Module 6: Genetic Engineering

BMES Cell Team

Winter 2021

Outline

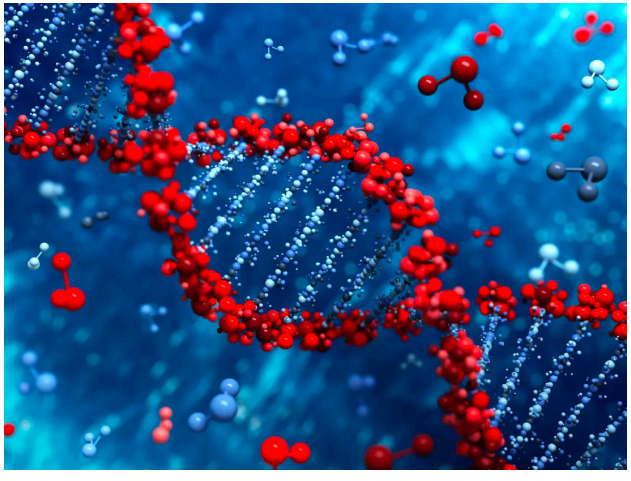
- Breakout Rooms to Socialize
- Quarter Long Group Projects Announcement
- Introduction to Genetic Engineering
- Laboratory Methods for Genetic Engineering
- Real Life Applications of Genetic Engineering
- Short Worksheet
- Winter Problem Set Discussion (Select Problems Only)

Introduction to Genetic Engineering

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

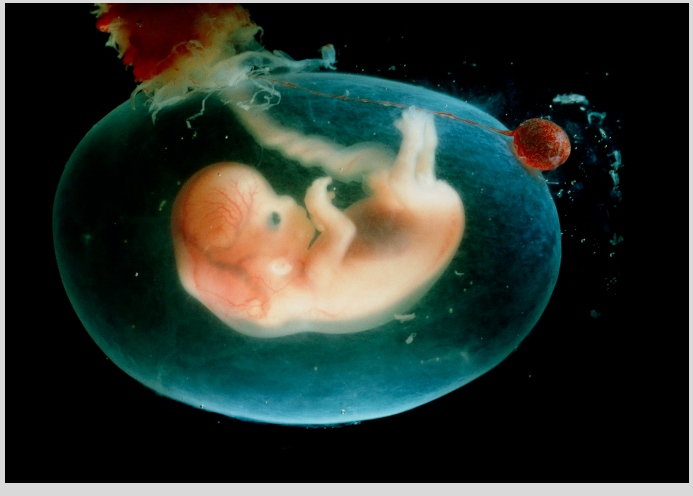
- There are four steps to genome editing:

