Technical characterization of CRISPR/Cas and the differences to conventional breeding

FGU Project genetic engineering and the environment

Dr. Katharina Kawall
Online conference 11/27/2020

Overview

- Technology characterization
- Process-mediated unintended alterations
- Possibilities of CRISPR/Cas to generate novel genotypes
- Impact of intended alterations on associated ecosystems

Overview of new genetic engineering techniques

Genome Editing: Collective term for new genetic engineering techniques that cause targeted alterations of specific regions in the genome

1. Site-directed nucleases (SDNs)
   - CRISPR/Cas9
   - TALENs
   - ZNF
2. Oligonucleotide-directed Mutagenesis (ODM)

CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats
Cas: CRISPR associated
TALEN: Transcription Activator Like Effector Nuclease
ZNF: Zinc Finger Nuclease

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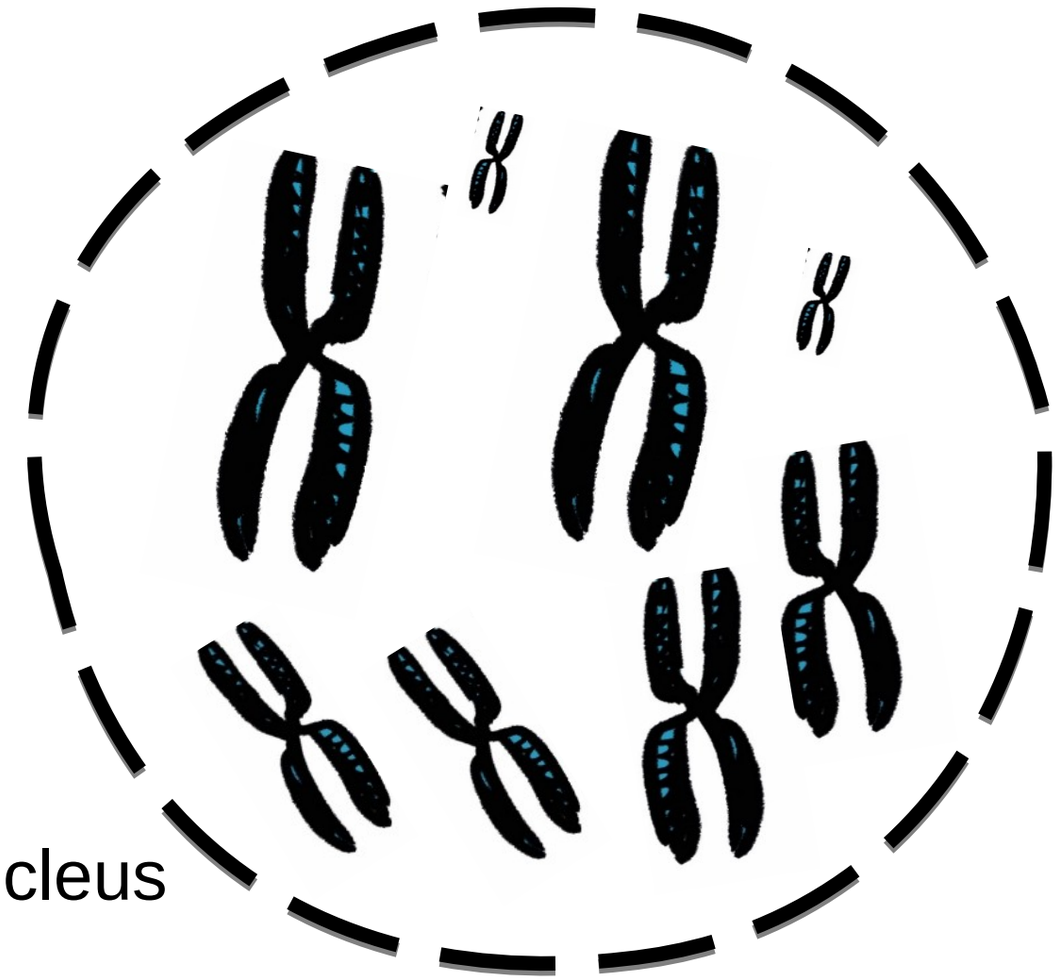
How does CRISPR/Cas9 work?

