Dow AgroSciences

Use of Zinc Finger Nucleases for plant breeding purposes

Meeting New Breeding Techniques Platform
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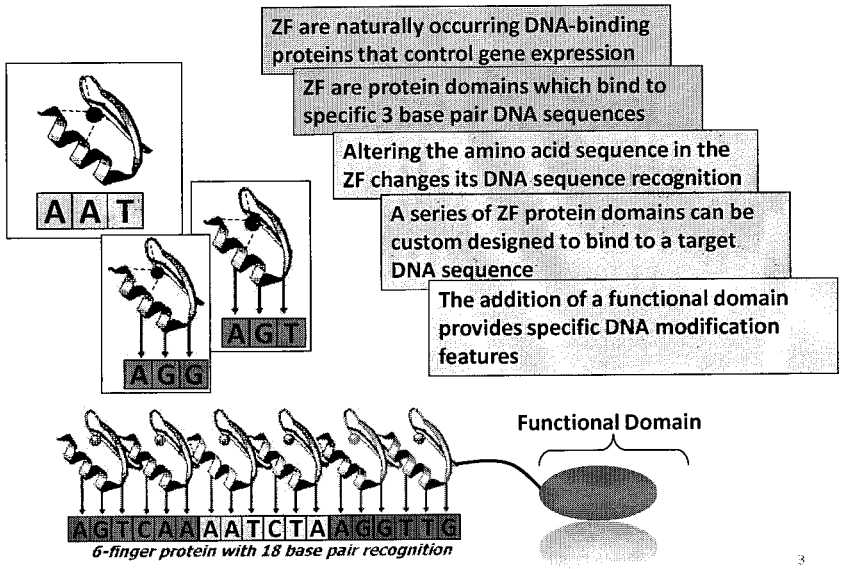
ZFN – Molecular tools with different types of applications

Zinc Finger Nucleases provide multiple methods to improve crops through precise genome modification:

ZFN-1 **Delete**	Targeted Mutagenesis or DNA Excision: Gene/sequence removal or functional knockout
ZFN-2 **Edit**	Targeted Editing (rewriting) of Genomic Sequence: Small deletions / additions / substitutions
ZFN-3 **Add**	Targeted Gene Addition: Single-gene traits, stacking, pathway engineering

Overview – How do ZFN work?

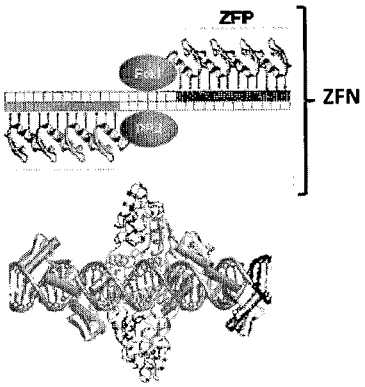


Overview – How do ZFN work?

Two independent, unique ZFPs bind the target DNA sequence to form a Zinc Finger Nuclease.

For DNA Cleavage to Occur:

- Two ZFPs must bind and be present on the DNA strand at the same time. A single ZFP has no cleavage activity.
- Once ZFPs bind the DNA, the two *FokI* domains dimerize to generate a functional *FokI* nuclease.

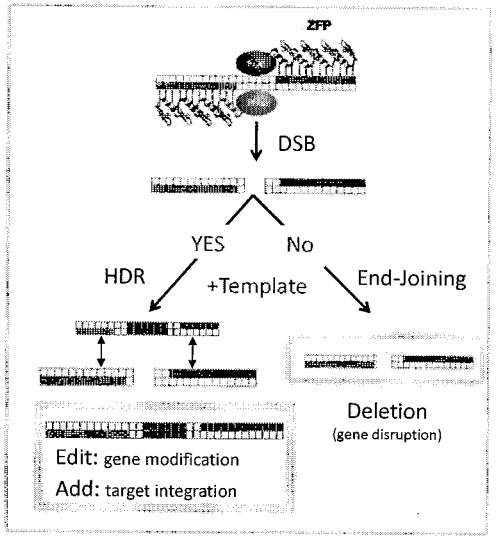


ZFN Targeting and Cleavage

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

Natural DNA Repair:

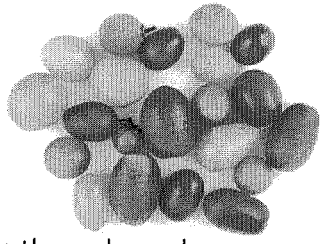
- After the double stranded break (DSB) is formed, the cell will naturally repair the DSB through 1 of 2 processes.
- Homology Directed Repair (HDR)
- Non-Homologous End Joining (HHEJ)