Insert → Delete → Modify → Replace

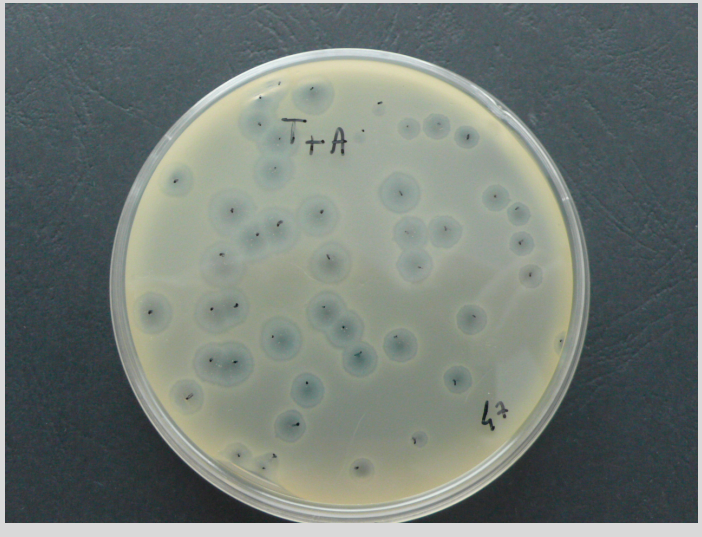


Introduction to Genetic Engineering

Applications of Genetic Engineering



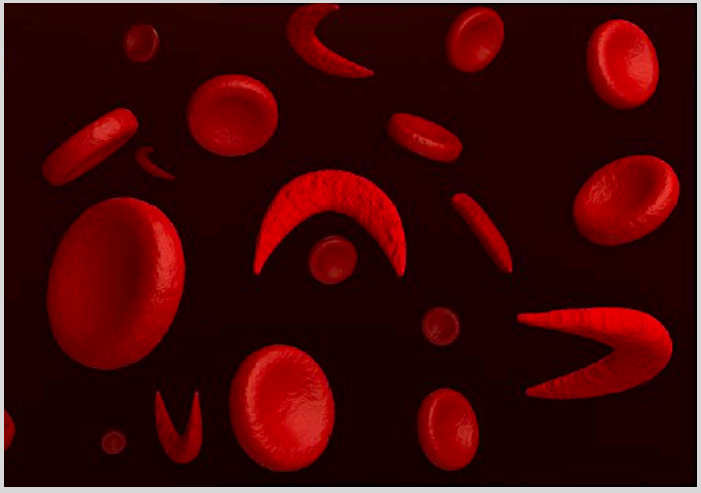
Designer Babies



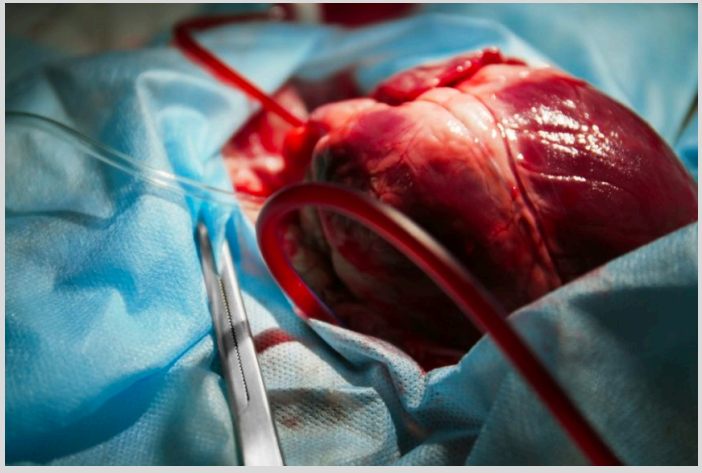
Epidemiology

Introduction to Genetic Engineering

Applications of Genetic Engineering



Sickle Cell Anemia



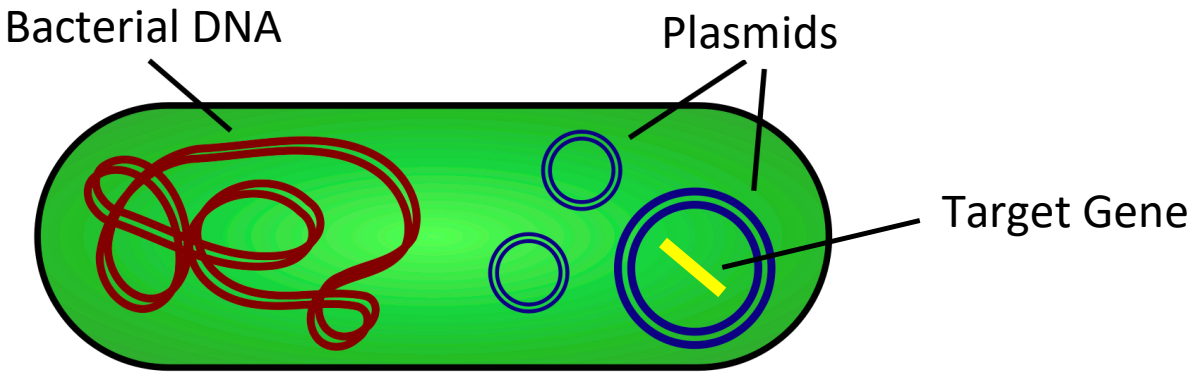
Organ Transplants

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.

