BS120(E)

Technology for the Treatment of Genetic Disorder

-CRISPR-

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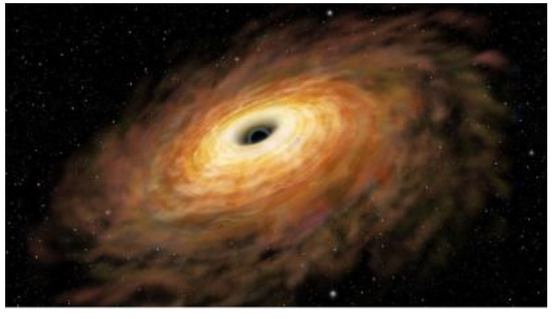
CHAPTER.1

INTRO

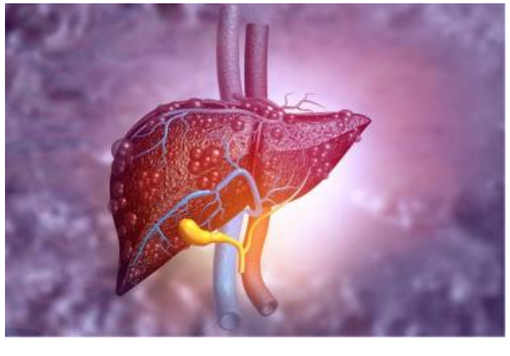
Genetic Disorders & Genetic Scissors

2020 Nobel Prizes

Physics



Physiology or Medicine



Chemistry

Genetic Disorder

A disease caused by harmful changes in genes

Type: autosomal inheritnace, sex-linked inheritance

Ex) Phenylketonuria(PKU), Huntington disease, Hemophilia, Red-green color blindness

Genetic Scissors

Technology that recognize certain part of DNA and cut & modify

Difference between Nucleic acid–degrading enzymes

– recognize certain part

–〉 can use to cure genetic disorder

Type: ZFNs, TALENs, CRISPR–Cas9

CHAPTER.2

CRISPR system

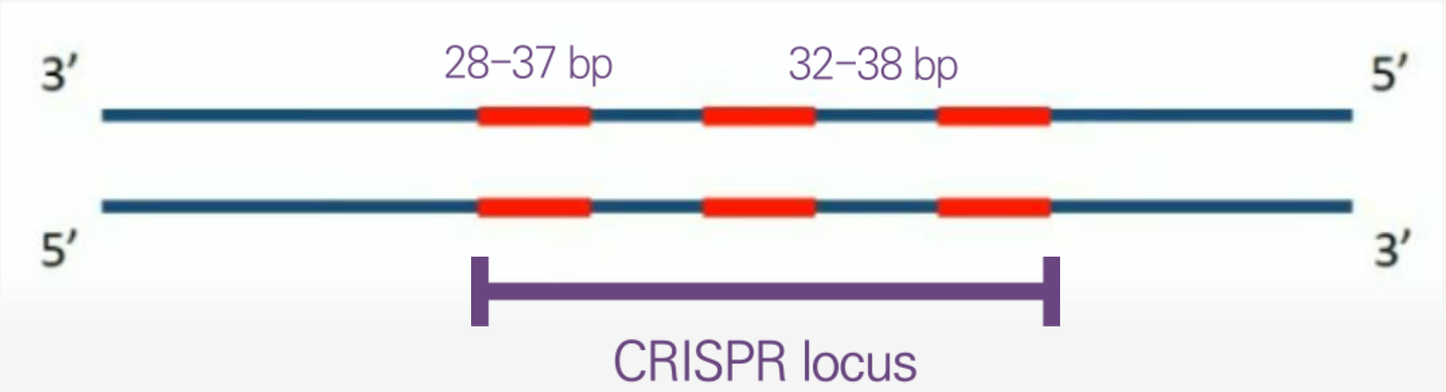
What is CRISPR and how does it act?

