

# Molecular Basis of Genome Editing



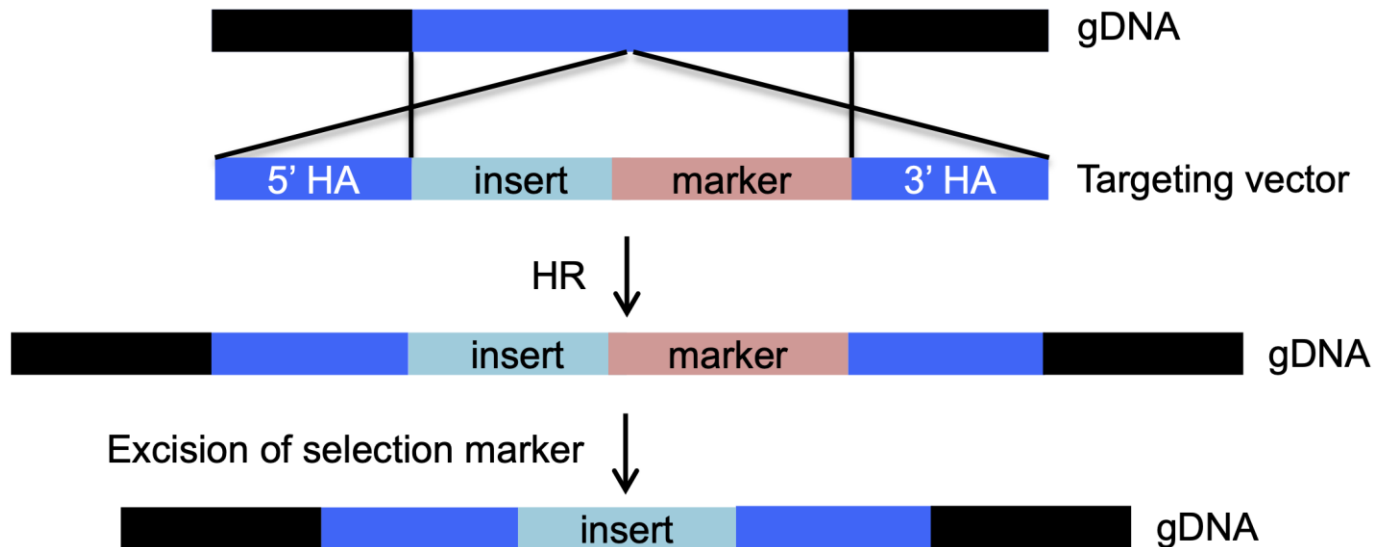
This course is designed to introduce students to basics and applications of genome editing and engineering.

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# Molecular Basis of Genome Editing

**Genome editing** refers to altering an organism's genetic code. Alterations include deleting nucleotides to knock-out a gene, adding nucleotides to knock-in a protein, or editing nucleotides to create a mutation. Gene editing can occur at the DNA, RNA, or epigenetic level.

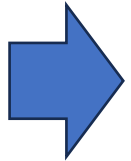
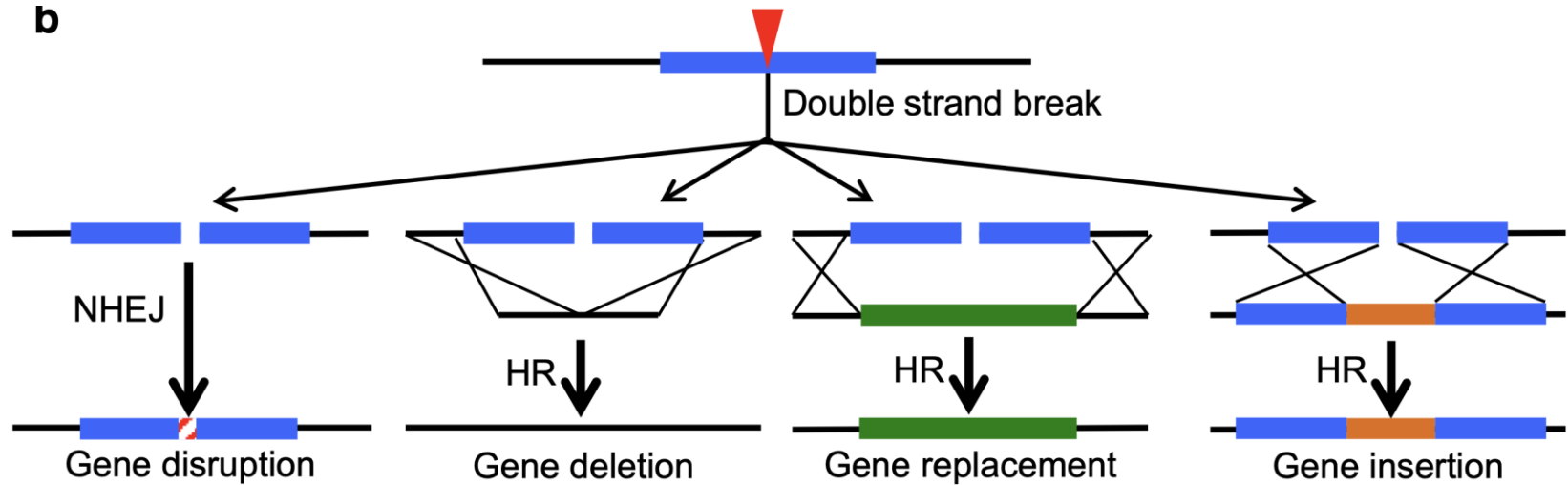
Historically, homologous recombination (HR), in which undamaged homologous DNA fragments are used as templates, has been the approach to realize targeted gene addition, replacement, or inactivation.



