

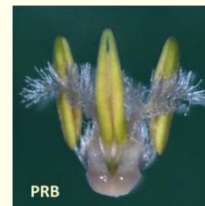
# Cas endonuclease technology in crops

## Establishment and applications

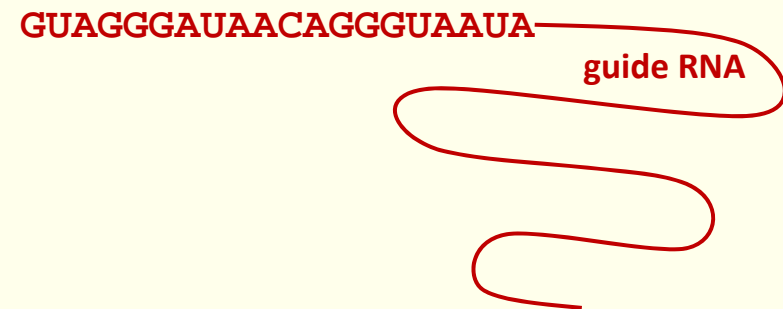
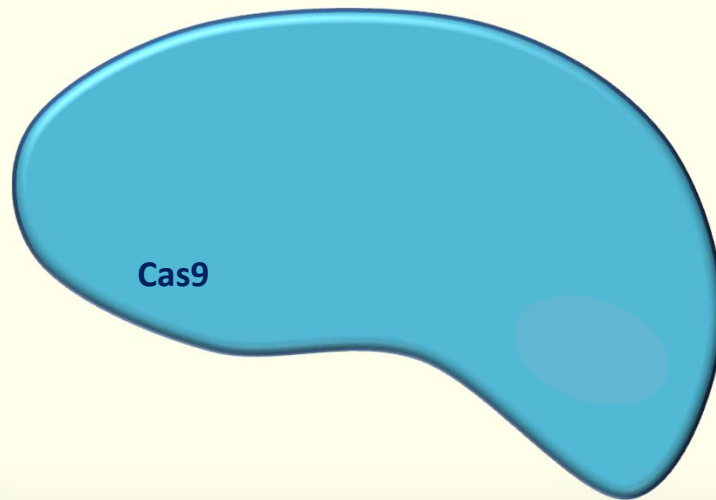
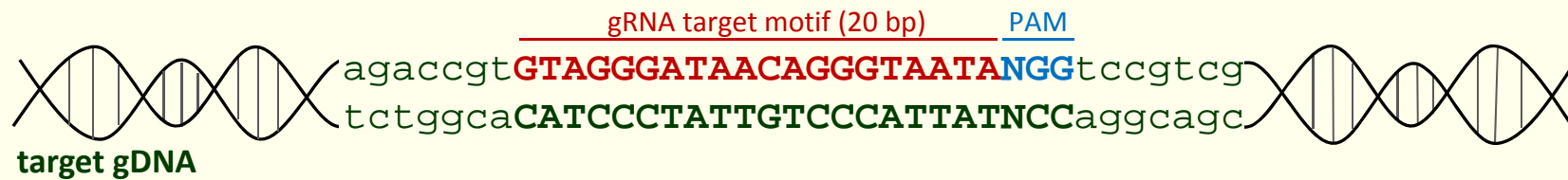
*Jochen Kumlehn*