ZFN-1 DELETE – Targeted Mutagenesis

ZFN-1 offers a new tool to conduct targeted mutagenesis, driving novel product development

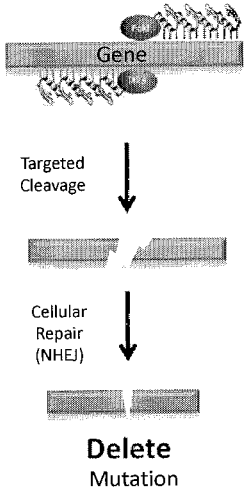
- Mutations have been driving advances in crop development for thousands of years.
- Classical mutational methods (radiation/chemical) have been safely used for close to a century, but introduce multiple unknown mutations throughout the plant.



- ZFN-1 offers a higher level of precision through modern targeted mutational applications

ZFN-1 DELETE – Targeted Mutagenesis

ZFN-1 Delete is a targeted mutagenesis technique:

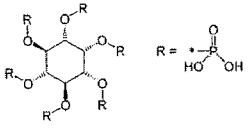


ZFN-1 Delete is simply a rapid, reliable and predictable process for generating targeted mutations in plants compared to traditional breeding and mutagenesis processes.

More efficient mutagenesis/selection

EXAMPLE of ZFN-1 DELETE

ZMIPK-1* : inositol-1,3,4,5,6-pentakisphosphate 2-kinase



Enzymatic product : phytic acid

- 75% of seed phosphorus – storage form for P
- Non-digestible by monogastric organisms
- Antinutrient: Chelates K, Mg, Ca
- Animals excrete into environment, pollutant

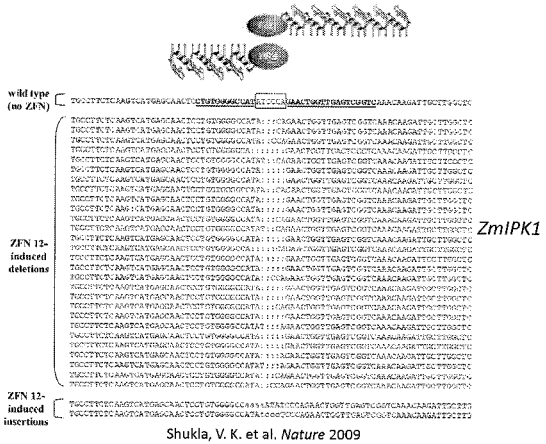
Deleting IPK-1 may reduce phytate production & increase the amount of available phosphate

*Sun et al. Plant Phys. 2007 paper

ZFN-1 DELETE: Maize IPK-1 Deletion

- **Targeted:** IPK-1 deletions / insertions only at the targeted DNA sequence

- **Highly Specific**
  - No InDels at IPK-2 (98% identical to IPK-1)

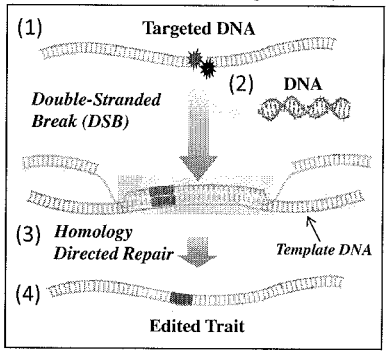


Shukla, V. K. et al. *Nature* 2009

Targeted mutagenesis of an endogenous gene with no "foreign" DNA present in the genome

ZFN-2 Edit

Derived from High K. *Nature*, 2005



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

Characteristics of ZFN-1/ZFN-2 – Targeted Mutagenesis

- 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 integrated in final product, only native plant DNA
- End product is the same as conventional mutagenesis

Benefits:

• Reduced probability of unintended effects on the genome -> higher level of precision and predictability.
•Time/cost savings during research and trait product development.

Benefits

Removal of Antinutrients /Allergens:

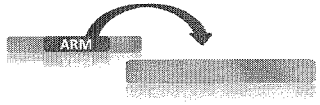
Remove undesirable plant traits for reduced environmental pollution and enhanced nutritional value: IPK in maize

Improved Nutritional Value

Edit native plant genes for enhanced nutritional value

Removal of Antibiotic Resistance Markers

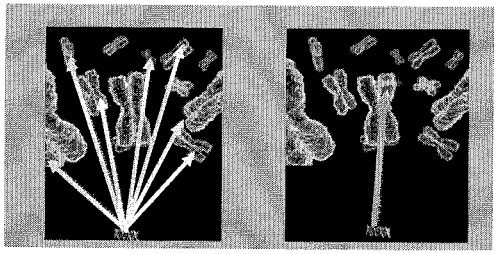
Removal of ARMs from existing products



New generation of traits

Delete / Edit native plant genes for enhanced nutritional value, conservation of environment and consumer needs without the introduction of cis / transgenes

