Restriction Endonucleases: The Molecular Scalpel

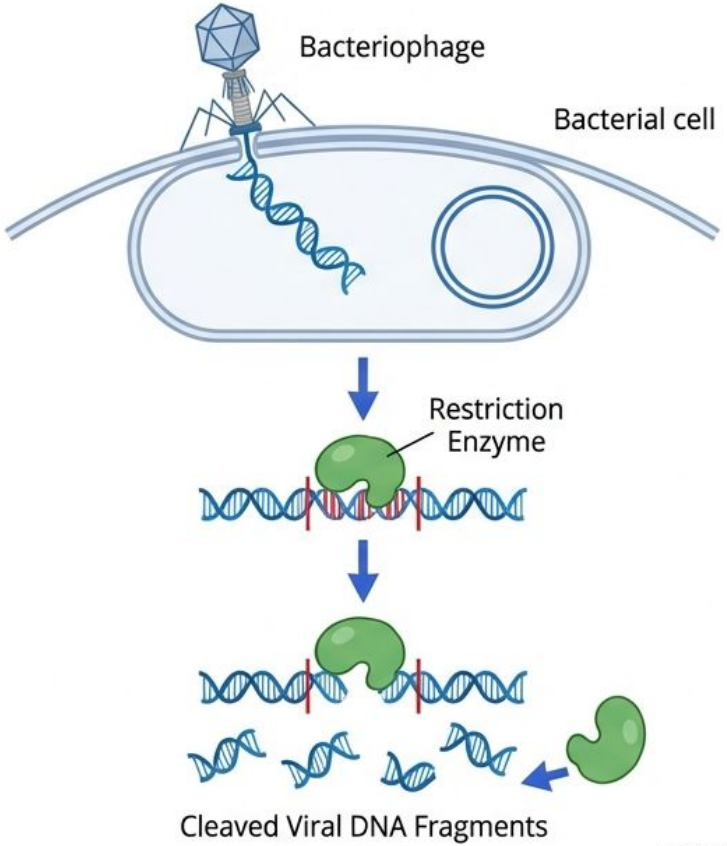
Principles, Mechanisms, and Clinical Applications

Comprehensive Review of Molecular Scissors

Definition & Biological Origin

- **Core Definition:** Restriction enzymes are site-specific endonucleases produced by bacteria and archaea that cleave the phosphodiester bond of DNA at specific recognition sequences.
- **Evolutionary Purpose:** A primitive bacterial immune system.
- **Target:** Bacteriophages (invading viruses).

- **Mechanism:** The enzyme “restricts” viral replication by degrading foreign DNA upon entry.

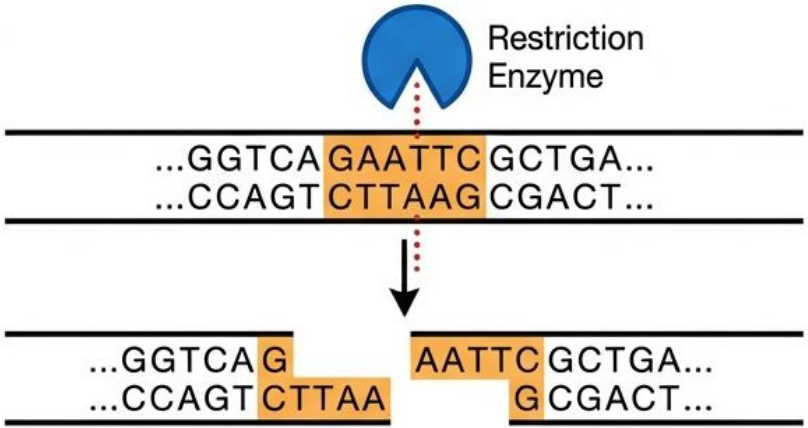


The Restriction-Modification (R-M) System

Sword and Shield

The Sword (Restriction)

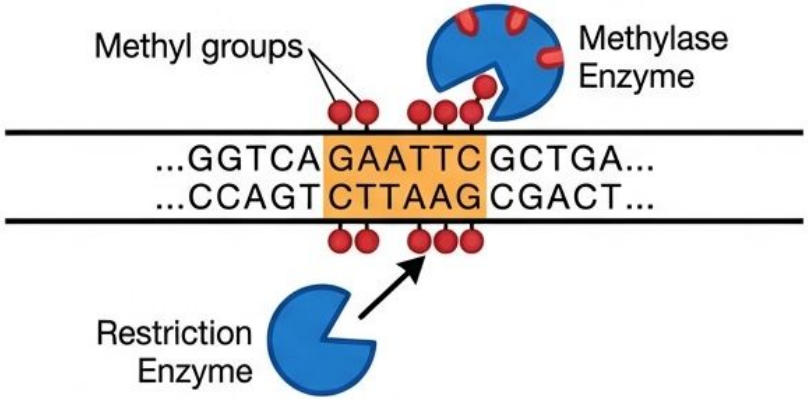
Foreign DNA (Unmethylated):
Recognized as a threat.



