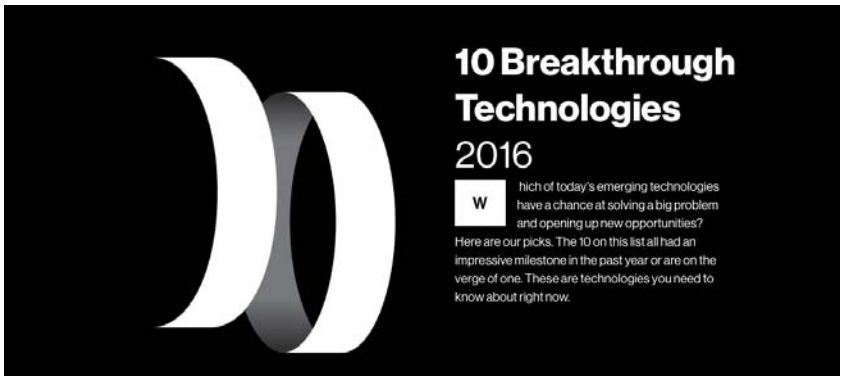
Genome editing as a new powerful tool for wheat breeding

Vladimir Nekrasov

15th WGIN Stakeholders' Meeting

ROTHAMSTED
RESEARCH



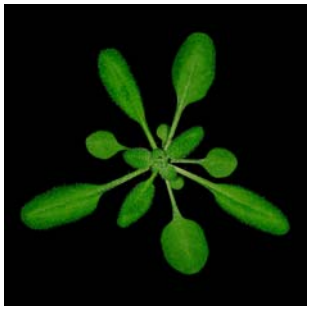
Precise Gene Editing in Plants

CRISPR offers an easy, exact way to alter genes to create traits such as disease resistance and drought tolerance.

Applying CRISPR-Cas9 technology in model and crop plants

Model plant:

Nicotiana benthamiana



Arabidopsis thaliana

Crop plants:

Tomato

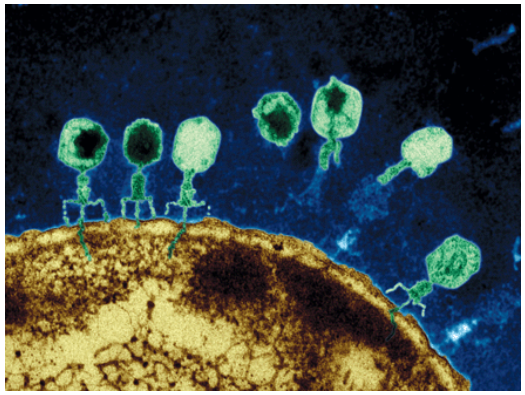
Barley

Maize

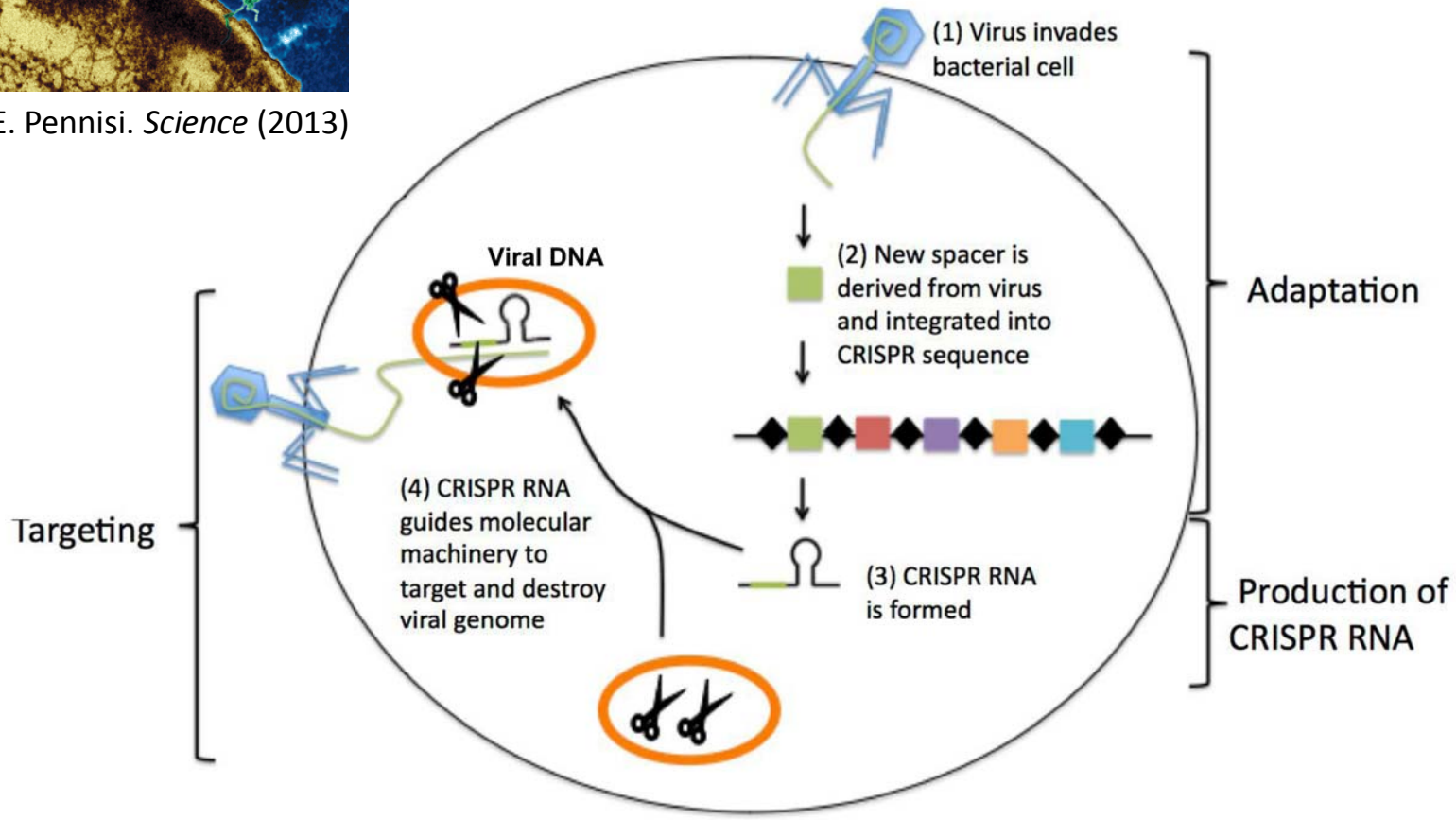
Brassica

Wheat

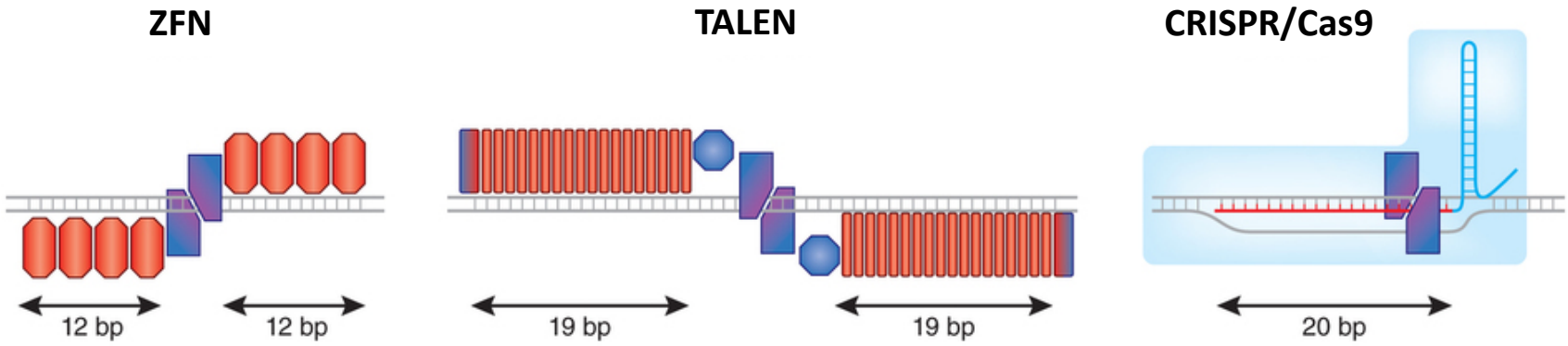
CRISPR/Cas system protects bacteria against phages



E. Pennisi. *Science* (2013)

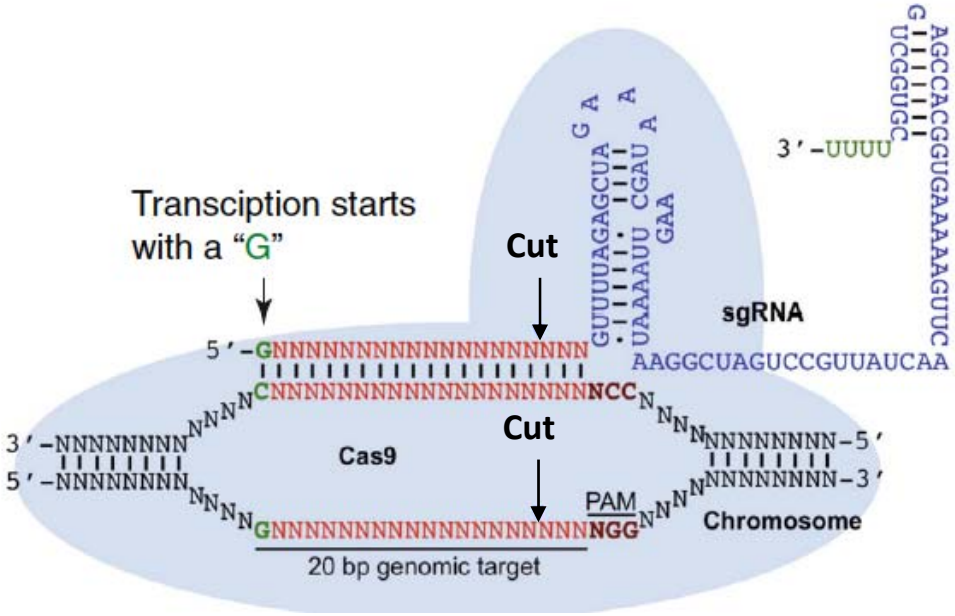


Site-directed nucleases (SDNs) are tools for inducing DNA double strand breaks (DSBs)



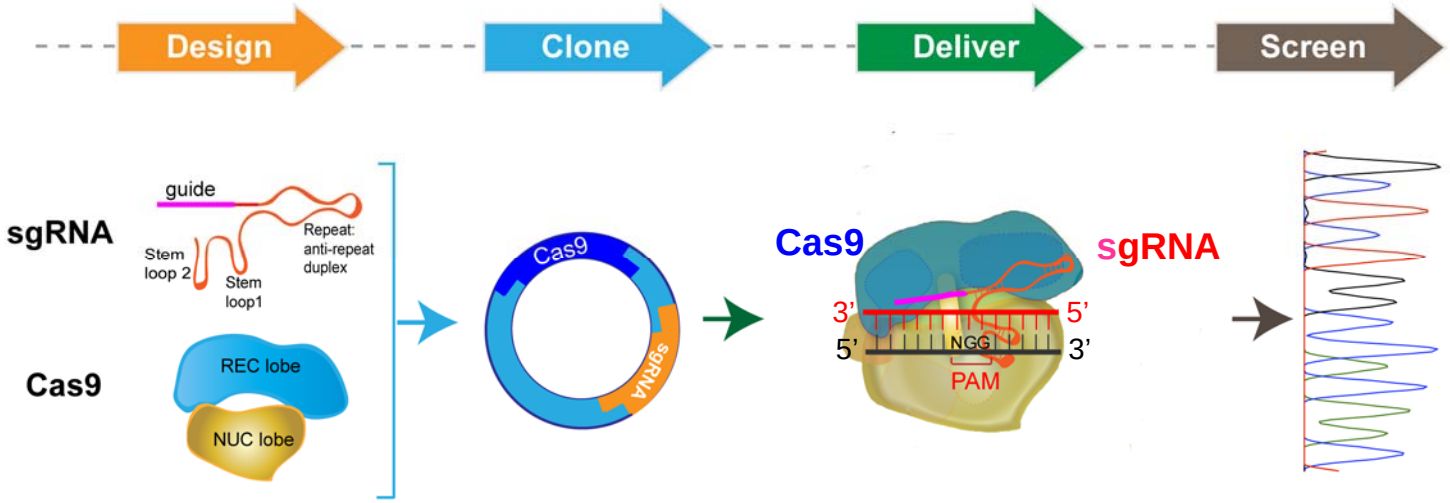
Nature Biotechnology **31**, 208–209 (2013)