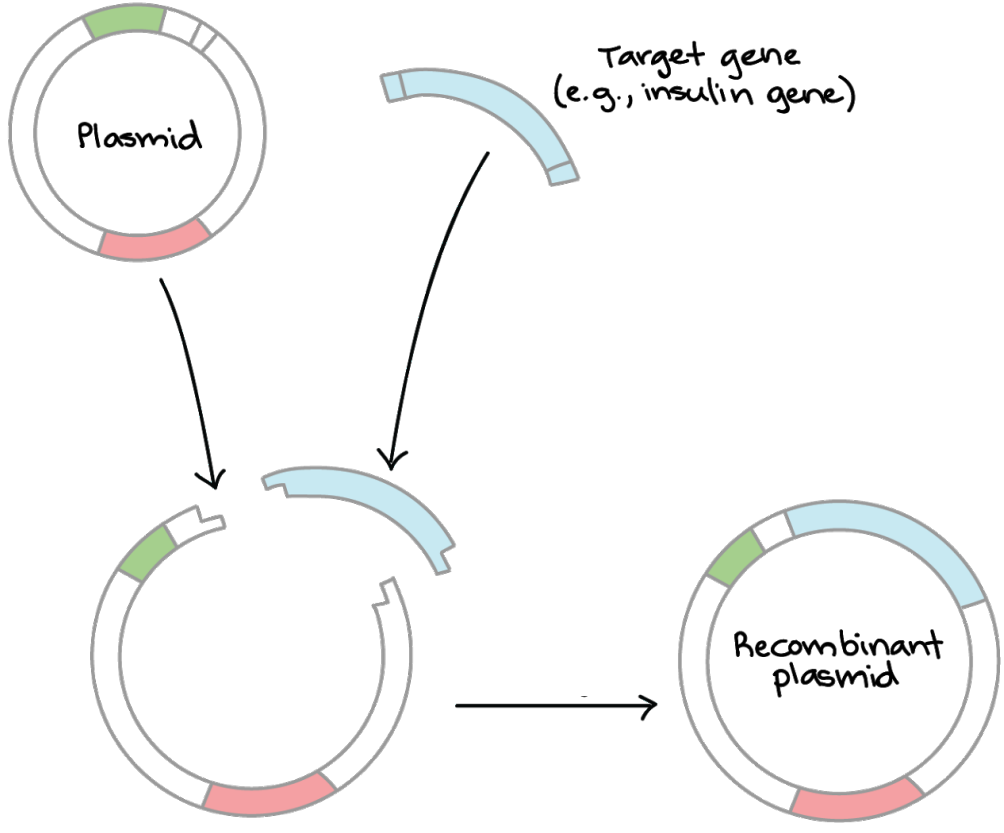


Laboratory Methods for Genetic Engineering

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)

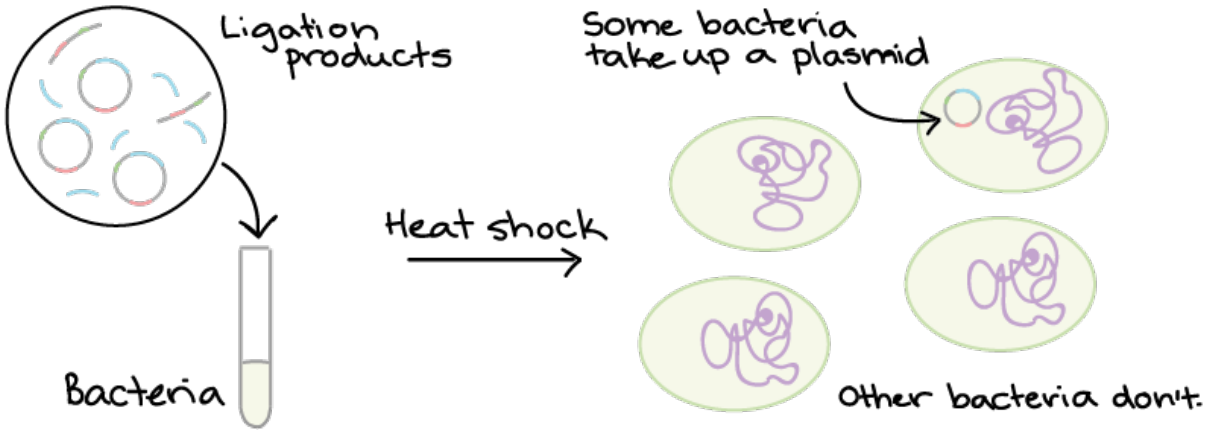


Laboratory Methods for Genetic Engineering

1. Cloning

Step 2:

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

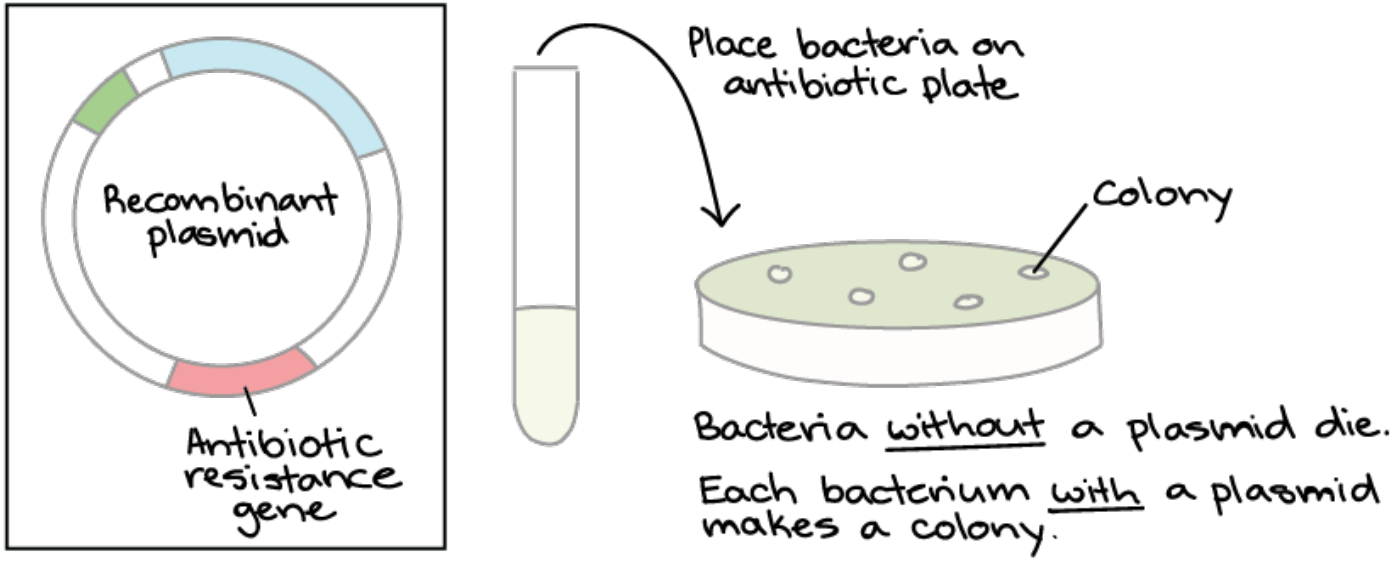


Laboratory Methods for Genetic Engineering

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

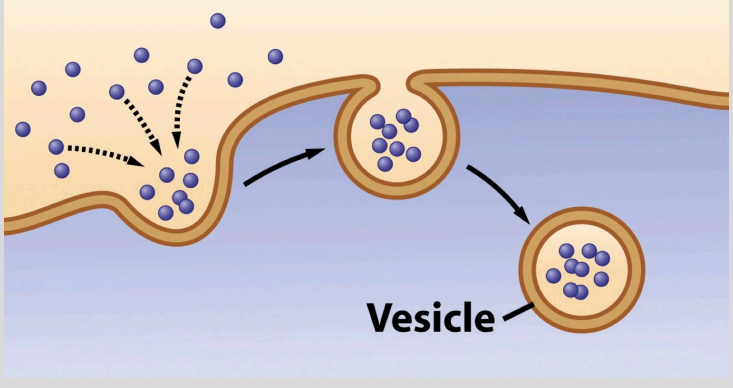
- **Definition:** Transfection is the process whereby the nucleic acid sequences are either introduced by biochemical or physical processes.