**Plant Reproductive Biology**

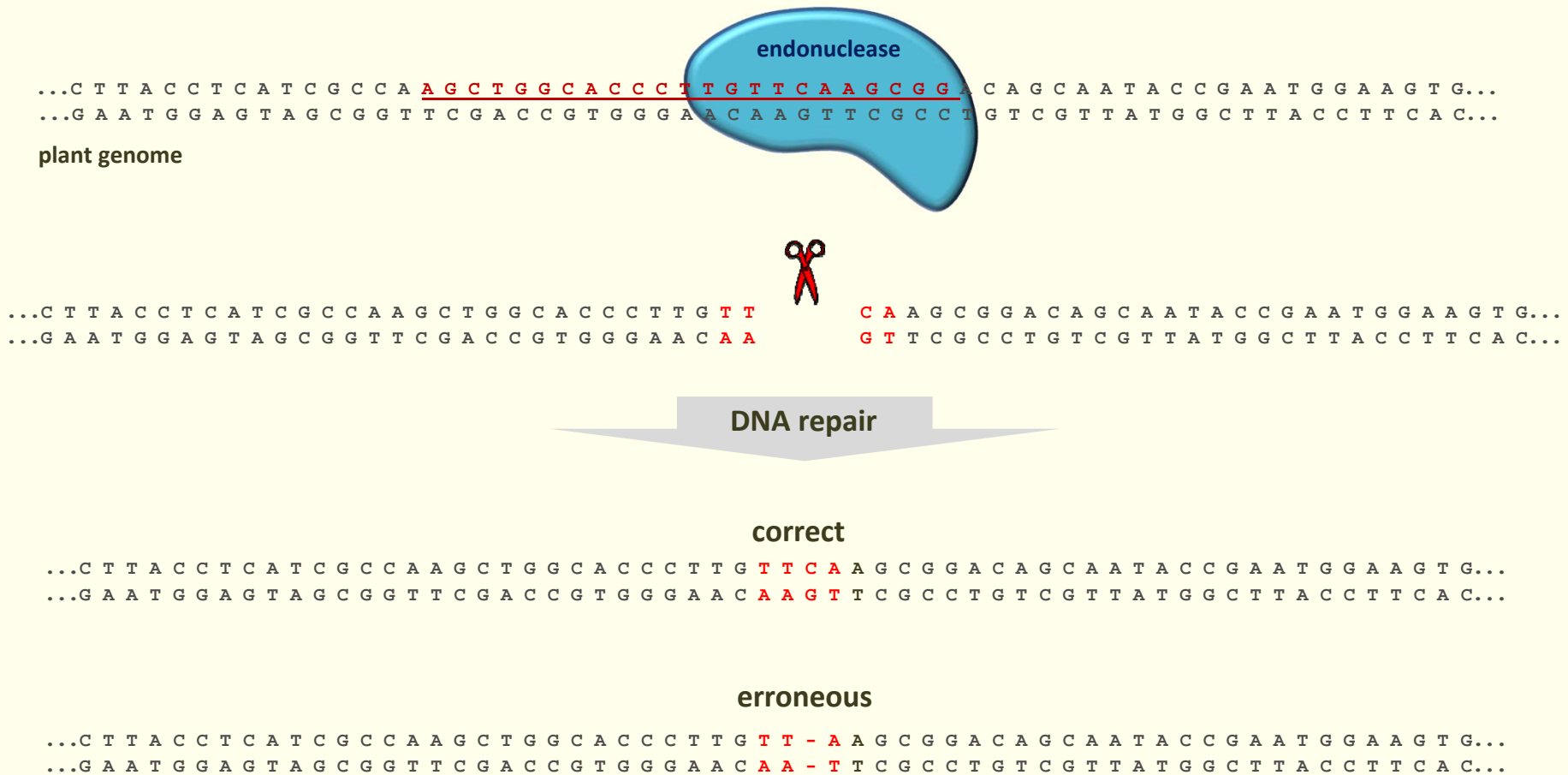
**Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben**



# RNA-guided Cas9 endonuclease



# Site-directed mutagenesis using customized Cas endonucleases



➡ Any genomic sequence of choice can be mutated *in planta*.

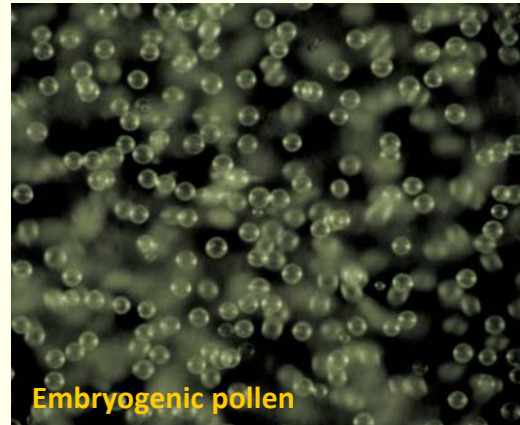
# Genetic transformation in cereals



Immature embryo

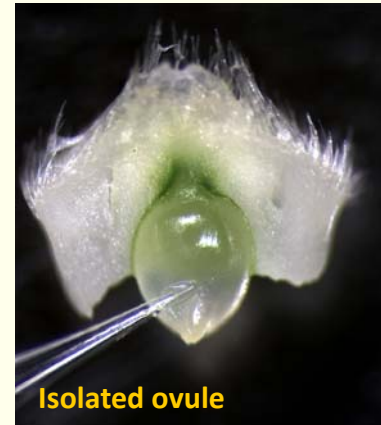
barley<sup>AB</sup>, *H. spontaneum*<sup>A</sup>,  
wheat<sup>AB</sup>, triticale<sup>A</sup>,  
maize<sup>A</sup>

