

# Module 6: Genetic Engineering

BMES Cell Team

Winter 2020



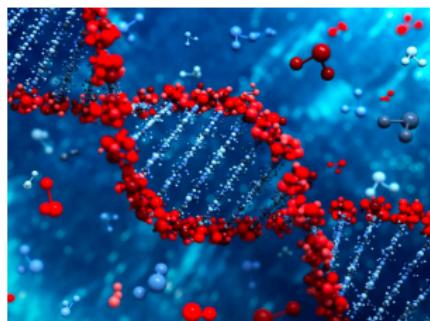
# Outline

- Breakout Rooms to Socialize
- Quarter Long Group Projects Announcement
- Introduction to Genetic Engineering
- Laboratory Methods for Genetic Engineering
- Real Life Applications of Genetic Engineering
- Short Worksheet
- Winter Problem Set Discussion (Select Problems Only)

# Introduction to Genetic Engineering

- **Definition:** **Genome editing** is a way of making specific changes to the DNA of a cell or organism.
- There are four steps to genome editing:

Insert → Delete → Modify → Replace



# Introduction to Genetic Engineering

## Applications of Genetic Engineering



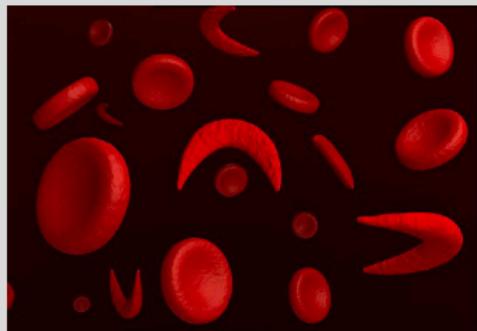
**Designer Babies**



**Epidemiology**

# Introduction to Genetic Engineering

## Applications of Genetic Engineering



Sickle Cell Anemia

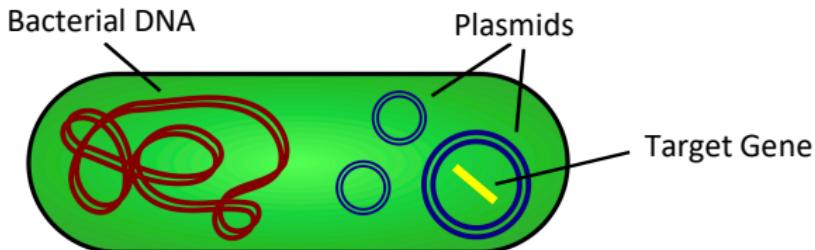


Organ Transplants

# Laboratory Methods for Genetic Engineering

## 1. Cloning

- **Definition:** **Cloning** is the process whereby a *target gene* is introduced into a plasmid.
- **Definition:** A **plasmid** is a circular piece of DNA that replicates independently of a cell's chromosomes.

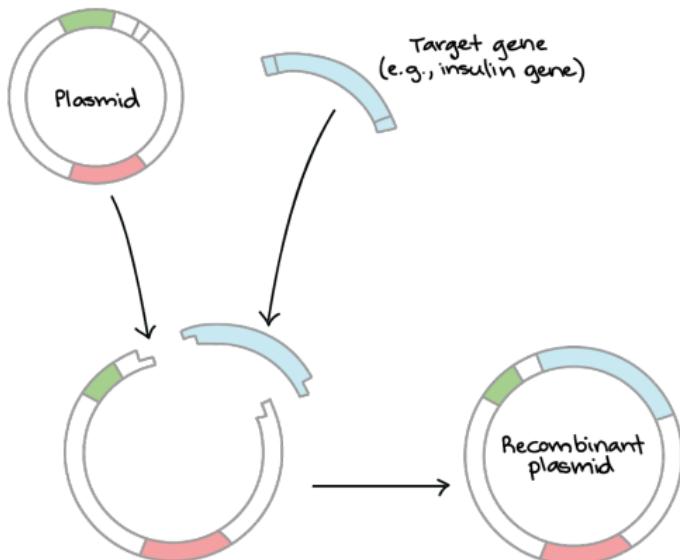


# Laboratory Methods for Genetic Engineering

## 1. Cloning

Step 1:

- Cut open the plasmid and "paste" in the gene
- This process relies on **restriction enzymes** (which cut DNA) and **DNA ligase** (which joins DNA)

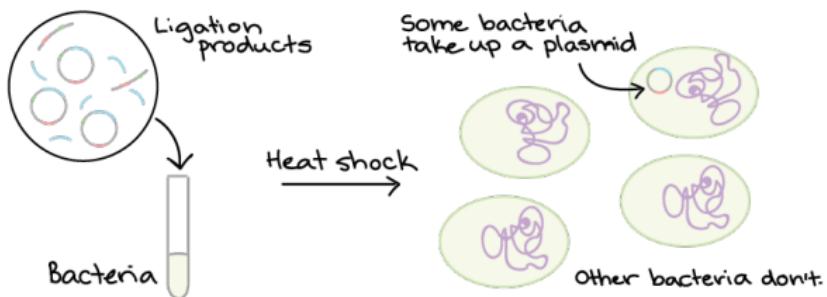


# Laboratory Methods for Genetic Engineering

## 1. Cloning

Step 2:

- Insert the plasmid into bacteria. Use antibiotic selection to identify the bacteria that took up the plasmid.

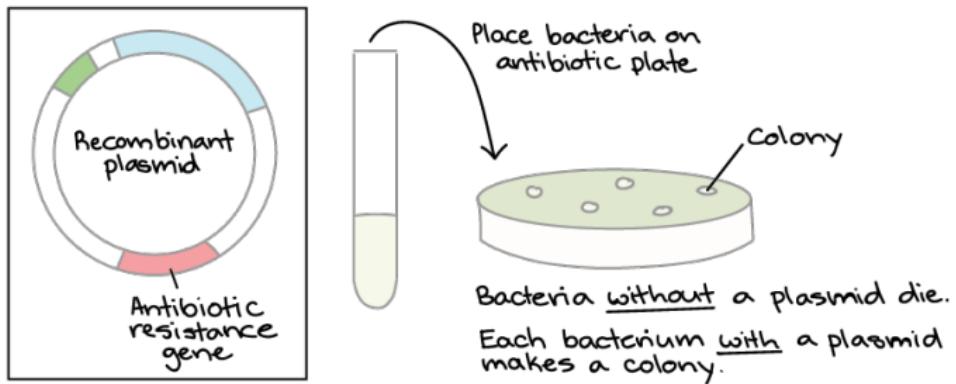


# Laboratory Methods for Genetic Engineering

## 1. Cloning

Step 3:

- Grow up lots of plasmid-carrying bacteria and collect either the plasmids or the proteins.



# Laboratory Methods for Genetic Engineering

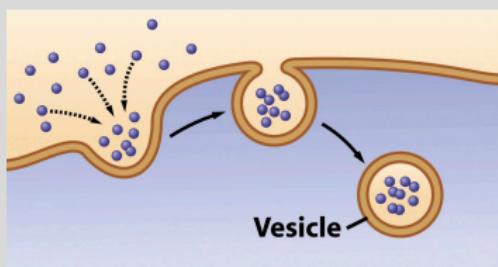
## 2. Transfection

- **Definition:** **Transfection** is the process whereby the nucleic acid sequences are either introduced by **biochemical** or **physical** processes.
- We will use **immortalized eukaryotic cell lines**, which can be *stable* or *transient*
  - **Stable:** Will continuously express transfected DNA and pass it onto daughter cells
  - **Transient:** Will express transfected DNA for a short time. Future generations will not be affected.

# Laboratory Methods for Genetic Engineering

## 2. Transfection

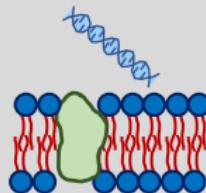
Liposome-Mediated Endocytosis



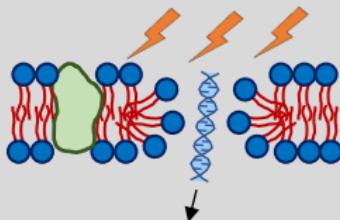
Biochemical

Electroporation

a.



b.



Physical

# Laboratory Methods for Genetic Engineering

## 3. Transduction

- **Definition:** **Transduction** is the process whereby the nucleic acid sequences are introduced by viral vectors.

