

Unintended genetic changes and effects in NGT plants that require risk assessment

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unintended genetic changes

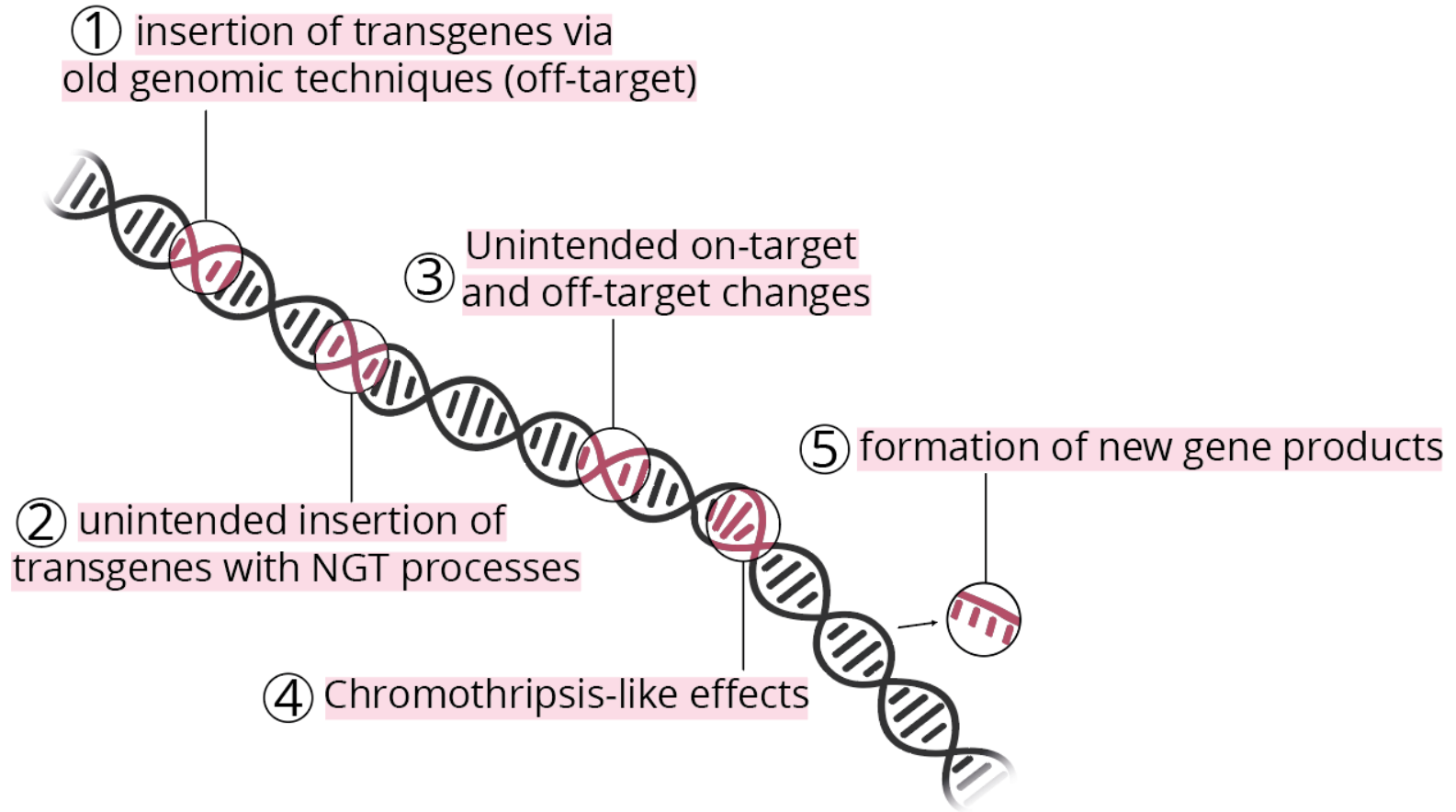
findings in regard to unintended genetic changes that can be caused by the application of NGTs*

***Koller, F. And Cieslak, M. (2023)** A perspective from the EU: Unintended genetic changes in plants caused by NGT – their relevance for a comprehensive molecular characterisation and risk assessment. *Front. Bioeng. Biotechnol.* 11:1276226. doi: 10.3389/fbioe.2023.1276226

