

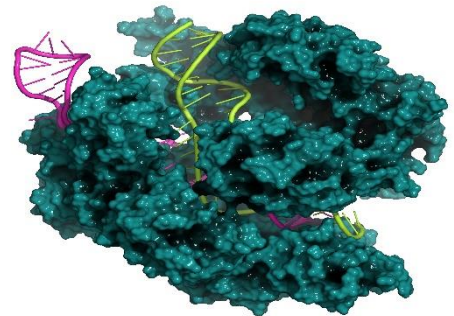


## CRISPR TECHNOLOGY

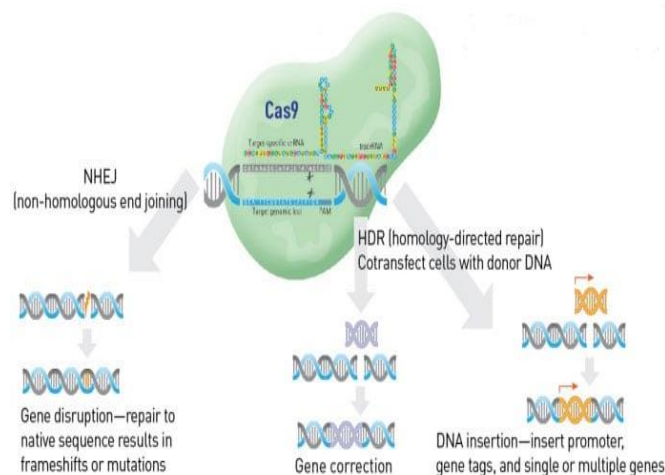
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### Introduction:

CRISPR-Cas9 technology has emerged as a groundbreaking tool in the field of genetic engineering, revolutionizing our ability to edit DNA with unprecedented precision and efficiency. Inspired by the bacterial immune system's ability to defend against viral invaders, CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) technology has rapidly transformed molecular biology, offering immense potential for applications across diverse fields ranging from agriculture and biotechnology to medicine and basic research.



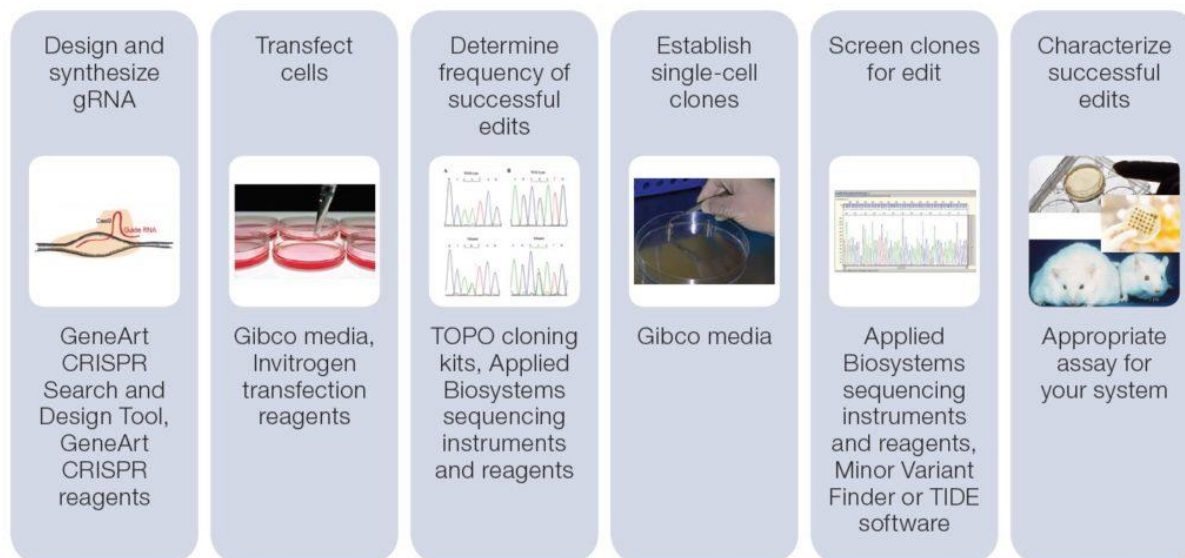
### WORKING PRINCIPLE



- The foundation of CRISPR technology lies in its elegant simplicity and adaptability. At its core, CRISPR consists of two key components: a guide RNA (gRNA) and a Cas protein, most commonly Cas9. The gRNA serves as a molecular GPS, directing the Cas protein to a specific target sequence within the genome. Once the Cas protein locates its target, it acts as molecular scissors, precisely cutting the DNA at the designated site.
- This targeted DNA cleavage triggers cellular repair mechanisms, which can lead to gene disruption through non-homologous end joining (NHEJ) or precise gene editing via homology-directed repair (HDR). The ability to selectively modify DNA sequences with such accuracy has opened up a world of possibilities for researchers, allowing them to investigate gene function, model human diseases, and develop novel therapeutic interventions with unprecedented speed and efficiency.

