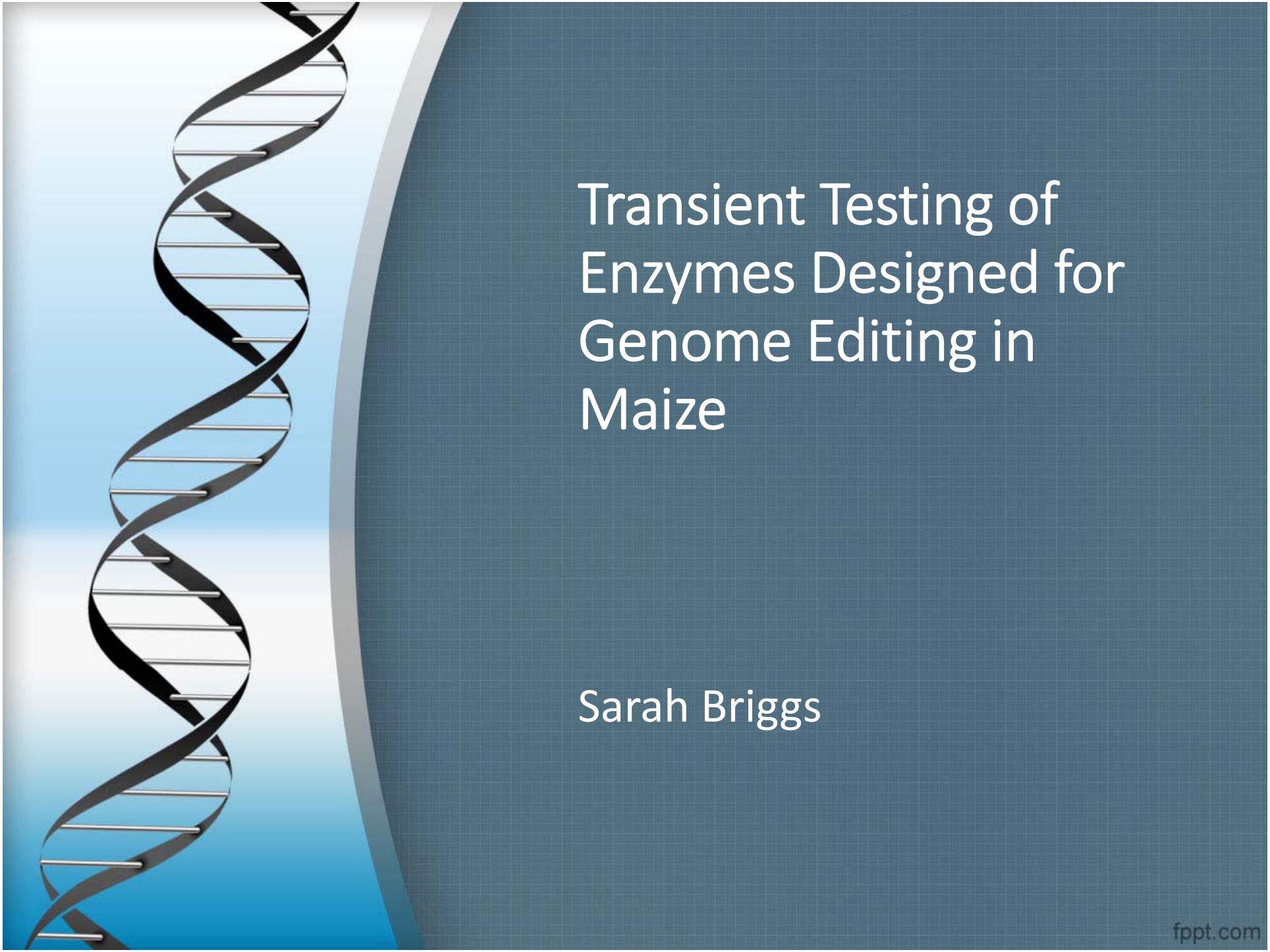


Transient Testing of Enzymes Designed for Genome Editing in Maize

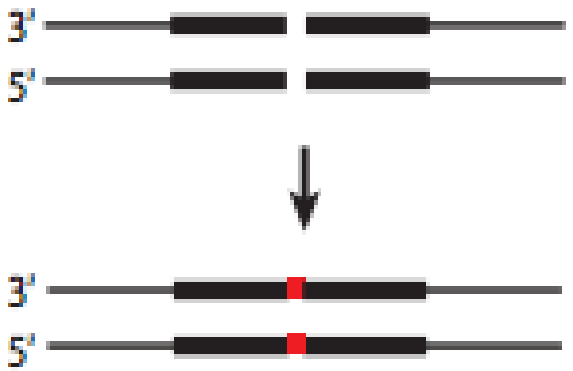
Sarah Briggs

Outline

- What is Genome Editing and why is it important
- Editing enzymes
- Challenges to Testing in Plants
- The Transient Testing System
- Experimental Procedure
- Our Findings

Genome Editing

- DNA is broken through a targeted double stranded break (DSB)
- Cell repair mechanisms (most common is NHEJ) repair DSB
- During repair, small changes in the DNA sequence are generated resulting in an edited genome.



