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Biotechnology — Genome editing

Part 1: Vocabulary

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Foreword

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This document was prepared by Technical Committee ISO/TC 276, *Biotechnology*.

A list of all parts in the ISO 5058 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Genome editing technology is a fast-growing and rapidly advancing global bioscience field with applications in many biotechnology sectors. Genome editing is used to modify the nucleic acids of a genetic code, which can be composed of DNA or RNA, in a site-specific manner. Modifications can include insertion, deletion or alteration of nucleic acids. The technology operates by biochemical principles generally applicable to every kind of cell. Examples of genome editing technology applications with global significance include human cell-based therapeutics, agriculture, microbial based therapeutics, synthetic biology and biomanufacturing.

While genome editing technology is being actively utilized, there is a need for international standardization in terms and definitions for this field, so as to enhance interpretation and communication of concepts, data and results.

This document has been developed to provide a unified standard set of terms and definitions that serve the needs of biotechnology stakeholders and act as a reference for genome editing technology. Standards in the field of genome editing are intended to harmonize and accelerate effective communication, technology development, qualification and evaluation of genome editing products. This document is expected to improve confidence in and clarity of scientific communication, data reporting and data interpretation in the genome editing field. Specific requirements for the application of genome editing technologies in agriculture and food are not included. For specific requirements, users can consult standards developed by appropriate ISO Technical Committees, e.g. ISO/TC 34/SC 16 *Horizontal methods for molecular biomarker analysis*, or ISO/TC 215 *Health informatics*.

This document provides a vocabulary that standardizes the use and meaning of terms associated with genome editing. This document is organized into categories and sub-categories as follows:

— genome editing concepts (see 3.1);
— genome editing tools (see 3.2):
  — general (see 3.2.1);
  — CRISPR specific (see 3.2.2);
  — meganuclease specific (see 3.2.3);
  — megaTAL specific (see 3.2.4);
  — TALEN specific (see 3.2.5);
  — ZFN specific (see 3.2.6);
— genome editing outcomes (see 3.3).

Terms within categories are listed alphabetically. The sub-category “General” contains terms that apply to all types of genome editing tools. Additional sub-categories contain terms specific to the sub-category of genome editing technology: “CRISPR specific”, “Meganuclease specific”, “megaTAL specific”, “TALEN specific” and “ZFN specific”. An alphabetical list of all terms is given in the index. Definitions follow English word order wherever possible.

It is also recognized that genome editing is a rapidly developing and evolving biotechnology, and additional terms and definitions will be needed as genome editing technologies mature.

INTERNATIONAL STANDARD ISO 5058-1:2021(E)

Biotechnology — Genome editing —

Part 1: Vocabulary

1 Scope

This document defines terms related to genome editing technology.

This document is applicable to general use of genome editing across species.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

3.1 Genome editing concepts

3.1.1
gene editing
techniques for *genome engineering* (3.1.3) that involve nucleic acid damage, repair mechanisms, replication and/or recombination for incorporating site-specific modification(s) into a gene or genes

Note 1 to entry: Gene editing is a subclass of *genome editing* (3.1.2).

Note 2 to entry: There are various genome editing tools (see 3.2 and Figure 1).

3.1.2
genome editing
techniques for *genome engineering* (3.1.3) that involve nucleic acid damage, repair mechanisms, replication and/or recombination for incorporating site-specific modification(s) into a genomic DNA

Note 1 to entry: *Gene editing* (3.1.1) is a subclass of genome editing.

Note 2 to entry: There are various genome editing tools (see 3.2 and Figure 1).

