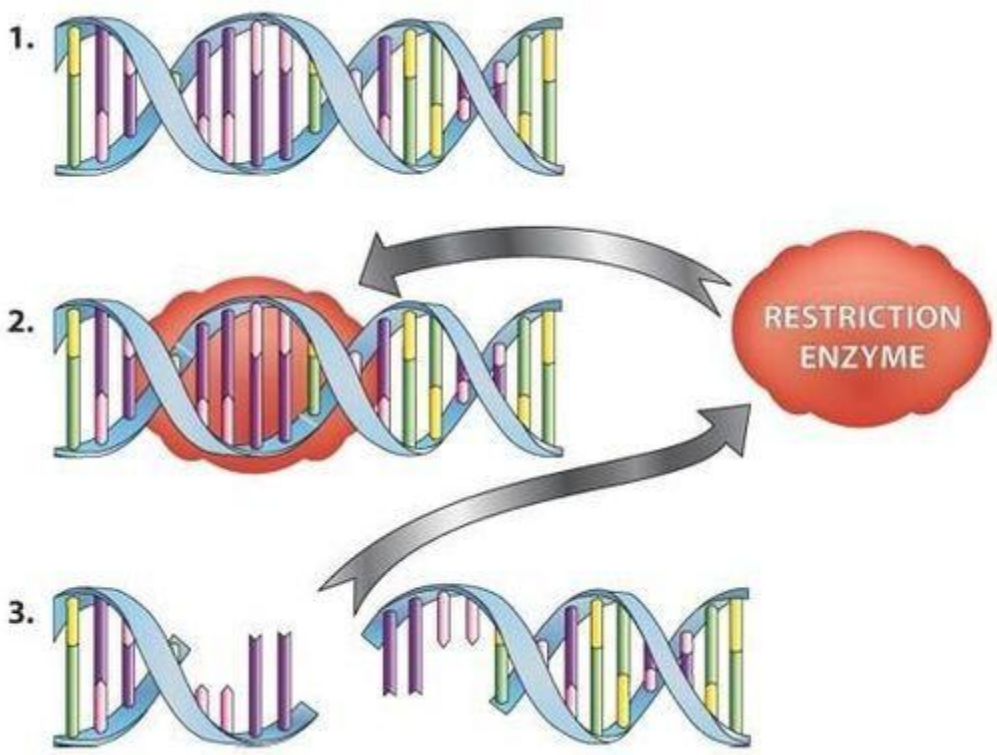
Lesson 17 – Genetic engineering: Restriction enzymes



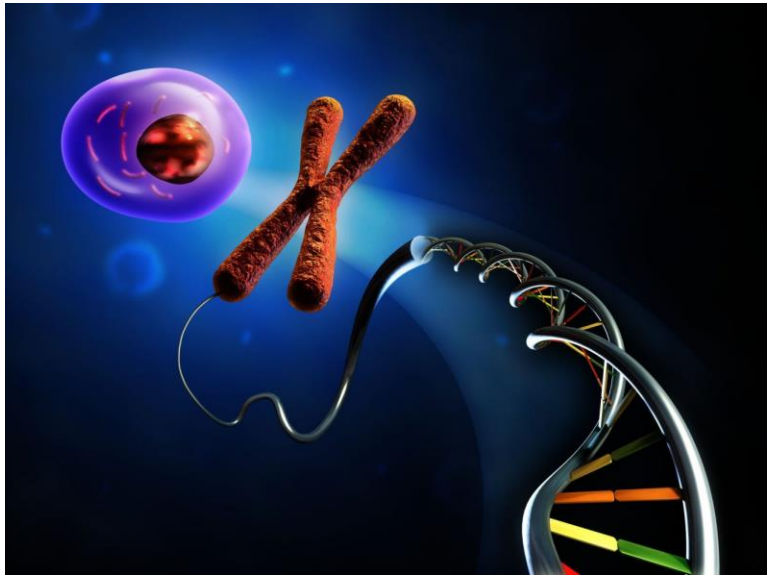
Summary of upcoming lectures

Genetic engineering

- Restriction enzymes (Lesson 17)
- Vectors and ligation (Lesson 18)
- Polymerase chain reaction (PCR) (Lesson 19)

Genetic engineering (RDNA)

- Genetic engineering = recombinant DNA technology (RDNA)
  - DNA construction made in the lab
- Molecular cloning = making lots of the same DNA (clones)
  - Why do you want to do this?
    - Obtain a lot of the corresponding protein (via transcription + translation)
      - Purify and study the protein as such or modify it as *e.g.*, a therapeutic agent
    - Study that particular DNA sequence – gene
    - …



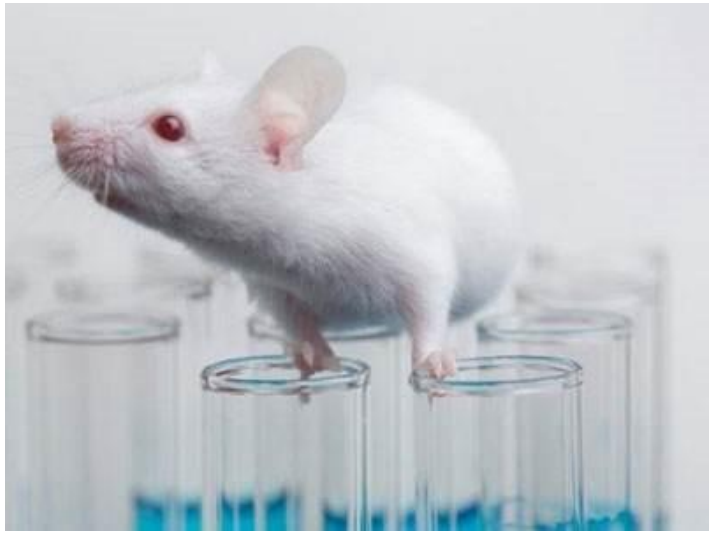
Genetic engineering - 1

Genetically engineered animals are produced for:

Nutrition (*e.g.*, cows that produce hypoallergenic milk or are resistant to bacterial infections

Research (*e.g.*, transgenic mice for disease studies)

Fun (*e.g.*, fishes that glow in the dark)



Genetic engineering - 2

Genetically engineered food are produced for, *e.g.*, :

More nutritious and/or tastier food

Disease- and drought-resistant plants that require fewer environmental resources (such as water and fertilizers)

Less use of pesticides

Increased supply of food with reduced cost and longer shelf life

Faster growing plants….

