Genome Engineering Methods

Michelle Yue
Bio-Bootcamp Part I
1/19/26

Overview of Genome Engineering Methods

Major strategies:

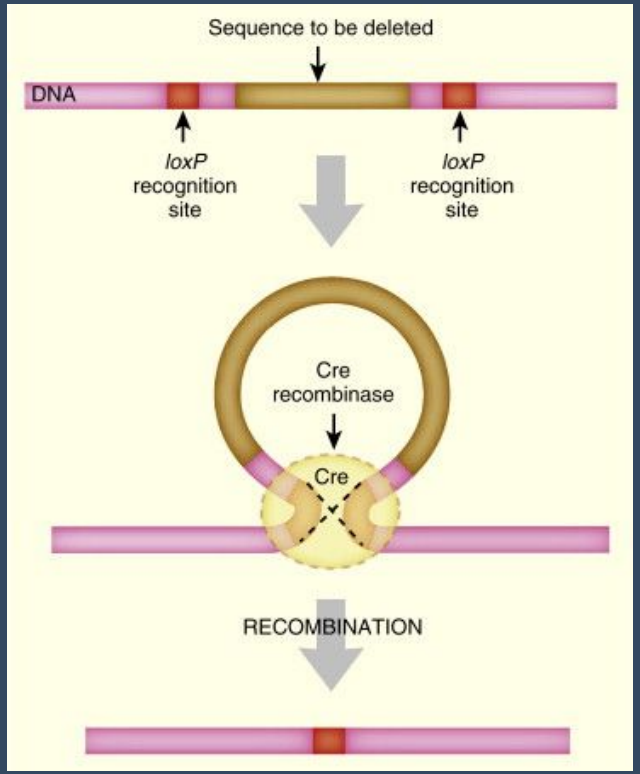
- ➔ Site specific recombinases
- ➔ Double-stranded break-based Nucleases

Site Specific Recombinase (SSR) Method:

1. SSRs recognize and bind two specific, short DNA sequences (recognition sites) inserted at a genomic region of interest
2. SSRs make a double-stranded break
3. SSRs recombine the DNA strands

Commonly Used SSRs:

- Cre-LoxP:
  Cre (SSR) recognizes LoxP sites
- Flp-FRT:
  Flp (SSR) recognizes FRT sites



Source: D.S.T. (2023) An introduction to genetic engineering, Fourth Edition, Cambridge University Press

Image sources: https://blog.addgene.org/plasmids-101-cre-lox
https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/site-specific-recombination

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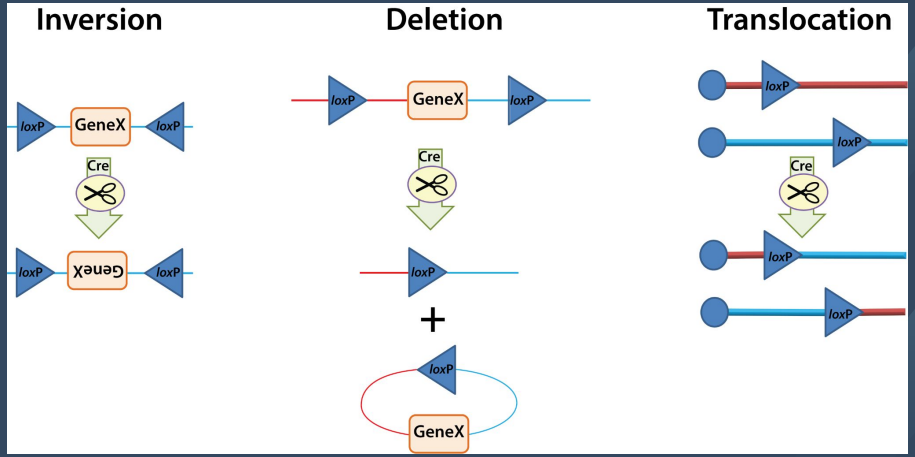

Types of Modifications:

- Inversions
- Insertions or Deletions
- Replacements
- Translocations

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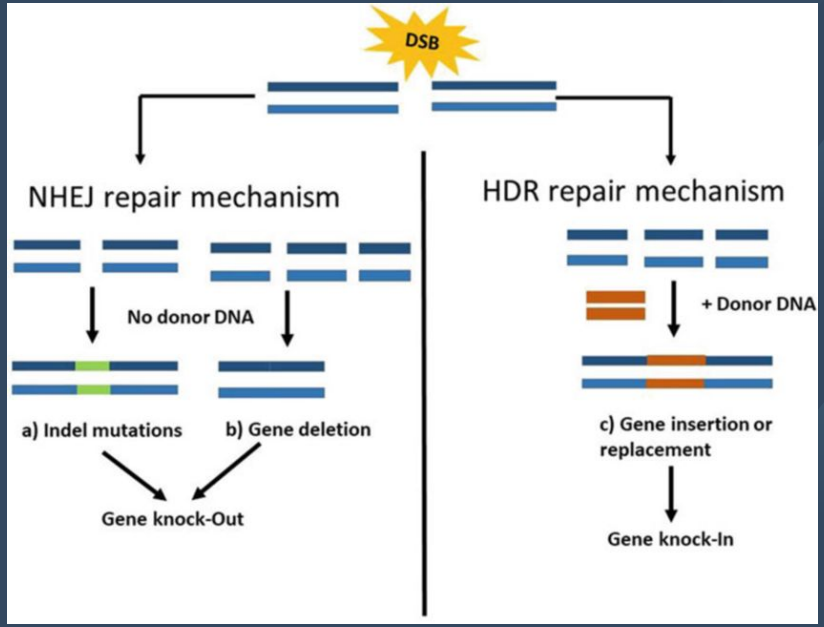


Double-stranded break-based Nucleases:

1. Nuclease creates double-stranded break (DSB) at specific DNA sequence
2. DSB triggers cellular repair mechanisms
    a. Non-Homologous End Joining (NHEJ)
    b. Homology-Directed Repair (HDR)
        i. Correct mutations
        ii. Create mutations

Examples:

1. Zinc-Finger Nucleases (ZFNs)
2. TALENs
3. CRISPR-Cas9

Source: D.S.T. (2023) An introduction to genetic engineering, Fourth Edition, Cambridge University Press

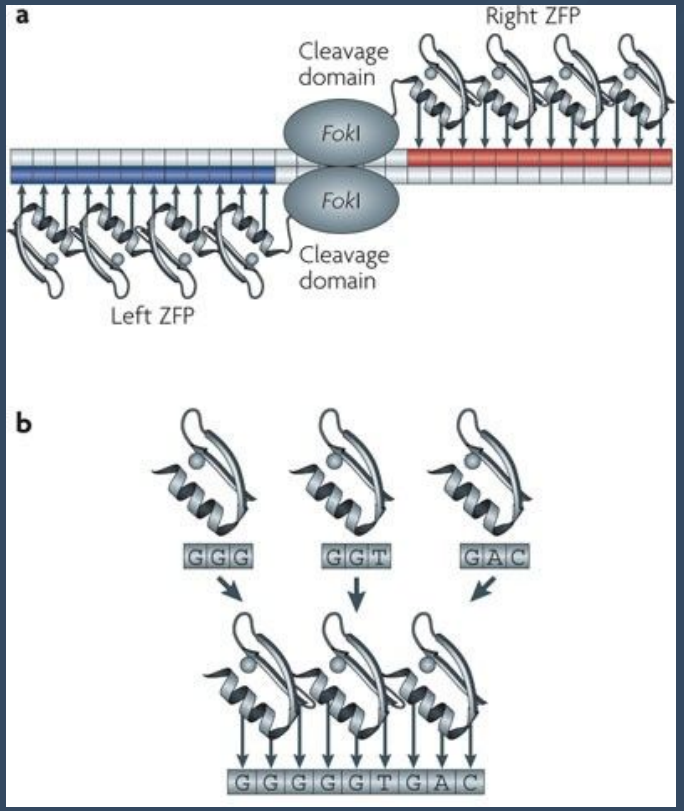
Image Source: https://www.researchgate.net/profile/Pallavi-Dhiman/publication/368520942/figure/fig1/AS:11431281178830526@1691083746202/Generalized-scheme-showing-non-homologous-end-joining-NHEJ-and-homologous-recombinant.png