- 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

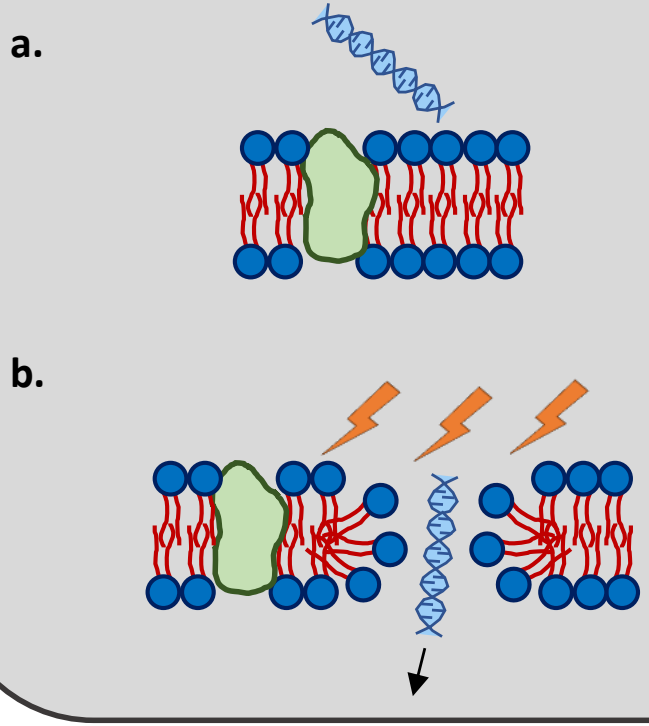
2. Transfection

Liposome-Mediated Endocytosis



Biochemical

Electroporation



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

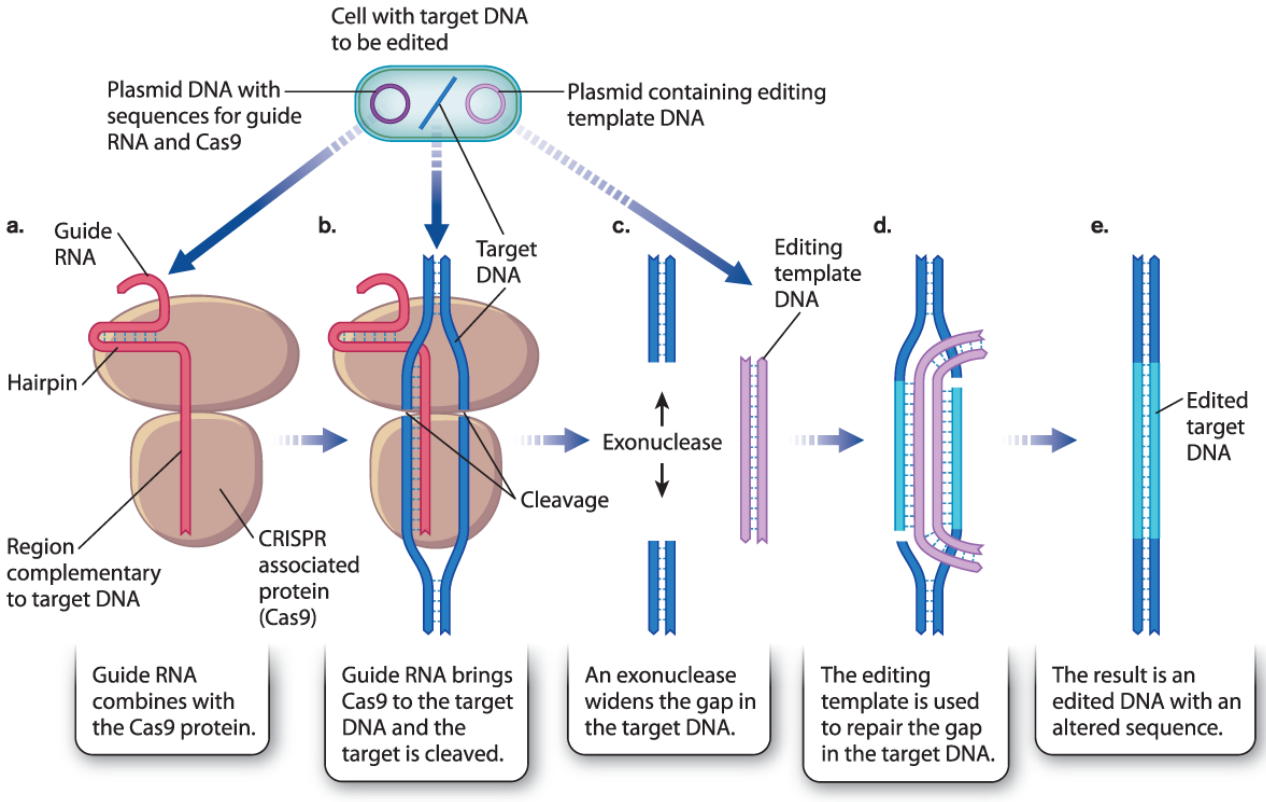
Laboratory Methods for Genetic Engineering