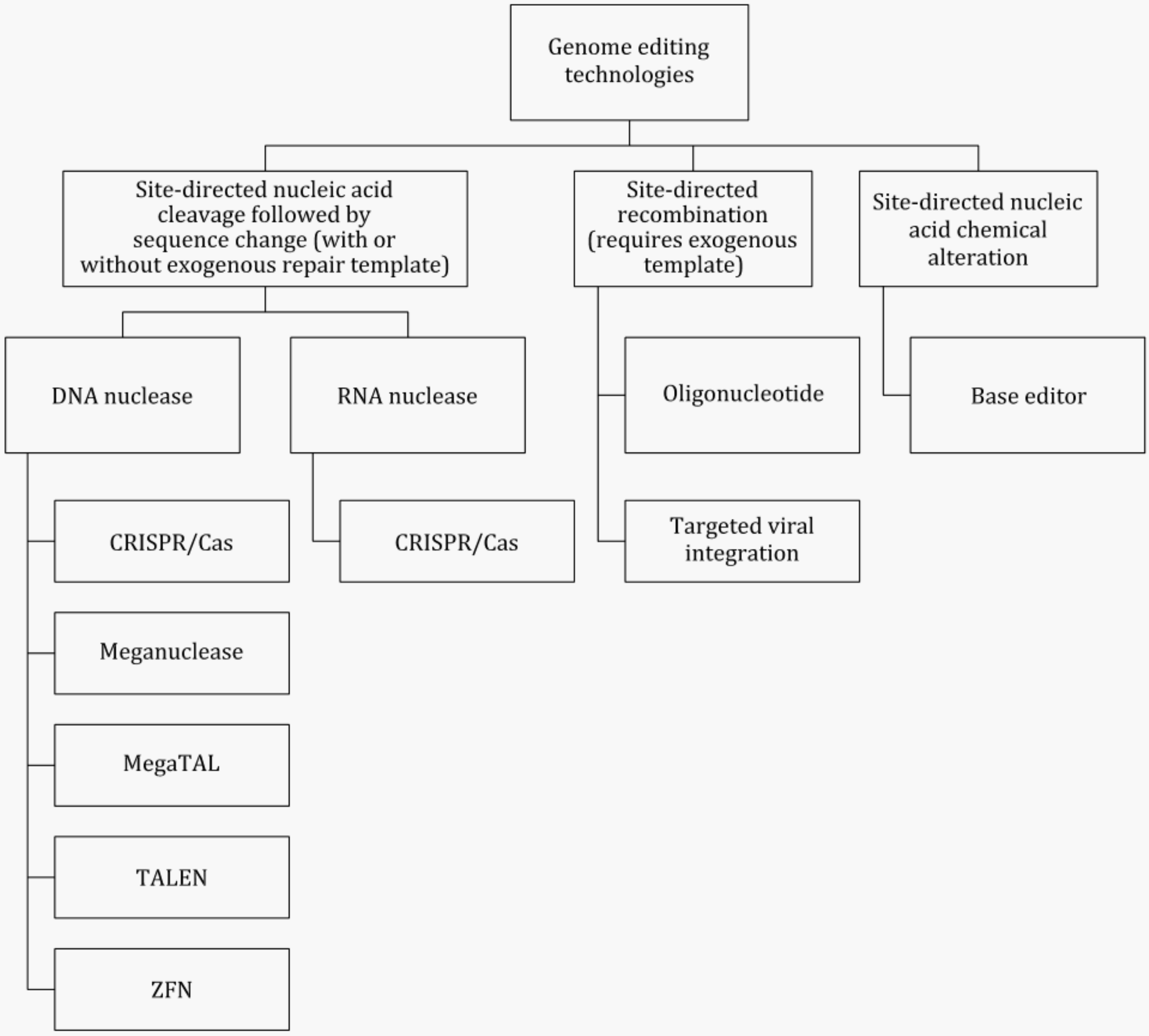

Figure 1 — Examples for genome editing technologies/tools

3.1.3
genome engineering
process of introducing intentional changes to genomic nucleic acid

Note 1 to entry: *Gene editing* (3.1.1) and *genome editing* (3.1.2) are techniques used in genome engineering.

3.1.4
off-target
genome editing off-target
genomic position and/or nucleic acid sequence distinct from the *target* (3.1.6)

EXAMPLE Off-target binding, off-target cleavage, off-target edit, off-target sequence change.

Note 1 to entry: An off-target edit is an example of an *unintended edit* (3.3.7).

3.1.5
specificity
genome editing target specificity
extent to which an editing agent or procedure acts only on its intended *target* (3.1.6)

Note 1 to entry: When using this term, the procedure is defined, the intended target is defined, the action or outcome is measured and reported, and limits of detection are reported.