Recent Applications

- Genome editing has important applications
  - -Agriculture
    - Bacterial resistance in rice
  - -Medicine
    - Cured liver disorder in mice

http://fin6.com/2013/12/mouse/

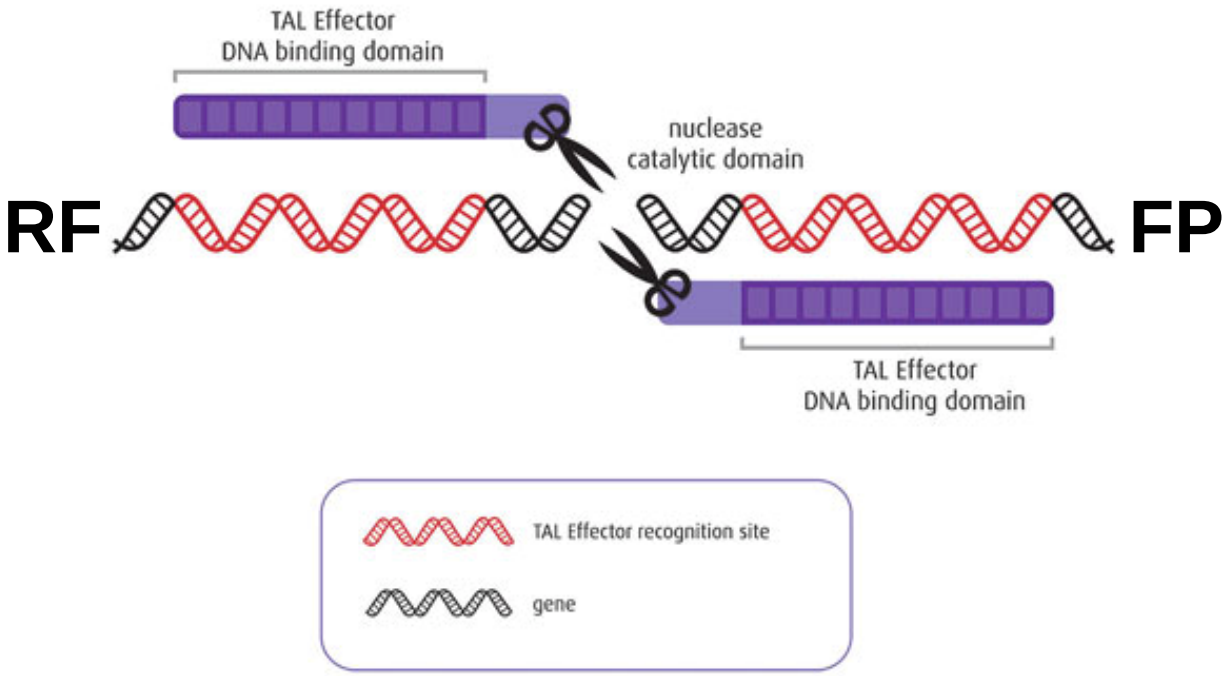
http://www.postgrad.com/files/editorial/subjects_-_agrculture1.jpg

Enzymes involved in genome editing

TALENs: Transcription activator-like effector nucleases- contains two domains

1. TAL DNA binding domain from bacteria. It was determined that specific residues of the protein correlate with the recognition of specific nucleotides in the DNA. This allows you to build a protein to bind to a specific DNA sequence you wish to target.
2. Nuclease domain to cut the DNA. This is derived from the cleavage domain of the bacterial restriction enzyme fokI).
3. The nuclease functions as a dimer so it takes a pair of TALENs to cut any specific target site in the DNA.

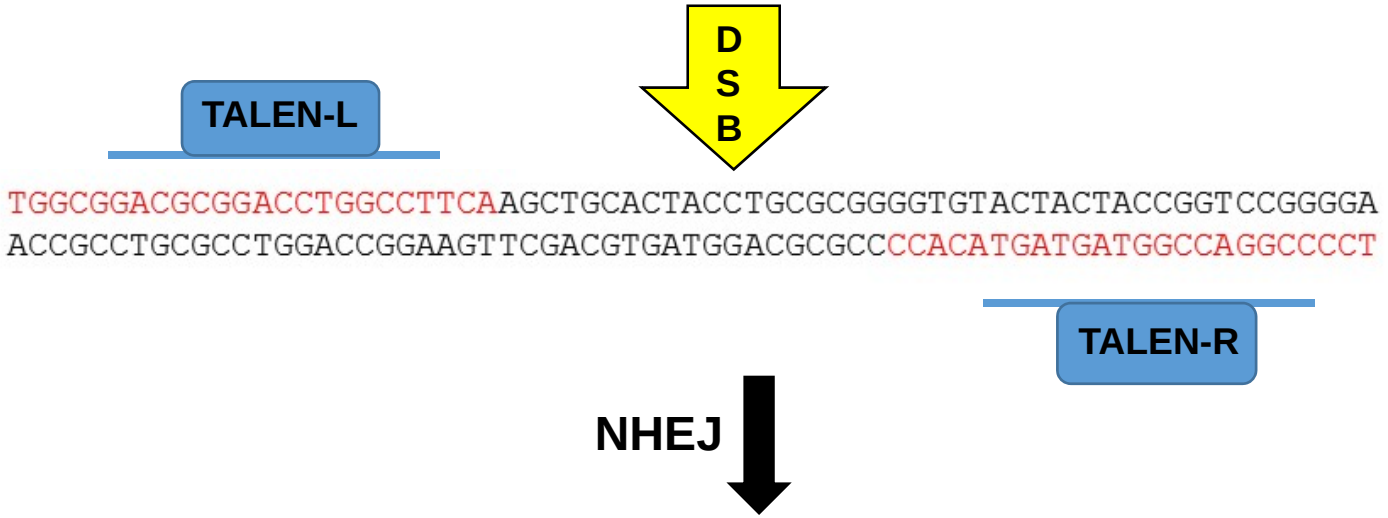
TALENs



http://www.genengnews.com/Media/images/Article Cellectis_TALEN_structure1815499084.jpg

TALEN-induced Mutagenesis

- TALENs are enzymes designed to cause DSB at the specific target site. NHEJ repairs the DNA but can result in changes in the DNA sequences.