NGTs

unintended genetic changes by NGTs

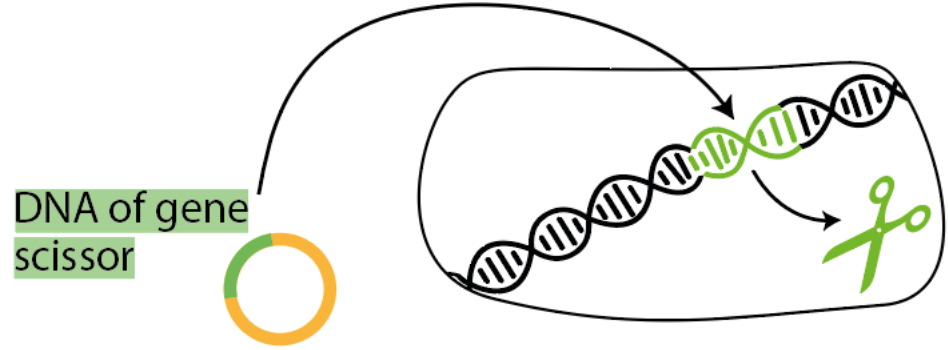
five categories of unintended genetic changes resulting from NGT processes based on scientific publications



① insertion of transgenes via old genomic techniques (off-target)



gene gun /
Agrobacterium tumefaciens

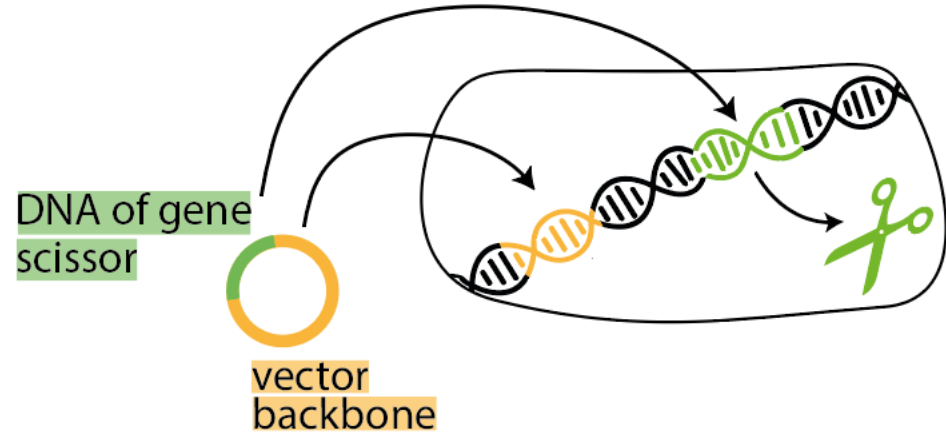


- non-targeted integration of transgenic DNA
- possible effects on epigenetic and disruption of genes
- remains of transgenic DNA after segregation breeding

① insertion of transgenes via old genomic techniques (off-target)



gene gun /
Agrobacterium tumefaciens

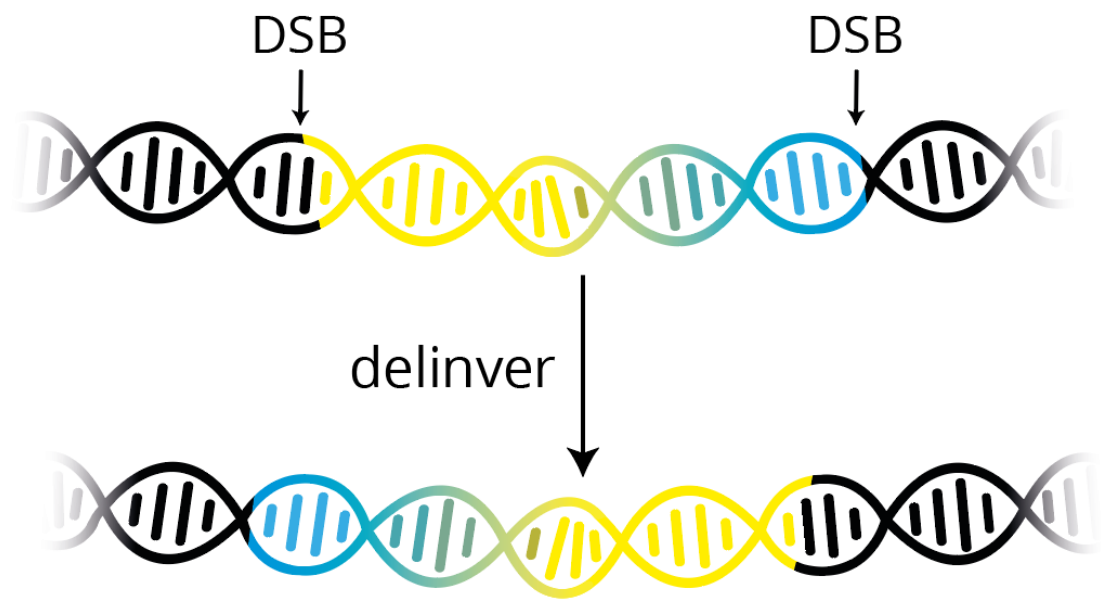
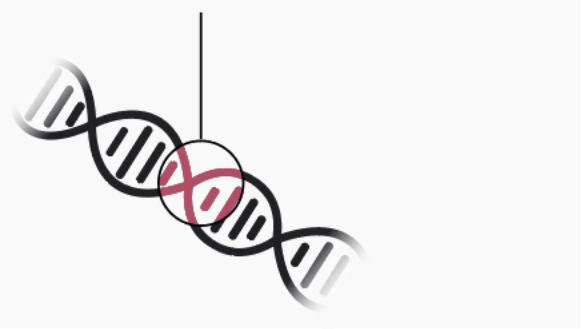