3.1.6
target
genome editing target
nucleic acid sequence subject to intentional binding, modification and/or cleavage during a *genome editing* (3.1.2) process

Note 1 to entry: See also *off-target* (3.1.4), *Cas nuclease target site* (3.2.2.2), *meganuclease target site* (3.2.3.4), *megaTAL target site* (3.2.4.3), *TALEN target site* (3.2.5.4) and *ZFN target site* (3.2.6.5).

3.2 Genome editing tools

3.2.1 General

3.2.1.1
repair template
nucleic acid sequence used to direct cellular DNA repair pathways to incorporate specific DNA sequence changes at or near a *target* (3.1.6)

3.2.1.2
site-directed DNA modification enzyme
enzyme capable of modifying DNA at a specific sequence

EXAMPLE *Site-directed nuclease* (3.2.1.3), site-directed adenosine deaminase.

3.2.1.3
site-directed nuclease
sequence-specific nuclease
enzyme capable of cleaving the phosphodiester bond between adjacent nucleotides in a nucleic acid polymer at a specific sequence

3.2.2 CRISPR specific

3.2.2.1
Cas nuclease
CRISPR associated nuclease
enzyme that is a component of CRISPR systems that is capable of breaking the phosphodiester bonds between nucleotides

EXAMPLE Cas3, Cas9, Cas12a, Cas13, CasX.

Note 1 to entry: Some but not all Cas nucleases interact with a *gRNA* (3.2.2.4). See also *crRNA* (3.2.2.3), *sgRNA* (3.2.2.7) and *tracrRNA* (3.2.2.9).

3.2.2.2
Cas nuclease target site
nucleotide sequence comprising the *PAM* (3.2.2.5), in most cases, and a region that hybridizes to the target sequence specific guide of a Cas *RNP* (3.2.2.6)

3.2.2.3
crRNA
CRISPR RNA
polyribonucleotide that includes sequence complementarity to the *target* (3.1.6) and a sequence that interacts with a Cas protein and optionally *tracrRNA* (3.2.2.9)

Note 1 to entry: crRNA is a component of g*RNA* (3.2.2.4) or a complete gRNA, depending on the CRISPR system.

Note 2 to entry: In some CRISPR systems, a portion of the crRNA will base-pair with the tracrRNA (e.g. Cas9). Other CRISPR systems lack tracrRNA (e.g. Cas12a/Cpf1). In systems that do not require tracrRNA, the gRNA is called a "CRISPR RNA" or simply "crRNA".

3.2.2.4
gRNA
guide RNA
polyribonucleotide containing regions sufficient for productive interaction with a *Cas nuclease* (3.2.2.1) or variant to direct interaction with the specific *target* (3.1.6)

Note 1 to entry: See *crRNA* (3.2.2.3), *tracrRNA* (3.2.2.9) and *sgRNA* (3.2.2.7).

Note 2 to entry: For Cas9-type proteins, the natural gRNA comprises a crRNA that imparts sequence specificity and the tracrRNA that interacts with and activates the protein. This is sometimes referred to as a "dual guide". Other Cas proteins can have different gRNA structures.

Note 3 to entry: sgRNA for Cas9 proteins are non-naturally occurring polyribonucleotides where the crRNA and tracrRNA are fused with an artificial linker.

Note 4 to entry: In some cases, chemical modifications of the polyribonucleotide are used, such as modifications to the phosphodiester linkages, bases or sugar moieties. These can include substitution of DNA (2′-deoxy) or 2′-methoxy nucleotides for RNA nucleotides, etc.

3.2.2.5
PAM
protospacer adjacent motif
short nucleotide motif in the targeted region of nucleic acid required for guided *Cas nuclease* (3.2.2.1) or variant binding

Note 1 to entry: PAMs are distinct from, but in close proximity to, nucleic acid sequence targeted by g*RNA* (3.2.2.4).

3.2.2.6
RNP
ribonucleoprotein
complex comprising protein bound to RNA

Note 1 to entry: In the context of CRISPR-based *genome editing* (3.1.2), RNP refers to the complex of Cas protein(s) and g*RNA* (3.2.2.4).