Cell

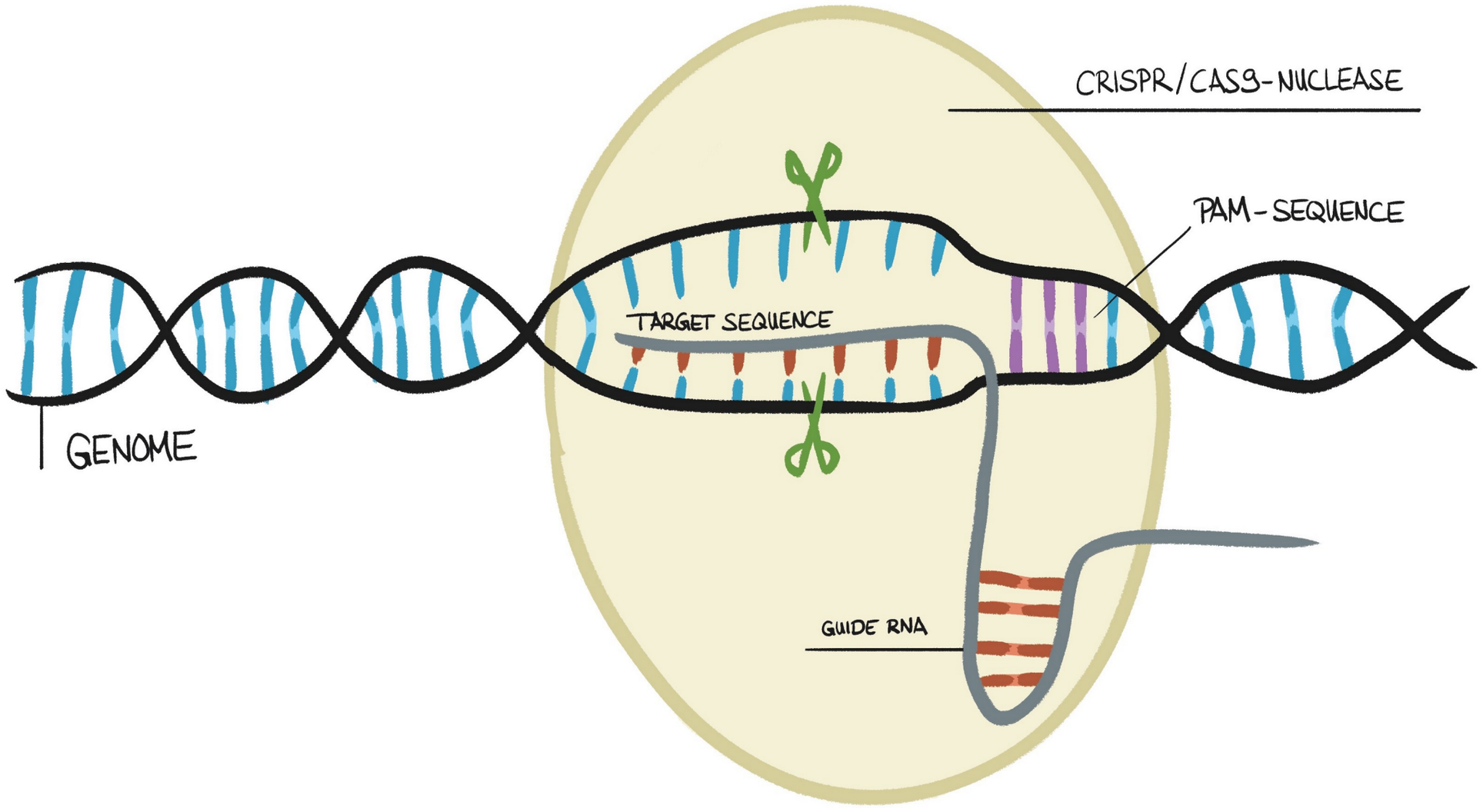


cell nucleus

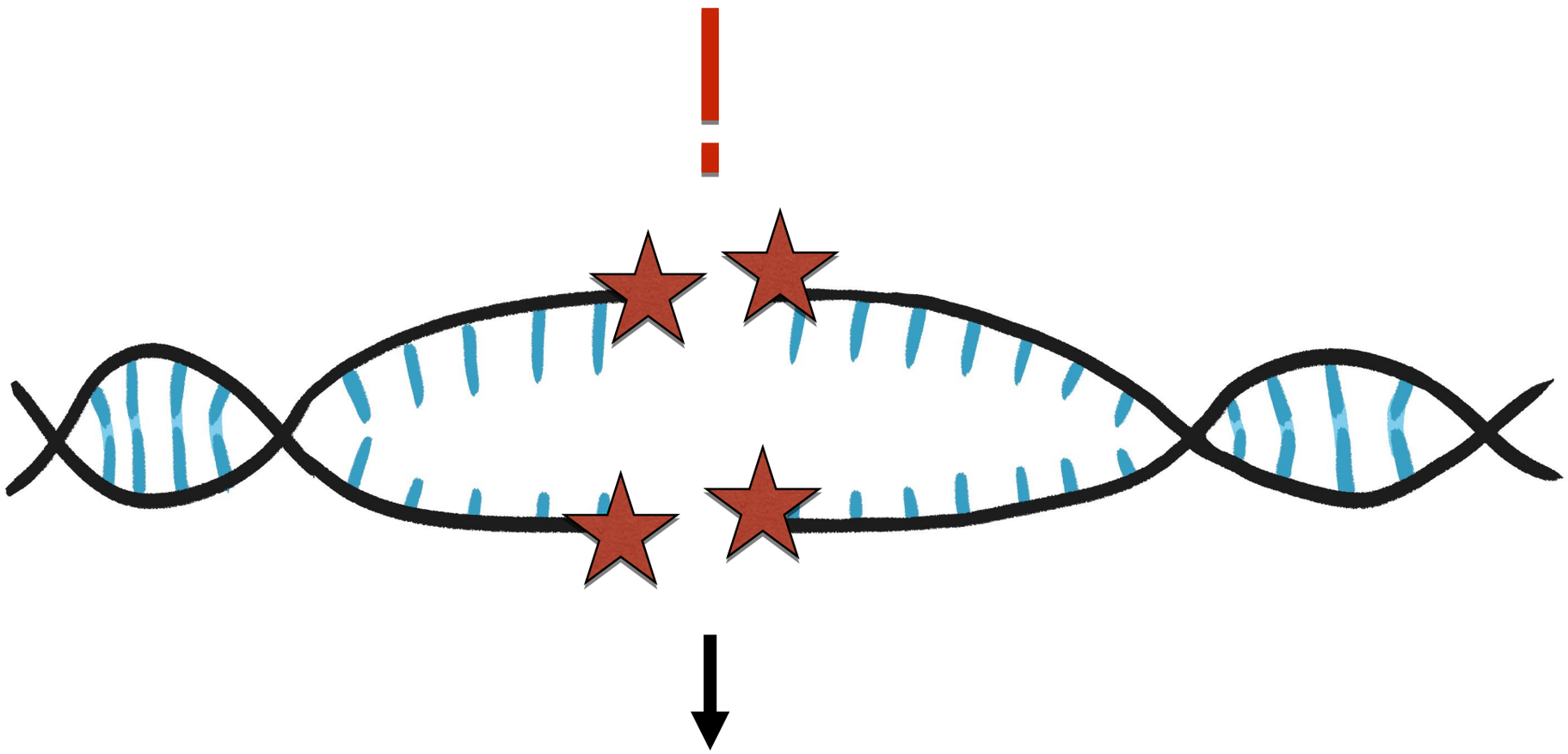
DNA/genome

4 letters of DNA: A, T, G, C

Mode of action of CRISPR/Cas9



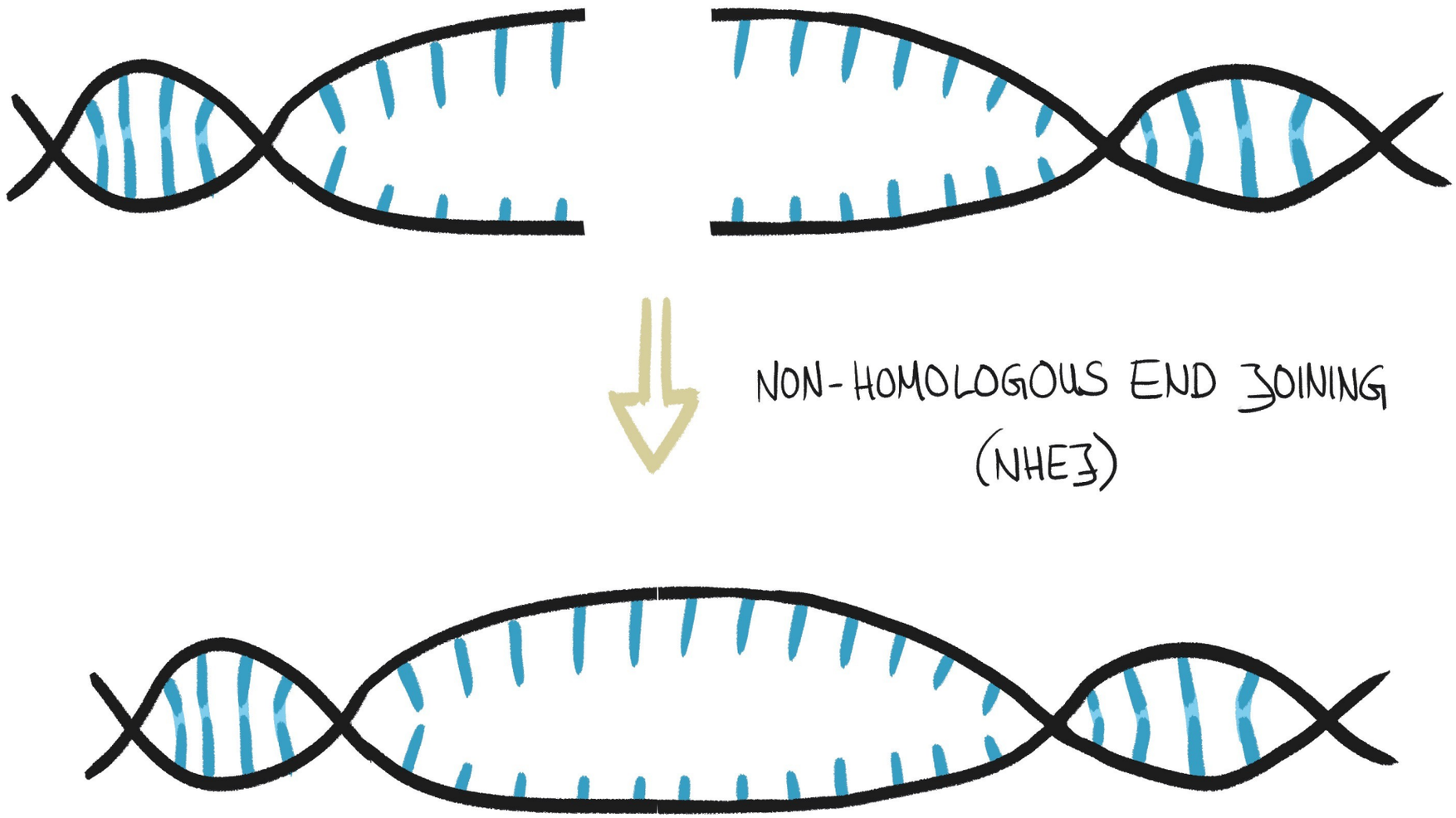
CRISPR/Cas9 generates DNA double strand breaks at specific positions of the genome.