② unintended insertion of transgenes with NGT processes



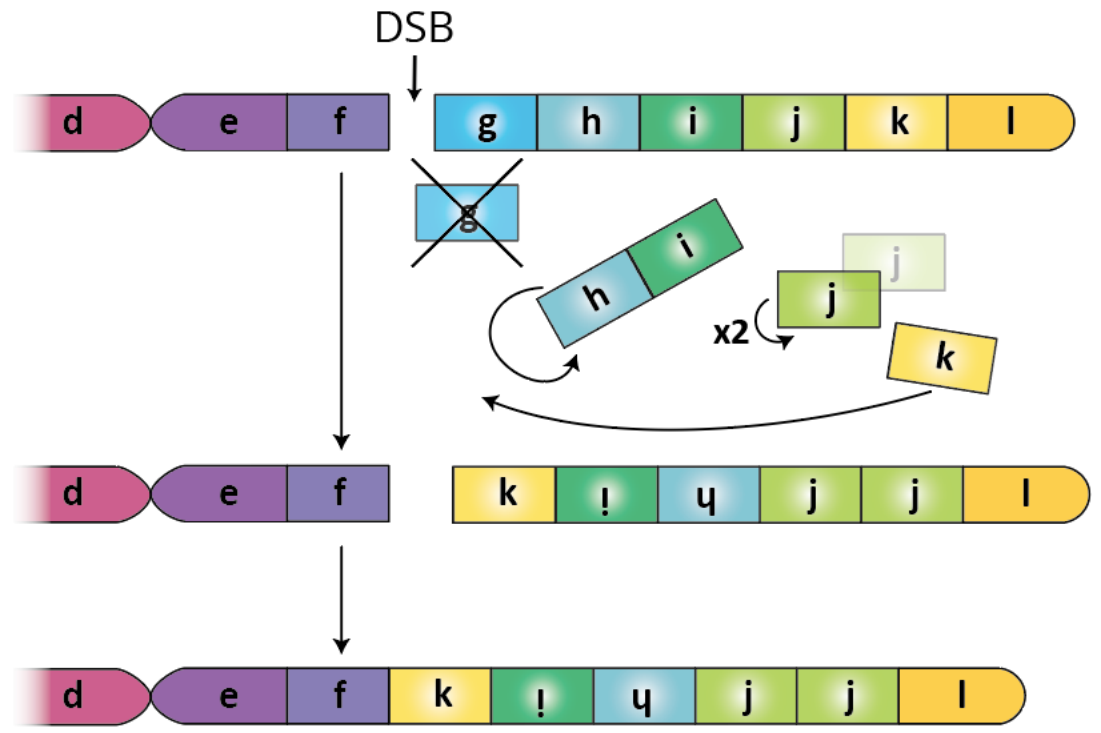
- unintended integration of parts of vector construct
- possible effects on epigenetic and disruption of genes
- partly difficult to detect

③ Unintended on-target and off-target changes



- off-target DNA cleavage of similar DNA sequences
- unintended genetic changes after DNA cleavage: large insertions, duplications
- partly difficult to detect
- occurrence at genomic sites that differ compared to conventional breeding