Golden rice
Engineered to produce β-carotene which is fundamental to prevent vitamin A deficiency in rice-based diet

Creating foods that can cause an allergic reaction or that are toxic
Unexpected or harmful genetic changes
Genes moving from one GM plant or animal to another plant or animal that is not genetically engineered
Foods that are less nutritious

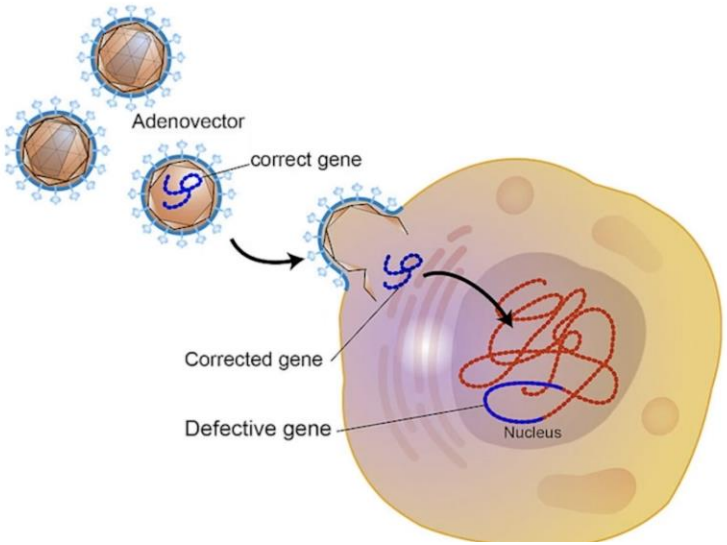
These concerns have proven to be unfounded. None of the GE foods used today have caused any of these problems (https://medlineplus.gov/ency/article/002432.htm)