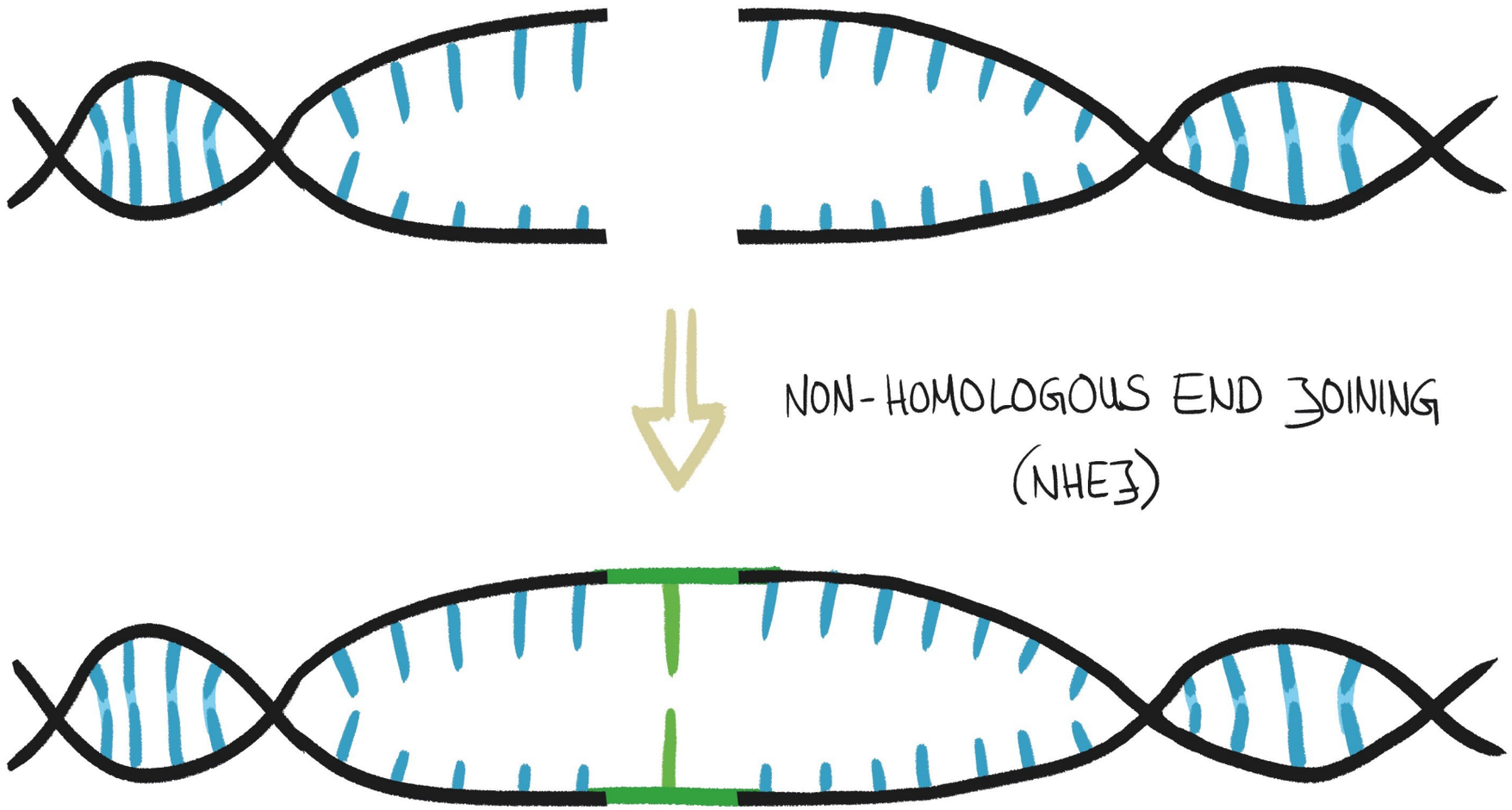
damage of the genome

activation of the cell‘s repair mechanisms

Repair back to original state



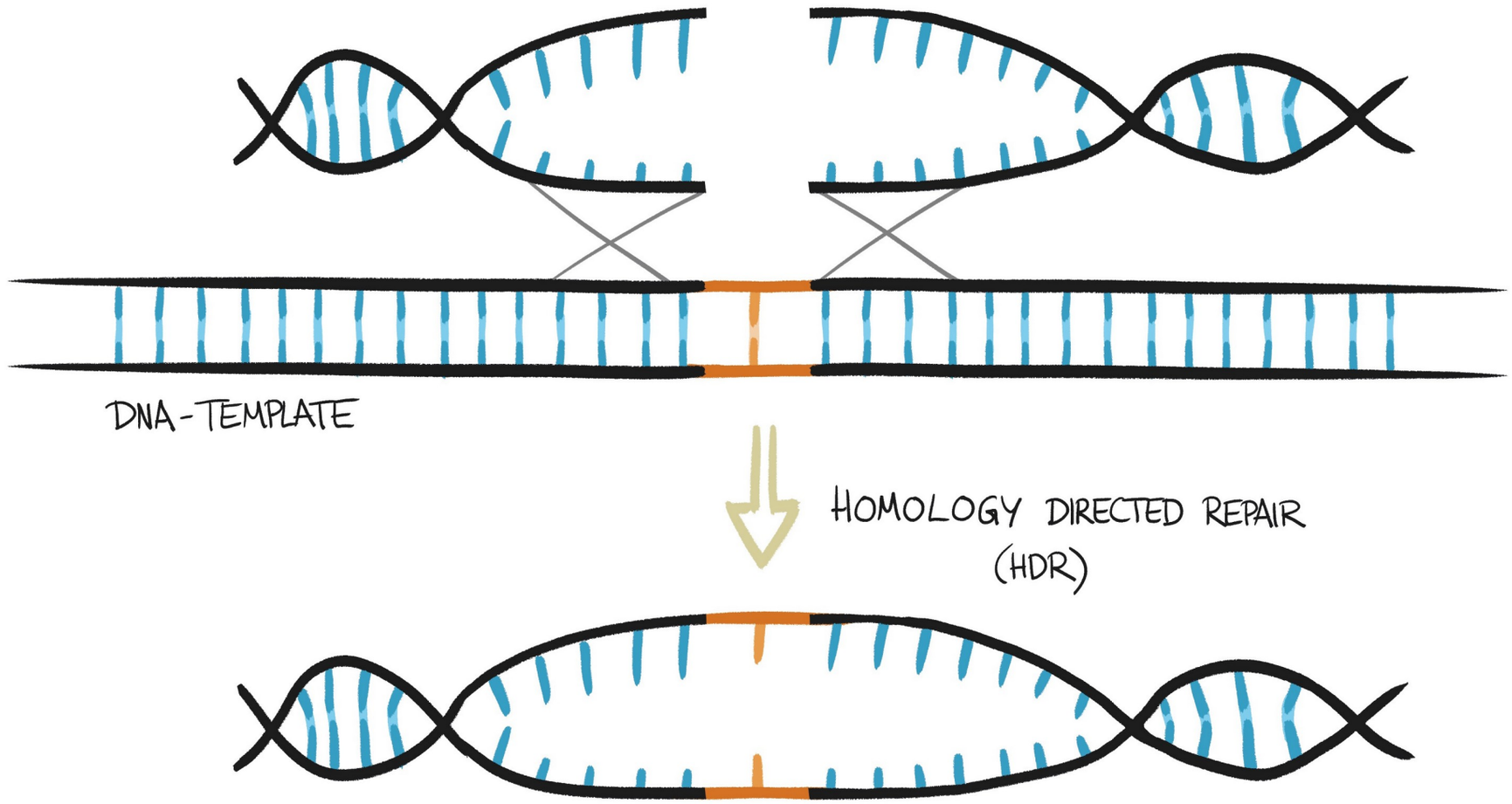
SDN-1 (Site Directed Nuclease-1)