CRISPR/Cas is an RNA-guided DNA endonuclease that includes Cas9 and sgRNA



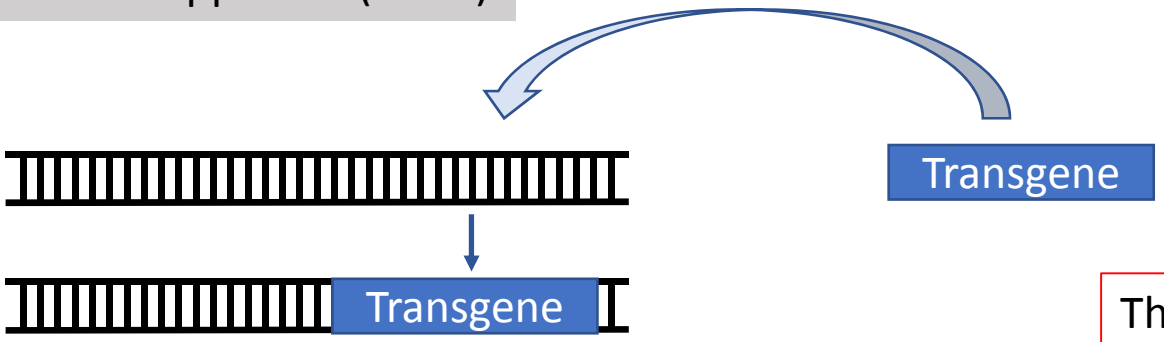
adapted from Belhaj et al. *Plant Methods* (2013)

The pipeline of targeted mutagenesis in plants using the CRISPR/Cas9 system



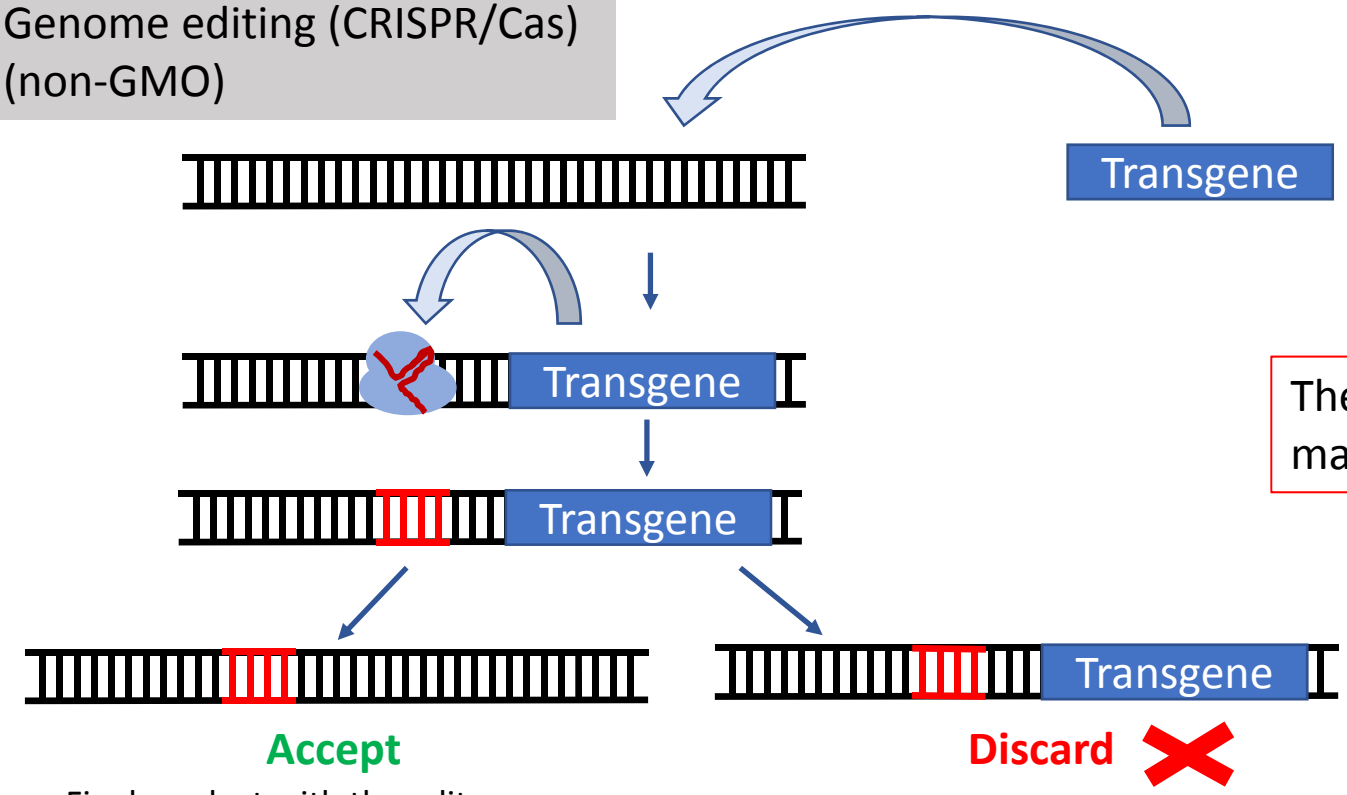
adapted from Belhaj et al. *Curr Opin Biotechnol* (2015)

Transgene based approach (GMO)



The **Transgene** insertion makes the product

Genome editing (CRISPR/Cas) (non-GMO)