3.2.2.7
sgRNA
single-guide RNA
fusion of *crRNA* (3.2.2.3) and *tracrRNA* (3.2.2.9)

Note 1 to entry: See g*RNA* (3.2.2.4).

3.2.2.8
target strand
CRISPR target strand
single-stranded nucleic acid sequence that is complementary to the g*RNA* (3.2.2.4) of a Cas protein or variant

3.2.2.9
tracrRNA
trans-activating CRISPR RNA
polyribonucleotide that base-pairs with the *crRNA* (3.2.2.3) and interacts with a *Cas nuclease* (3.2.2.1) to enable sequence-specific interaction of the *target* (3.1.6)

Note 1 to entry: tracrRNA is an optional component of g*RNA* (3.2.2.4).

3.2.3 Meganuclease specific

3.2.3.1
meganuclease
variant of the LAGLIDADG subtype of homing endonucleases engineered to recognize a 15 to 40 base pair DNA *target* (3.1.6) different from the site recognized by the parent endonuclease

Note 1 to entry: The LAGLIDADG consensus sequence represents an alpha helix that serves as a dimerization interface and key component in the DNA cleavage site in this family of meganucleases.

3.2.3.2
meganuclease linker
natural or artificially derived polypeptide sequence that links two LAGLIDADG domains to one another to form a single polypeptide chain

Note 1 to entry: The LAGLIDADG consensus sequence represents an alpha helix that serves as a dimerization interface and key component in the DNA cleavage site in this family of *meganucleases* (3.2.3.1).

3.2.3.3
meganuclease single chain
meganuclease (3.2.3.1) composed of two LAGLIDADG domains joined by either a natural or artificially derived polypeptide linker in order to be expressed as a single polypeptide chain

Note 1 to entry: The LAGLIDADG consensus sequence represents an alpha helix that serves as a dimerization interface and key component in the DNA cleavage site in this family of meganucleases.

3.2.3.4
meganuclease target site
DNA sequence recognized by *meganucleases* (3.2.3.1)

Note 1 to entry: Meganuclease target sites are 15 to 40 base pair DNA sequence consisting of two equal length half sites separated by a 4 base pair middle sequence (also known as “central 4”). Cleavage occurs at the junction of the half sites and the middle site on each DNA strand leaving a 4 nucleotide 3′ overhang.

3.2.4 megaTAL specific

3.2.4.1
megaTAL
artificial chimeric nucleases composed of an array of transcription activator-like (TAL) effector (TALE) [1] DNA binding domains, a *megaTAL linker* (3.2.4.2) and a *meganuclease* (3.2.3.1)

3.2.4.2
megaTAL linker
amino acid sequence that links an array of TAL DNA binding domains and a *meganuclease* (3.2.3.1)

3.2.4.3
megaTAL target site
intended DNA binding site of a *megaTAL* (3.2.4.1), encompassing the DNA sequence targeted by both the TAL array and the *meganuclease* (3.2.3.1)

3.2.5 TALEN specific

3.2.5.1
RVDs
repeat variable diresidue
two amino acid sequence in TAL repeats that imparts DNA binding specificity

3.2.5.2
TALEN
transcription activator-like effector nuclease
artificial nuclease composed of an endodeoxyribonuclease fused to DNA-binding domains of TALEs[1] that cleave DNA at a defined distance from TALE recognition sequences

Note 1 to entry: A TALEN can refer to a pair of TALE-FokI fusion proteins that dimerize on opposite strands of DNA adjacent to a *target* (3.1.6) for cleavage.

3.2.5.3
TALEN linker
polypeptide sequence that links an array of TAL DNA binding domains and an endodeoxyribonuclease, typically FokI

3.2.5.4
TALEN target site
DNA sequence recognized by *TALENs* (3.2.5.2)

Note 1 to entry: Typical TALEN target sites are recognized by a pair of TALENs and contain a central spacer region flanked by upstream and downstream sequences that are each recognized by one TALEN. This pair is designed in such a way that two TALEN nuclease domains dimerize to cleave DNA within the spacer region.

3.2.6 ZFN specific

3.2.6.1
zinc finger
Cys2His2 zinc finger
DNA binding domain that folds via coordination of zinc into a compact structure consisting of two beta strands and one alpha-helix (β β α)

Note 1 to entry: Zinc finger DNA binding domains typically contain 28 amino acids.