- **CRISPR**

Clustered Regularly Interspaced Short Palindromic Repeats

- **CRISPR locus**

An array of short direct **DNA repeats** interspersed with **spacer DNA**



CRISPR MECHANISM

- Immune system

1. Space Aquisition
2. crRNA Processing
3. Interference

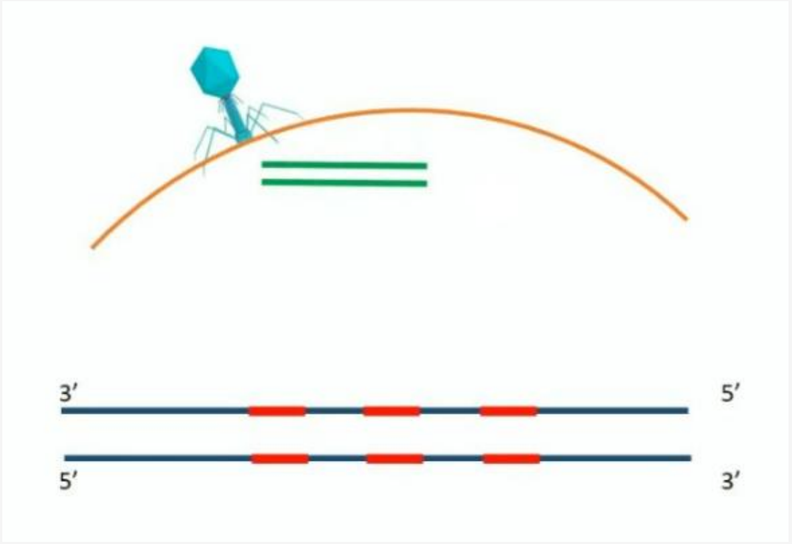
- types of CRISPR system

type 1

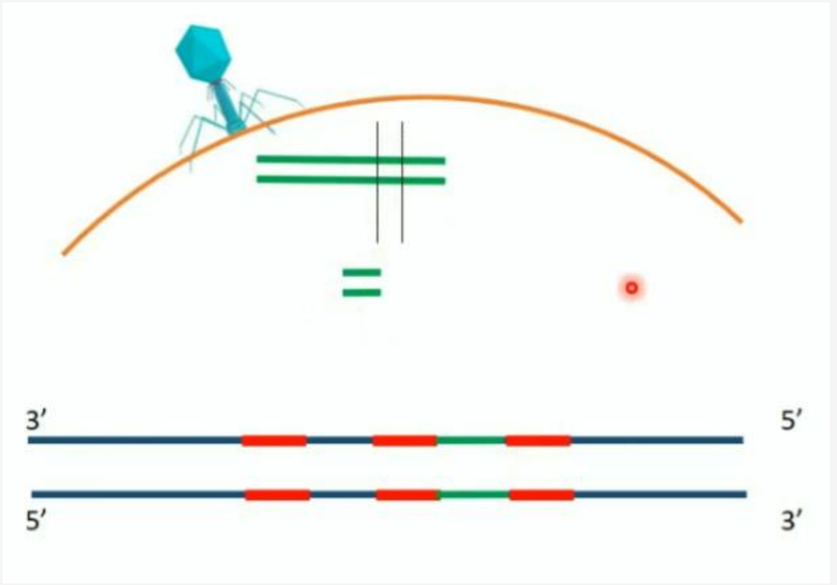
type 2 ⇒ mostly used for gene engineering

type 3

1. Spacer Acquisition

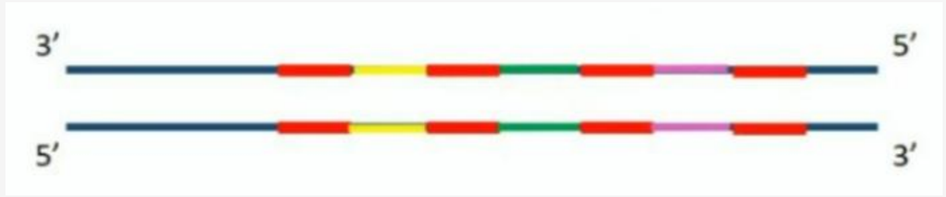


A bacteriophage infected cell for the first time



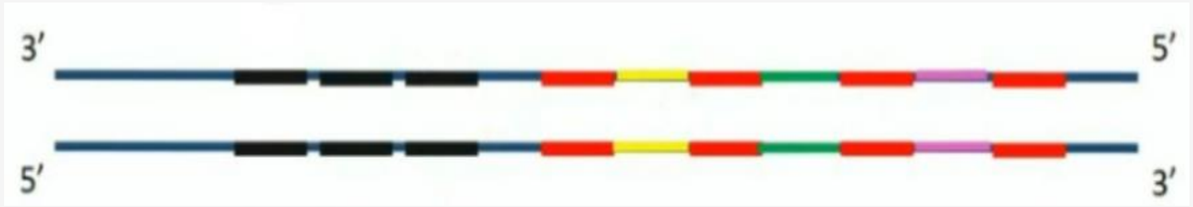
Bacterial cell chops up the viral genome,
take a piece of it,
insert this piece into the spacer DNA