undirected repair

Alterations at the target site can emerge due to the error prone repair of non-homologous end joining.

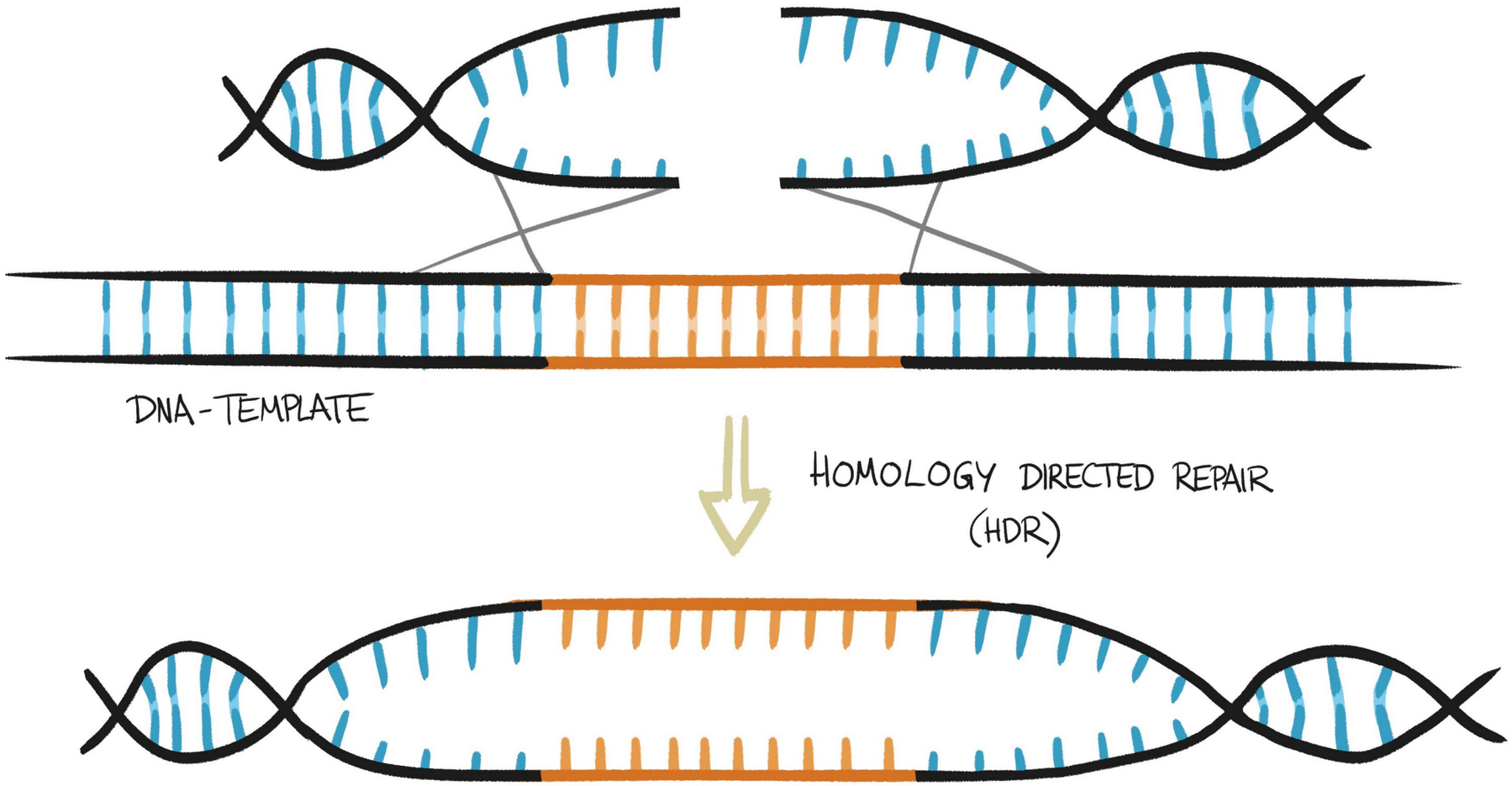
SDN-2 (Site Directed Nuclease-2)



directed repair

Homology directed repair can use a DNA template to repair the break introducing a specific alteration at the target sequence.