### Steps:

1. **Transfection:** Introduce the desired plasmid and “packaging proteins” into a producer cell which constructs viruses containing the plasmid gene sequence
2. **Collect the virus produced** and dispose of the producer cells
3. **Transduction:** Add the virus to your desired cells to induce expression of the plasmid gene sequence

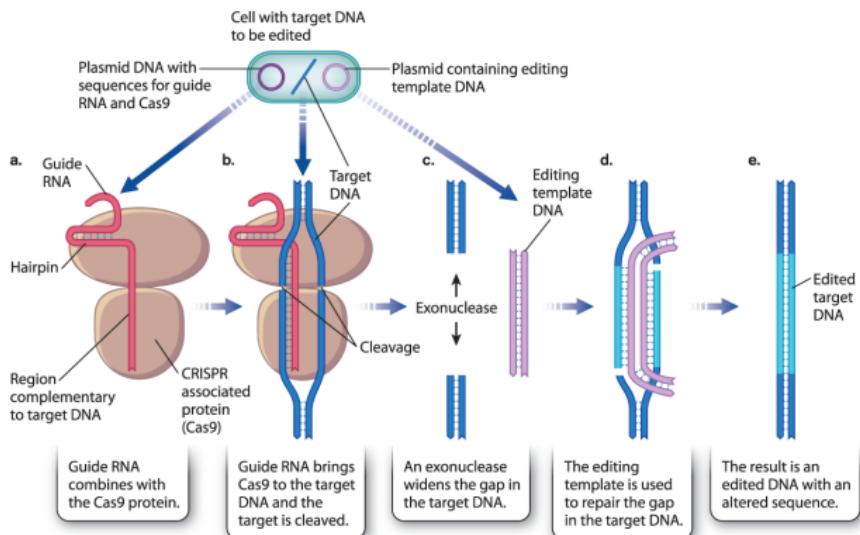
# Laboratory Methods for Genetic Engineering

## 4. CRISPR-Cas9

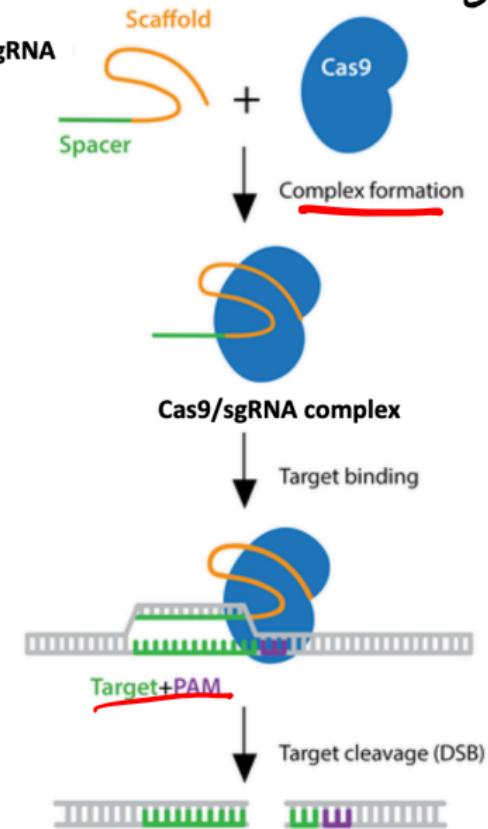
- The **Gold Standard** for modern genome editing
- Once researchers have identified a gene they want to edit, they need:
  - A **guide RNA** that is engineered to be *complementary* to the target DNA
  - A **gene for a protein** (Cas9) that cleaves DNA when it associates with the guide RNA
  - A **piece of DNA** that acts as a template for the new desired sequence