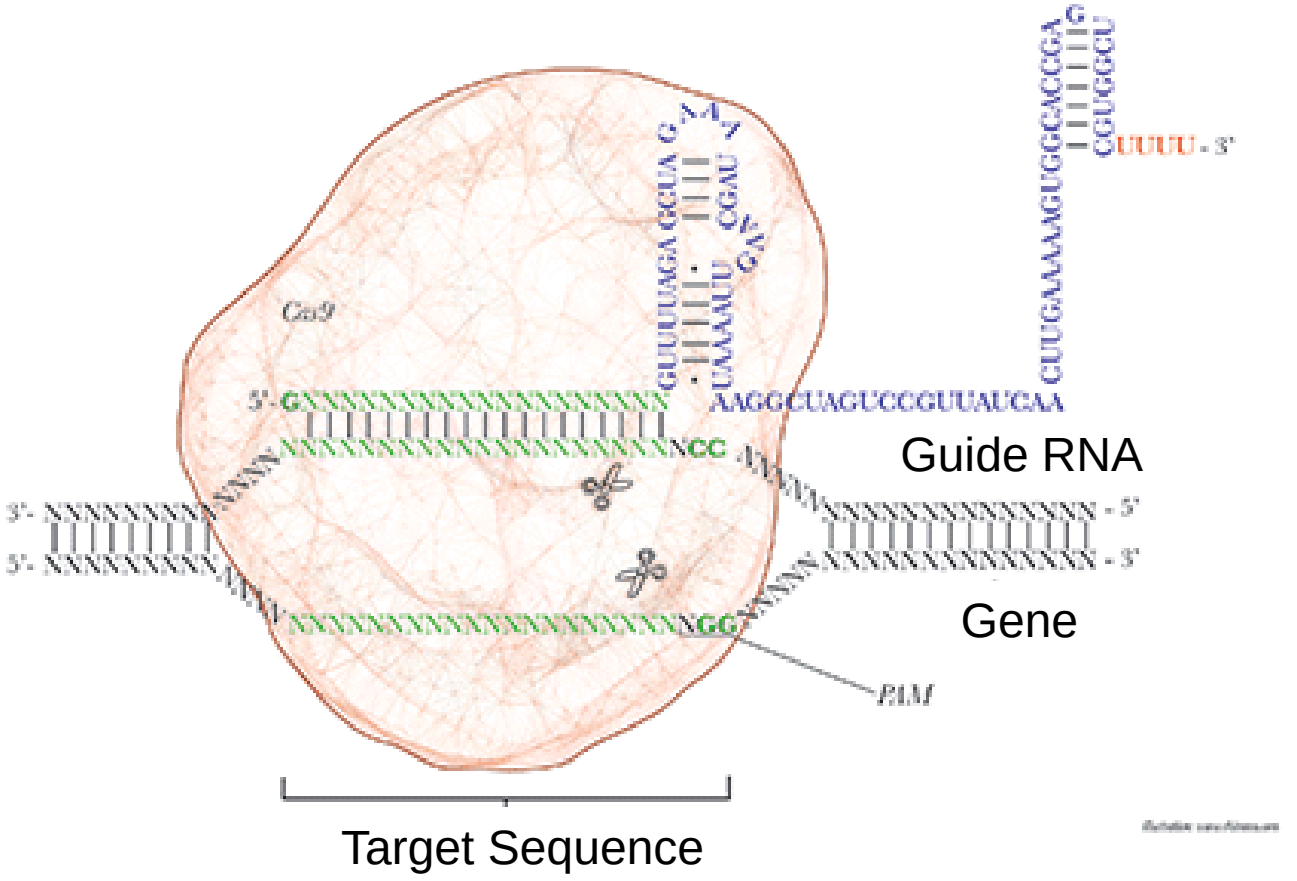

```
TGGCGGACGCGGACCTGGCCTTCAAGCTGCACTACCTGCGCGGGGTGTACTACTACCGGTCCGGGGA   WT
TGGCGGACGCGGACCTGGCCTTCAAGCTGCACTAC---CGCGGGGTGTACTACTACCGGTCCGGGGA   -3
TGGCGGACGCGGACCTGGCCTTCAAGCTGCACTAC----GCGGGGTGTACTACTACCGGTCCGGGGA   -4
TGGCGGACGCGGACCTGGCCTTCAAGCTGCACT-----CGCGGGGTGTACTACTACCGGTCCGGGGA   -5
TGGCGGACGCGGACCTGGCCTTCAAGCTGCA------GCGCGGGGTGTACTACTACCGGTCCGGGGA   -6
TGGCGGACGCGGACCTGGCCTTCAAGCTGCACT-------CGGGGTGTACTACTACCGGTCCGGGGA   -7
TGGCGGACGCGGACCTGGCCTTCAAGCTGC---------GCGGGGTGTACTACTACCGGTCCGGGGA   -9
```