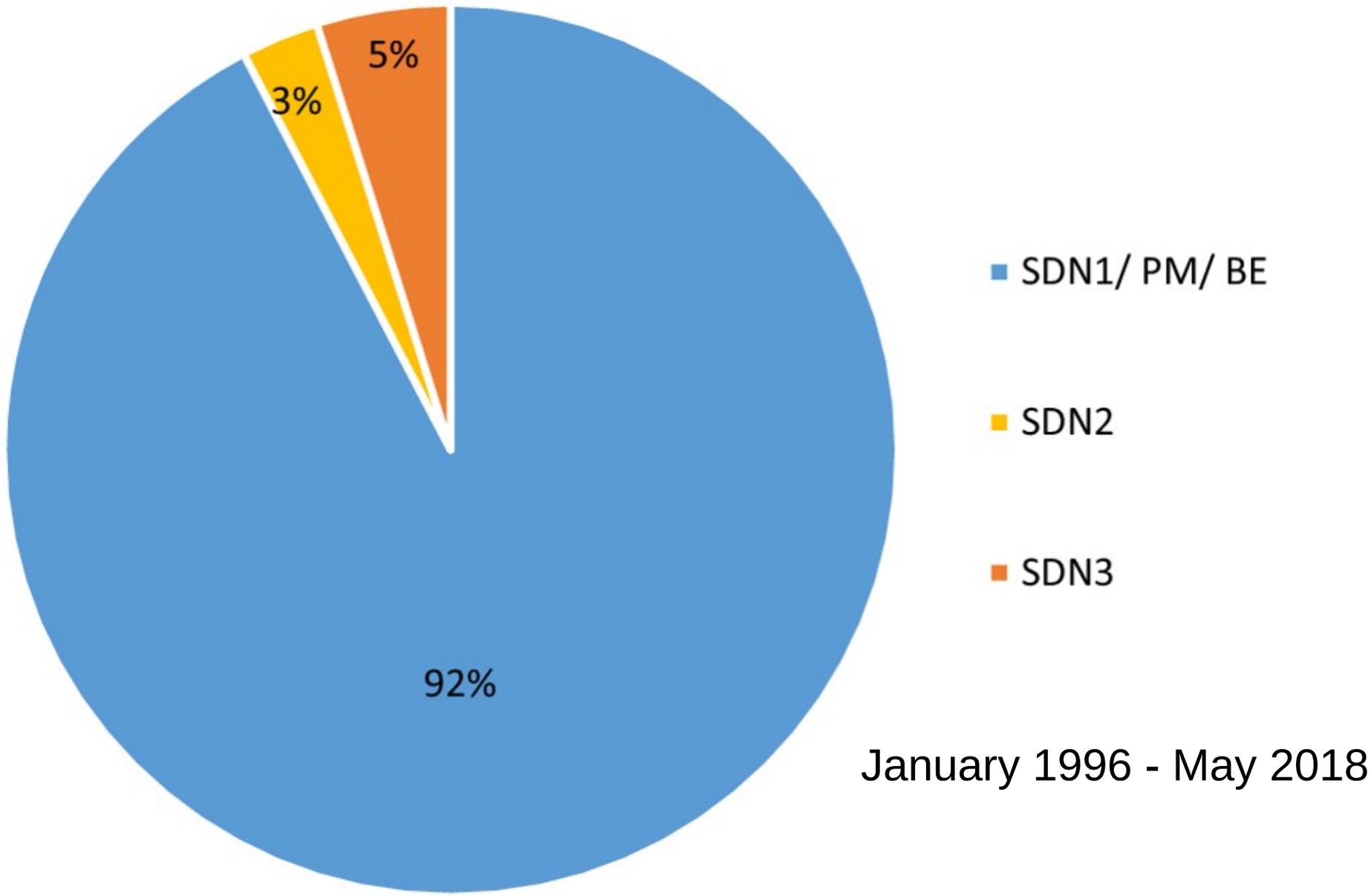
SDN-3 (Site Directed Nuclease-3)



directed repair, insertion of gene(s)

Homology directed repair can use a DNA template to repair the DNA double strand break introducing a large DNA fragment at the target sequence.

Distribution of the types of alterations introduced in plant genomes by the application of genome-editing



January 1996 - May 2018

SDN: Site-directed nuclease, PM: Point Mutation, BE: Base Editors

aus: Modrzejewski , D., et al.(2019) Environ Evid.

Alterations mediated by genome editing

Genes can be…

…knocked out.

…enhanced in their function(s).

…activated or silenced.

…deleted.

…structurally altered.

…knocked in.

Technical failures: What can go wrong?

What unintended effects are already known?

Process-induced unintended alterations

- **Specific for CRISPR/Cas**
- Specific for old genetic engineering techniques

Kawall K, Cotter J, Then C (2020) Broadening the GMO risk assessment in the EU for genome editing technologies in agriculture. Environmental Sciences Europe 32 (1):106. doi:10.1186/s12302-020-00361-2

Unintended effects specific for CRISPR/Cas

1. Off-target effects

CRISPR/Cas cuts at unintended sites of the genome.

NHEJ repair can introduce unintended changes at these sites.

These alterations can cause gene knockouts, changes in the sequence of a gene (function of proteins) or in the expression of genes (depends on the context) and need to be investigated even if they are rare.

Occurrence of off-target effects depends on the experimental design.

Found in rice, maize, barley, wheat, animals and human cell lines.

Unintended effects specific for CRISPR/Cas

2. On-target effects

Unintended alterations like large deletions, translocations and inversions either at or near the target site.

Found in human, animal and plant cells.

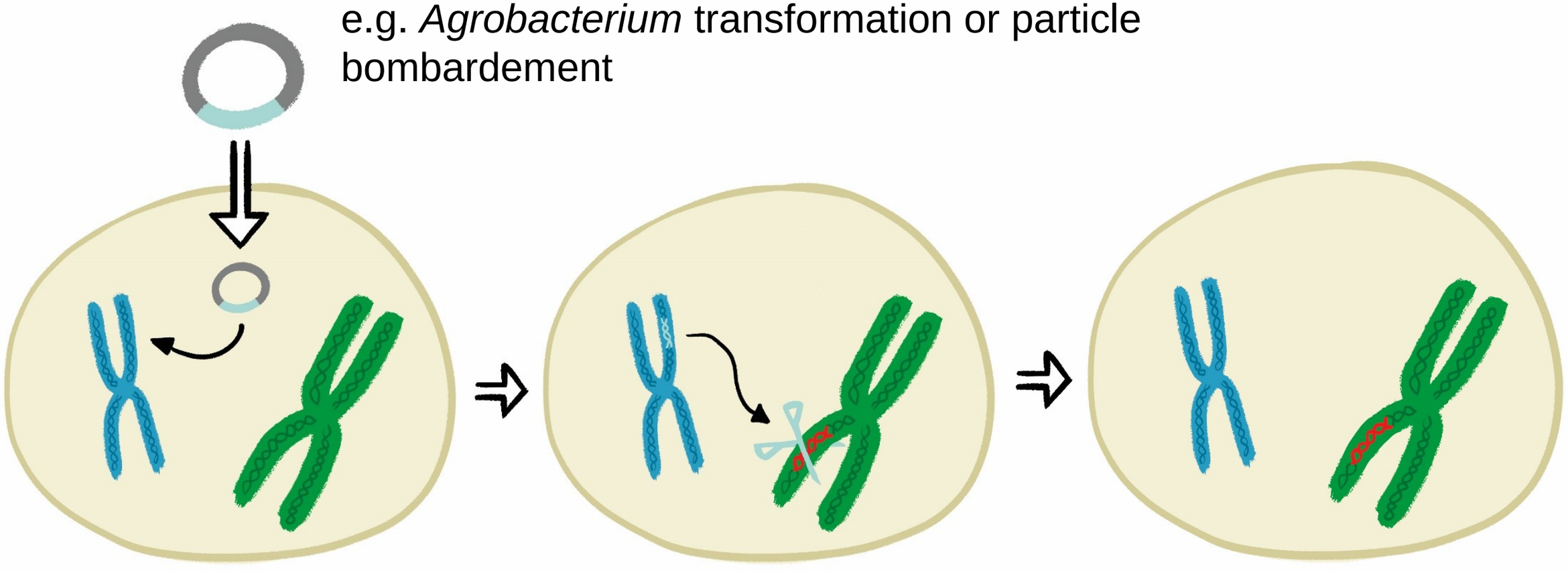
Unintended effects specific for CRISPR/Cas

3. Insertion of DNA-fragments at the target site

- Unintended integration of DNA donor templates (SDN-2/SDN-3)
- Unintended integration of endogenous DNA fragments
- Unintended integration of DNA-fragments derived from experimental procedure

Unintended effects specific for old genetic engineering techniques

Old genetic engineering techniques are still often used for delivery of CRISPR/Cas components.



Unintended effects specific for old genetic engineering techniques

- Unintended integration of DNA sequences (at multiple sites of the genome)
- Genomic DNA rearrangements as well as rearrangements of the transgenic loci
- Insertions, deletions
- Changes to the epigenome

What becomes possible through CRISPR/Cas?