Zinc Finger Nucleases (ZFNs)

Major strategies:

- ➔ Site specific recombinases
- ➔ Double-stranded break-based Nucleases

- Modular zinc finger (ZF) protein domains, each with a zinc-ion coordination center
- ZFs recognize DNA triplets → multiple ZFs linked) bind longer DNA sequences
- ZF + type II endonuclease (FokI) = ZFN
- ZFNs act as dimers:
  - Left and right ZFNs bind opposite DNA strands
  - Dimerization of FokI nuclease domains induces DSB
- DSB is prepared by cellular repair mechanisms (NHEJ or template + HDR)

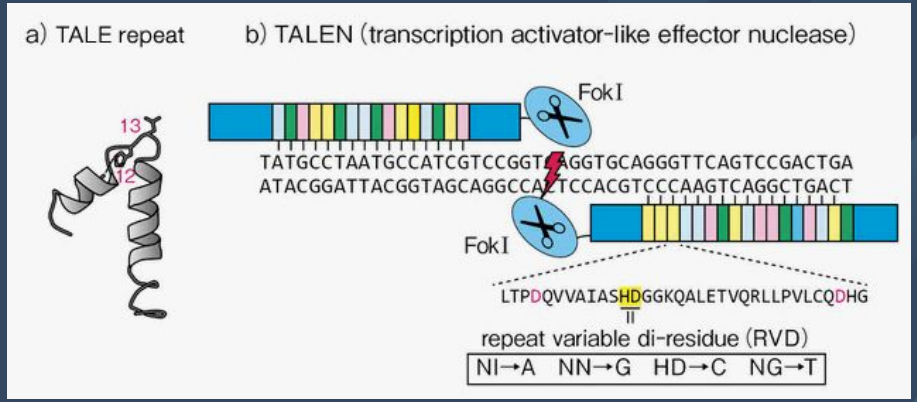


Source: D.S.T. (2023) An introduction to genetic engineering, Fourth Edition, Cambridge University Press
Image Source: https://www.nature.com/articles/nrg2842

Transcription Activator-Like Effector Nucleases (TALENs)

Major strategies:

- ➔ Site specific recombinases
- ➔ Double-stranded break-based Nucleases



- TALEs:
  - DNA-binding proteins
  - TALE repeats have conserved amino acid residues (except 2 AA)
    - Each repeat contains a repeat variable diresidue (RVD) that recognizes and binds to a specific DNA base
- TALE sequence specificity can be customized by changing RVD order
- TALE + FokI endonuclease = TALEN
- TALENs act as dimers:
  - Left and right ZFNs bind opposite DNA strands
  - Dimerization of FokI nuclease domains induces DSB
- DSB is prepared by cellular repair mechanisms (NHEJ or template + HDR)

Source: D.S.T. (2023) An introduction to genetic engineering, Fourth Edition, Cambridge University Press
Image Source: https://www.mls.sci.hiroshima-u.ac.jp/smg/GEIN/en/map/tale/index.html

