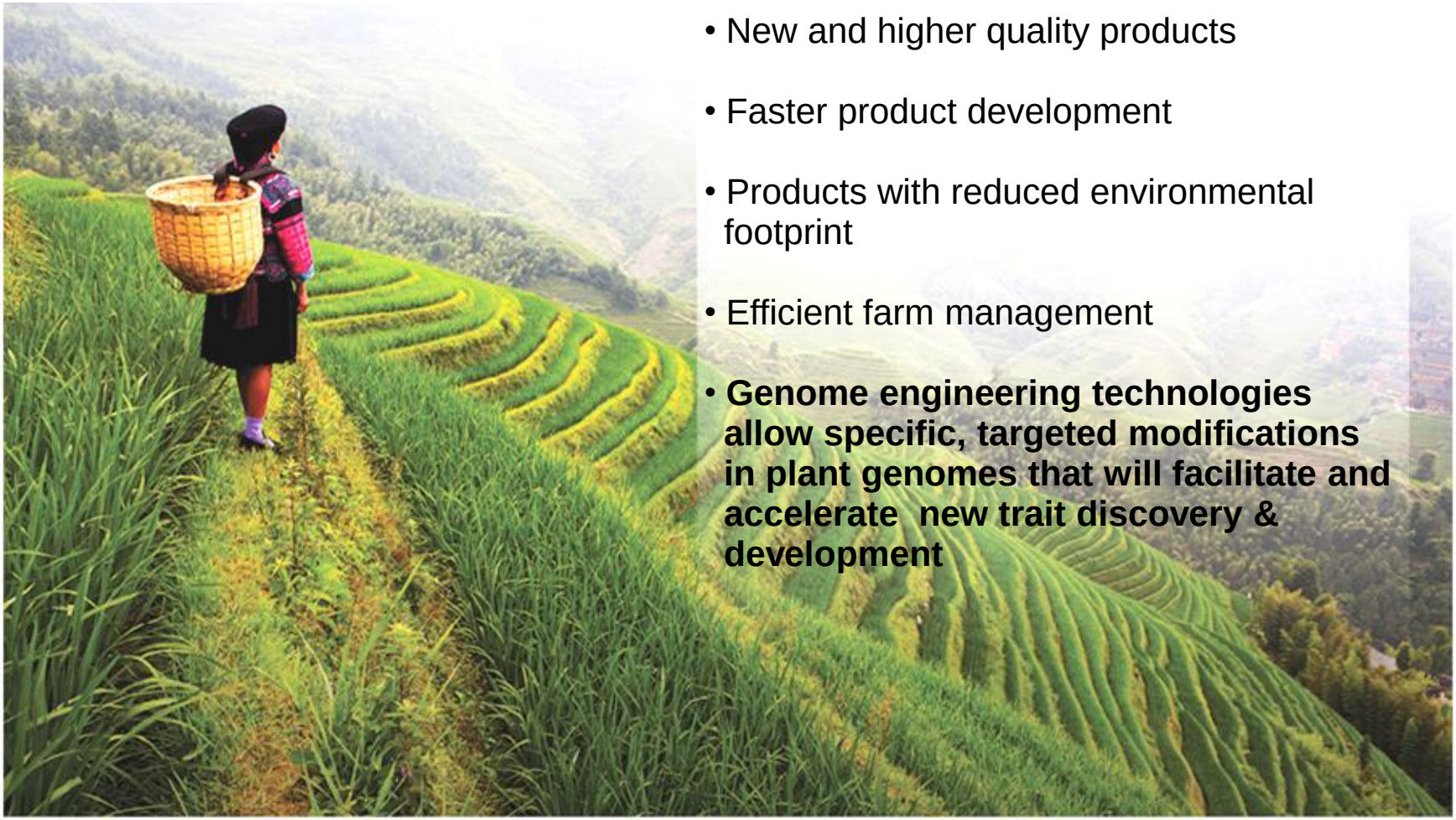
Demand Drivers	Needed Result	Global Constraints
Population Growth Nutritional Deficit Protein Demand	**Increased Food Production**	Limited Arable Land Limited Water Supply