1. Spacer Acquisition

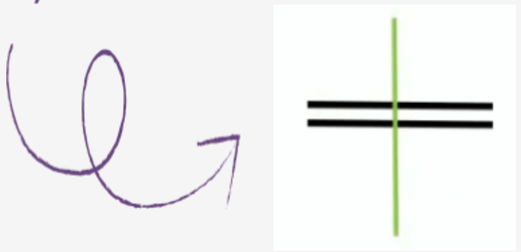


Each time a bacteriophage infects the cell,

cell takes a piece of it and inserts it into the spacer DNA



Cas Enzymes



mostly nucleases

cas1

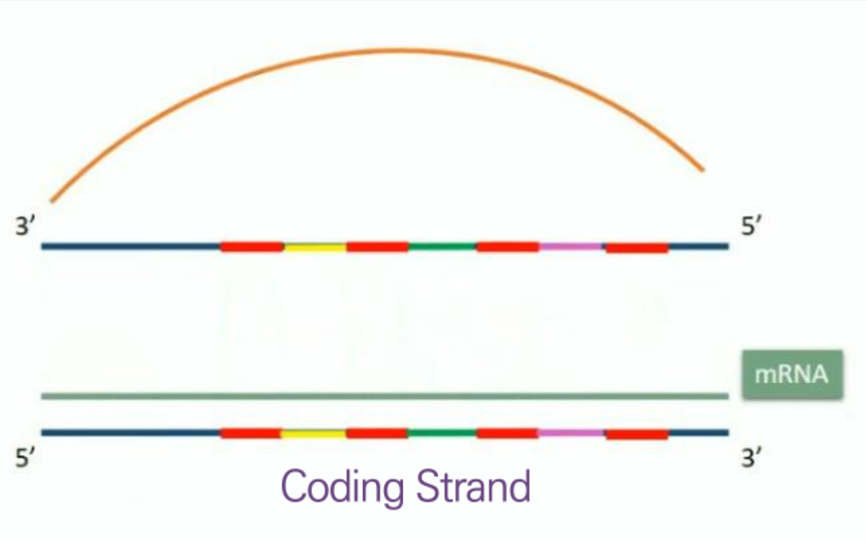
nucleases

integrase

cas2

endoriboucleases

2. crRNA Processing



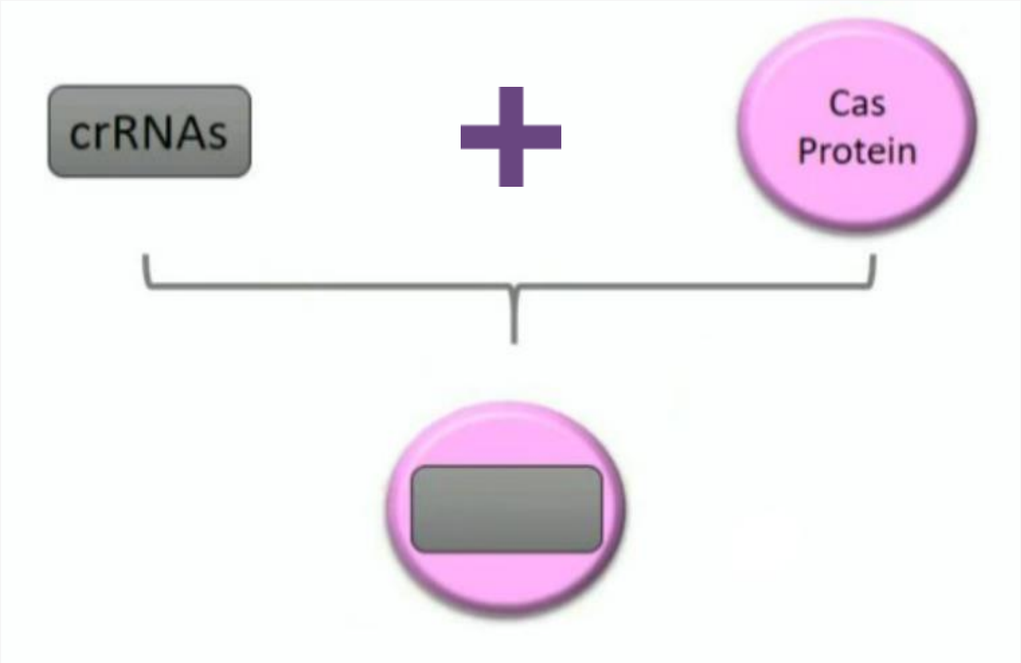
One of the two strands of baterial DNA is transcribed into mRNA