Application of CRISPR/Cas can result in novel genotypes

- Alteration of multiple, identical DNA-sequences
- Multiplexing (simultaneous alteration of multiple, different DNA-sequences)
- Alteration of protected parts of the genome
- Alteration of genetically linked genes (overcoming the linkage drag)

Kawall K (2019) New Possibilities on the Horizon: Genome Editing Makes the Whole Genome Accessible for Changes. Front Plant Sci 10:525. doi:10.3389/fpls.2019.00525

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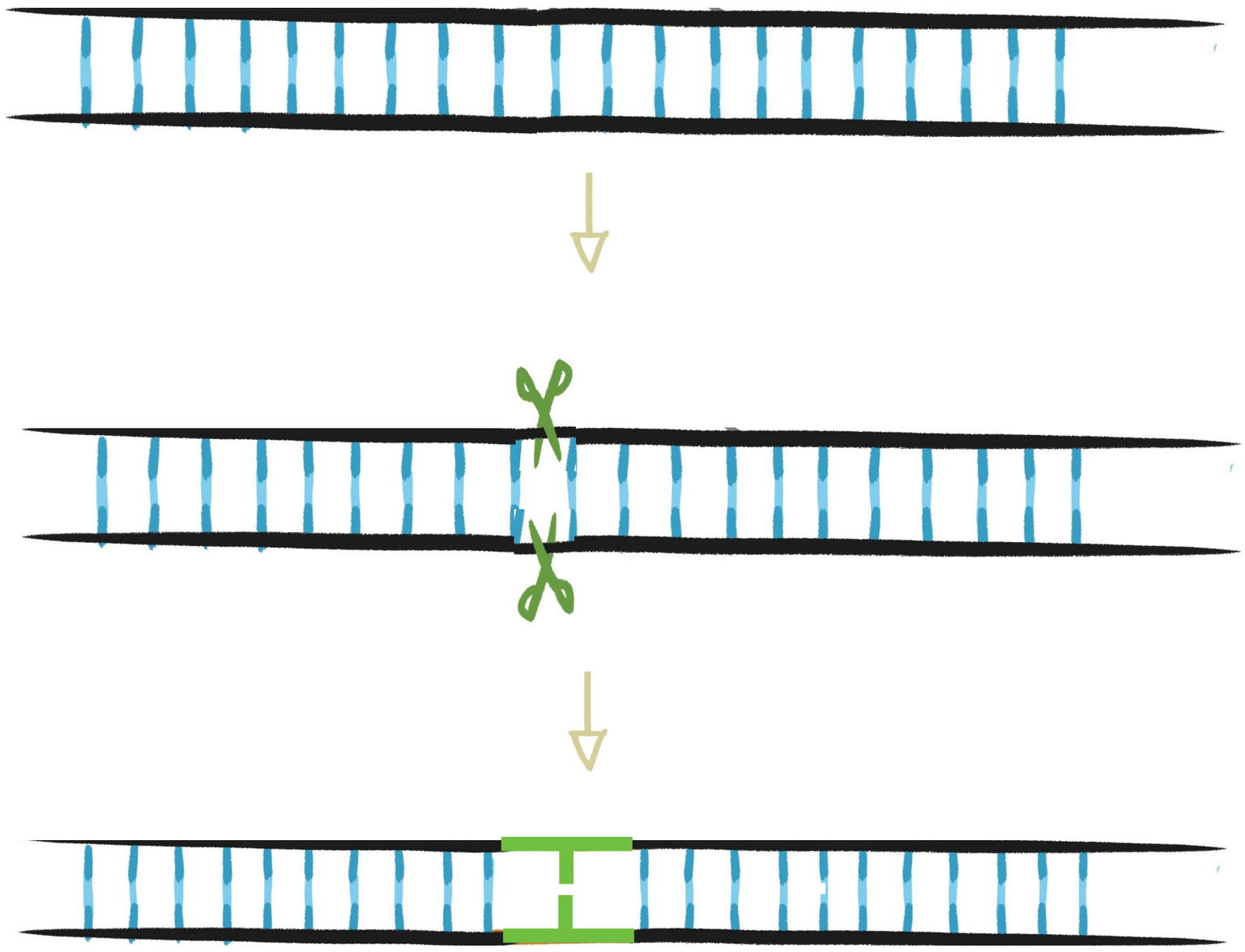
CRISPR/Cas can alter all DNA-regions containing the same target sequence



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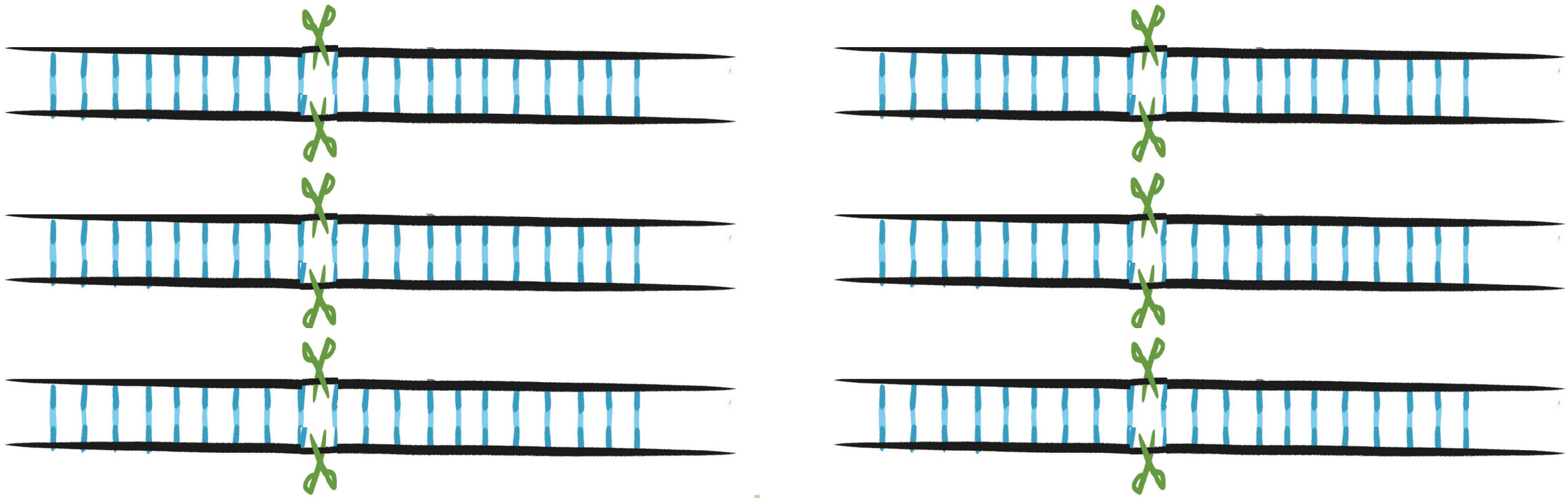
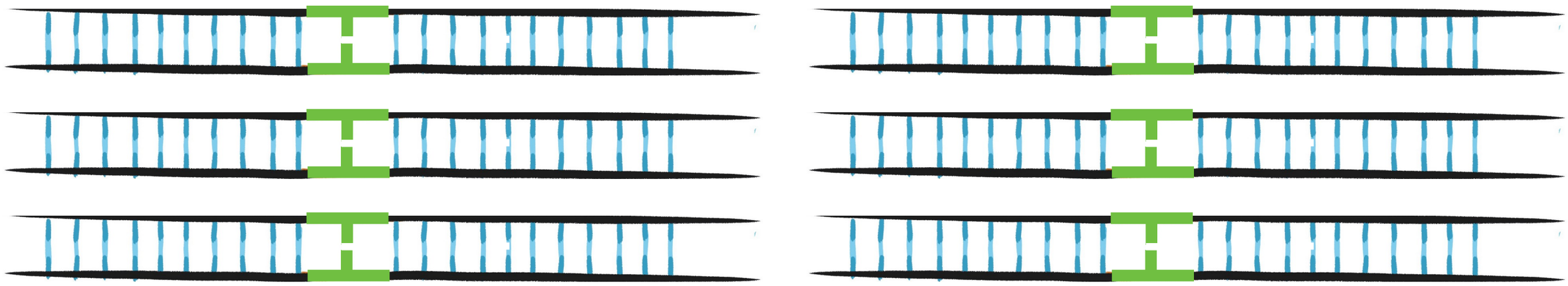


example: hexaploid wheat



CRISPR/Cas can alter all DNA-regions containing the same target sequence

- Gene knockouts in crops with multiple chromosome sets
- Gene copies/duplications (gene families)
- Gene dosage