The **Transgene** delivers what makes the product

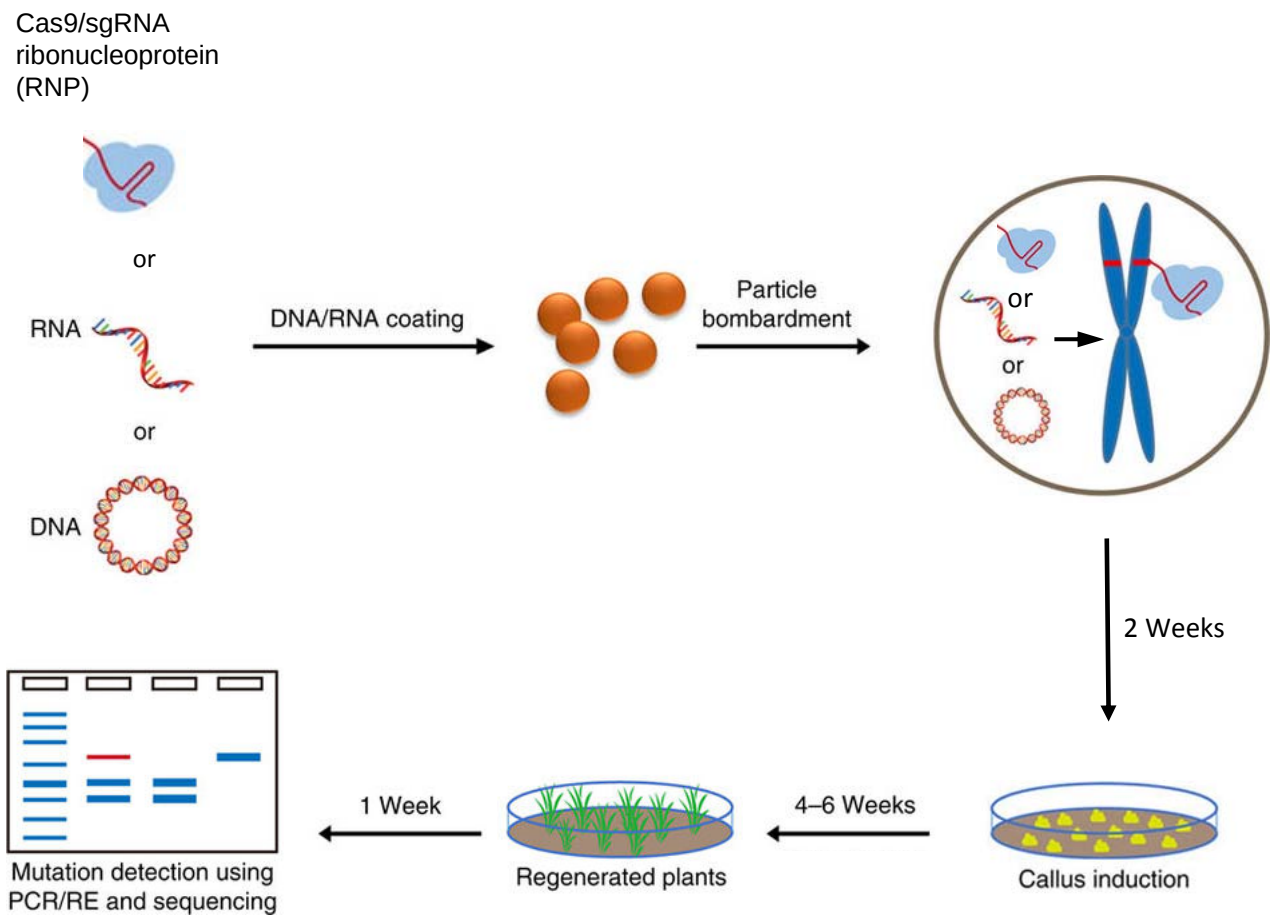
Segregation in T1

Accept

Final product with the edit but no transgene

Discard

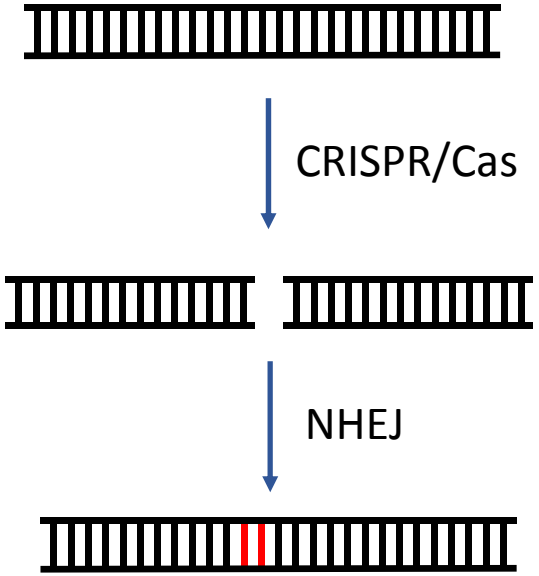
Testing alternative ways to deliver CRISPR/Cas reagents into wheat



adapted from Zhang et al. *Nat Commun.* (2016)

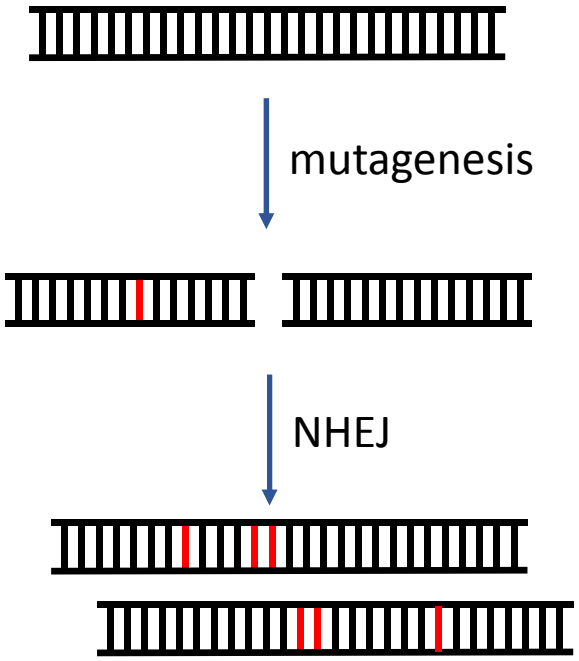
Site-directed nuclease type 1 (SDN-1) activity