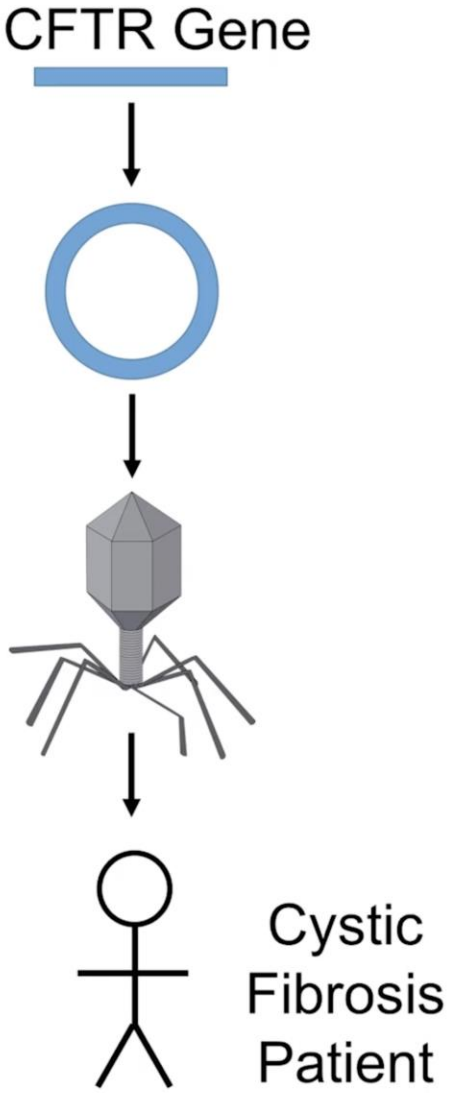
Genetic engineering - 3

MEDICINE

CRISPR gene replacement

CRISPR

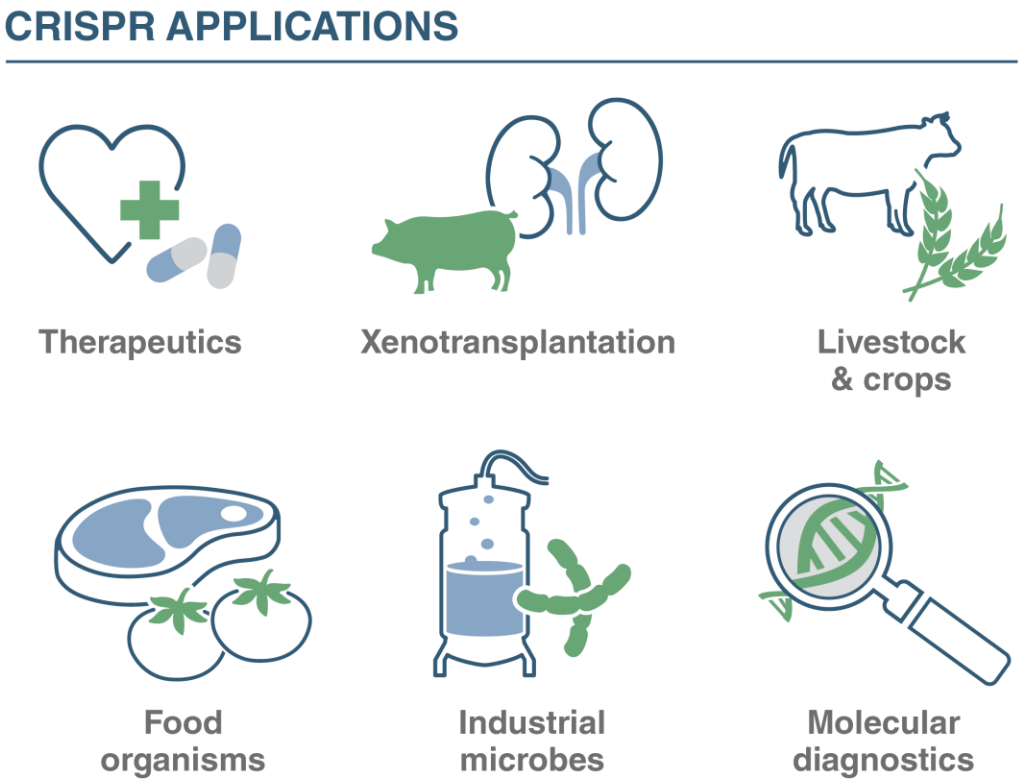




Cystic Fibrosis Patient

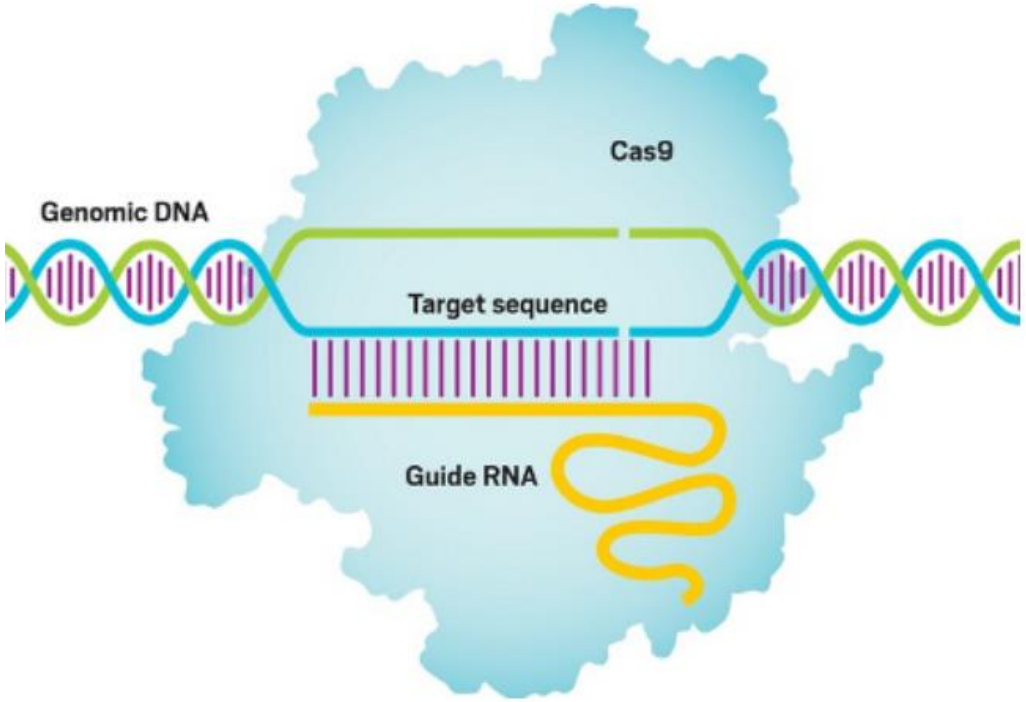
Genetic engineering - 4

- **CRISPR-Cas9** is a gene-editing tool derived from bacterial immune defense
- It allows precise modification of DNA by cutting at specific sites
- Used in biotechnology, medicine, and genetic engineering



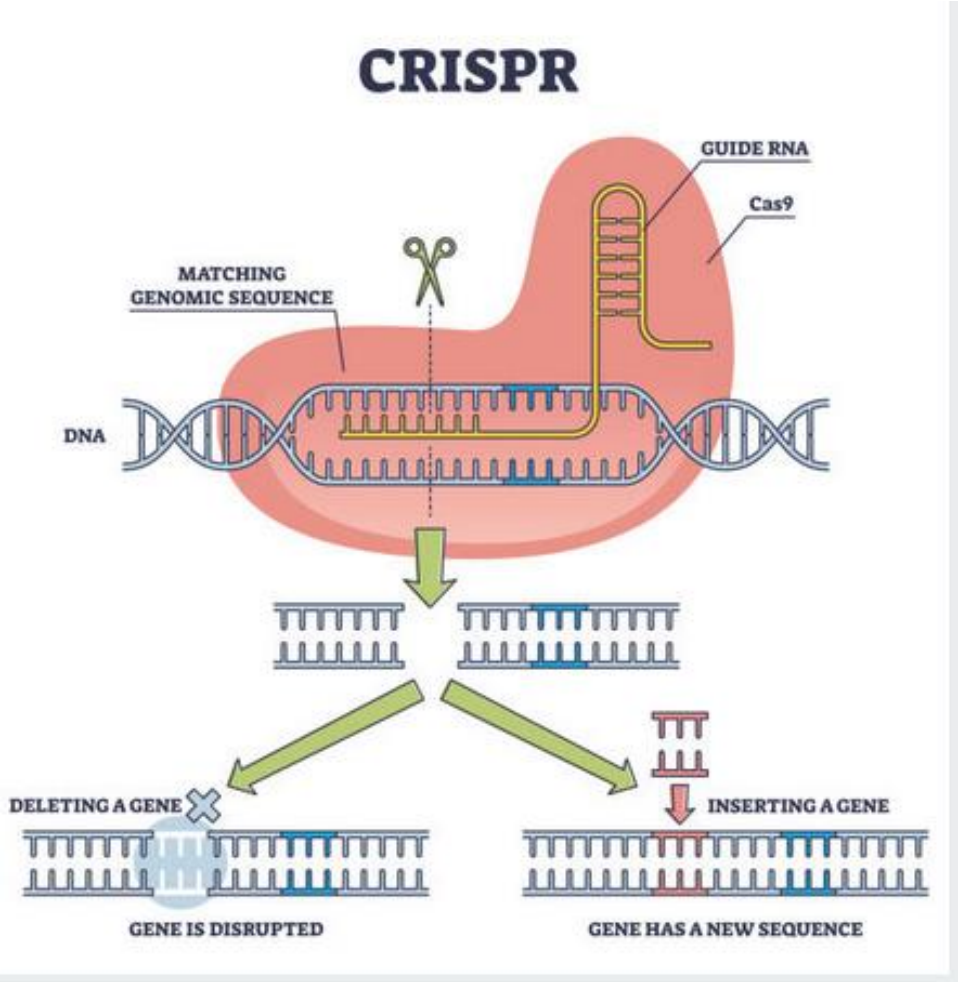
Genetic engineering - 4

- The **genetic scissor system CRISPR-Cas9** (Charpentier and Doudna, NP 2020) is inspired by a mechanism used by bacteria involving an enzyme called **Cas9**
- Think of CRISPR-Cas9 as a high-precision genetic editing tool, similar to a pair of molecular scissors that can cut and modify DNA in living organisms
- CRISPR-Cas9 is inspired by a natural defense system in bacteria
  - Bacteria are constantly attacked by viruses
  - To protect themselves, they store small pieces of the virus's DNA in their own genetic code (like keeping a "mugshot" of the enemy)
  - If the same virus attacks again, the bacteria use this stored information to cut and destroy the virus’s DNA before it can harm them