Enzymes involved in genome editing

CRISPR/cas9: (**clustered regularly interspaced short palindromic repeats**) are DNA loci associated with a cas9 gene. They are present in bacteria and provide a mechanism for the bacterial cell to recognize and mutate foreign (viral) DNA in its genome. For genome editing in eukaryotic cells, you need to have 2 components:

1. Cas9 gene. This a nuclease that derives its specificity from a RNA guide molecule.
2. sgRNA (single molecule guide RNA) This is a gene for a synthetic non-coding RNA. This makes an RNA molecule with a specific secondary structure to direct the cas9 nuclease to the specific target site in the genome.

CRISPRs (Cas9+sgRNA)



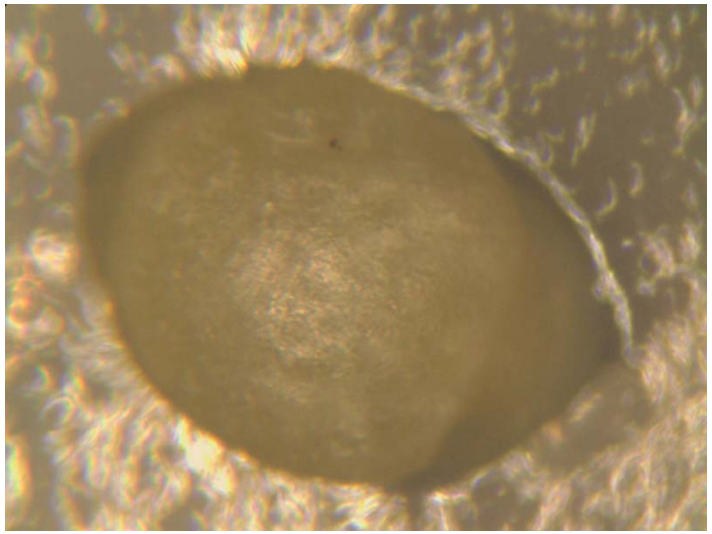
Modified from
http://www.addgene.org/static/cms/images/CRISPR_Church.png

Challenges to Testing/Using gene editing tools in corn

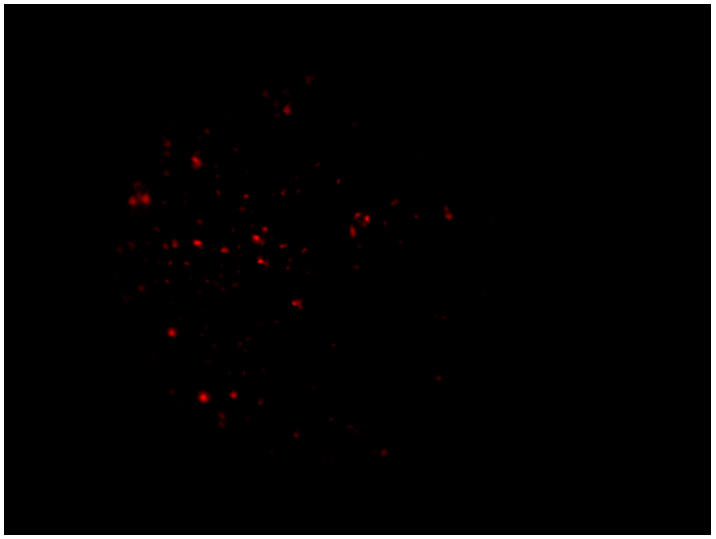
- Low transformation efficiency
- Long generation time for transgenic plants
- Editing enzymes may be toxic to cells continually expressing them
- Optimal gene expression conditions for editing enzymes not well understood

Project goal: Develop a system to rapidly test genome editing enzymes in corn cells