SDN-1:
Error-prone repair via the non-homologous end joining (NHEJ) mechanism



mismatches (SNPs)
or indels at a specific site

Breeding Equivalent:
Mutation breeding



mismatches (SNPs)
or indels at a random sites

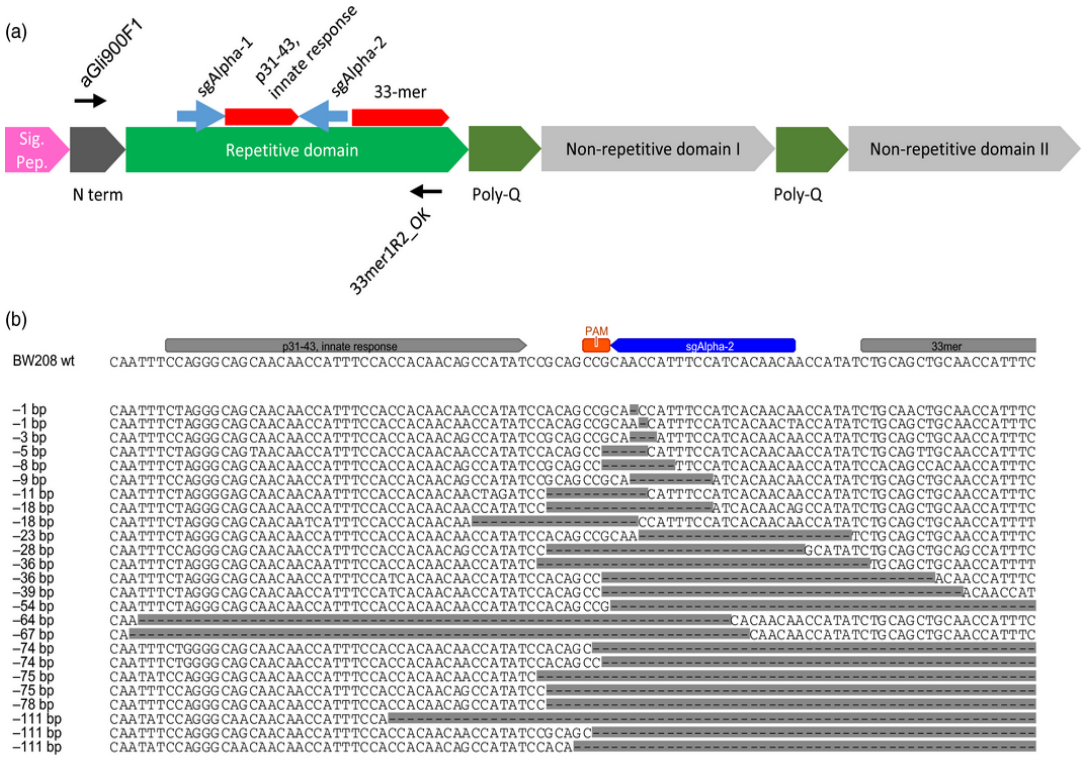
Plant Biotechnology Journal

Plant Biotechnology Journal (2017), pp. 1–9 doi: 10.1111/pbi.12837

Low-gluten, nontransgenic wheat engineered with CRISPR/Cas9

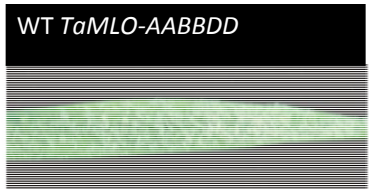
Susana Sánchez-León[1,#], Javier Gil-Humanes[2,*,#], Carmen V. Ozuna[1], María J. Giménez[1], Carolina Sousa[3], Daniel F. Voytas[2] and Francisco Barro[1,*]

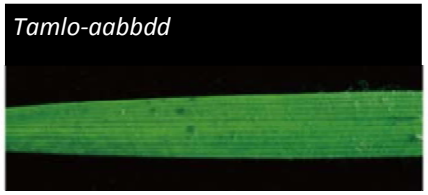
Up to 35 out 45 gliadin genes mutated in one of the edited wheat lines



Engineering powdery mildew resistance in wheat by targeting the *TaMlo* locus

Gene editing approach (TALENs)





Genetically edited KO line (TALENs)

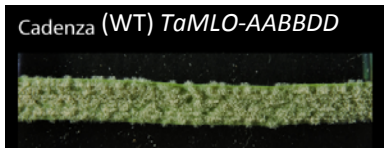
nature biotechnology

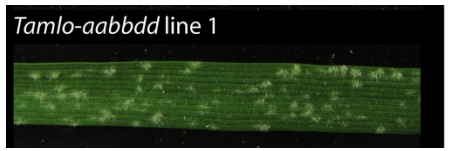
Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew

Yanpeng Wang, Xi Cheng, Qiwei Shan, Yi Zhang, Jinxing Liu, Caixia Gao & Jin-Long Qiu

Nature Biotechnology **32**, 947–951 (2014) Received: 02 April 2014

TILLING approach





Tamlo-A1 P325L, *Tamlo-B1* G319R
Tamlo-D1 P335L TILLING line

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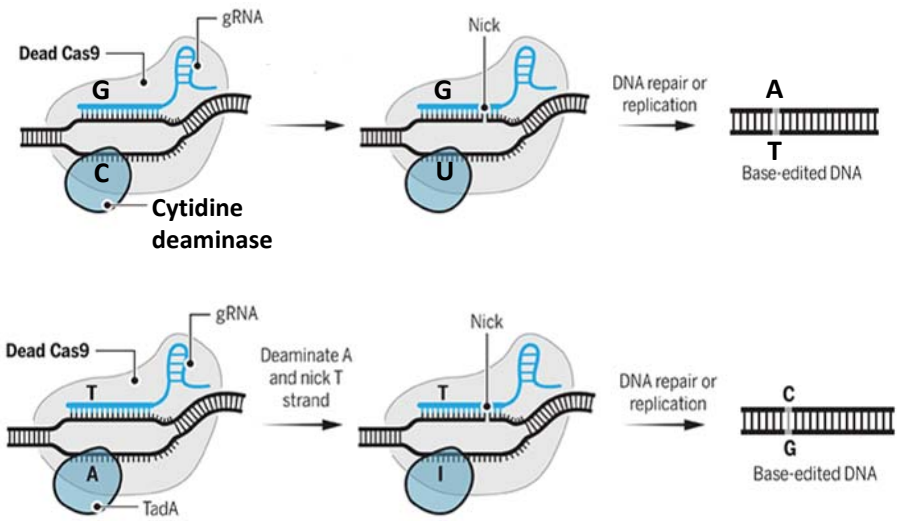
Research Article