- Since its discovery, CRISPR technology has catalyzed a paradigm shift in genetic engineering. Moreover, its potential extends far beyond the laboratory, addressing pressing global challenges such as food security, infectious diseases, and genetic disorders.

## CRISPR WORKFLOW



1. **Designing gRNA**: The process starts with identifying the target DNA sequence that needs to be modified. A guide RNA (gRNA) is designed to be complementary to the target DNA sequence.
2. **CRISPR-Cas9 Complex Formation**: The gRNA is then combined with the Cas9 protein to form a CRISPR-Cas9 complex. Cas9 is an enzyme that acts as molecular scissors, cutting the DNA at the target site specified by the gRNA.
3. **Delivery into Cells**: The CRISPR-Cas9 complex is delivered into the cells that need to be modified. This can be done using various methods such as viral vectors, liposomes, or electroporation.
4. **Target DNA Cleavage**: Once inside the cell, the CRISPR-Cas9 complex locates the target DNA sequence and the Cas9 protein cuts both strands of the DNA at the precise location specified by the gRNA.
5. **DNA Repair Mechanisms**: After the DNA is cut, the cell's natural DNA repair mechanisms come into play. There are two main repair pathways: Non-Homologous End Joining (NHEJ) and Homology-Directed Repair (HDR).
  - **NHEJ**: This is an error-prone repair mechanism that often results in small insertions or deletions (indels) at the site of the cut. These indels can disrupt the function of the target gene, leading to gene knockout.
  - **HDR**: This repair mechanism uses a template DNA molecule to repair the cut DNA sequence precisely. This allows for the introduction of specific genetic modifications, such as point mutations or insertions, at the target site.
6. **Verification of Editing**: The edited cells are then screened to verify the desired genetic modifications. This can be done using various molecular biology techniques such as PCR, sequencing, or functional assays.
7. **Cell Expansion and Characterization**: Once verified, the edited cells are expanded and characterized to ensure the desired phenotype or genotype has been achieved.
8. **Application-Specific Assays**: Depending on the application, further assays or tests may be conducted to assess the functionality or behaviour of the edited cells or organisms.
9. **Iterative Optimization (if necessary)**: If the desired outcome is not achieved in the initial editing round, the process may be repeated with modifications to improve efficiency or specificity.

# APPLICATIONS

- **Basic Research:**
  - Gene function studies: CRISPR allows researchers to selectively disrupt or modify genes in model organisms, providing insights into gene function and biological processes.
  - **Disease modelling:** CRISPR can be used to introduce disease-associated mutations into cellular or animal models, enabling the study of disease mechanisms and the development of potential therapies.
- **Biotechnology:**
  - **Crop improvement:** CRISPR can be employed to enhance crop traits such as yield, disease resistance, and nutritional content by precisely editing plant genomes.
  - Livestock breeding: CRISPR-mediated genome editing can facilitate the development of livestock with desirable traits such as disease resistance and improved productivity.
  - Therapeutic Applications:
    - **Gene therapy:** CRISPR holds promise for treating genetic disorders by correcting disease-causing mutations in patient cells or tissues. Cancer treatment: CRISPR-based approaches, such as gene editing to enhance immune cell function or disrupt cancer-promoting genes, are being explored for cancer immunotherapy and targeted therapy.
- **Drug Discovery and Development:**
  - **Target validation:** CRISPR can aid in identifying and validating potential drug targets by selectively disrupting genes associated with disease pathways. Drug screening: CRISPR-based screening techniques enable high-throughput identification of genes involved in drug response or resistance, facilitating drug discovery and personalized medicine.
- **Infectious Disease Control:**
  - **Antiviral strategies:** CRISPR systems can be engineered to target and destroy viral genomes, offering potential applications in combating viral infections such as HIV, hepatitis, and COVID-19. Disease vector control: CRISPR-mediated gene drive technology holds promise for controlling vector-borne diseases by spreading genetic modifications through insect populations to reduce disease transmission.
- **Environmental Applications:**
  - Environmental remediation: CRISPR-based approaches can be used to engineer microorganisms capable of degrading pollutants or remediate contaminated environments.
  - **Conservation:** CRISPR technology has potential applications in conservation efforts, including genetic rescue of endangered species and management of invasive species.
  - These applications represent just a subset of the diverse ways in which CRISPR technology is being utilized to address fundamental scientific questions, develop novel therapeutics, and tackle pressing societal challenges. As research in this field continues to advance, the potential applications of CRISPR are expected to expand even further, shaping the future of biotechnology, medicine, and beyond.