tracr RNAs bind to the CRISPR repeats on the mRNA

mRNA is chopped up by **cas9** and **RNase3**

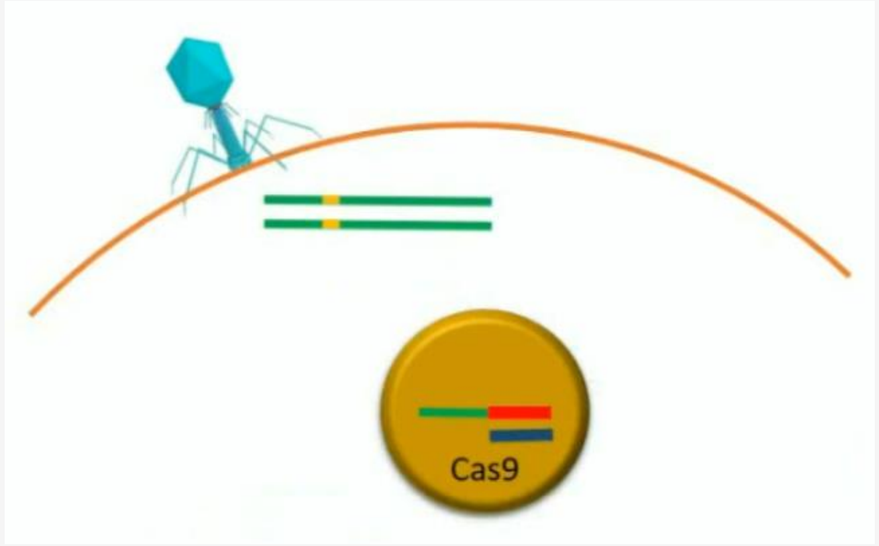
crRNA

3. Interference

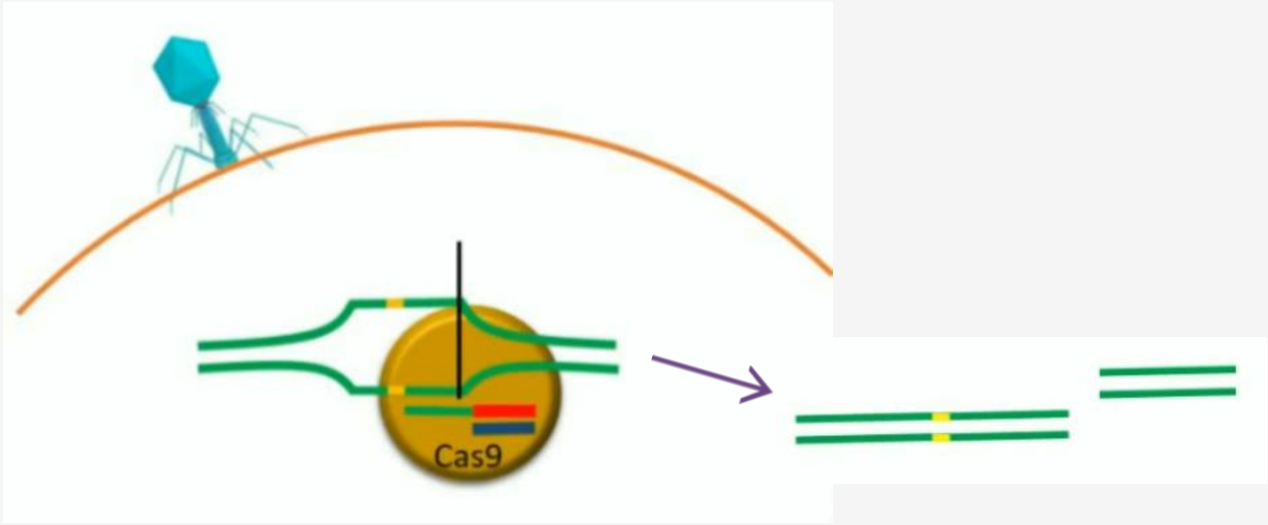


crRNAs and specific cas protein are merged together to form the complex above

3. Interference



cas9 enzyme recognizes PAM sequence of viral genome and RNA sequence(green part) recognizes **DNA sequence** of viral genome



Viral genome binds to the RNA and cas enzyme undergoes **double strand break**

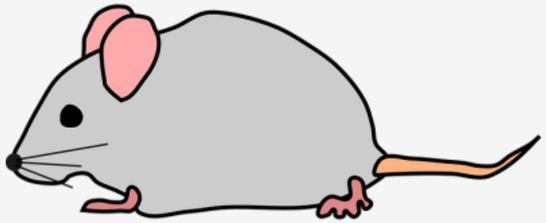
[HNH
RuvC] RnasH-like endonuclease domains