Genetic engineering - 4

- Scientists have hijacked this bacterial system to edit genes in any living organism. The process works like this:
- ***Finding the Target***
  - Scientists design a guide RNA (sgRNA) to match the exact DNA sequence they want to modify
  - This guide RNA leads Cas9 to the right spot in the DNA
- ***Cutting the DNA***
  - Cas9, a special protein that acts like molecular scissors, cuts the DNA at the target site
- ***Fixing the DNA***
  - Once the DNA is cut, the cell repairs the break in one of two ways:
    - Non-Homologous End Joining (NHEJ) → The cell sticks the broken DNA ends back together, often introducing small errors (useful for turning off genes).
    - Homology-Directed Repair (HDR) → Scientists provide a template DNA sequence, and the cell copies it into the break, allowing precise changes (gene therapy)



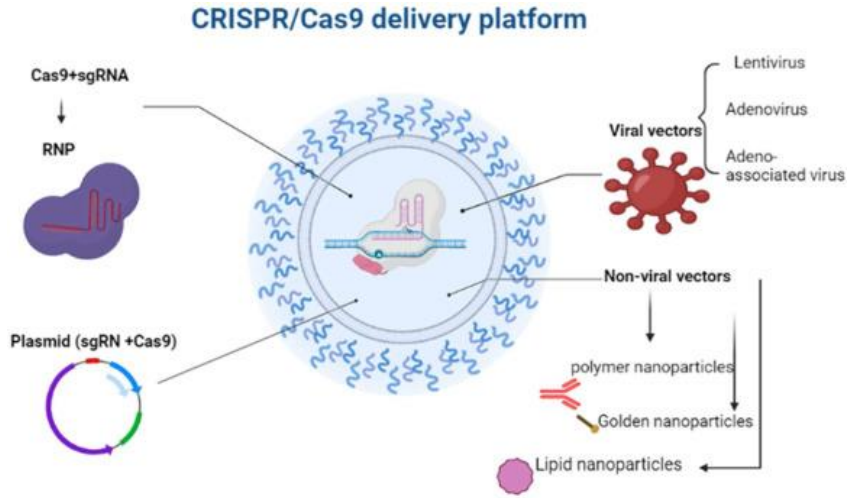
Genetic engineering - 4

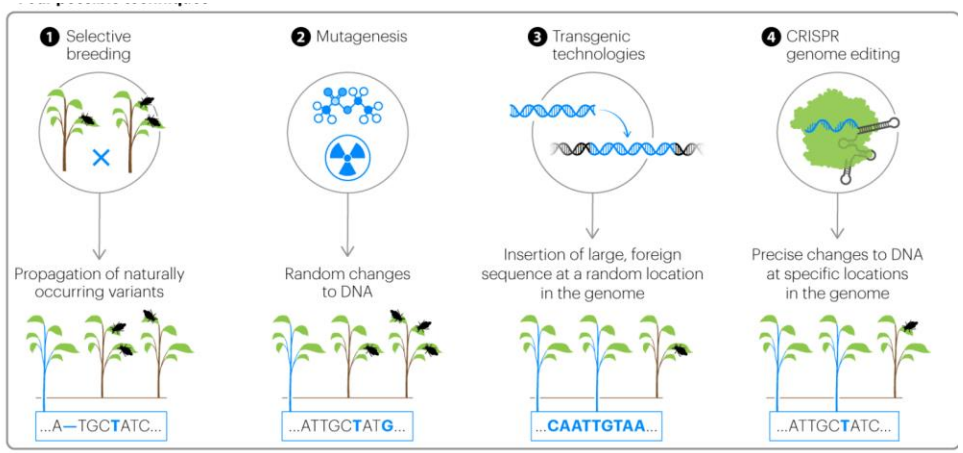
- CRISPR-Cas9 is a **game-changer** because it allows scientists to edit genes **quickly, cheaply, and with high precision**. Some key applications include:

✅ **Medicine**: Treating genetic diseases like sickle cell anemia and cystic fibrosis

✅ **Agriculture**: Creating disease-resistant crops and improving food production

✅ **Biotechnology**: Developing new therapies and studying diseases more effectively

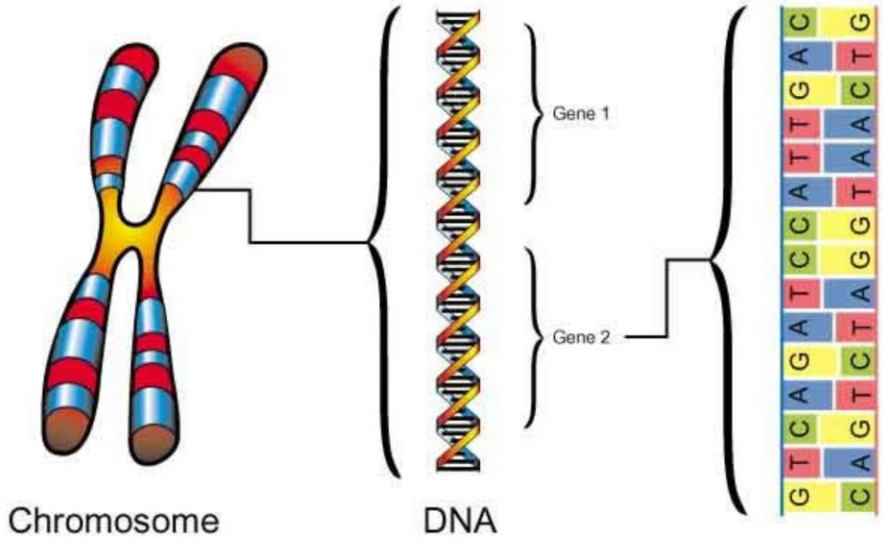




Building recombinant DNA

- Basic step of building recombinant DNA
  - Recombinant DNA = DNA that comes from 2 or more sources