A: Agrobacterium-mediated  
B: ballistic



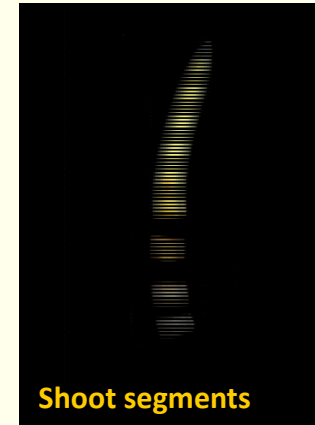
Embryogenic pollen

barley<sup>AB</sup>



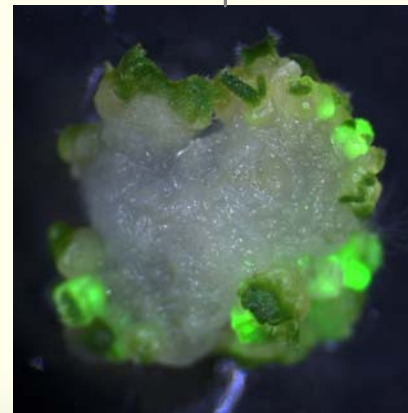
Isolated ovule

barley<sup>A</sup>

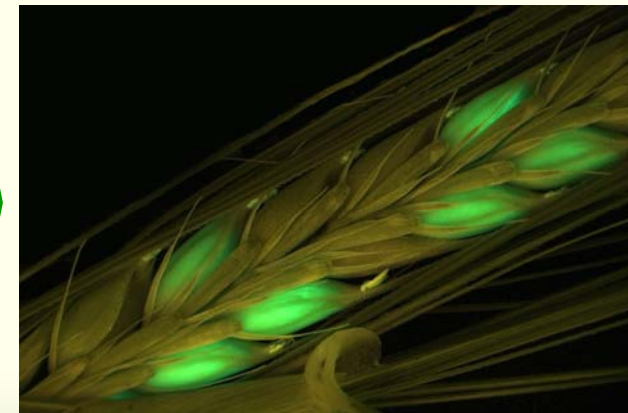


Shoot segments

*Brachypodium*<sup>A</sup>



Callus and shoot formation



T<sub>0</sub> spike, GFP segregation in T<sub>1</sub> grains

# Generation of naked barley via targeted mutagenesis of *HvNUD*

S. Gerasimova, C. Hertig, S. Hiekel, S. Sommerfeld, A. Korotkova, E. Kolosovskaya, A. Kochetov, E. Khlestkina

Hulled (WT) barley depends on the *NUD* gene which encodes an ERF, a regulator of lipid biosynthesis (Taketa et al., *PNAS* 2008)

<b>WT Nud exon1</b>	TTCGCGGCGTCAGGCAGCG	<b>CCACTGGG</b>	<b>GCTCCTGGGTCTCC</b>	GAGATCAGGCATCCTCTC	
297 (A12)	TTCGCGGCGTCAGGCAGCGCCACTGGG	<b>G</b>	GCTCCTGGGTCTCCGAGATCAGGCATCCTCTC		<b>+1</b>
257 (A7)	TTCNCGGCGTCAGGCAGCGCCACTGG-		GCTCCTGGGTCTCCGAGATCAGGNNTCCTCTC		<b>-1</b>
258 (B7)	TTCGCGGCGTCAGGCAGCGCCACTGG-		GCTCCTGGGTCTCCGAGATCAGGCATCCTCTC		<b>-1</b>
283 (C10)	TTCGCGGCGTCAGGCAGCGCCACTGG-		GCTCCTGGGTCTCCGAGATCAGGCATCCTCTC		<b>-1</b>
270 (F8)	TTCGCGGCGTCAGGCAGCGCCACTGG-		<b>--</b> TCCTGGGTCTCCGAGATCAGGCATCCTCTC		<b>-3</b>



Golden Promise  
WT

BG776-E01  
(1 bp deletion,  
homozygous)