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

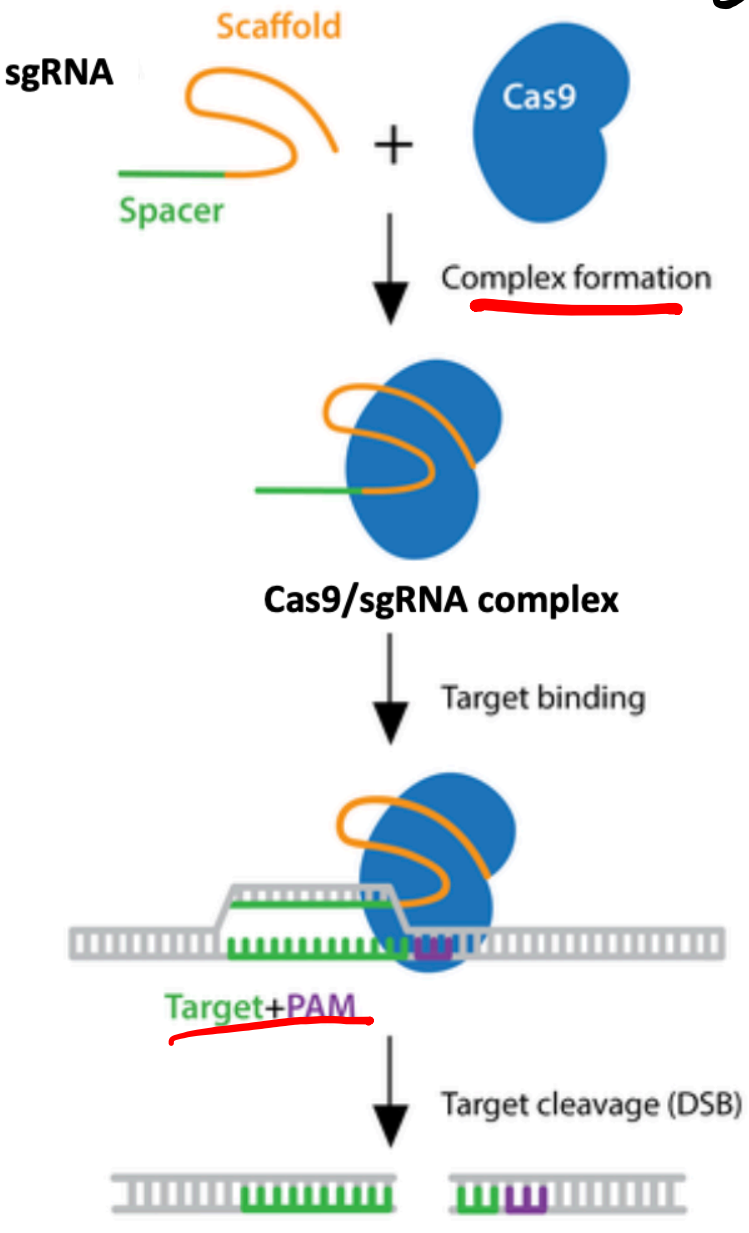
Laboratory Methods for Genetic Engineering

4. CRISPR-Cas9



Biology: How Life Works (3e)

How does CRISPR/Cas9 work in the lab?



Cutting the target DNA

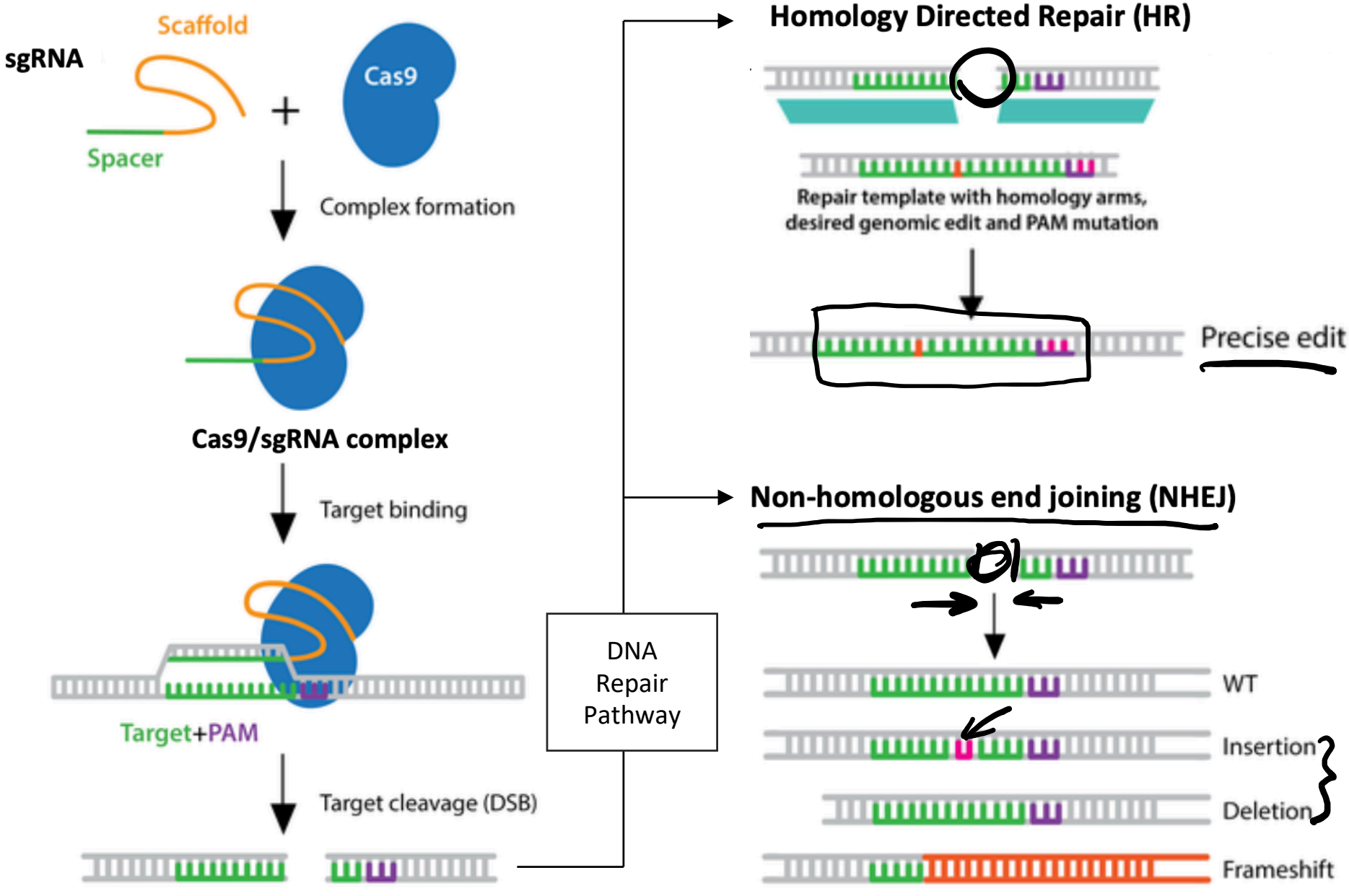
1. Cas9 protein binds to the small guide RNA (sgRNA)