CHAPTER.3

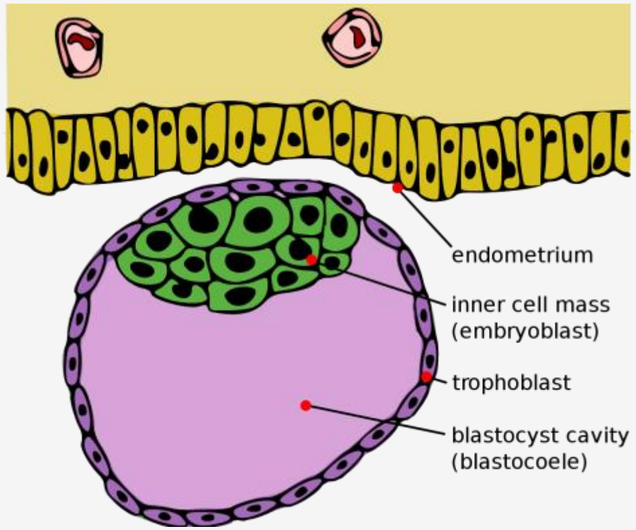
CRISPR-cas9 technique

Gene Engineering

GENE KNOCKOUT



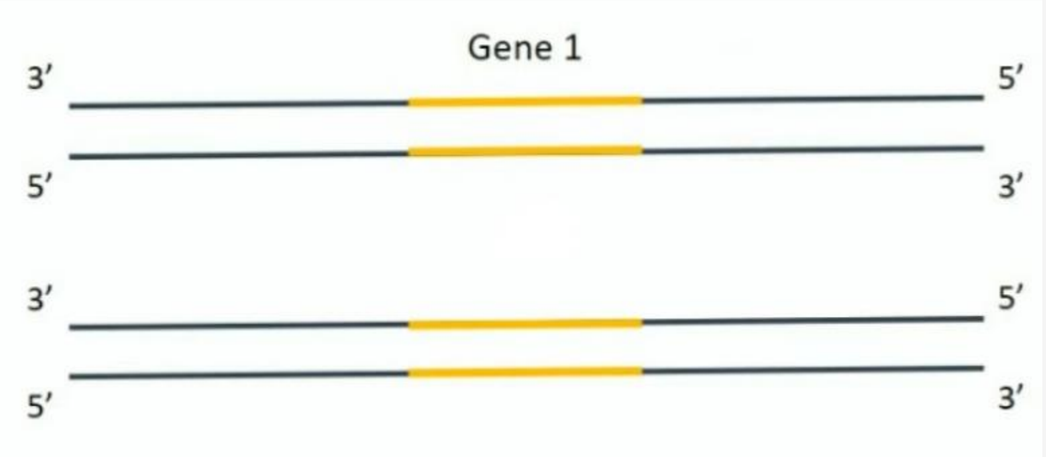
- Diploid
- 40 chromosomes (20 pairs)



- Embryonic development
- Blastocyst
- Embryonic stem cells
- Pluripotent stem cells