Microprojectile bombardment delivers plasmid DNA into Maize Cells



Bright field

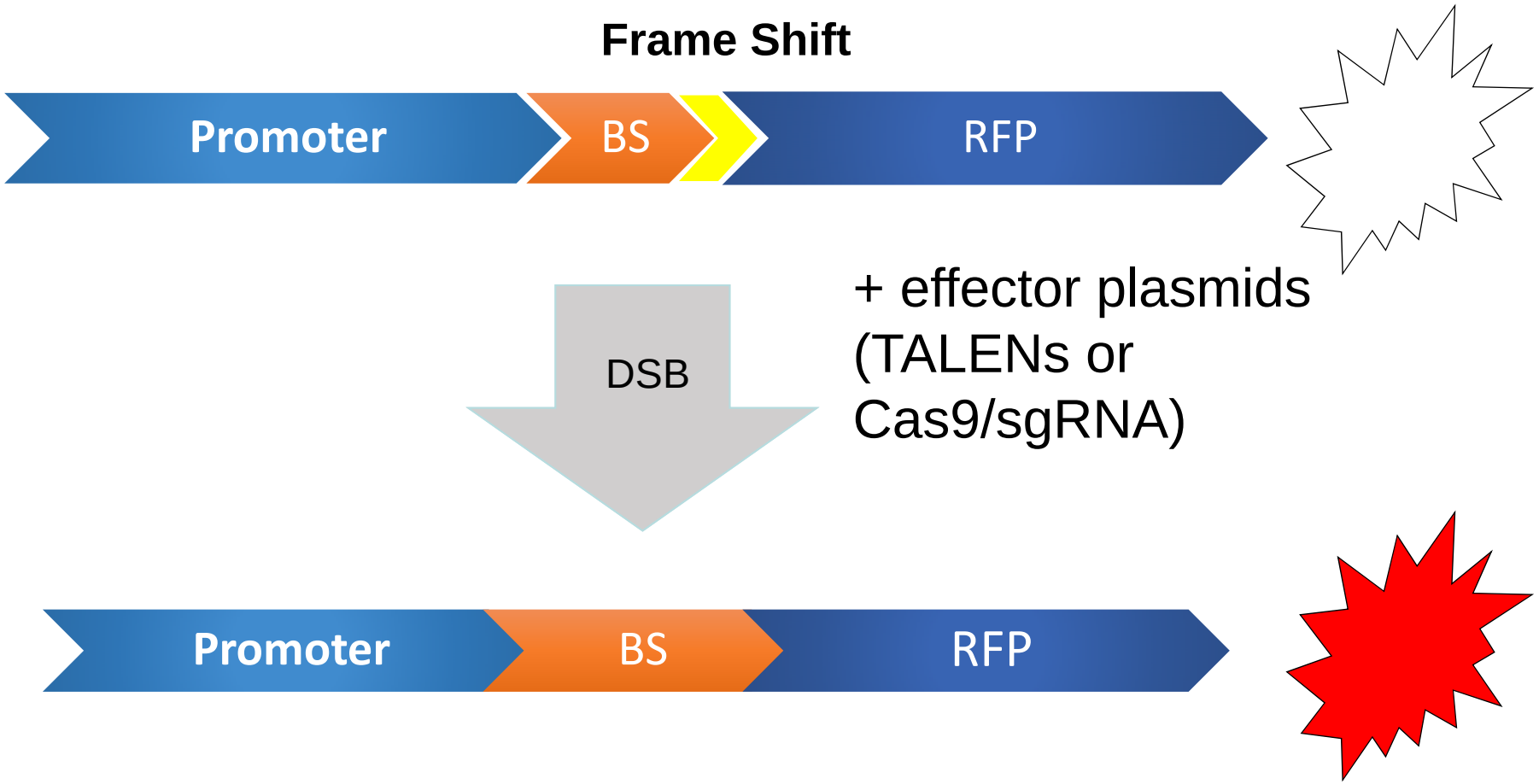


RFP

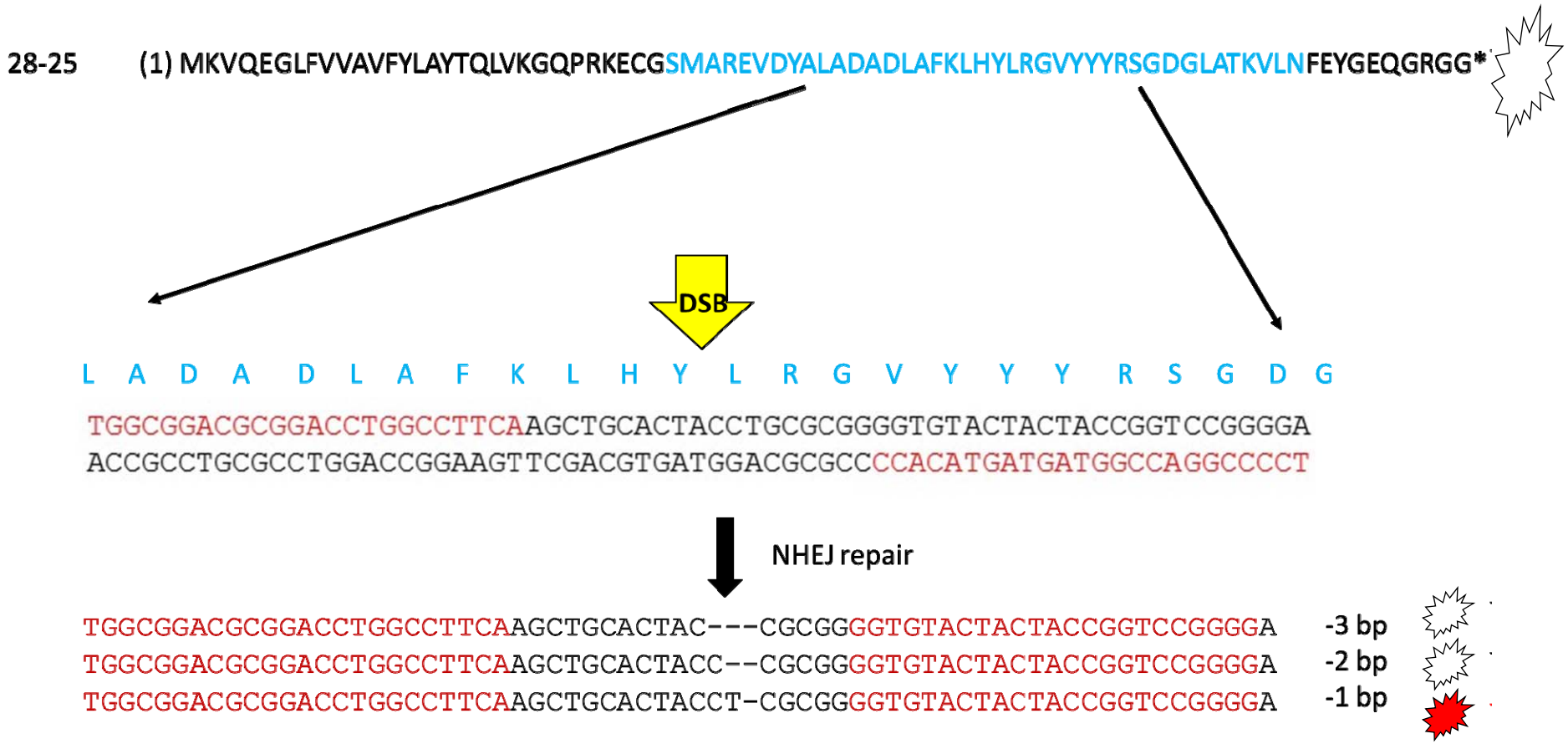
Transient Testing System

- Introduce plasmid DNAs encoding the editing enzymes (Effectors) and a gene whose expression is altered after editing (Reporter) into plant cells
- Assay the effects within a few days or a week
- Enables quick assessment of efficacy of editing enzymes in plants

The Reporter



Gene editing can change a non-functional RFP to a functional fluorescent reporter

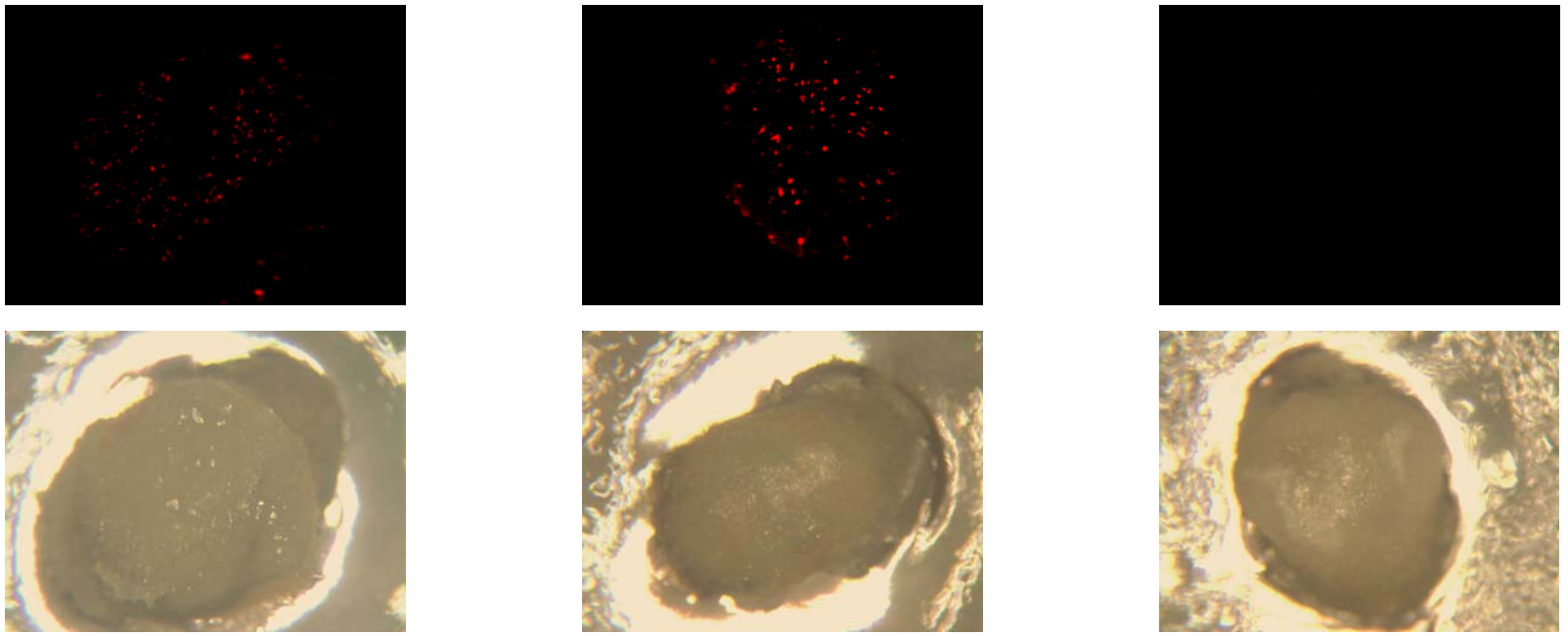


Testing reporter gene expression

ERmcherry (1) MKVQEGLFVVAVFYLAYTQLVKGQPRKECG----------SMVSKGEEDNMAIIKEF

28-13 (1) MKVQEGLFVVAVFYLAYTQLVKGQPRKECGSMAREVDYALADADLAFKLHYLRGVYYYRSGDGLATKVLNSSMVSKGEEDNMAIIKEF

28-25 (1) MKVQEGLFVVAVFYLAYTQLVKGQPRKECGSMAREVDYALADADLAFKLHYLRGVYYYRSGDGLATKVLNFEYGEQGRGG*



ERmcherry 28-13 28-25

Experimental Procedure

Day1: dissect immature embryos. Rest them on culture media (callus maintenance) for 1-2 days
Day3: Transfer embryos to a high osmotic medium (4 hr before bombardment)
Microprojectile bombardment of plasmids DNAs
Day4: Move embryos off osmotic medium to callus maintenance medium
Day5: Visualize/image RFP expression
Day7: Visualize/image RFP expression and stain samples for GUS expression
Day8: image GUS expression