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?



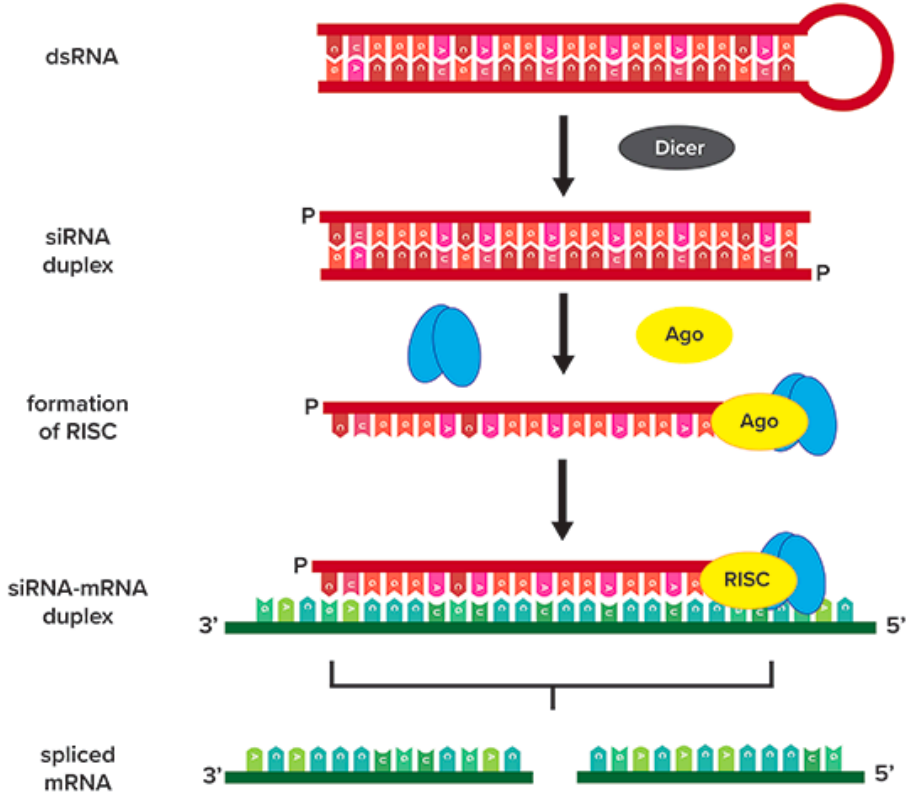
How does CRISPR/Cas9 work in the lab?