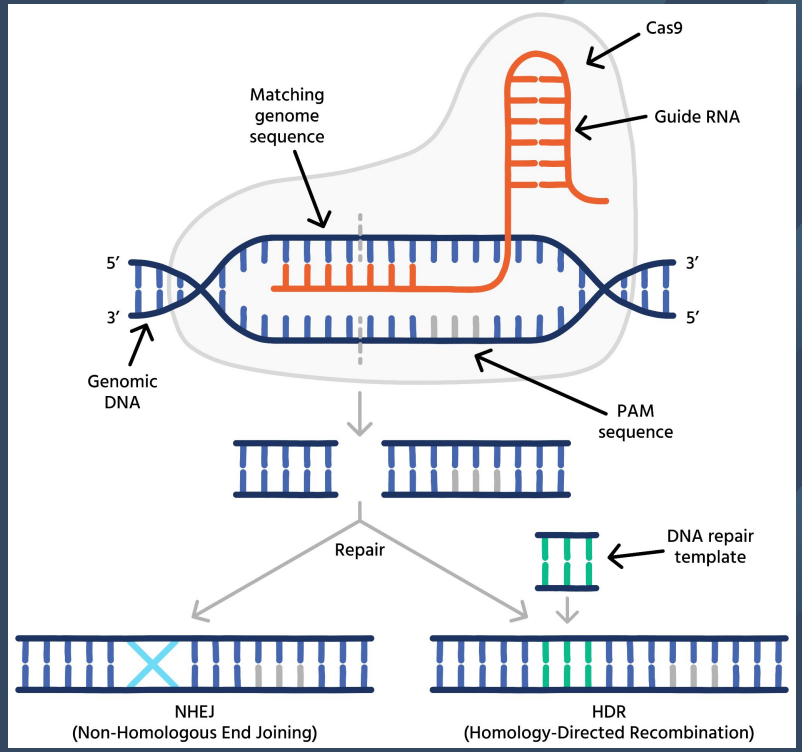
CRISPR-Cas9

Major strategies:

- ➔ Site specific recombinases
- ➔ Double-stranded break-based Nucleases

- Cas9 (endonuclease)
  - Recognizes Protospacer Adjacent Motif (PAM) in target DNA
  - Single guide RNA (sgRNA) directs it to target DNA

- sgRNA = CRISPR RNA + trans-activating RNA (tracrRNA)
  - CRISPR = clustered regularly interspaced short palindromic repeats
  - CRISPR RNA = complementary and binds to target DNA
  - tracrRNA: binds to Cas9 complex



Source: D.S.T. (2023) An introduction to genetic engineering, Fourth Edition, Cambridge University Press
Image Source: https://iotasciences.com/wp-content/uploads/2024/06/CRISPR-Cas9_mechanism.jpg

- sgRNA binds to the target DNA → Cas9 creates a double-stranded break (DSB) in DNA
- DSB is repaired by cellular repair mechanisms (NHEJ or template + HDR)

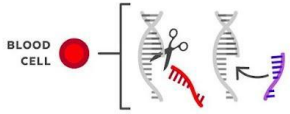
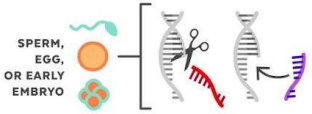
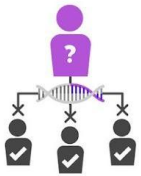
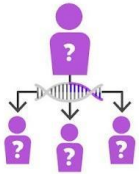
Types of Gene Function Modifications

Gene function can be modified in various ways.

Some types of gene modifications include...

Type of Modification	Description
Gene Knockout	Complete or partial loss of gene function. Achieved by deleting the gene, introducing disruptive mutations, or inserting non-functional sequences.
Gene Knock-in	Introduction of a specific genetic modification at a defined locus. Achieved by inserting a transgene (foreign DNA) or other functional sequences to alter gene activity (gain-of-function).
Gene Knockdown/Silencing	Reduction or temporary suppression of gene expression. Achieved by blocking transcription initiation or elongation (e.g., CRISPRi) or targeting mRNA for degradation (e.g., RNAi or antisense oligonucleotides).
Gene Activation	Increased or temporary activation of gene expression. Achieved by targeting regulatory regions (e.g., promoters or enhancers) with transcriptional activators (e.g., CRISPRa or VP64 fusion proteins).

Gene Editing — Timing Matters!

	SOMATIC GENE EDITING		VS.	GERMLINE GENE EDITING	
EDIT		Somatic therapies target genes in specific types of cells (blood cells, for example).			Germline modifications are made so early in development that any change is copied into all of the new cells.
COPY		The edited gene is contained only in the target cell type. No other types of cells are affected.			The edited gene is copied in every cell, including sperm or eggs.
RISKS **NEXT GENERATION**		Any changes, including potential off-target effects, are limited to the treated individual. The edited gene is not passed down to future generations.			If the person has children, the edited gene is passed on to future generations.
CONSENSUS		Somatic cell therapies have been researched and tested for more than 20 years and are highly regulated.			Human germline editing is new. Heritability of germline changes presents new legal and societal considerations.

Thank You!

Next up: 5 minute break

Then, Lisa will discuss phage therapy!