U.N. Food and Agriculture Organization

Impact of New Technologies on Food Production



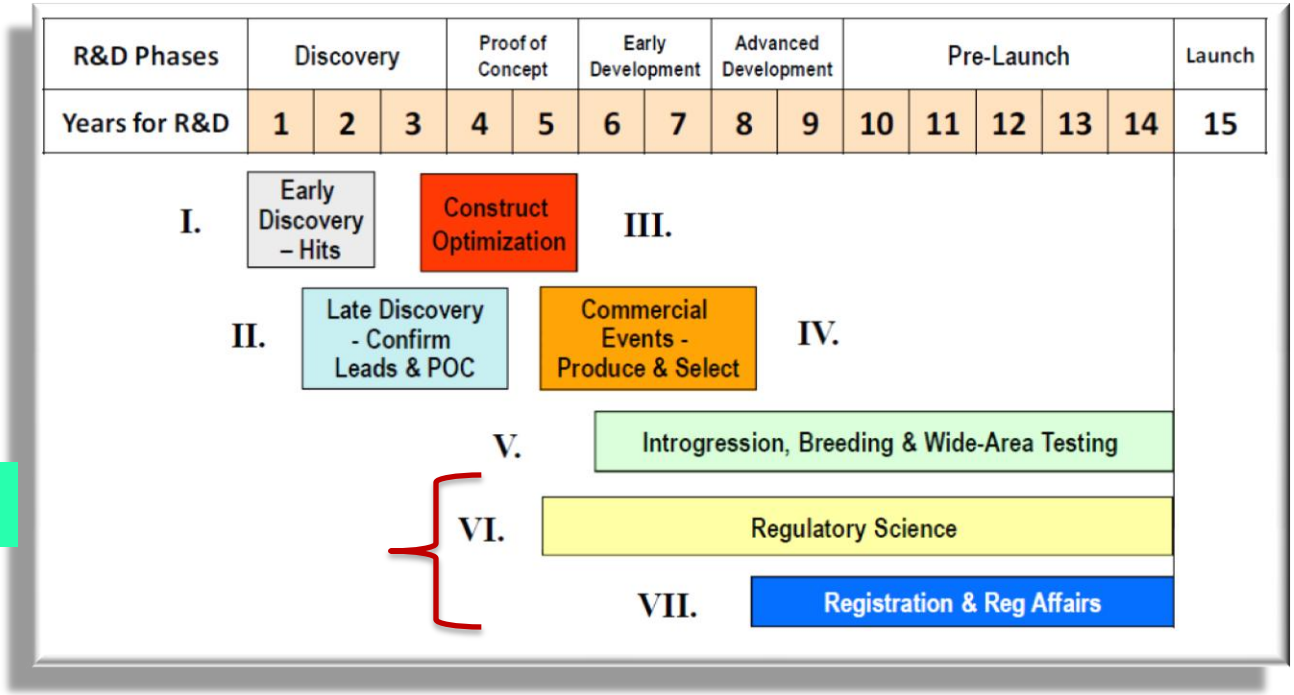
- New and higher quality products
- Faster product development
- Products with reduced environmental footprint
- Efficient farm management
- **Genome engineering technologies allow specific, targeted modifications in plant genomes that will facilitate and accelerate new trait discovery & development**

The Cost of Bringing A Biotech Crop to the Market

$136 M

13 YRS

Registration & Reg Affairs	$17.2 m
Reg Science	$17.9 m
Total	**$35.1 m**



Lack of regulatory clarity and / or overregulation of products:

Unjustifiably inhibits innovation

Delays and inhibits uptake of technology by developers

Stigmatizes new technologies

Phillips McDougall, *Crop Life International - The cost and time involved in the discovery, development and authorisation of a new plant biotechnology derived trait*, 2011.

A Global Research & Development Bases Agricultural Company

Sustainable crop protection and plant biotechnology solutions are helping to enhance yields and crop quality and address challenging issues in global agriculture.

We have leaders in:

- **Crop Protection;**
- **Seeds, Traits & Oils;**
- **Vegetation Management;**
- **Range & Pasture;**
- **Turf & Ornamentals; and**
- **Pest Management.**

All to meet the food, feed, fiber and fuel of the world.

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Impact of Technologies

Technology helps address sustainability, food security & public health:

- Increase food production / improved nutrition
- Crops tolerant to biotic and abiotic stresses
- Reduced labor and costs

Technology helps contribute to environmental protection:

- Targeted use of crop protection products
- Reduced environmental footprint (land, water, nutrients, waste, etc…)
- Preserves and protects biodiversity

Ability to quickly respond to tomorrow's global challenges:

- Reduced product development time
- More targeted and precise breeding process

Plant Breeding Technologies

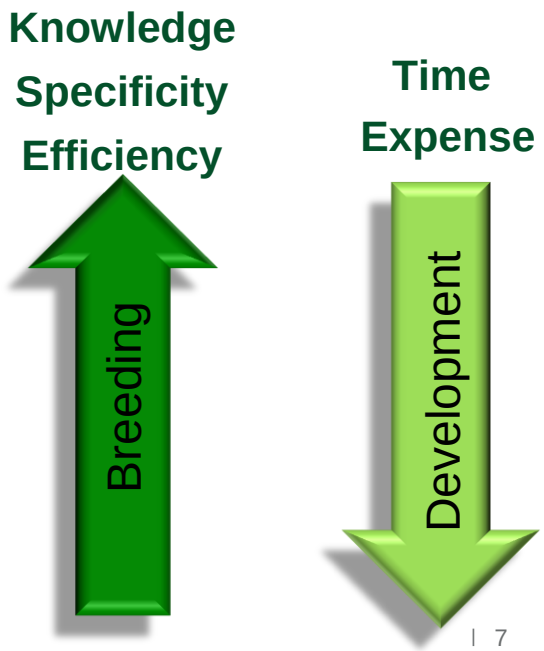
Over the past several decades multiple plant breeding techniques have been developed…

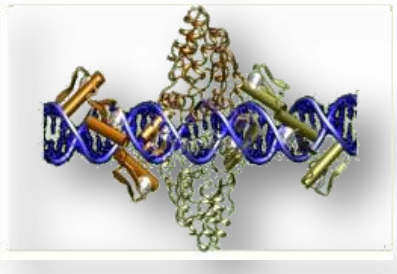
- innovative improvements and refinements of existing breeding methods
- Resulting products in many cases are indistinguishable / similar to existing products produced by traditional breeding
- Enhance the efficiency and specificity of breeding, with more knowledge and understanding of the final product than ever before
- Adaptable to a variety of crops, including trees and vegetables, by researchers from all sectors (public and private, large and small)

"NEW BREEDING TECHNIQUES"

1. **ODM**
2. **SDN (-1,-2, -3)**
3. **Cisgenesis/Transgenesis**
4. **Grafting (GMO root)**
5. **Agro-infiltration**
6. **RdDM (RNAi dpt. DNA Methylase)**
7. **Reverse Breeding**