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”

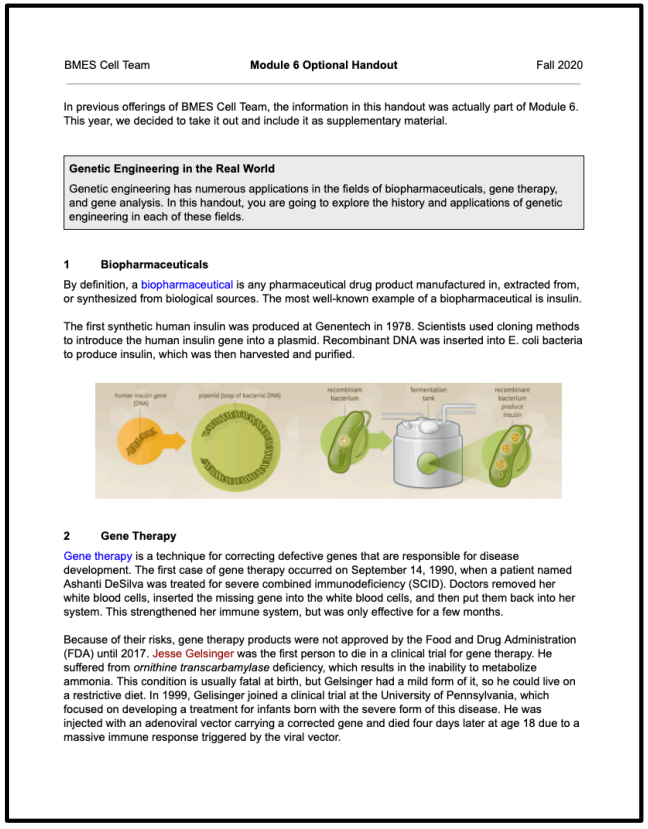
5. siRNA

- 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

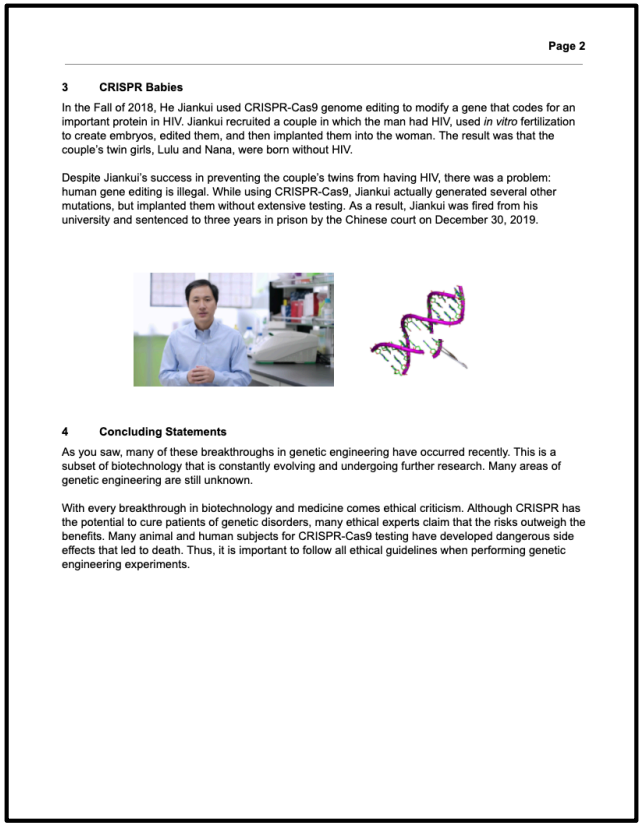
By definition, a biopharmaceutical is any pharmaceutical drug product manufactured in, extracted from, or synthesized from biological sources. The most well-known example of a biopharmaceutical is insulin.

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 Ashanti DeSilva was treated for severe combined immunodeficiency (SCID). Doctors removed her white blood cells, inserted the missing gene into the white blood cells, and then put them 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. Jesse Gelsinger was the first person to die in a clinical trial for gene therapy. He suffered from *ornithine transcarbamylase* deficiency, which results in the inability to metabolize ammonia. This condition is usually fatal at birth, but Gelsinger had a mild form of it, so he could live on a restrictive diet. In 1999, Gelisinger joined a clinical trial at the University of Pennsylvania, which focused on developing a treatment for infants born with the severe form of this disease. He was injected with an adenoviral vector carrying a corrected 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 important protein in HIV. Jiankui recruited a couple in which the man had HIV, used *in vitro* fertilization to create embryos, edited them, and then implanted them into the woman. The result was that the couple's twin girls, Lulu and Nana, were born without 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 disorders, many ethical experts claim that the risks outweigh the benefits. Many animal and human subjects for CRISPR-Cas9 testing have developed dangerous side effects that led to death. Thus, it is important to follow all ethical guidelines when performing genetic engineering experiments.