http://plant-tc.cfans.umn.edu/maize-tc/shotemb.jpg

http://upload.wikimedia.org/wikipedia/commons/d/d3/Genegun.jpg

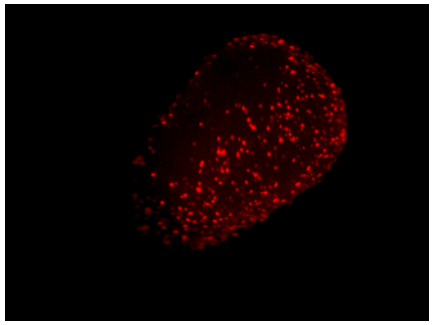
Testing DSB Enzymes in Maize Cells

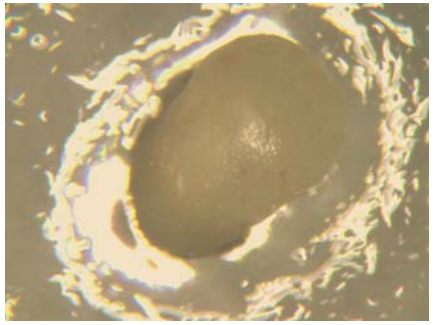
order for bomb.	reporter	effector	GUS	Average number of spots (2 days post BB) ±SEM	Average number of spots (5 days post BB)±SEM	Average number of spots (7 days post BB)±SEM	Number of embryos bombarded
1	28-25	NONE	960	0	0	0	17
2	28-25	TALEN1	960	8.7±1.27	11.8±2.5	16.3±4.1	21
3	28-25	TALEN2	960	0	7.4±1.9	4.9±1.4	17
4	28-25	cas9+sgRNA	960	0	0	0	15
5	28-13	none	960	>125	>125	>125	19

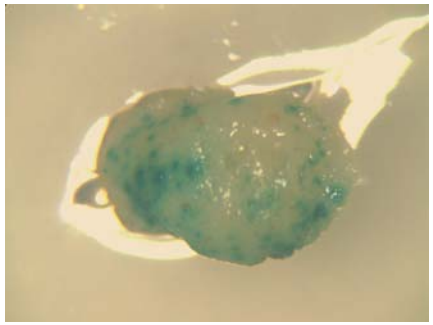
These results suggest TALEN1 may be more efficient than TALEN2, but we don't yet have enough replicates to conclude definitive differences between TALEN1 and TALEN2.