foster further innovation in plant breeding…



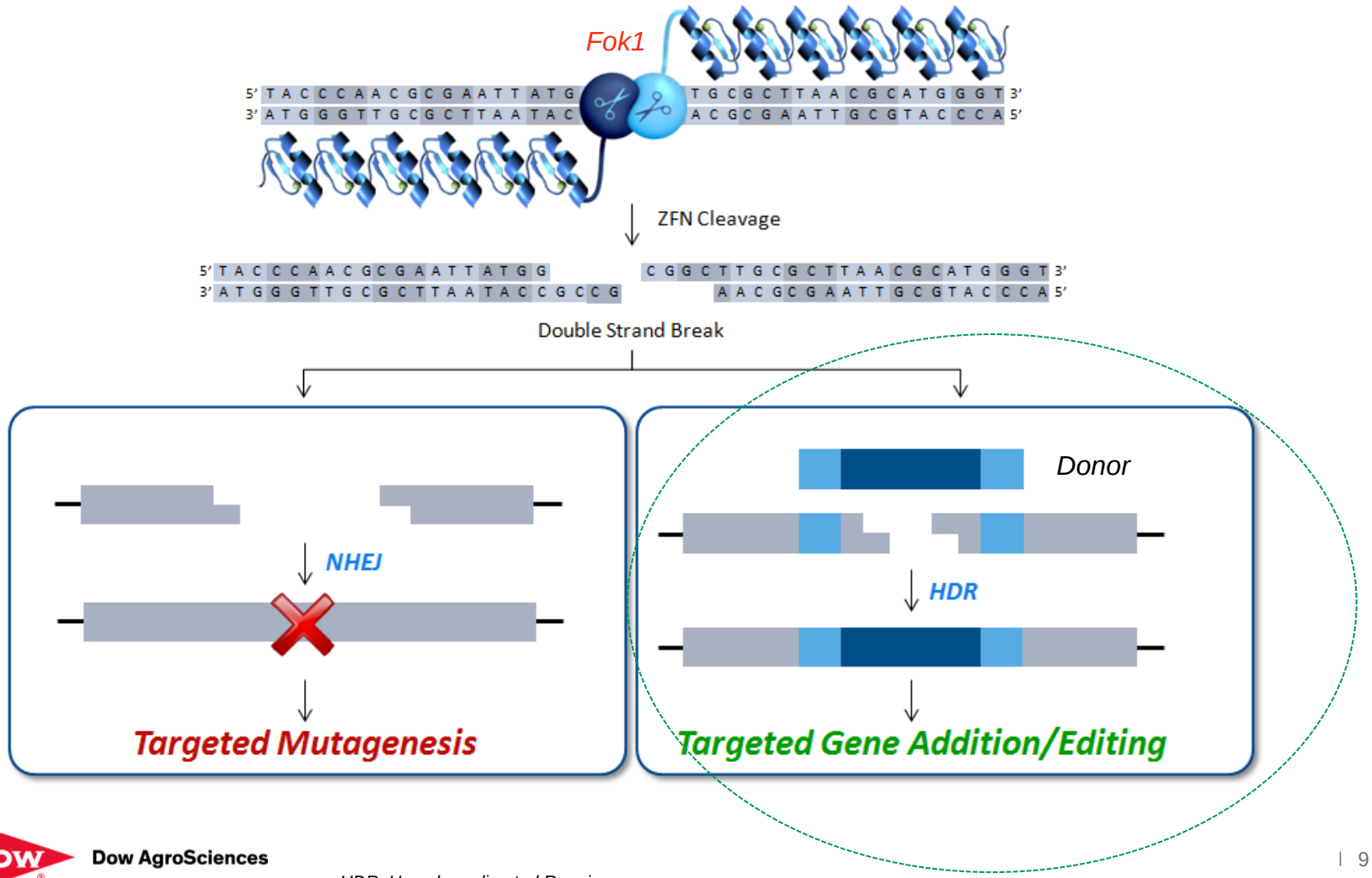


EXZACT™
Precision Technology

Engineering Plant Genomes with ZFNs for Trait Product Development

EXZACT™ Precision Technology

- ZFN based proprietary genome editing technology
- Validated in monocots & dicots, model and crop species



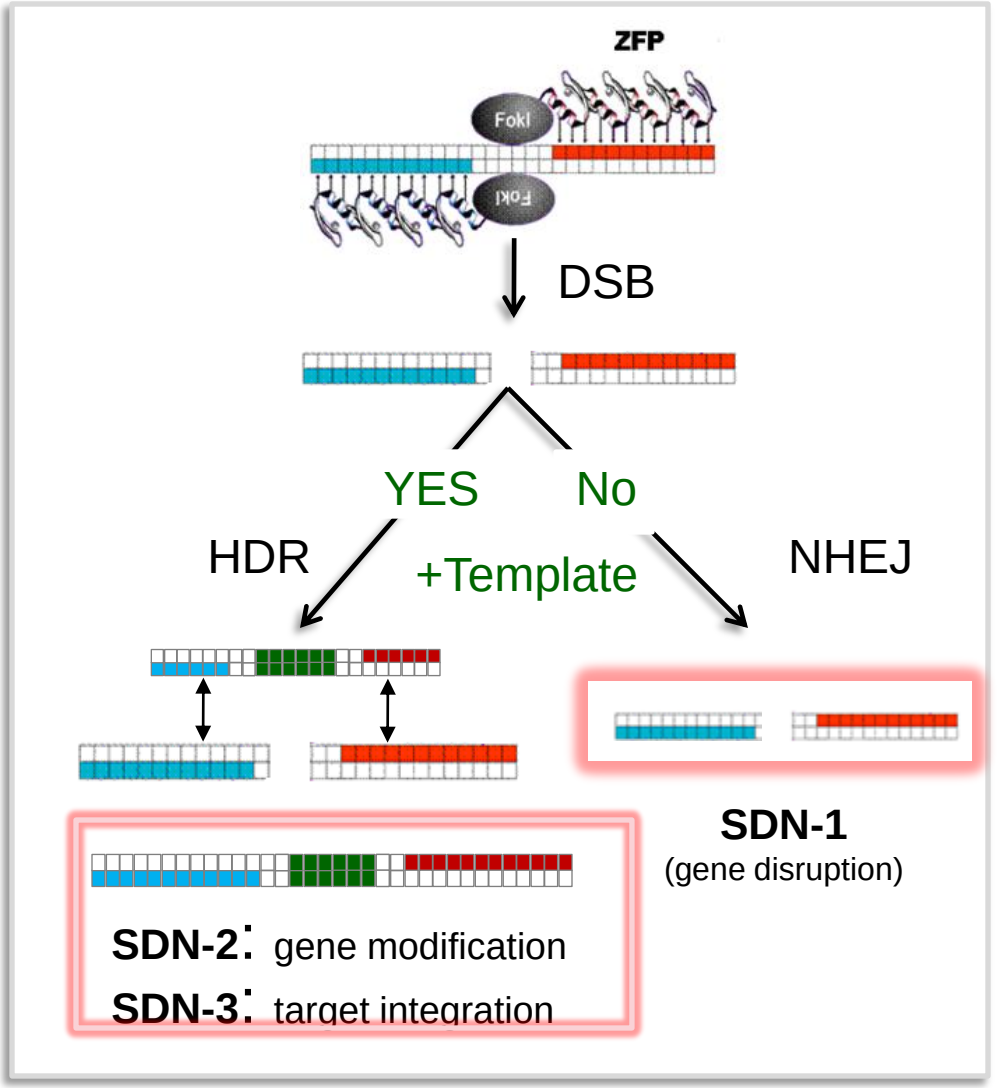
HDR: Homology directed Repair
NHEJ: Non Homologous End Joining