④ Chromothripsis-like effects

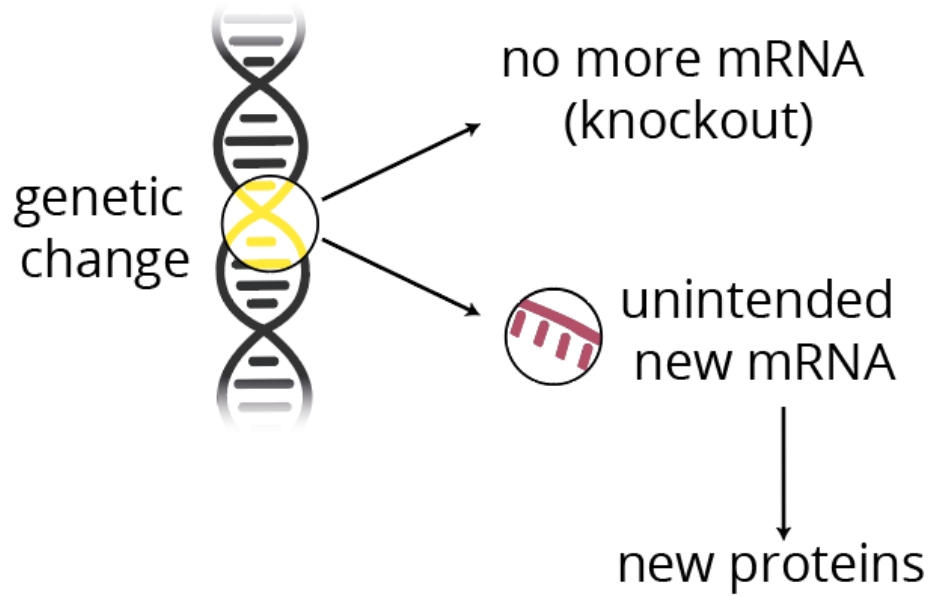
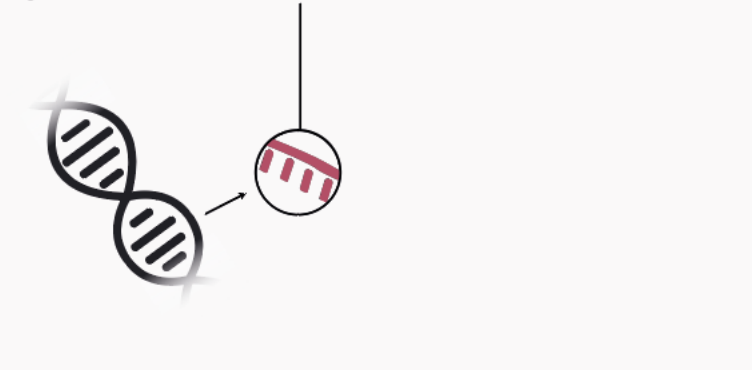


→ rearrangements after DSB

→ loss of DNA

→ probability of chromothripsis at specific genomic sites

⑤ formation of new gene products



→ formation of new mRNA caused by (un)intended genetic change

→ unpredictable function of new mRNA and new proteins

NGTs

unintended genetic changes by NGTs

- can affect small and large sections of chromosomes and result in unintended gene products
- the **sites of the mutations**, their **genomic context** and their **frequency (in regard to specific sites)** can differ when compared to conventionally bred plants
- the biological effects (phenotypes) can therefore be different and may cause specific risks for health and the environment

consequences for a comprehensive **molecular characterisation** and **risk assessment**:

- combination of **multiple approaches** to comprehensively identify and assess the unintended changes throughout the genome
- additional methods (**Omics**) to identify associated unintended biological effects

Thank you for your attention!

Liu et al (2023) Hidden prevalence of deletion-inversion bi-alleles in CRISPR-mediated deletions of tandemly arrayed genes in plants. *Nat Commun* 14, 6787

Samach et al (2023) CRISPR/Cas9-induced DNA breaks trigger crossover, chromosomal loss, and chromothripsis-like rearrangements. *The Plant Cell*, 35/11

Further references can be found in

Koller & Cieslak (2023) A perspective from the EU: Unintended genetic changes in plants caused by NGT – their relevance for a comprehensive molecular characterisation and risk assessment. *Front. Bioeng. Biotechnol.* 11:1276226.