¶Two Days After Bombardment

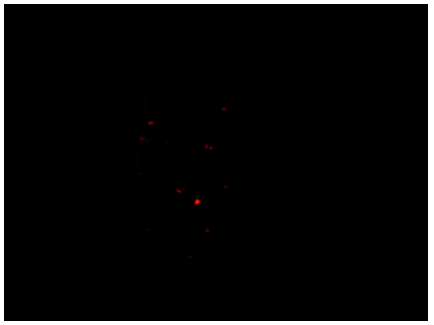
28-13+GUS

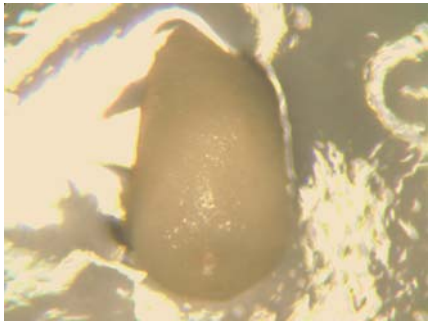


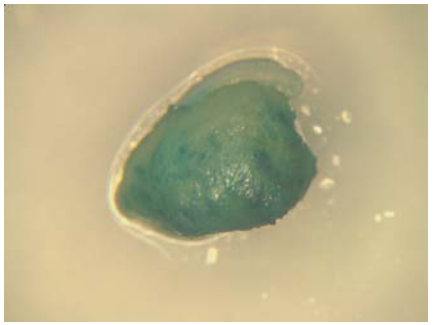




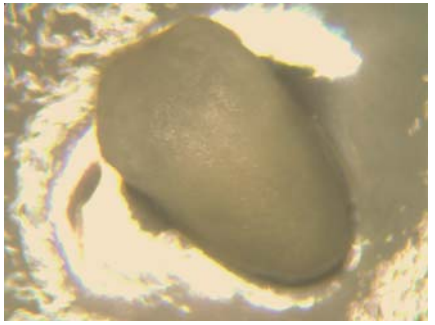
28-25+T1+GUS

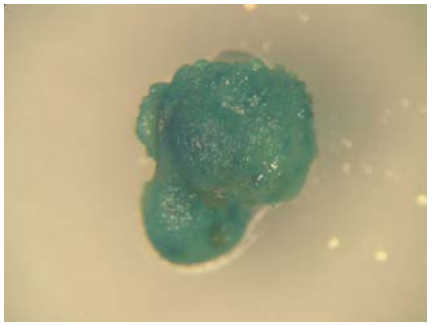






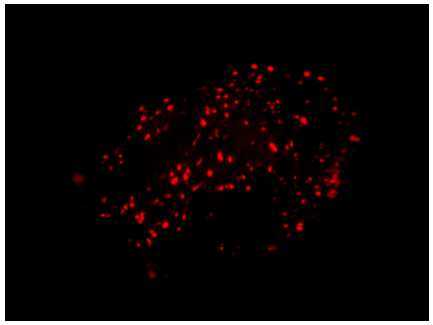
28-25+T2+GUS

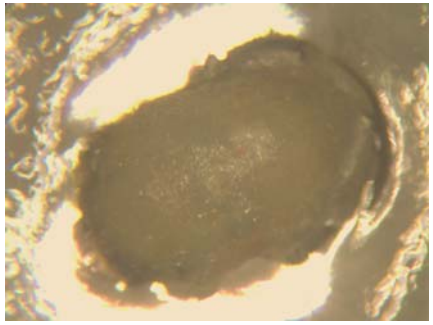


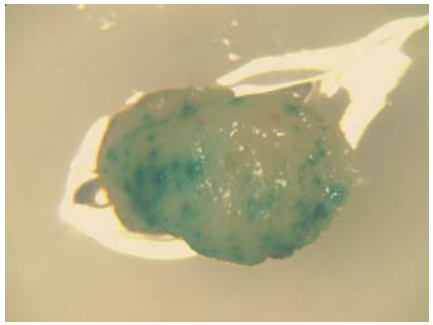


Five Days after Bombardment

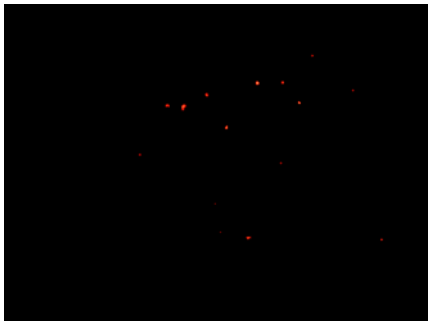
28-13+GUS

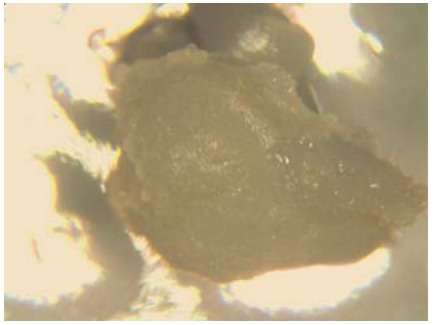


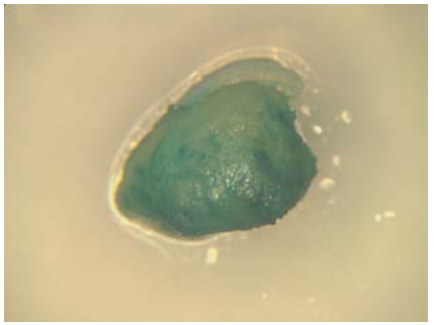




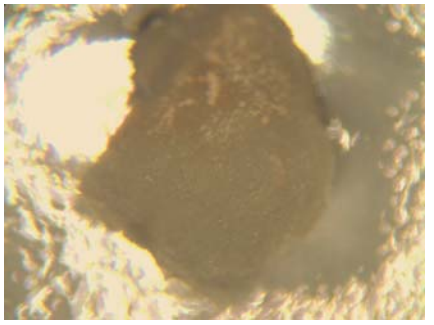
28-25+T1+GUS

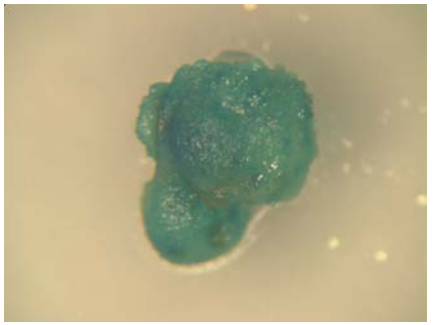






28-25+T2+GUS





Summary

- We developed a system to evaluate editing enzymes in corn.
- Our data suggest TALEN1 is better at editing this target than TALEN2 or cas9.
- Future experiments are needed to confirm results and further investigate cas9.

Acknowledgements

Dr. Erica Unger-Wallace

Dr. Erik Vollbrecht

Josh Strable

Wimalanathan Kokulapalan (Gokul)

Bronwyn Frame

Dr. Bing Yang

Dr. David Wright

Works Cited

Belanger, Karl. "Using TALENs and CRISPRs in Crop Development." Seed World. N.p., Jan. 2014. Web. 08 Apr. 2014.

Belhaj, Khaoula, Angela Chaparro-Garcia, Sophien Kamoun, and Vladimir Nekrasov. "Plant Genome Editing Made Easy: Targeted Mutagenesis in Model and Crop Plants Using the CRISPR/Cas System." Plant Methods 9.39 (2013): n. pag. Plant Methods. 11 Oct. 2013. Web. 08 Apr. 2014.

Li, Ting, Bo Liu, Martin H. Spalding, Donald P. Weeks, and Bing Yang. "High-efficiency TALEN-based Gene Editing Produces Disease-resistant Rice." Nature Biotechnology 30 (2012): 390-92.

Massachusetts Institute of Technology. "Erasing a genetic mutation: Researchers reverse a liver disorder in mice by correcting a mutated gene." ScienceDaily. ScienceDaily, 30 March 2014