ZFN Targeting and Cleavage

Once a double stranded break is generated, the function of the ZFN is completed

Natural DNA Repair:

- After the double stranded break (DSB) is formed, the cell will naturally repair the DSB through 1 of 2 processes.
- Homology Directed Repair (HDR)
- Non-Homologous End Joining (NHEJ)



EXZACT ™ Zinc Finger Nuclease Applications

Technology Platform provides multiple methods of genome engineering using ZFNs

Mutational Applications

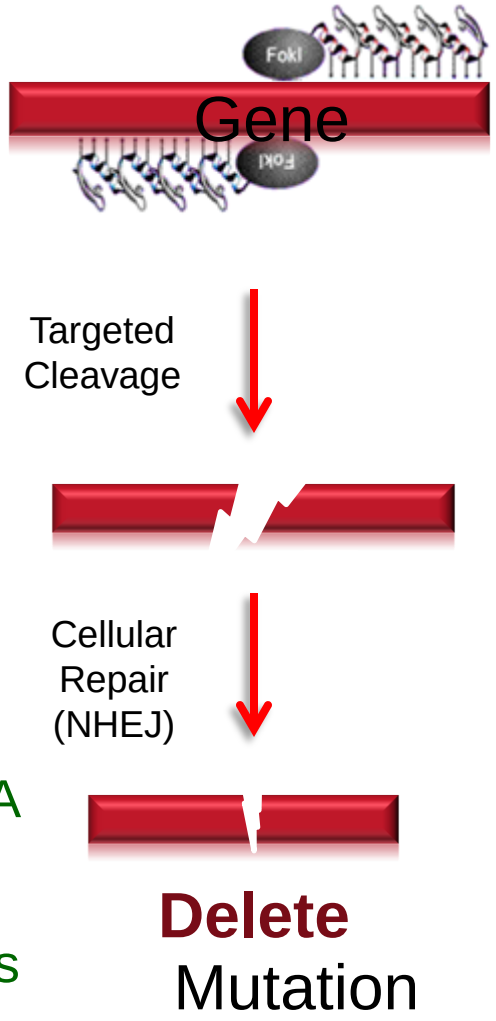
EXZACT™ Add	• Targeted Gene Addition
EXZACT™ Delete	• Targeted gene deletion / mutagenesis
EXZACT™ Edit	• Targeted Editing (rewriting/mutation) of Genomic Sequence

EXACT DELETE – Targeted Mutagenesis

EXZACT Delete is a targeted mutagenesis technique:

EXZACT Delete is simply a rapid, reliable and predictable process for generating targeted mutations in plants compared to traditional breeding and mutagenesis processes.

- No genetic material is introduced into the genome of the host via genetic recombination
- Mutations are known and pre-determined: Unlike random mutagenesis techniques, ZFN are designed to generate mutations only at the predetermined targeted DNA location
- ZFNs are absent in final product
- No foreign DNA in final product, only native plant DNA
- End product is the same as conventional mutagenesis