CRISPR/Cas can alter all DNA-regions containing the same target sequence

Strawberry (octoploid):

Alteration of all alleles of FaTM6: Altered anther development

Martín-Pizarro C, Triviño JC, Posé D (2019) Functional analysis of the TM6 MADS-box gene in the octoploid strawberry by CRISPR/Cas9-directed mutagenesis. Journal of Experimental Botany 70 (3):885-895. doi:10.1093/jxb/ery400

Camelina (hexaploid):

Different allelic combinations of CsFAD2: alteration of fatty acid content

Morineau C, Bellec Y, Tellier F, Gissot L, Kelemen Z, Nogue F, Faure JD (2017) Selective gene dosage by CRISPR-Cas9 genome editing in hexaploid Camelina sativa. Plant Biotechnol J 15 (6):729-739. doi:10.1111/pbi.12671

Application of CRISPR/Cas can result in novel genotypes

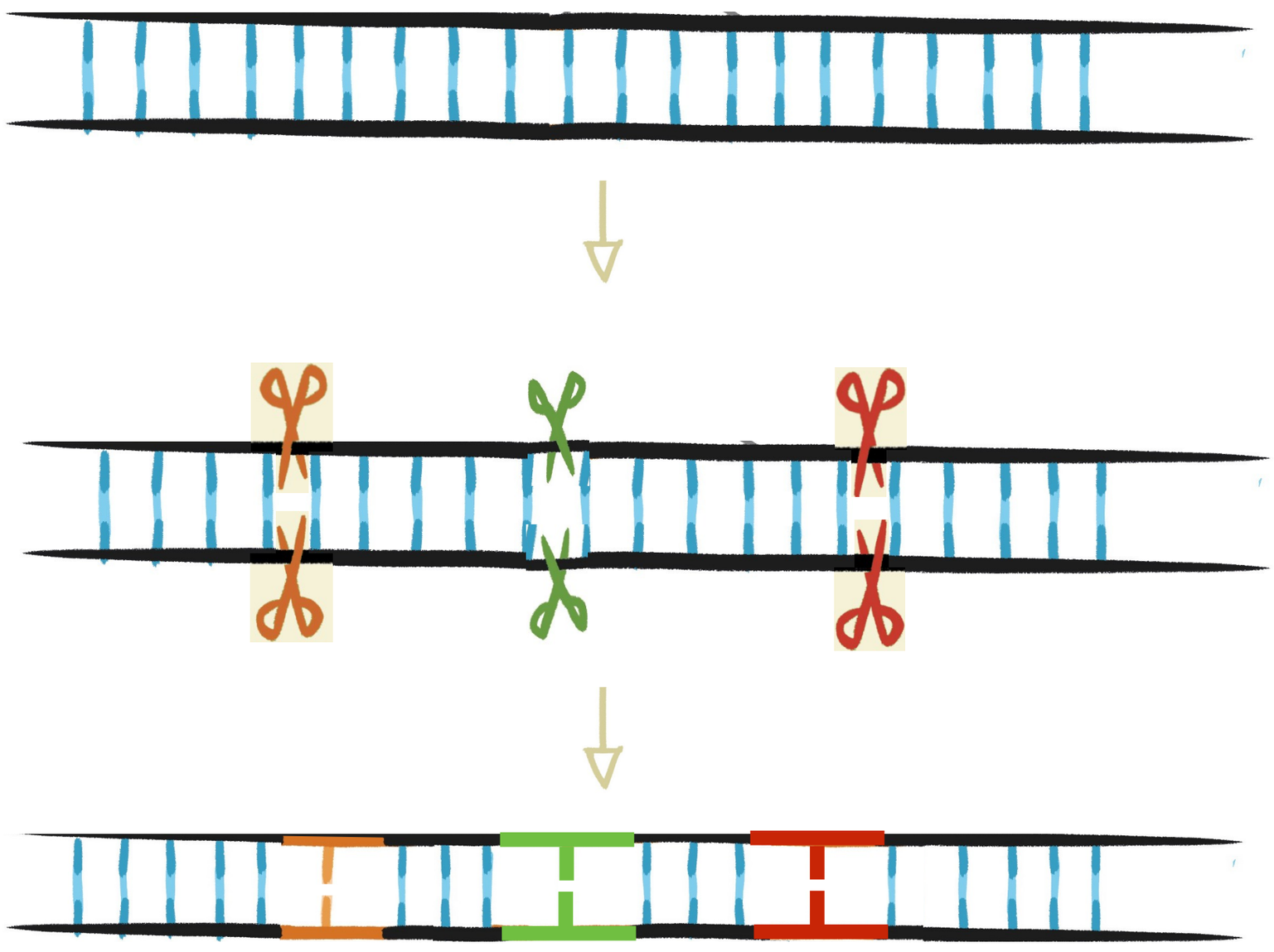
teration of multiple, identical DNA-sequences

ultiplexing (simultaneous alteration of multiple, different DNA-sequence

teration of protected parts of the genome

teration of genetically linked genes (overcoming the linkage drag)