3.2.6.2
ZFN
zinc finger nuclease
chimeric protein consisting of an array of *zinc fingers* (3.2.6.1) linked to a DNA cleavage domain

Note 1 to entry: FokI is prevalently used as the DNA cleavage domain bound to a zinc finger.

Note 2 to entry: Binding of two ZFNs to a pair of appropriately spaced DNA target sites enables nuclease domain dimerization and DNA cleavage between the targets.

3.2.6.3
ZFN linker
polypeptide sequence that links an array of *zinc finger* (3.2.6.1) binding domains and a DNA cleavage domain

Note 1 to entry: FokI is prevalently used as the DNA cleavage domain bound to a zinc finger.

3.2.6.4
ZFN recognition helix
seven residue positions within a *zinc finger* (3.2.6.1) that are most directly responsible for its DNA binding preference

Note 1 to entry: The seven residues comprise the first six residues of the alpha helix, along with the residue immediately preceding the N-terminal of the helix. They are typically referred to as positions +1 to +6 (within the alpha helix) and position (–1) (immediately preceding the helix).

3.2.6.5
ZFN target site
DNA sequence recognized by a pair of *ZFNs* (3.2.6.2)

Note 1 to entry: Typical ZFN target sites contain a central spacer region flanked by DNA sequences that are each recognized by an array of *zinc fingers* (3.2.6.1) oriented such that the ZFN nuclease domains dimerize and cleave within the spacer.

3.2.6.6
ZFP
zinc finger protein
DNA binding protein consisting of a tandem array of multiple *zinc fingers* (3.2.6.1)

3.3 Genome editing outcomes

3.3.1
edit
DNA edit
RNA edit
epigenome edit
DNA, RNA or epigenome edit
modification to nucleic acid sequence resulting from the application of *genome editing* (3.1.2) components

EXAMPLE Insertion, deletion, substitution, deamination, methylation, demethylation.

Note 1 to entry: Genome editing components can include a nuclease and *repair template* (3.2.1.1).

3.3.2
HDR
homology-directed repair
mechanism of recombinational DNA repair[2] where repair is templated by a polynucleotide with regions corresponding to sequences flanking the *target* (3.1.6)

EXAMPLE Single-stranded DNA oligonucleotide templated HDR.

Note 1 to entry: *Repair templates* (3.2.1.1) can be exogenously introduced to achieve sequence changes in *genome editing* (3.1.2) approaches.

3.3.3
indel
InDel mutation
sequence change caused by the insertion and/or deletion of nucleotides

3.3.4
intended edit
designed modification to a *target* (3.1.6) resulting from the application of *genome editing* (3.1.2) components

Note 1 to entry: See *edit* (3.3.1).

Note 2 to entry: Genome editing components can include a nuclease and *repair template* (3.2.1.1).

3.3.5
MMEJ
microhomology-mediated end joining repair
mechanism of DNA end-joining repair[3] where the DNA ends are rejoined to each other using short regions of homology flanking the initiating double-stranded break to align the ends for repair

Note 1 to entry: MMEJ repair of DNA breaks in *genome editing* (3.1.2) approaches can result in deletion between pairs of microhomology regions.

Note 2 to entry: Short regions of homology for MMEJ are typically 2 to 25 base pairs.

3.3.6
NHEJ
non-homologous end joining
mechanism of DNA end-joining repair[3] in which DNA ends are joined in a homology-independent manner

Note 1 to entry: NHEJ repair of DNA breaks in *genome editing* (3.1.2) workflows can result in *indel* (3.3.3) formation.

3.3.7
unintended edit
modification to nucleic acid at an *off-target* (3.1.4) resulting from the application of *genome editing* (3.1.2) components

Note 1 to entry: See *edit* (3.3.1).

Note 2 to entry: Genome editing components can include a nuclease and *repair template* (3.2.1.1).

4 Abbreviated terms

DNA	deoxyribonucleic acid
CRISPR	clustered regularly interspaced short palindromic repeats
RNA	ribonucleic acid
TAL	transcription activator-like
TALE	transcription activator-like effector

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