EXACT Edit

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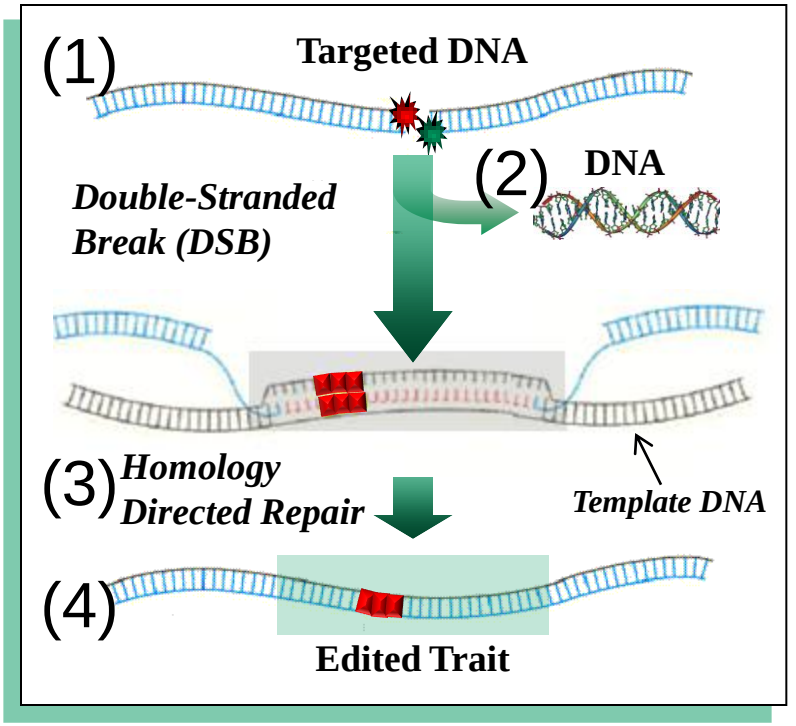
Derived from High K. *Nature,* 2005



Mechanism is based on homology-directed repair using an "edited" gene sequence donor

EXZACT™ Targeted Gene Addition: Precision Transformation

Benefits for trait product development

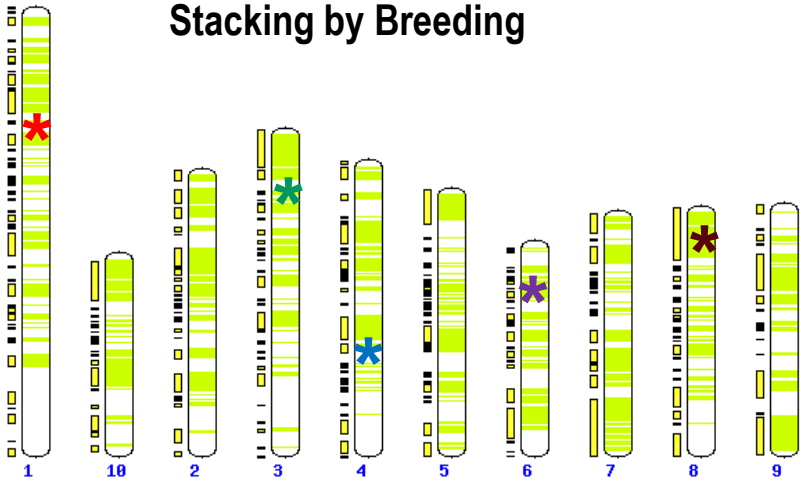
(compared to conventional methods)

- Gene addition to a specific genetic locus (safe harbor/high performance)
  - *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
  - *Accrued cost savings*
- New gene stacking options for multi-trait product development

Multi-Gene Stacking Technologies

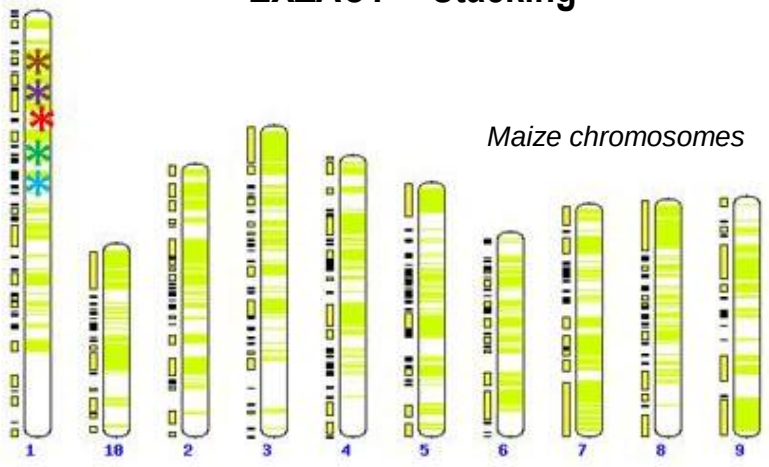
- **EXZACT™ Benefits**
  - Insertion of multiple transgenes at a single locus
  - Add genes to existing events (reuse of assets)