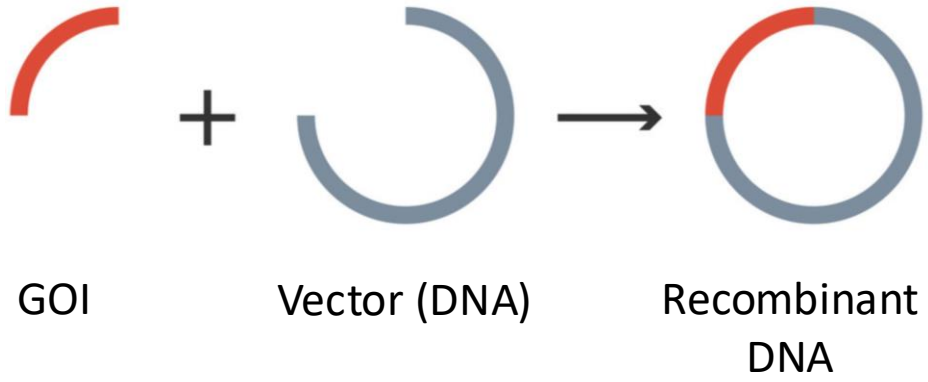
1. Identify your **gene of interest (GOI)** that you want to reproduce on the corresponding chromosome



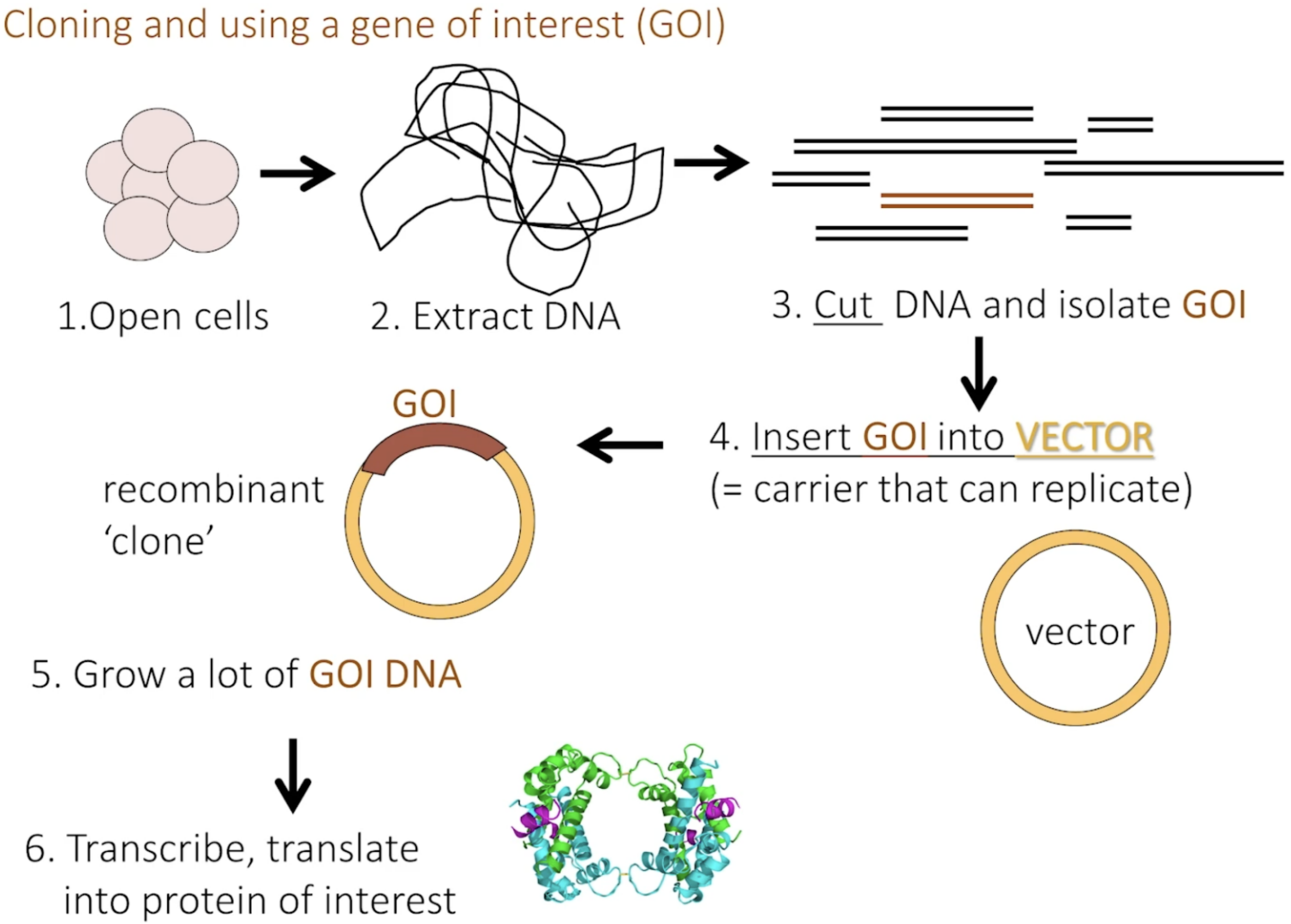
Building recombinant DNA

- Basic step of building recombinant DNA
  - Recombinant DNA = DNA that comes from 2 or more sources

1. Identify your gene of interest (GOI) that you want to reproduce on the corresponding chromosome
2. Cut your GOI DNA from a larger piece and paste it into a **vector**
   - **Vector = carrier = DNA that replicates very avidly**
     - Grows lots of your clone DNA