Golden Promise

BG776-E01

# Generation of naked barley via targeted mutagenesis of *HvNUD*

S. Gerasimova, C. Hertig, S. Hiekel, S. Sommerfeld, A. Korotkova, E. Kolosovskaya, A. Kochetov, E. Khlestkina

## Grains of homozygous *nud* M1 lines, after mechanical threshing



Golden Promise  
WT



Line 16-6, -17 bp



Line 16-14, -3 bp



Line 31-12, -6 bp



Line 33-4, -1 bp

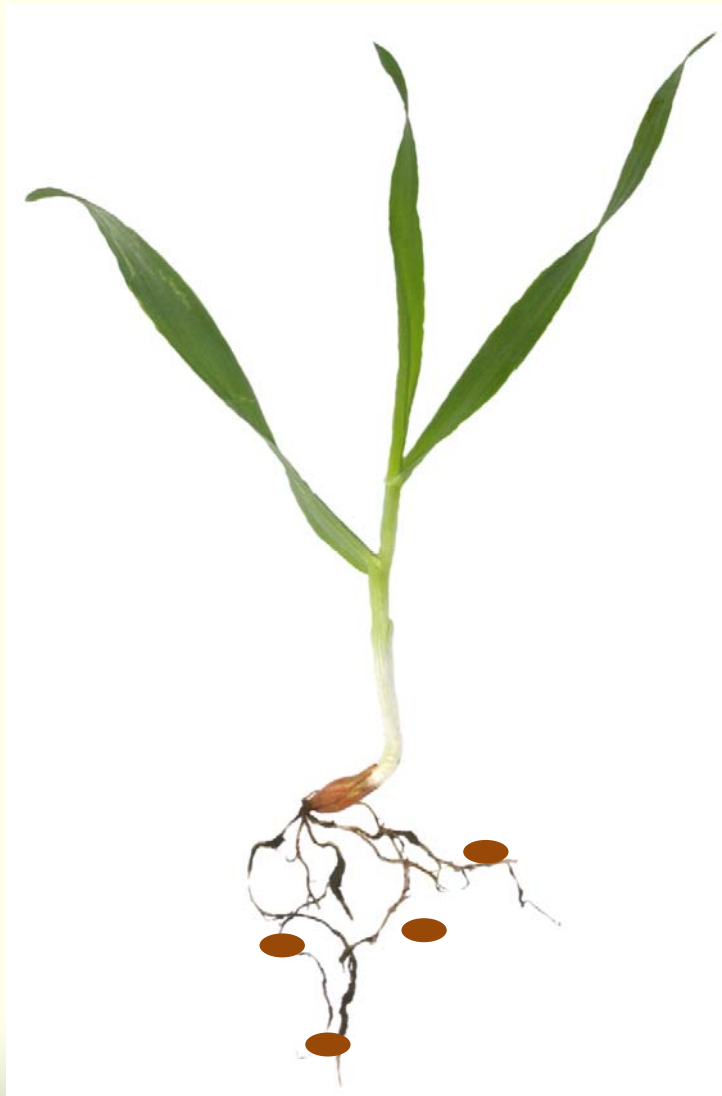


Line 33-10, -1 bp



Line 33-24, -1 bp

# Resistance to the Barley Yellow Mosaic Virus Disease



**Barley Yellow Mosaic Virus (BaYMV 1/2)**

**Barley Mild Mosaic Virus (BaMMV)**

- ss(+)RNA-Virus, Bymovirus
- transmitted via the roots  
by the soil-borne fungus *Polymyxa graminis*



**Symptoms**

- yellow mosaic on leaves
- later: necrosis
- yield losses up to 50 %

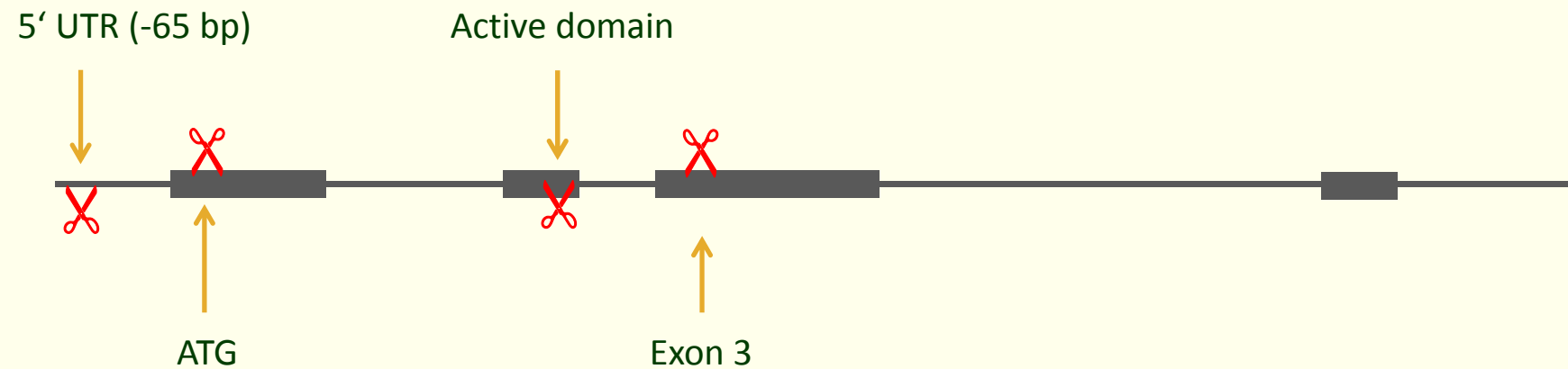
**Plant Disulfide Isomerase-Like (PDIL5-1)**