Stacking by Breeding



Multi-locus stacked product = Complex breeding

EXZACT™ Stacking



Single locus = Simpler breeding

EXZACT ™ Precision Technology Platform

operable platform

Precision Transformation (gene addition to a safe harbor locus)
Gene Stacking in Corn

EXZACT™ Precision Technology Platform

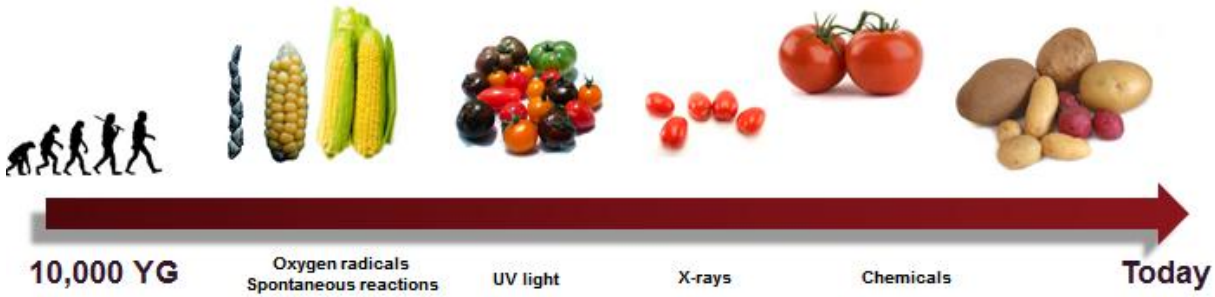
- Key External Collaborations
  - Sangamo BioSciences, USA
  - Agriculture Victoria Services (AVS, Australia)

Sangamo BioSciences

AGRICULTURE VICTORIA SERVICES

Example: Mutations (SDN-1, SDN-2)

MUTAGENESIS IS FUNDAMENTAL TO EVOLUTION & CROP BREEDING-SELECTION

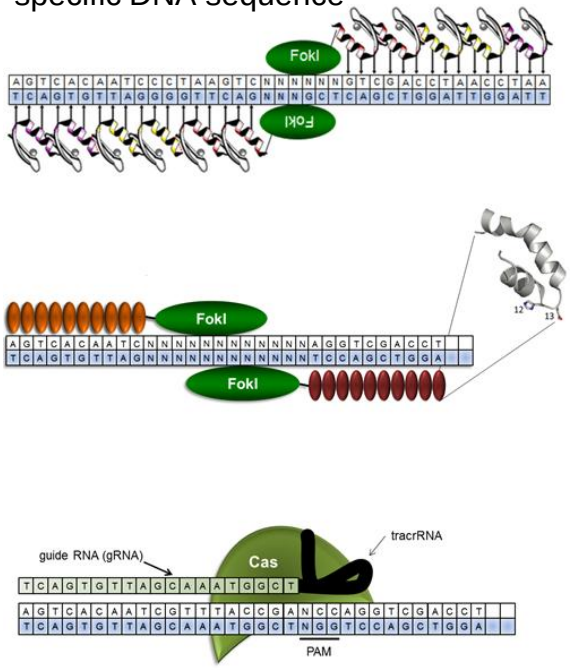


- Mutational products have a long history of safe use. Over 3,200 cultivars have been used commercially and are globally adopted
- SDNs continue the history of improving crop development through modern targeted mutational applications
- SDN-1/-2 allow, for the first time, mutations to be targeted to a specific, desired location in the plant genome

SITE DIRECTED NUCLEASES

1. **Zinc Finger Nucleases**
2. **Meganucleases**
3. **TALENs**
4. **CRISPRs**
5. ***ETC…***

DNA binding and restriction proteins which can be designed to recognize a specific DNA sequence