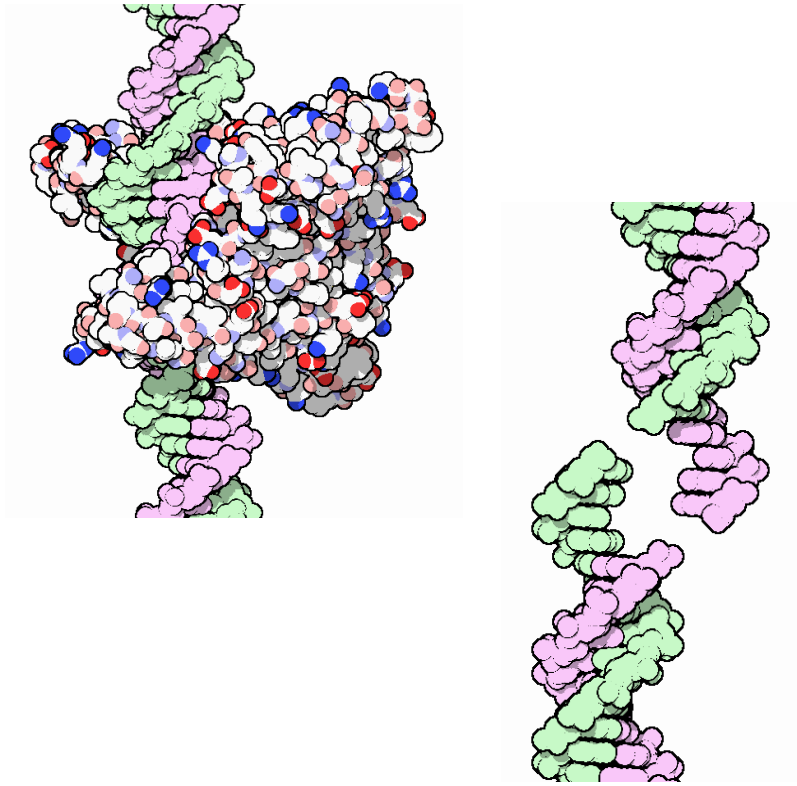


Building recombinant DNA



Cutting DNA

- DNA cutting is performed using specific proteins
  - **Restriction endonucleases (REs)** (enzymes)
- REs precisely cut DNA at specific locations ("molecular scissors")
  - **REs:**
    1. Recognize particular DNA sequences
    2. Bind to these sequences
    3. Break the double-stranded DNA at these specific locations



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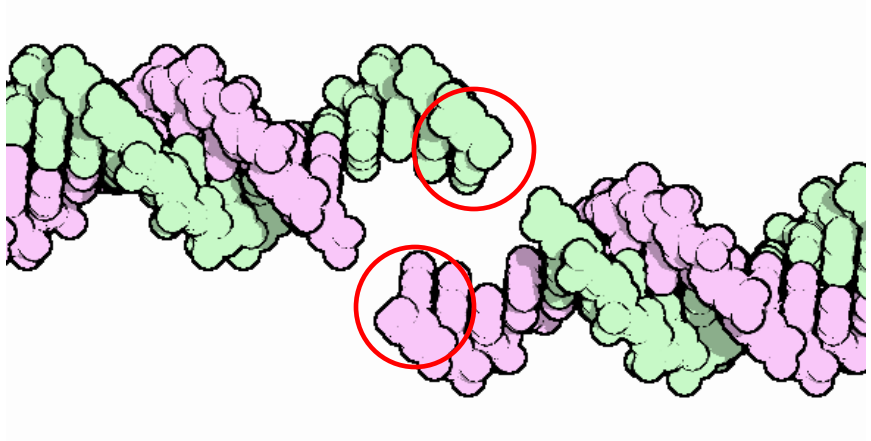
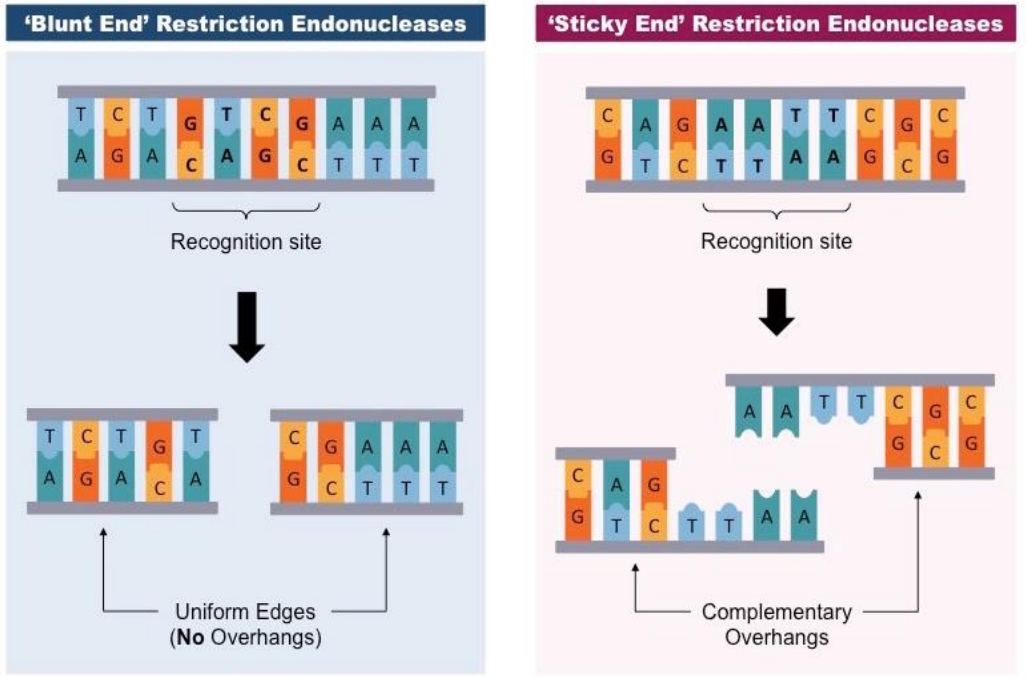
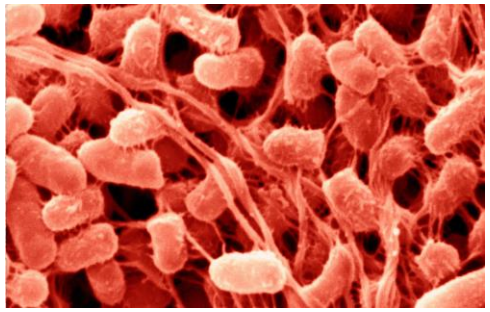
- An RE cut may leave two types of DNA ends:
  - **Blunt ends**
  - **Sticky ends**