GENE KNOCKOUT

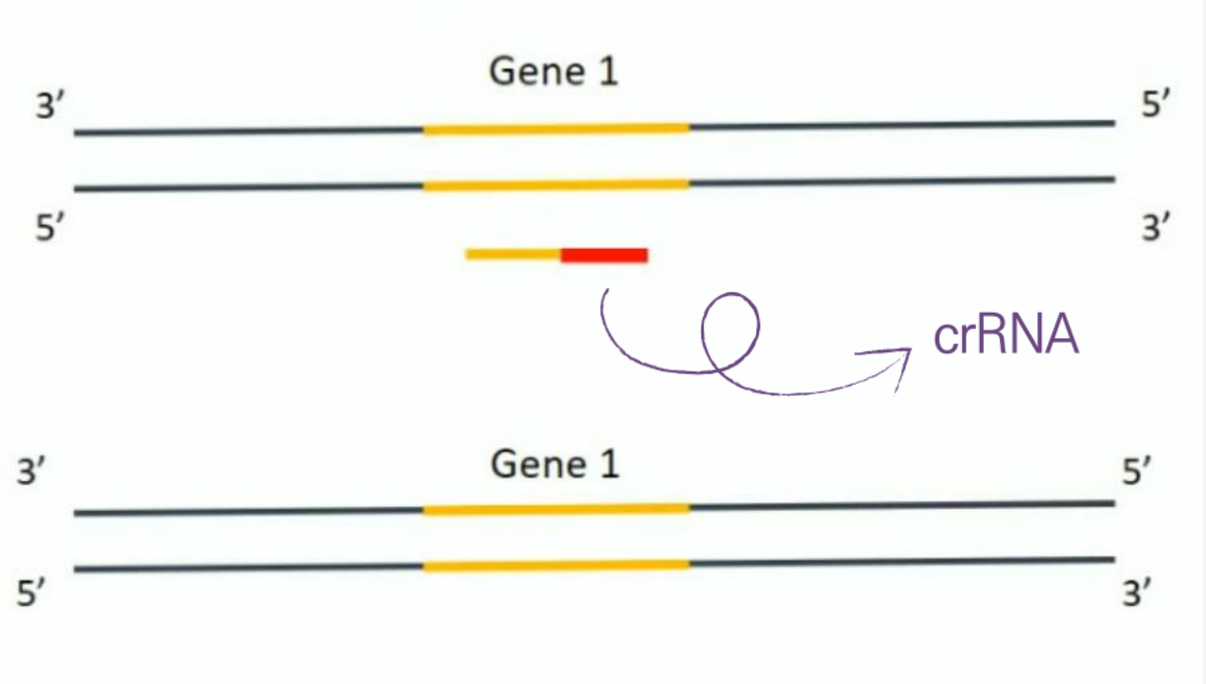
Embryonic stem cells



Suppose that Gene 1 causes certain disease,

so we want to knock out this gene

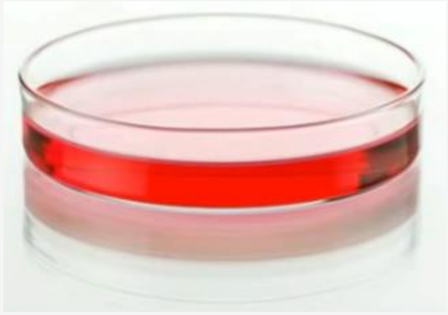
GENE KNOCKOUT



To knock out the Gene 1, we need crRNA that contains CRISPR repeat and RNA sequence that is complementary to the coding strand

GENE KNOCKOUT

Embryonic stem cells



crRNA

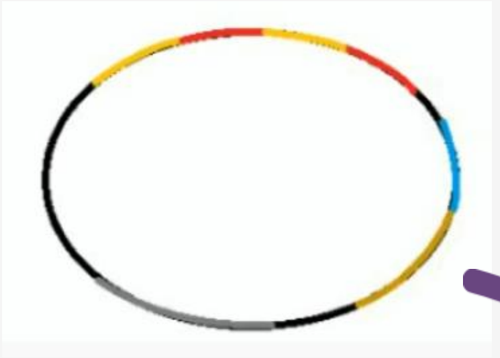
tracrRNA

Cas9

DNA Repair Template

By the form of **plasmid**, all of the above components are delivered into the embryonic cell