***mlo*-based powdery mildew resistance in hexaploid bread wheat generated by a non-transgenic TILLING approach**

Johanna Acevedo-Garcia, David Spencer, Hannah Thieron, Anja Reinstädler, Kim Hammond-Kosack, Andrew L. Phillips, Ralph Panstruga

First published: 25 September 2016 Full publication history

Base editors allow conversion of C-G base pairs into T-A and vice versa



Base editors:

dCas9-cytidine deaminase (C-> T; G-> A)

dCas9-TadA (A-> G; T-> C)

adapted from Cohen *Science* (2017)

nature biotechnology

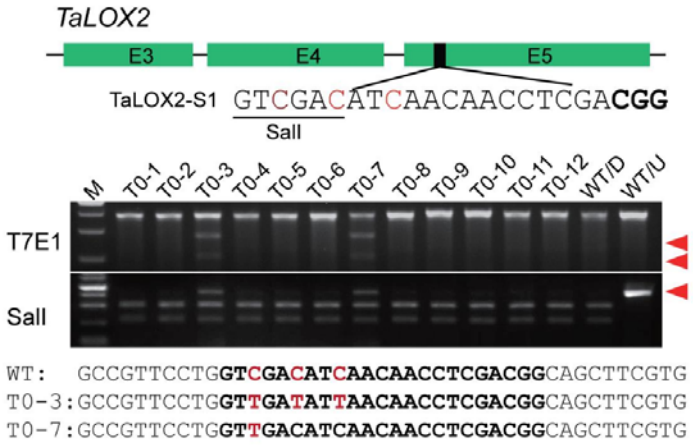
Brief Communication

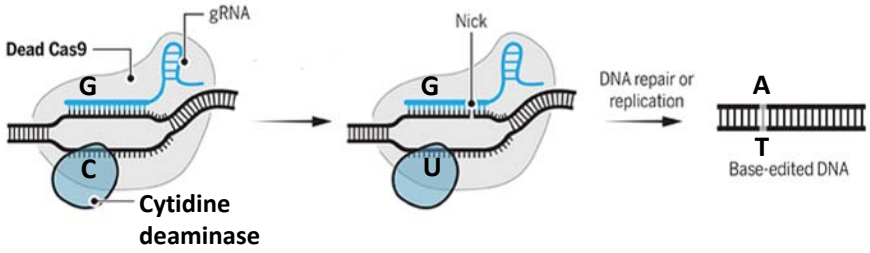
Precise base editing in rice, wheat and maize with a Cas9-cytidine deaminase fusion

Yuan Zong, Yanpeng Wang, Chao Li, Rui Zhang, Kunling Chen, Yidong Ran, Jin-Long Qiu, Daowen Wang & Caixia Gao

Nature Biotechnology **35**, 438–440 (2017)
doi:10.1038/nbt.3811

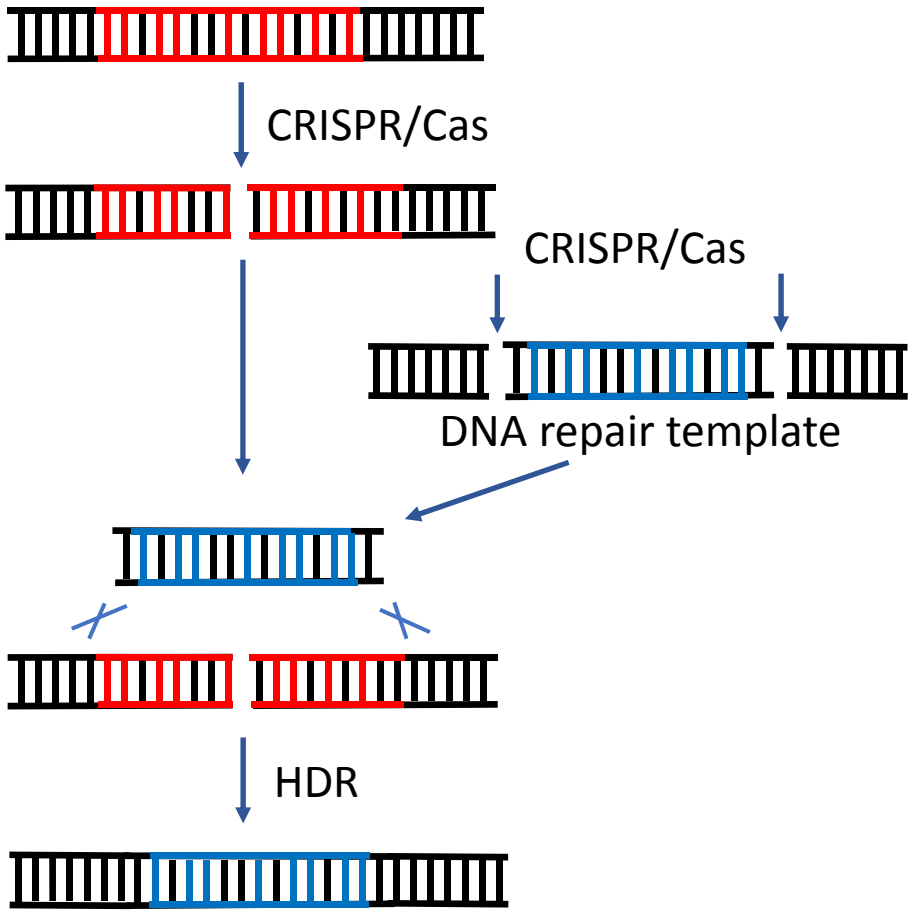
Received: 31 October 2016
Accepted: 05 February 2017