- susceptibility factor
- recruited by Bymoviruses
- encoded at the *rym11* locus  
(Yang et al., PNAS 2014)

# Resistance to Barley Yellow Mosaic Virus Disease

Robert Hoffie

## *Knockout of HvPDIL5-1 in winter barley*



**4 target motifs addressed**



# Resistance to Barley Yellow Mosaic Virus Disease

Robert Hoffie, Ingrid Dubsky, Antje Habekuß, Dragan Perovic

## *Analysis of mutant progeny*



**Phenotype and  
immuno-detection**

**16 plants infested**  
11 susceptible  
5 resistant

**19 plants infested**  
all resistant

**Genotype**

10 sequenced  
2 heterozygous

all 19 sequenced  
all mutated

# The importance of plant height in cereals



Pieter Bruegel the Elder 1565



Harvest 2018

## Green Revolution

- lodging resistance thanks to reduced plant height
- hence, more effective use of N-fertilizer
- and improved relation of grain and straw
- most plant height genes play a role in GA biosynthesis or perception

same principle in all cereals  
nutritional basis of modern society

# Generation of new genetic diversity in plant height

## BRASSINOSTEROID-INSENSITIVE 1

- encodes a brassinosteroid receptor
- *bri1* mutants cannot implement the hormonal signal into shoot growth
- the *uzu* mutant has reduced *BRI1* function (used in Asia)



Bowman (WT)



*uzu1*

# Generation of allelic diversity in the *BRI1* gene

Nagu Budhagatapalli, Stefan Hiekel, Götz Hensel, Sabine Sommerfeld, Sibylle Freist