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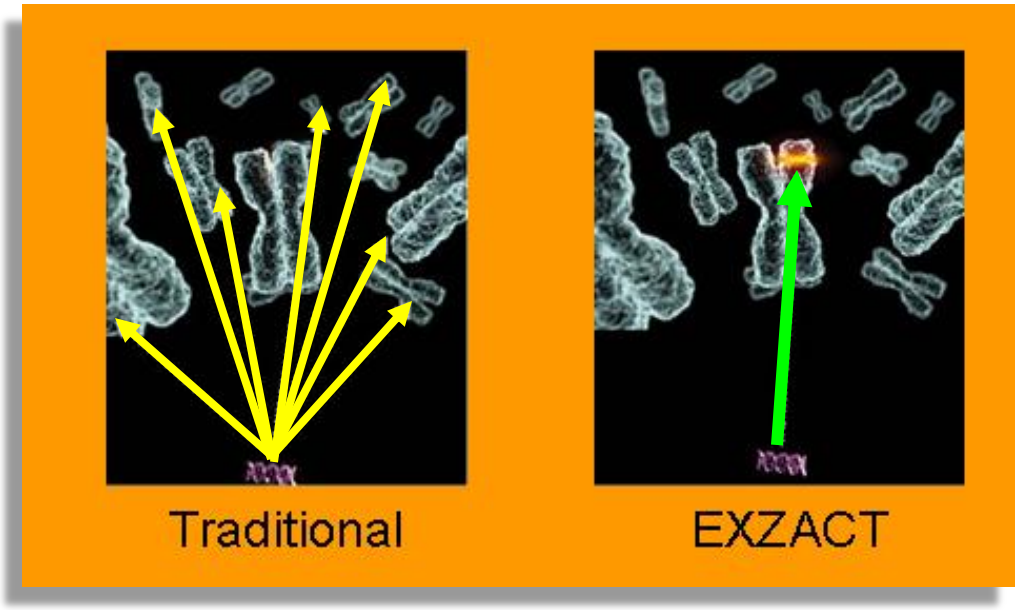
Genome Editing Techniques



Traditional: DNA inserts / mutations introduced **randomly** in genome
– screening for desirable product is expensive and time-consuming

EXZACT: DNA changes are at a pre-determined, **targeted** location

- Higher quality events with minimal unintended side effects and higher probability of success
- Reduced time and cost for trait development

Benefits

IN CONTRAST TO CONVENTIONAL MUTAGENESIS …

SDN-1/-2 - Targeted Mutagenesis

- Targeted mutations at specific sequences only
- More efficient mutagenesis / selection
- No foreign DNA in the genome, akin to a *conventional variety*
- End-product is indistinguishable from traditional breeding lines

- Reduced probability of unintended effects on the genome -> reduced variability
- Time/cost savings during research and trait product development

Plant Breeding Technologies

EMBO reports

science & society

Transgenic or not? No simple answer!

New biotechnology-based plant breeding techniques and the regulatory landscape

Nancy Podevin, Yann Devos, Howard Vivian Davies & Kaare Magne Nielsen

>GMO

PROCESS ? **PRODUCT**

Base regulations on HOW the product was developed

Base regulations on the nature of the final product

Global harmonized approach

Timely guidance

Fosters innovation

Science Based

What is the time and cost involved in achieving regulatory approval for a biotech product that is not transgenic / GMO ?

Genome Editing technologies are developed using biotechnology, but the resulting traits may be identical to non-biotech products!

Phillips McDougall, *Crop Life International - The cost and time involved in the discovery, development and authorization of a new plant biotechnology derived trait*, 2011.

Global Regulatory Landscape

Country Regulatory Discussions

- Country specific workshops and discussions have / are occurring in Argentina, Australia, Brazil, Japan & US…

- February 2014 – OECD (Organisation for Economic Co-operation and Development) meeting:
  - ❑ General agreement that some products (i.e. mutations) developed through NPBTs should fall outside the scope of current regulations
  - ❑ Current regulations are outdated and NPBTs cannot afford the regulatory burdens seen for transgenic crops
  - ❑ Need for harmonious decision making

- Working groups in EU and Australia have booth determined that mutational products developed through NPBTs should be excluded from regulations and pose no additional risk compared to traditional mutational products

Conclusions

- NPBTs are innovative improvements and refinements of existing breeding methods. It is the characteristics of the plant (product) that determines its safety
- The public, private and scientists alike have significant opportunities to employ NPBTs in their breeding programs
- The adoption of these technologies will be highly dependent on the regulatory requirements imposed on the products produced through NPBTs.

  Non-scientific, unnecessary and non-harmonious oversight / requirements will result in...

  - *undue, costly burdens*
  - *stifle innovation*
  - *prevent the uptake / limit use of NPBTs*
  - *Disrupt trade*
  - *Loss of public confidence*

Conclusions

- All governments are encouraged to adopt a globally harmonized approach towards NPBTs, and avoid unnecessary oversight of products developed through NPBTs

- Governments are encouraged to provide predictable, timely guidance regarding the oversight of any NPBT products for developers to foresee appropriate investment and commercialization.

- Agencies and policy advisors are encouraged to carefully consider legislations and regulations to ensure clarity of definitions. When drafting or reviewing legislation be sure that the language is clear and unambiguous. Think ahead to how that legislation may be interpreted in a decade….if you can