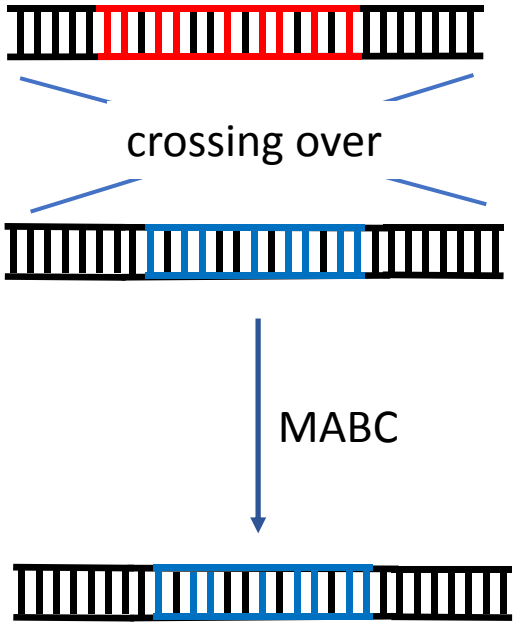
Site-directed nuclease type 2 (SDN-2) activity

SDN-2:
insertion of orthologue sequence
via homology-directed repair (HDR)



allele replacement

Breeding Equivalent:
variety upgrading by
marker assisted backcrossing
(MABC)

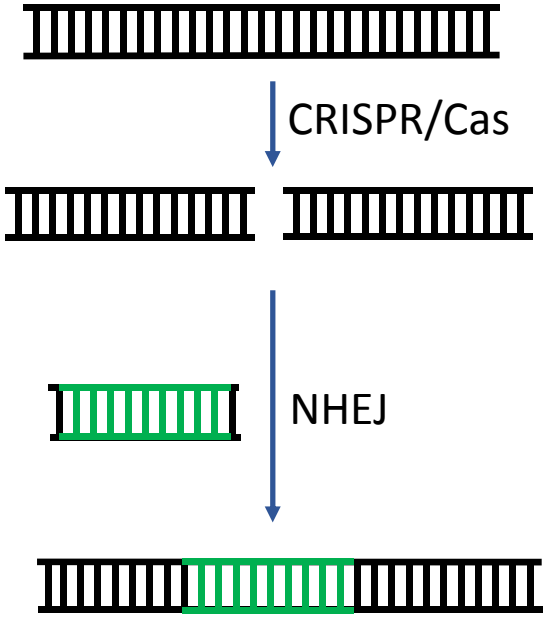


near isogenic lines (NILs)

Site-directed nuclease type 3 (SDN-3) activity

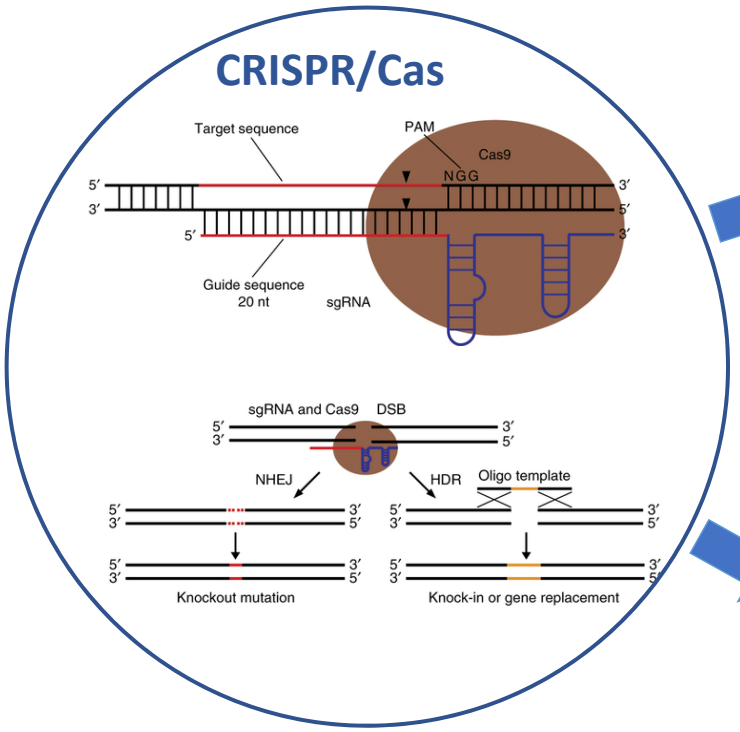
SDN-3:
insertion of foreign DNA via NHEJ or HDR

Breeding Equivalent:
None, except for GMO (e.g. Bt transgene)

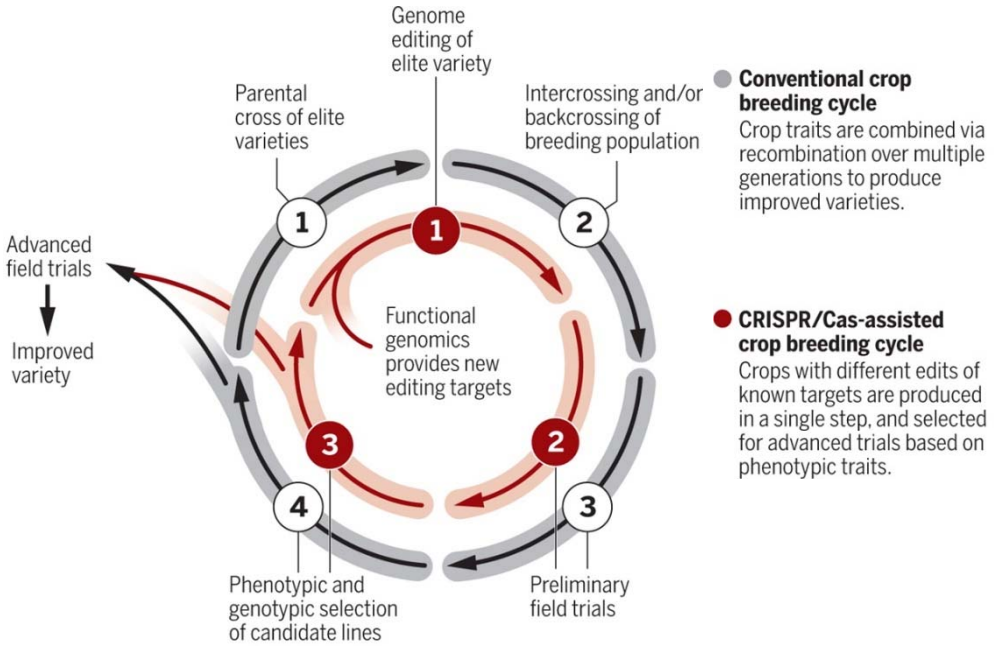


targeted insertion of cis- or trans-genes

CRISPR/Cas applications in wheat



Crop Improvement: engineering beneficial traits and speeding up the breeding cycle



Gene function studies:

Gene knockout ➡ Phenotype???

adapted from Scheben et al. *Science* (2017)

Are genetically edited crops GM or non-GM?