## gRNA/Cas9-triggered mutagenesis of *BRI1* in barley



WT (GP) *bri1* KO mutants



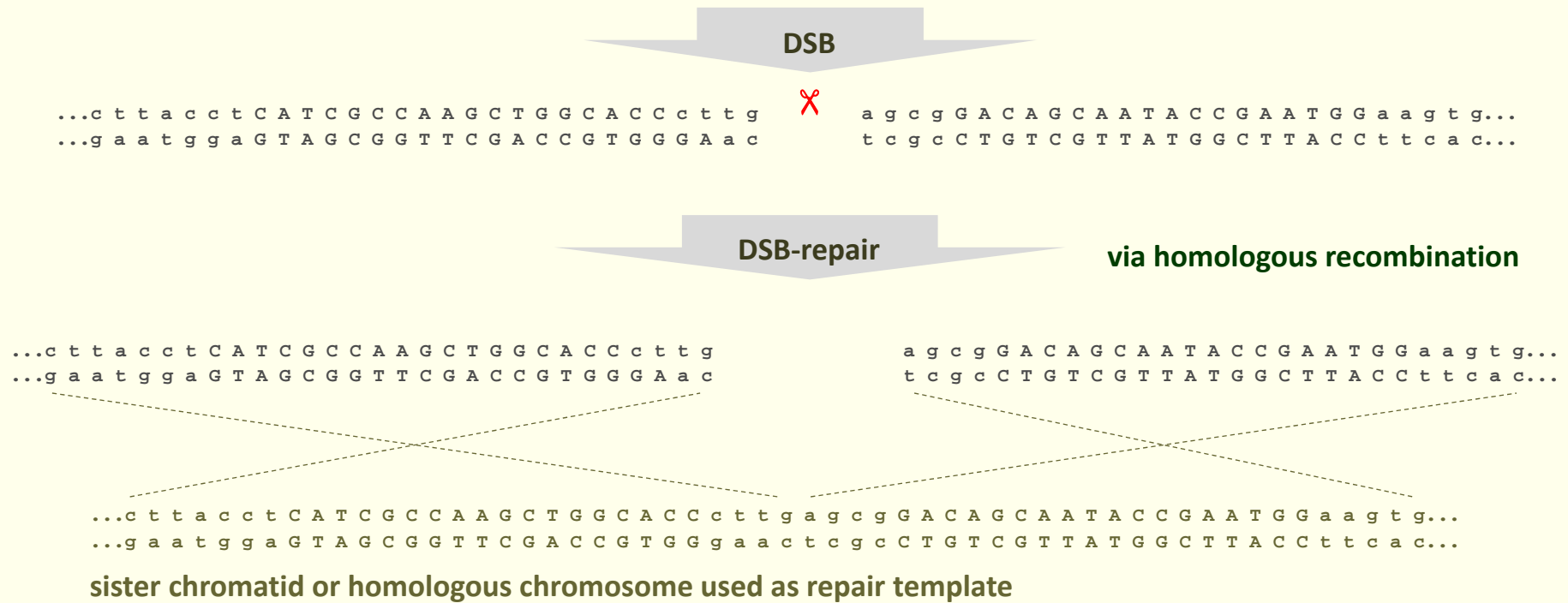
WT *BRI1* in-frame mutant



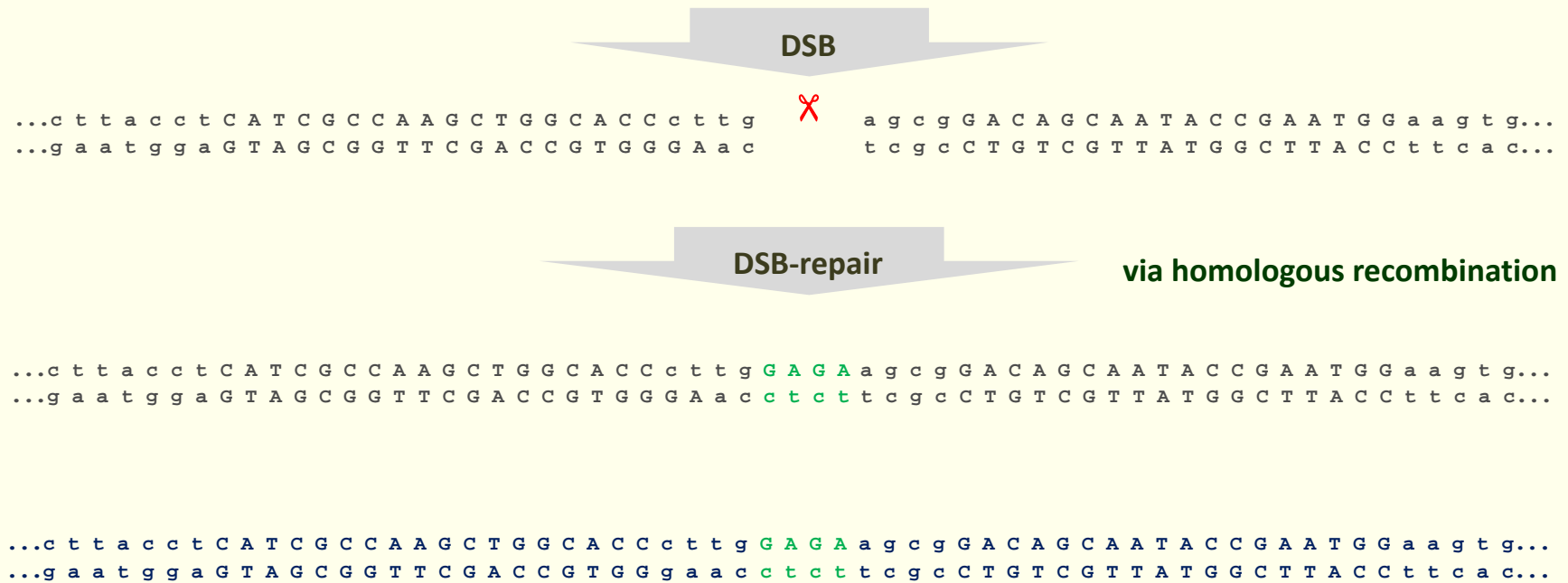
WT *BRI1* in-frame mutant

- site-directed mutagenesis in elite material may provide new plant height diversity for breeders

# Homology-directed precise genome editing



# Homology-directed precise genome editing



synthetic DNA repair template including **modification of choice**

## Options

- resultant seqs can be predefined
- precise editing of single nucleotides and amino acids
- exchange of whole alleles in correct genomic position

# Plant Reproductive Biology



## Scientists

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Aleksandra Grabowska  
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