Multiplexing



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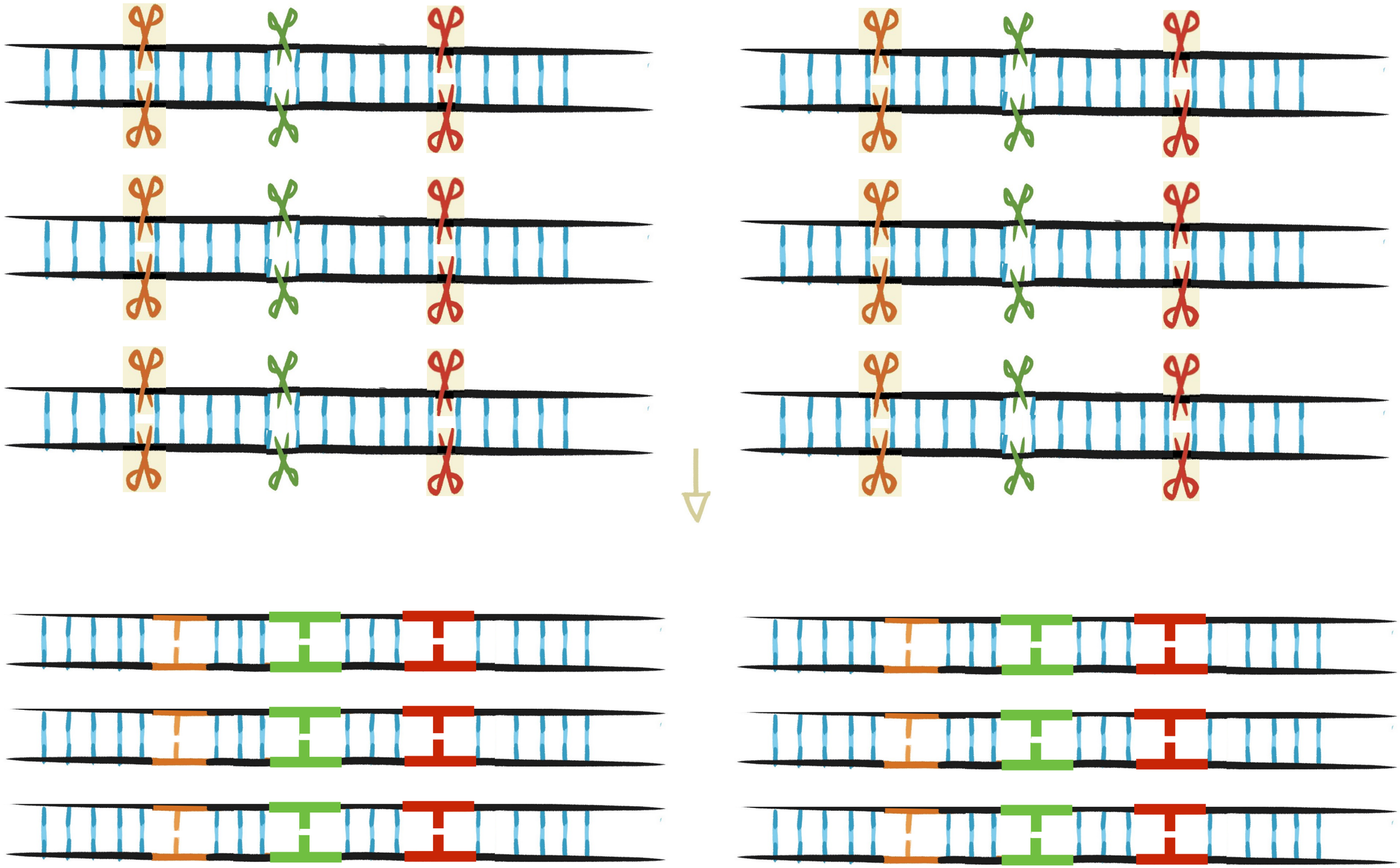
teration of genetically linked genes (overcoming the linkage drag)

Multiplexing plus altering all gene copies of a target gene



Multiplexing

- Increased complexity of the applications of CRISPR/Cas

  → powerful tool
- Many traits depend on the interplay of multiple genes
- Metabolic reprogramming (synthetic biology)

Multiplexing

Wheat (hexaploid):

Alteration of 3 different genes: higher yield

Wang, W., Akhunova, A, Chao, S, Trick H, Akhunov, E (2018). Transgenerational CRISPR-Cas9 activity facilitates multiplex gene editing
in allopolyploid wheat. The CRISPR Journal 1(1). doi: 10.1089/crispr.2017.0010.

Rice (diploid):

Alteration of 8 different genes : higher yield, altered growth, fragrance

Shen L, Hua Y, Fu Y, Li J, Liu Q, Jiao X, Xin G, Wang J, Wang X, Yan C, Wang K (2017) Rapid generation of genetic diversity by multiplex CRISPR/Cas9 genome editing in rice. Sci China Life Sci 60 (5):506-515. doi:10.1007/s11427-017-9008-8

Application of CRISPR/Cas can result in novel genotypes

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- **Alteration of protected parts of the genome**
- Alteration of genetically linked genes (overcoming the linkage drag)

Classical evolutionary theory says that the mutation rate is unbiased and independent of gene function and fitness consequences. New mutations can occur anywhere in the genome irrespective of the fitness consequence.

Mutation bias shapes gene evolution in *Arabidopsis thaliana*

Monroe, J. Grey[1,2†], Srikant, Thanvi[1], Carbonell-Bejerano, Pablo[1], Exposito-Alonso, Moises[3,4], Weng, Mao-Lun[5], Rutter, Matthew T.[6], Fenster, Charles B.[7], Weigel, Detlef[1†]

Research

DNA mismatch repair preferentially protects genes from mutation

Eric J. Belfield,[1,5] Zhong Jie Ding,[2,5] Fiona J.C. Jamieson,[1] Anne M. Visscher,[1,3] Shao Jian Zheng,[2] Aziz Mithani,[4] and Nicholas P. Harberd[1]

[1]Department of Plant Sciences, University of Oxford, Oxford OX1 3RB, United Kingdom; [2]State Key Laboratory of Plant Physiology and Biochemistry, College of Life Sciences, Zhejiang University, Hangzhou, 310058, China; [3]Comparative Plant and Fungal Biology, Royal Botanic Gardens, Kew, Wakehurst Place, Ardingly, West Sussex RH17 6TN, United Kingdom; [4]Department of Biology, Syed Babar Ali School of Science and Engineering, Lahore University of Management Sciences (LUMS), DHA, Lahore 54792, Pakistan

JBC ARTICLE

H3K36me3-mediated mismatch repair preferentially protects actively transcribed genes from mutation

Received for publication, March 9, 2018, and in revised form, March 26, 2018 Published, Papers in Press, April 2, 2018, DOI 10.1074/jbc.RA118.002839

Yaping Huang[‡], Liya Gu[§], and Guo-Min Li[‡§1]

CRISPR/Cas can induce mutations at any part of the genome

- The mutation rate across the genome is not random.
- Some functionally constrained regions of the genome mutate less.
- Mutation rate is dependent of certain repair mechanisms, the distance between certain gene regions and epigenetic modifications.

Using CRISPR/Cas it is possible now to induce mutations in gene coding regions that are naturally associated with lower mutation rates.

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Genetically linked genes

Tomato:

Decoupling of the fruit shape and the abscission zone.

Roldan MVG, Perilleux C, Morin H, Huerga-Fernandez S, Latrasse D, Benhamed M, Bendahmane A (2017) Natural and induced loss of function mutations in SlMBP21 MADS box gene led to jointless-2 phenotype in tomato. Sci Rep 7 (1):4402. doi:10.1038/s41598- 017-04556-1

Maize:

Reduction of the impact of linkage drag on yield.

Dong, L., Qi, X., Zhu, J., Liu, C., Zhang, X., Cheng, B., et al. (2019). Supersweet and waxy: meeting the diverse demands for specialty maize by genome editing. Plant Biotechnol J 17(10), 1853-1855. doi: 10.1111/pbi.13144.

Intended alterations can cause unintended effects.

Consequences for the genome-edited organism

- influence on the development
- alteration of the nutritional composition
- altered reaction under stress conditions
- reduced/enhanced fitness
- influence on the circadian rhythm
- altered flowering induction

How do these novel genotypes impact associated ecosystems?

It can be expected that in a short period of time several genome-edited organisms with novel traits will be released in case they are deregulated. The combinatorial and cumulative effects are hardly predictable.

Impact of the intended alterations on the associated ecosystem

- altered defense mechanisms
- altered intraspecific communication/communication between different species
- pollinators
- food web
- toxic effects
- altered composition of the microbiome

Summary

- CRISPR/Cas is a powerful, potent biotechnological tool
- CRISPR/Cas is mainly used for SDN-1 applications in plants.
- The application of CRISPR/Cas can cause unintended effects.
- CRISPR/Cas enables the generation of novel genotypes.
- Ecological risks associated with a release of genome-edited crops are not predictable.

Thank you

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