In the **USA**, until recently, USDA has been granting permissions for genetically edited crops not to be treated as GMO as long as they don't carry transgenic DNA

nature biotechnology

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NATURE BIOTECHNOLOGY | NEWS

CRISPR-edited crops free to enter market, skip regulation

Emily Waltz

Nature Biotechnology **34**, 582 (2016) | doi:10.1038/nbt0616-582
Published online 09 June 2016

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DuPont Pioneer's high amylopectin corn is the first CRISPR-edited plant likely to bypass USDA oversight.

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NATURE | NEWS

Gene-edited CRISPR mushroom escapes US regulation

A fungus engineered with the CRISPR–Cas9 technique can be cultivated and sold without further oversight.

Emily Waltz

14 April 2016

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Jose A. Bernat Bacete/Getty Images

The common white button mushroom (*Agaricus bisporus*) has been modified to resist browning.

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The U.S. Department of Agriculture wants to reconsider how to regulate some genetically engineered crops.

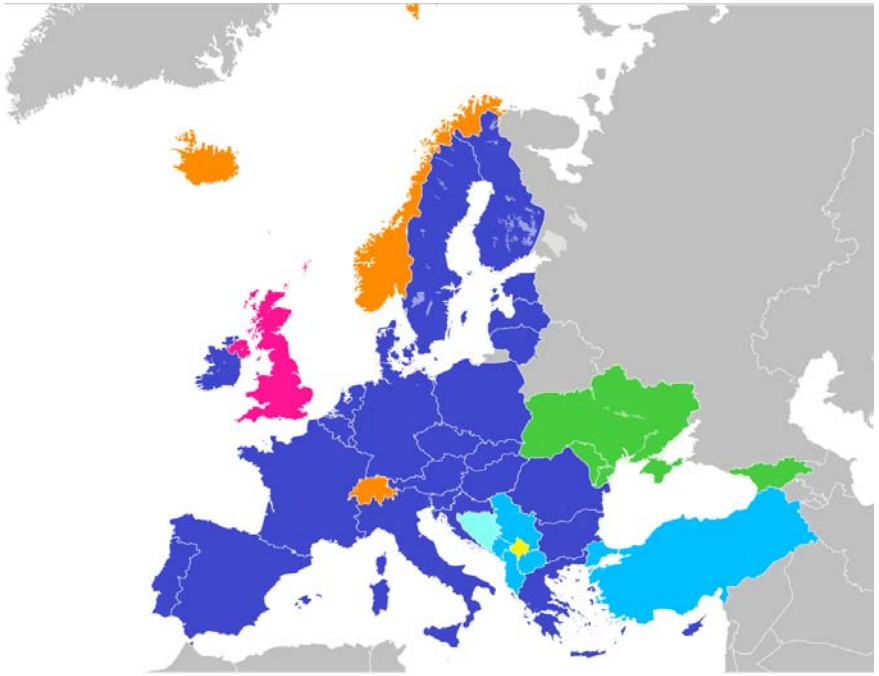
Wayne Stadler/Flickr (CC BY-NC-ND 2.0)

Trump's agriculture department reverses course on biotech rules

By **Kelly Servick** | Nov. 6, 2017 , 5:32 PM

The U.S. Department of Agriculture (USDA) has withdrawn a plan to overhaul how it regulates biotechnology products such as genetically engineered (GE) crops.

In the **EU**, the GMO legislation is entirely based on the process. So, a crop variety produced using any recombinant nucleic acid, would be considered GMO.



European Court of Justice to provide legal opinion on the interpretation of the EU directive 2001/18/EC – started in October 2016. Ruling expected in 2018.

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Alison Huttly

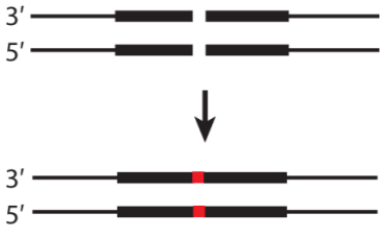
Kostya Kanyuka
Kim Hammond-Kosack

Rowan Mitchell

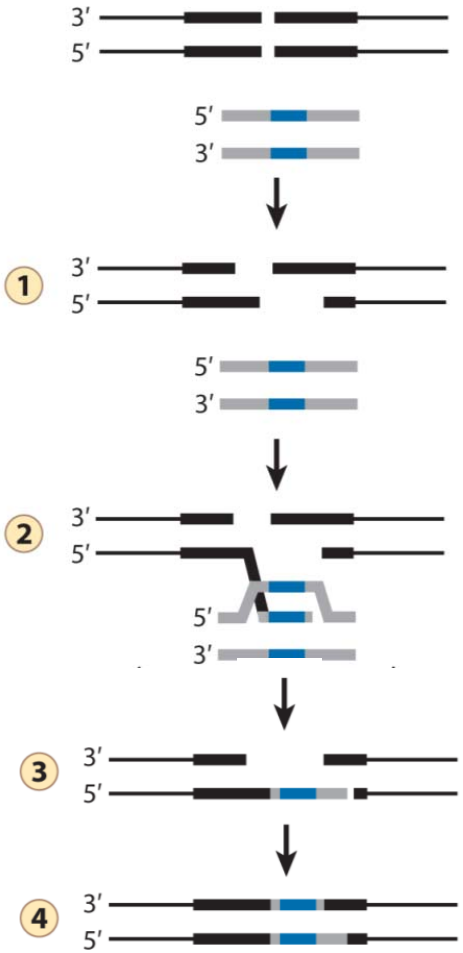
Keith Edwards

Targeted modification of a plant genome is achieved via harnessing DNA double strand break (DSB) repair pathways:

1. Non-Homologous End Joining (NHEJ): small random deletions or insertions introduced



2. Homology-Directed Repair (HDR): specified locus modification introduced



The key to targeted genome modification is to have means of introducing a DSB at a specific locus

Adapted from Voytas D. *Annu. Rev. Plant Biol.* (2013)