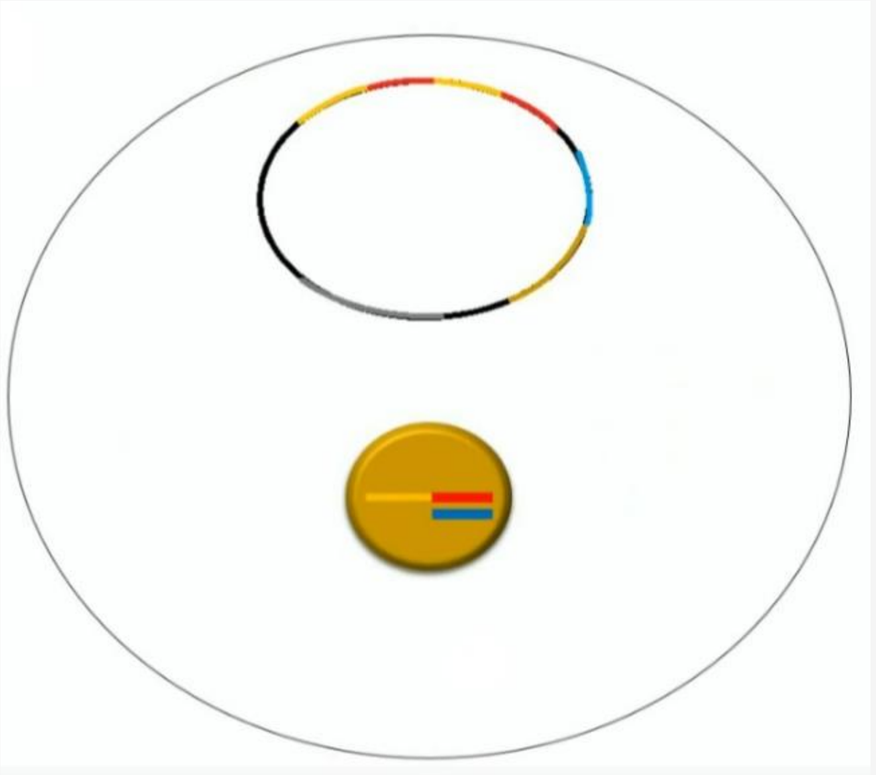
GENE KNOCKOUT



Delivered by two ways

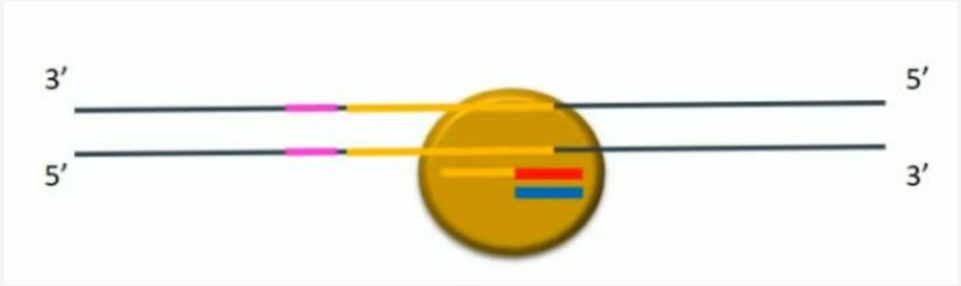
: Electroporation

: Viruses (lentivirus, adenovirus)



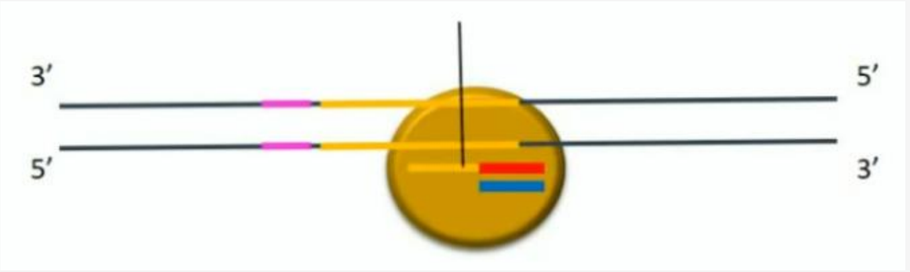
crRNA, tracrRNA, and cas9 form CRISPR-cas9 complex in the embryonic cell

GENE KNOCKOUT



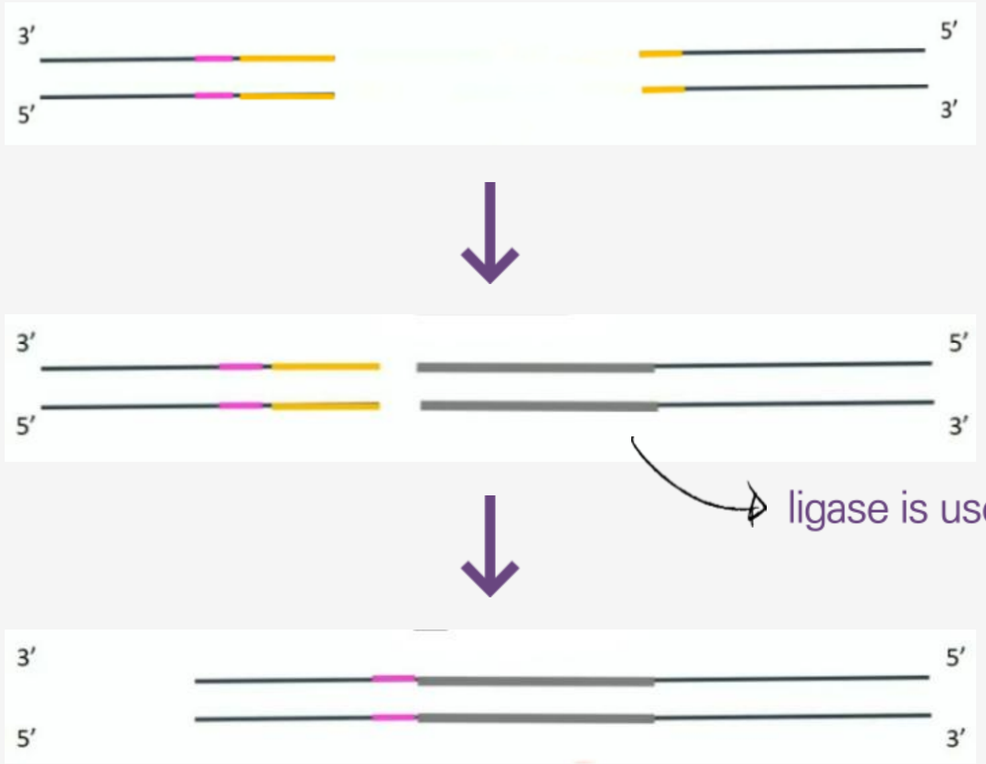
Target sequence is recognized by the RNA sequence and PAM sequence it recognized by cas9 enzyme