ZFN-3 ADD: Targeted Gene Addition

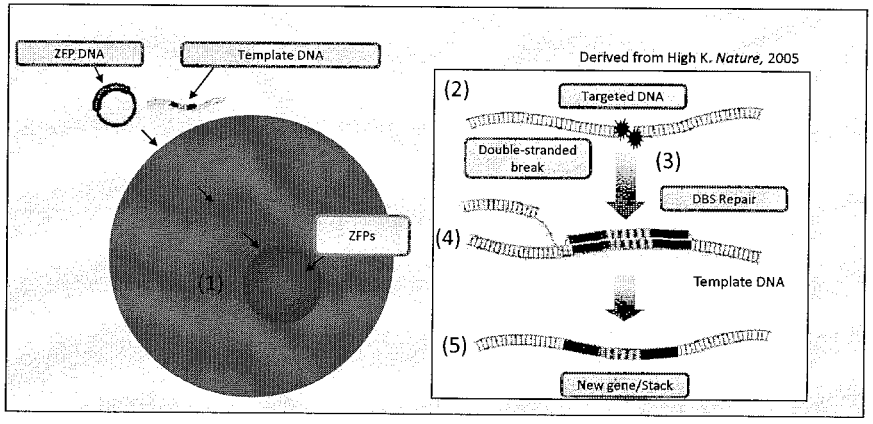


Traditional ZFN-3

- **Traditional:** DNA inserts randomly in genome – screening for desirable events is expensive and time-consuming.
- **ZFN-3:** DNA inserts at a pre-determined, specific site — lowering the potential for unintended effects and reducing the time and cost of screening.

ZFN-3 ADD: Precise Gene Insertion

Template and ZF DNA introduced to cell; gene added is stable and heritable.



ZFN-3: Benefits - Precise Gene Insertion

Precise Gene Insertion Results In:

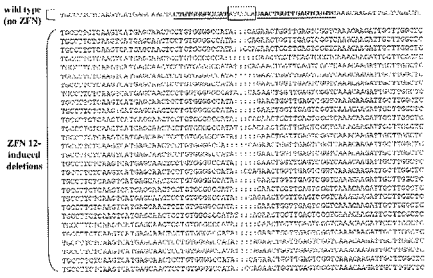
- Reduced numbers of events to assess
- Mitigation of unintended consequences associated with random integration
- High quality data on events generated early in development phase.
- More efficient trait stacking

We can produce high quality products with high quality data in less time and with less costs

Regulatory status

Based on the USDA regulatory guidelines, the USDA has determined that "GE plants containing targeted deletions, caused by naturally-occurring DNA repair after the targeted break is made by zinc-finger nucleases, and in which no genetic material is inserted into the plant genome, are not regulated articles".

- The location of mutation is known and pre-determined
- No genetic material is introduced into the genome
- Outcome is identical to existing products exempt from regulation



ZmIPK1

Regulatory status

OGTR (Australia) has stated that plants bred using ZFN-1 Delete are not considered GMOs...Therefore those plants would not be subject to regulation under the GT Act.

- ZFN applications 2 and 3 were not yet part of the reviewing process by regulatory authorities.

ZFN Conclusions

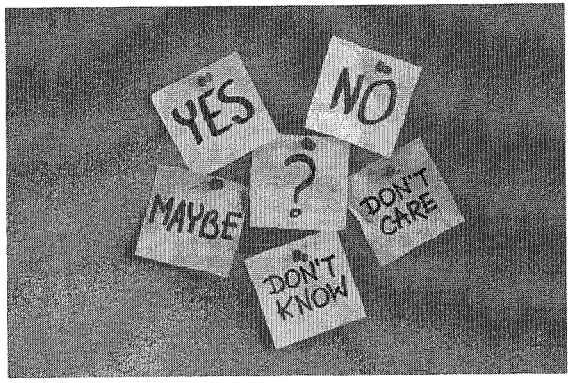
Next steps for Zinc Finger Nucleases

- Dow AgroSciences is collaborating with academics, small companies, & government research institutes to make ZFN technology widely available
- Develop further examples for testing with regulatory authorities worldwide
- Integrate use of ZFN in R&D process

More info:

See Nature Paper for targeted insertion of PAT into maize (Shukla et. al., *Nature* 459, 337-341 (2009)

Uptake will depend on the regulatory status



Thank you for your attention