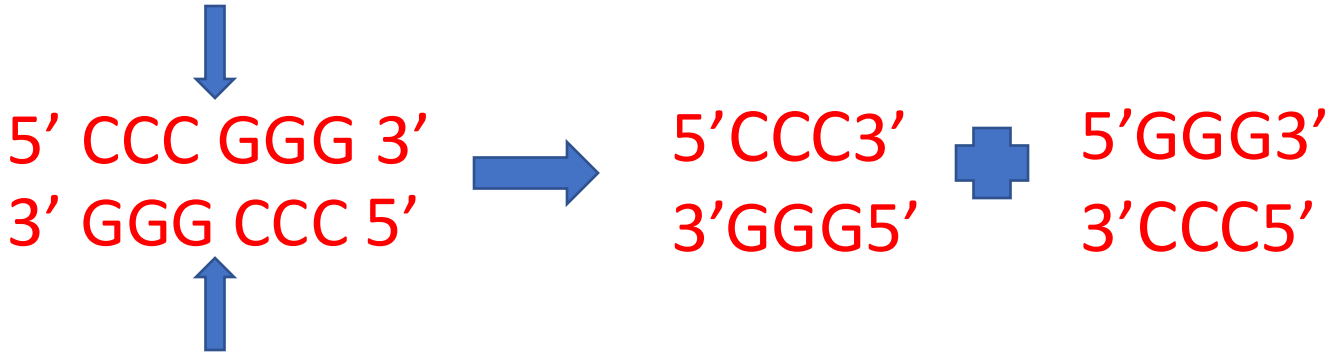




Blunt end (BE) restriction endonucleases



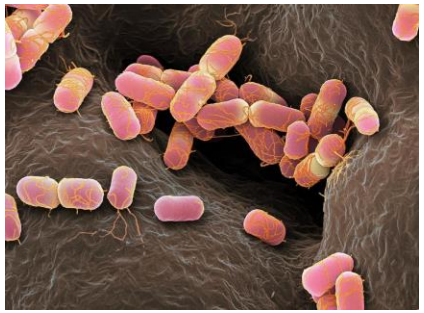
- Prototypical example: **Sma1** (aka **SmaI**)
  - Derived from the bacterium *Serratia marcescens* (hospital opportunistic infections)
    - REs are an important part of bacterial defense system
      - *e.g.*, after a DNA virus attack, the bacterial REs destroy the invader DNA by cutting into pieces
- Sma1 DNA recognition site:



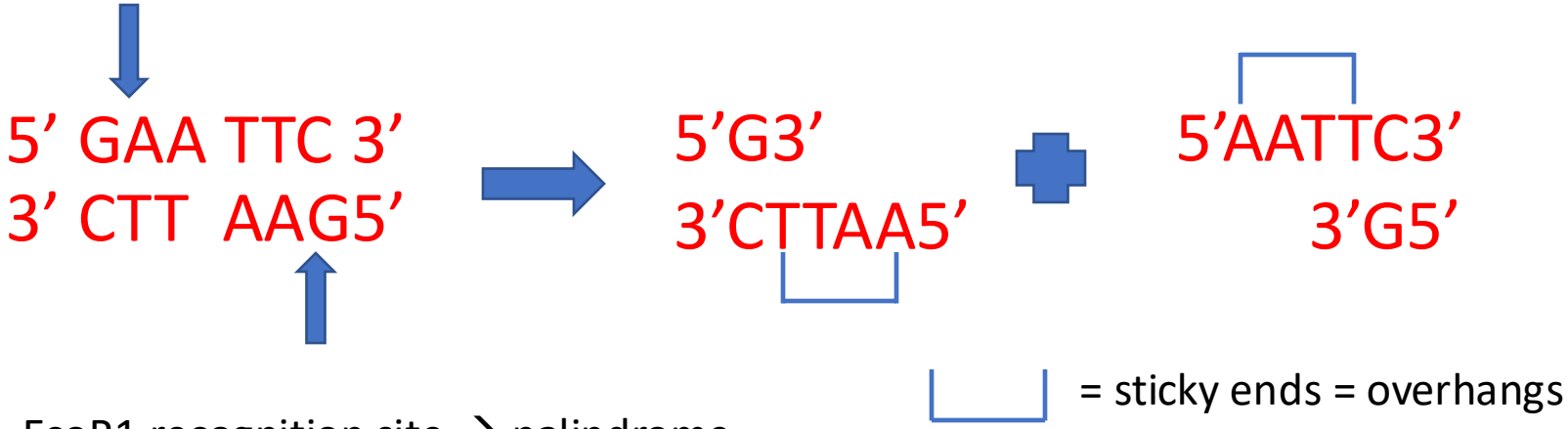
All sequence ends have paired nucleotides
NO unpaired nucleotides (blunt ends)

Sma1 recognition site → palindrome → many RE recognition sites are palindromic

Sticky end (SE) restriction endonucleases



- Prototypical example: EcoR1 (aka EcoRI)
  - Derived from the bacterium *Escherichia coli*
    - *E. coli* forms part of the digestive flora and is always present in fecal matter
    - *E. coli* infections cover a range of severities of urinary tract infection
- EcoR1 DNA recognition site:

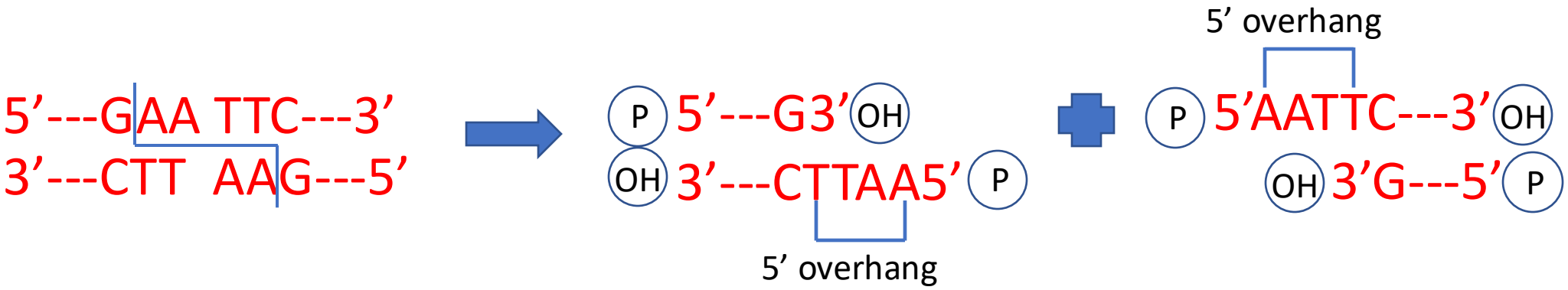


EcoR1 recognition site → palindrome

Single-stranded (ss) DNA ends result after the cut
These ss DNAs can base pair with complementary sequences
sticky ends

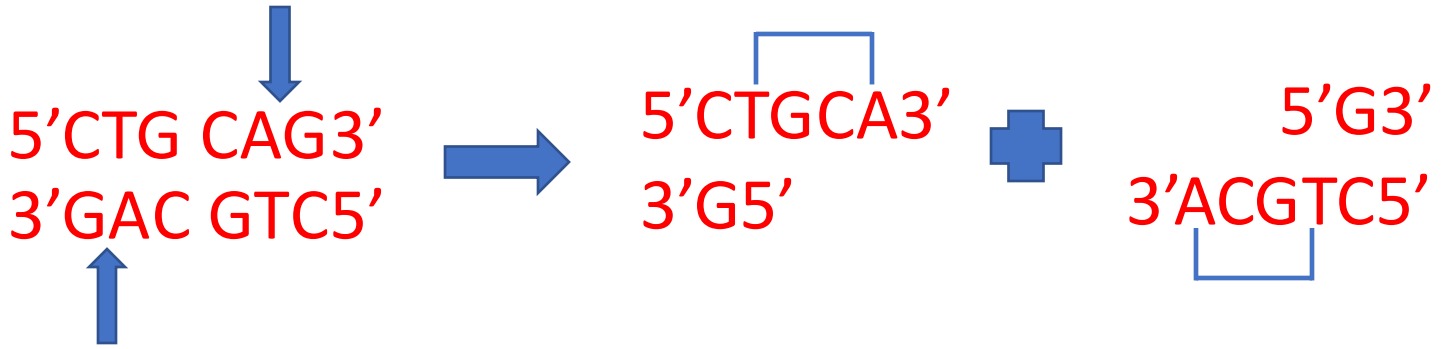
Sticky end (SE) restriction endonucleases

- EcoR1 leaves **5’ overhangs**
  - *If the single-stranded bases end with a 5’ phosphate, the RE enzyme is said to leave 5’ overhangs*



Sticky end (SE) restriction endonucleases

- There are also SE RE enzymes that leave 3’ overhangs:
- Prototypical example: Pst1 (often PstI)
  - Derived from the bacterium *Providencia stuartii*
    - *P. Stuartii* is an opportunistic pathogen seen in patients with severe burns or urinary infections
- Pst1 DNA recognition site:

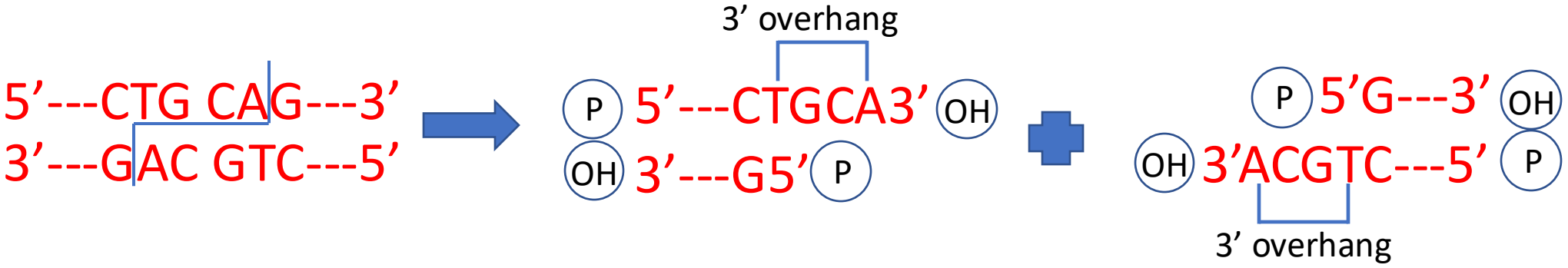


Pst1 recognition site → palindrome

= sticky ends = overhangs

Sticky end (SE) restriction endonucleases

- PST1 leaves **3’ overhangs**
  - *If the single-stranded bases end with a 3’ hydroxyl, the enzyme is said to leave 3’ overhangs*



REs - recap

- There are > 3,000 REs, named after the bacterium they were discovered in
- REs database:
  - You can find an RE that cuts the DNA exactly where you want to

Enzyme	Source organism	Restriction recognition site	Structure of the cleaved products
EcoRI	*Escherichia coli*	5′—G—A—A—T—T—C—3′ 3′—C—T—T—A—A—G—5′	5′—G A—A—T—T—C—3′ 3′—C—T—T—A—A G—5′ 5′ overhang
PstI	*Providencia stuartii*	5′—C—T—G—C—A—G—3′ 3′—G—A—C—G—T—C—5′	5′—C—T—G—C—A G—3′ 3′—G A—C—G—T—C—5′ 3′ overhang
SmaI	*Serratia marcescens*	5′—C—C—C—G—G—G—3′ 3′—G—G—G—C—C—C—5′	5′—C—C—C G—G—G—3′ 3′—G—G—G C—C—C—5′ Blunt ends