→ RNA sequence binds to its complementary sequence



Cas9 enzyme undergoes double strand break or single strand break

1. Blunt Ends (double strand break)



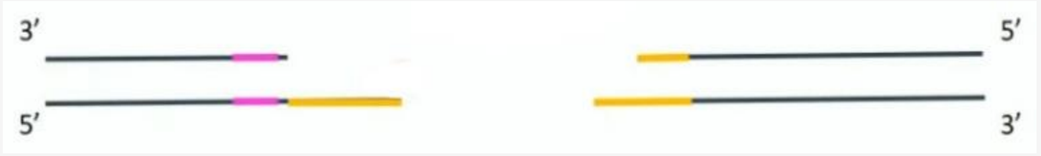
To prevent homology-directed repair,

DNA Repair Template is used

Rest of the Gene1 on the right part is removed and DNA Repair Template binds to the broken sequence

Rest of the Gene1 on the left part is degraded and each strand is linked with DNA Repair Template

2. Sticky Ends (single strand break)



Rest of the Gene1 on left up one is chopped up

DNA invasion: left part of the strands invade the DNA Repair Template and be copied according to the DNA Repair Template

Rest of the Gene1 is chopped up and the gap is filled using the DNA polymerase

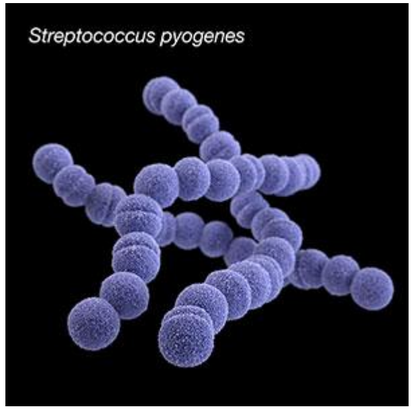
Rest of the Gene1 on right down one is chopped up and the two strands are bound together using the ligase

CHAPTER.4

Side Effects of Genetic Scissors

Three sides effects & The ways to overcome

1. Cause unwanted immune responses



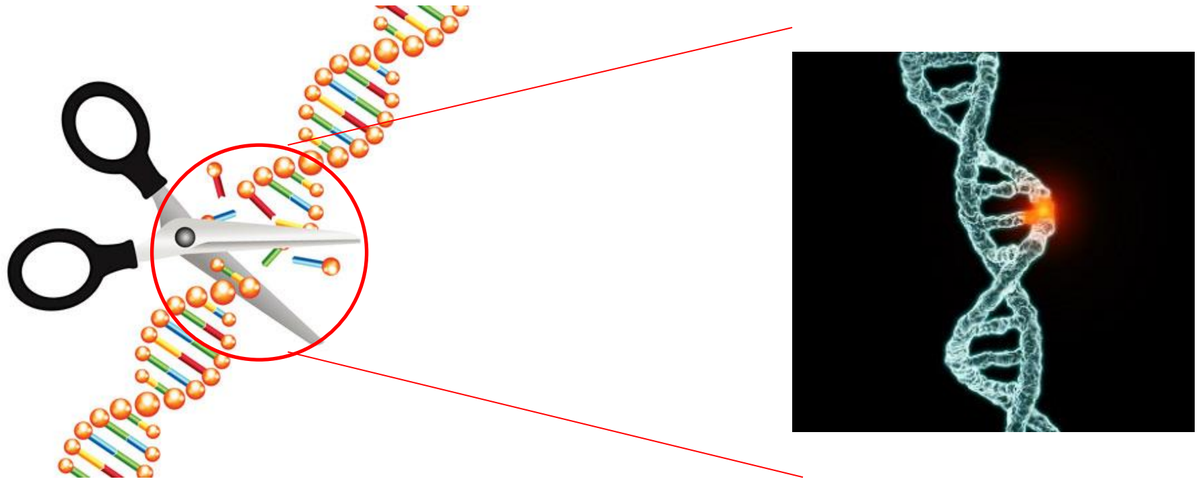
- CRISPR/Cas9 is from beacterium called streptococcus

- Get infected with this bacterium -> have an immune system

- T cell react to Cas9 -> affect the stability and effectiveness of genetic scissors

2. Induce unwanted changes in genes

– Even if genetic scissors are precisely found at target point and cut, un expected genetic changes can occur near the amputation point



– New mutation can cuase another genetic disorder or have harmful eff ects on human body

3. Higher cancer risks

– p53 gene take part in the recovery precess in the event of DNA dama ge and suppresses tumors

– Genetic scissors must cut the double helix structure of DNA –〉 p53 int erferes –〉 genetic scissors work much better in abnormal cell where p5 3 does not function

– Cells that have under gone genetic scissors can eliminate tumor suppr essor gene p53 so increase the risk of cancer

How to overcome side effects?

- 4th generation genetic scissors(prime editter) are under study

- Prime editing can prevent cuts that occur outside the target sequence

- Replace NHEJ or HDR by cutting out only one strand of DNA and inser t the correct sequence

Any Questions?
Feel free to ask!

Q&A