However, the utility of HR is heavily limited due to its inefficiency.

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Then, it was discovered that DSBs could **raise the incidence of HDR** by multiple orders of magnitude.



**Targeted nucleases** have been found as an alternative approach to increase the efficiency of HDR-mediated genetic alteration. Once a targeted DSB has been made, HDR may reconstruct the cleaved DNA using an exogenous DNA template analog to the break site sequence.

# **Molecular Basis of Genome Editing**

## **Syllabus:**

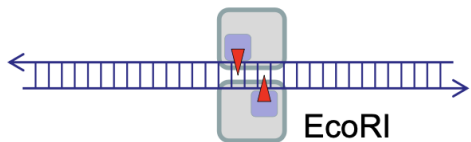
- Introduction to genes and genomes
- Physical structure of the genomic material
- Breakage and repair of genomic DNA
- DNA recombination
- DNA repair

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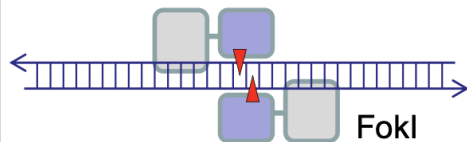
- Conventional approaches to genome editing (homologous recombination), chemical methods and techniques based on homing endonucleases
- Genome editing based on modern methodologies: using proteins: mega-nucleases, zinc-finger nucleases, Transcription Activator-Like Effector Nucleases (TALENs)
- Using RNA-based systems: Clustered regularly interspaced short palindromic repeats (CRISPR-CAS systems).

Natural  
endonucleases

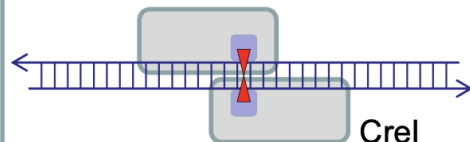
(A) Type II-Restriction nuclease



(B) Type IIS-Restriction nuclease

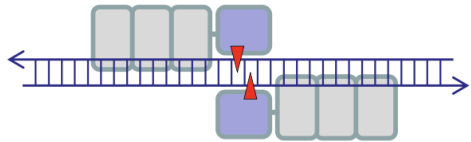


(C) Homing endonuclease

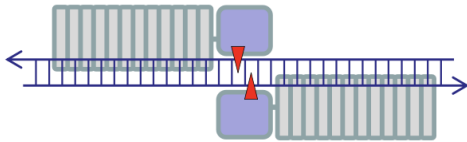


Synthetic  
FokI-fusions

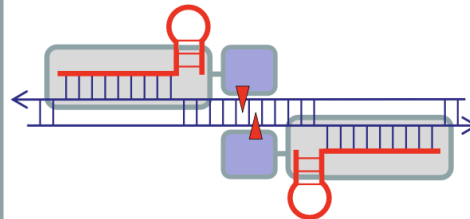
(D) Zinc Finger-FokI (ZFN)



(E) TALE-FokI (TALEN)

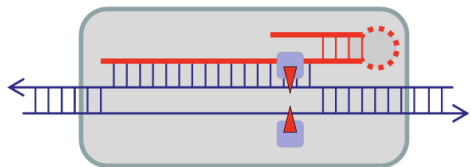


(F) CRISPR-Cascade-FokI

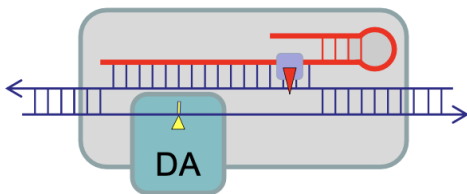


CRISPR-Cas9  
variants

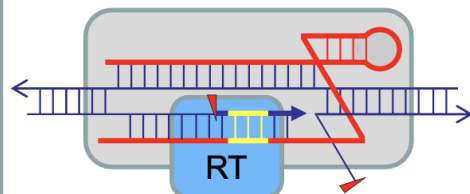
(G) CRISPR-Cas9



(H) nCas9-Base Editor (BE)



(I) nCas9-Prime Editor (PE)



# Molecular Basis of Genome Editing



Examples of genomic editing applications

Ethical implications & challenges

# Molecular Basis of Genome Editing

- The course consists of **7 CFU**: 5 CFU (40 hrs) of classroom lectures and 2 CFU (32 hrs) of hands-on laboratory.
- The lab part will take place in a laboratory with individual workstations. Each student is required to perform the practical laboratory activities individually. The lecturer will provide a theoretical introduction to the laboratory and ongoing technical assistance.
- The course will take place in the **first semester** of 25/26 AA
- Readings/Bibliography: some chapters of textbooks will be suggested (**Zlatanova, Jordanka; Kensal E. Holde, Molecular Biology, 2nd Edition - Structure and Dynamics of Genomes and Proteomes. Garland Science, 2023**); reviews and scientific articles indicated by the teacher; PowerPoint files (MOODLE: <https://stem.elearning.unipd.it/>)
- The final exam consists of an **oral interview**. **Three questions covering different parts of the syllabus, including topics from the lab part**, will be asked. For each question, the student will receive a grade, based on the demonstrated ability to concisely and completely present the specific topics. The different partial grades contribute to the final grade.