# Laboratory Methods for Genetic Engineering

## 4. CRISPR-Cas9



# How does CRISPR/Cas9 work in the lab?



cutting the target DNA

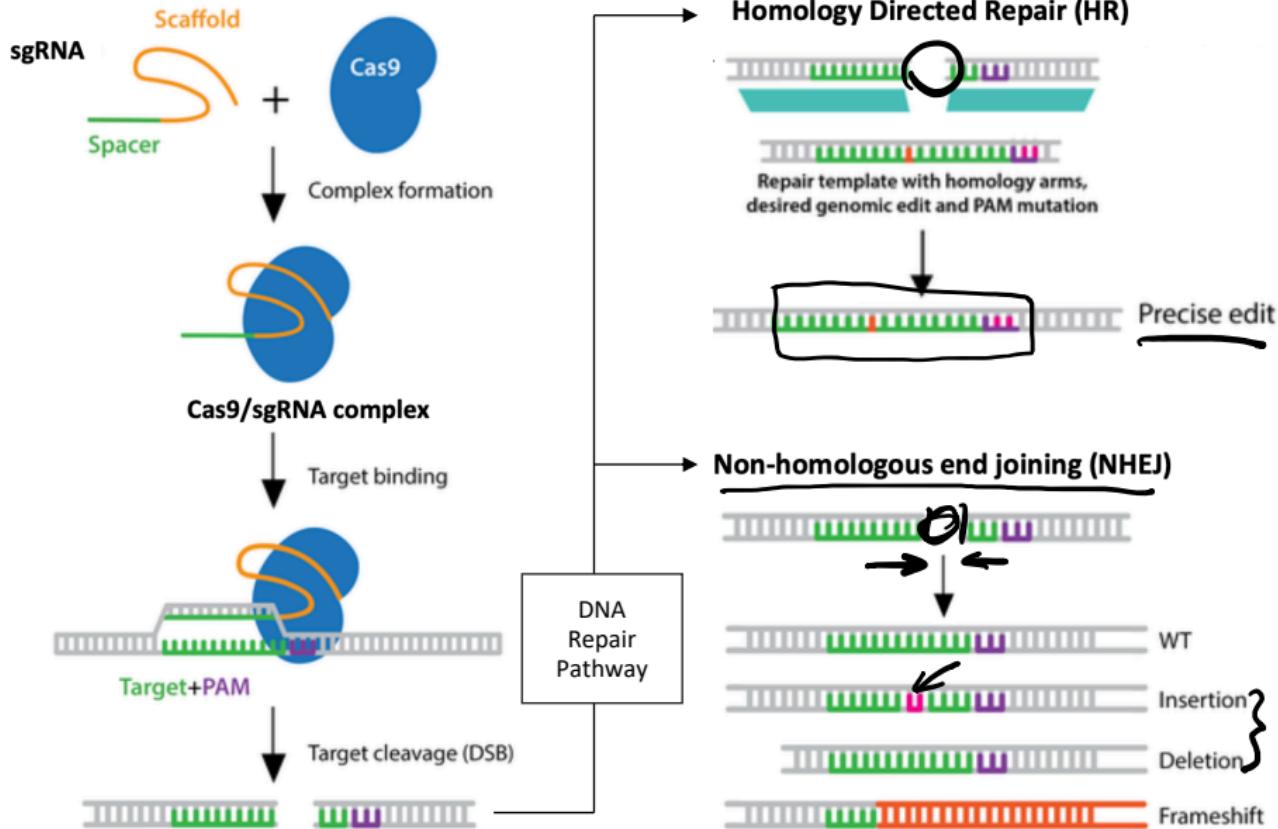
1. Cas9 protein binds to the small guide RNA (sgRNA)

2. Cas9/sgRNA complex scans the DNA for the target sequence

3. sgRNA hybridizes with the target DNA

4. Cas9 protein cuts the target DNA to create a double-stranded break

# How does CRISPR/Cas9 work in the lab?



# How does CRISPR/Cas9 work in the lab?

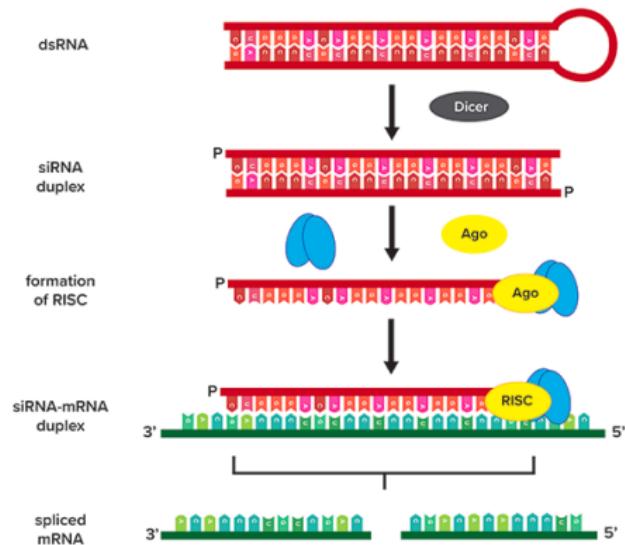
As shown, the two repair mechanisms for CRISPR are HR and NHEJ:

- **Homology Repair    HR or HDR**
  - Used for CRISPR experiments that require extreme precision
- **Non-Homologous End Joining**
  - Typically introduces mutations within genetic material
  - Also known as “sloppy repair”

# Laboratory Methods for Genetic Engineering

## 5. siRNA

- Small-Interfering RNA (siRNA) is used to inhibit gene expression by blocking translation



# Real-Life Applications of Genetic Engineering

- If you are interested in learning more about the uses of Genetic Engineering, please read the optional handout posted on the website

BMES Cell Team      Module 6 Optional Handout      Fall 2020

In previous offerings of BMES Cell Team, the information in this handout was actually part of Module 6. This year, we decided to take it out and include it as supplementary material.

**Genetic Engineering in the Real World**  
Genetic engineering has numerous applications in the fields of biopharmaceuticals, gene therapy, and gene analysis. In this handout, you are going to explore the history and applications of genetic engineering in each of these fields.

**1 Biopharmaceuticals**  
By definition, a **biopharmaceutical** is any pharmaceutical drug product manufactured in, extracted from, or synthesized from biological sources. The most well-known example of a biopharmaceutical is insulin.

The first synthetic human insulin was produced at Genentech in 1978. Scientists used cloning methods to introduce the human insulin gene into a plasmid. Recombinant DNA was inserted into E. coli bacteria to produce insulin, which was then harvested and purified.



**2 Gene Therapy**  
**Gene therapy** is a technique for correcting defective genes that are responsible for disease development. The first case of gene therapy occurred on September 14, 1990, when a patient named Adeline Dertinger was treated for severe combined immunodeficiency (SCID). Doctors removed her white blood cells, inserted the normal gene into the cells, and then put the cells back into her system. This strengthened her immune system, but was only effective for a few months.

Because of their risks, gene therapy products were not approved by the Food and Drug Administration (FDA) until 2017. Jessie Geltlinger was the first person to die in a clinical trial for gene therapy. He suffered from ornithine transcarbamoylase deficiency, which results in the inability to metabolize arginine. He had been given a gene that would allow him to produce the enzyme, but he still had to live on a restrictive diet. In 1999, Geltlinger joined a clinical trial at the University of Pennsylvania, which focused on developing a treatment for infants with the severe form of this disease. He was injected with the gene, but his body rejected the gene and died four days later at age 18 due to a massive immune response triggered by the viral vector.

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**3 CRISPR Babies**  
In the Fall of 2018, He Jiankui used CRISPR-Cas9 genome editing to modify a gene that codes for an HIV protein in HIV. Jiankui recruited a couple in which the man had HIV, used in vitro fertilization to create embryos, edited the DNA, and then implanted them into the woman. The result was that the couple's two daughters, Lulu and Nana, became born with HIV.

Despite Jiankui's success in preventing the couple's twins from having HIV, there was a problem: human gene editing is illegal. While using CRISPR-Cas9, Jiankui actually generated several other mutations, but implanted them without extensive testing. As a result, Jiankui was fired from his university and sentenced to three years in prison by the Chinese court on December 30, 2019.



**4 Concluding Statements**  
As you saw, many of these breakthroughs in genetic engineering have occurred recently. This is a subset of biotechnology that is constantly evolving and undergoing further research. Many areas of genetic engineering are still unknown.

With every breakthrough in biotechnology and medicine comes ethical criticism. Although CRISPR has the potential to cure patients of genetic diseases, some argue that the risks outweigh the benefits. Millions of dollars and human subjects for CRISPR-Cas9 testing have developed dangerous side effects that led to death. Thus, it is important to follow ethical guidelines when performing genetic engineering experiments.