Cleavage.

The Shield (Modification)

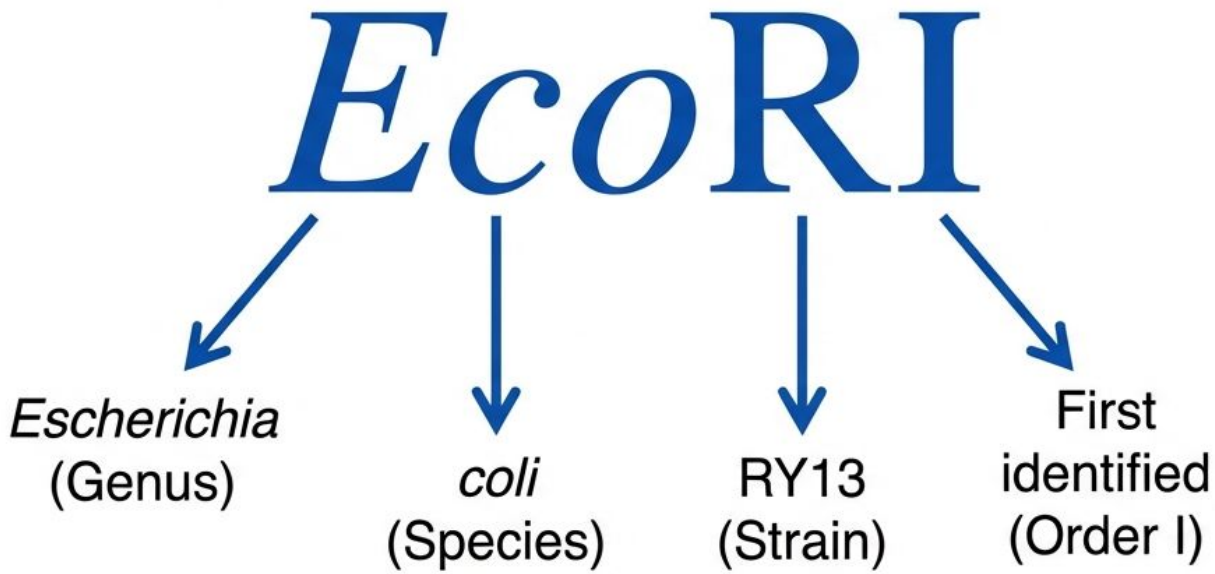
Host DNA (Methylated):
Protected from cleavage.



Protection.

Key Concept: Methylation masks the recognition site, preventing 'suicide' by the bacterium's own enzymes.

Nomenclature Rules



**Other Examples:

HindIII: *Haemophilus influenzae* (strain Rd), 3rd enzyme.

BamHI: *Bacillus amyloliquefaciens* (strain H), 1st enzyme.

Classes of Restriction Enzymes

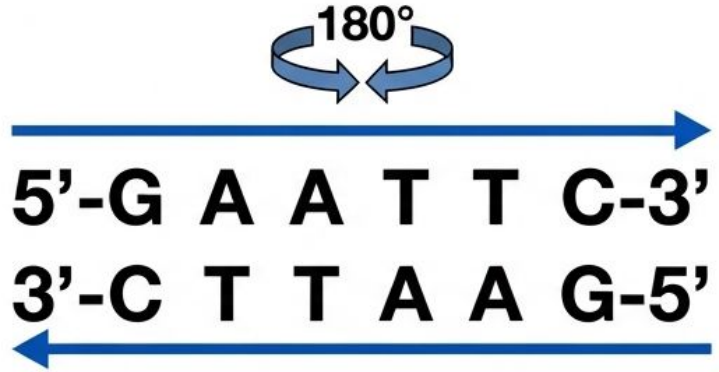
Type I	Type II (The Lab Standard)	Type III
Cut Site: Random (>1000bp away)	**Cut Site:** At specific recognition site	**Cut Site:** 25-27bp downstream
Cofactors: ATP, Mg^{2+}, SAM	**Cofactors:** Mg^{2+} Only (No ATP)	**Cofactors:** ATP, Mg^{2+}
Utility: Low (Non-specific)	**Utility:** High (Precise & Reproducible)	**Utility:** Low

Type II enzymes are the workhorses of molecular biology because they cut exactly at the recognition sequence.

Recognition Sites & Palindromes

The Concept

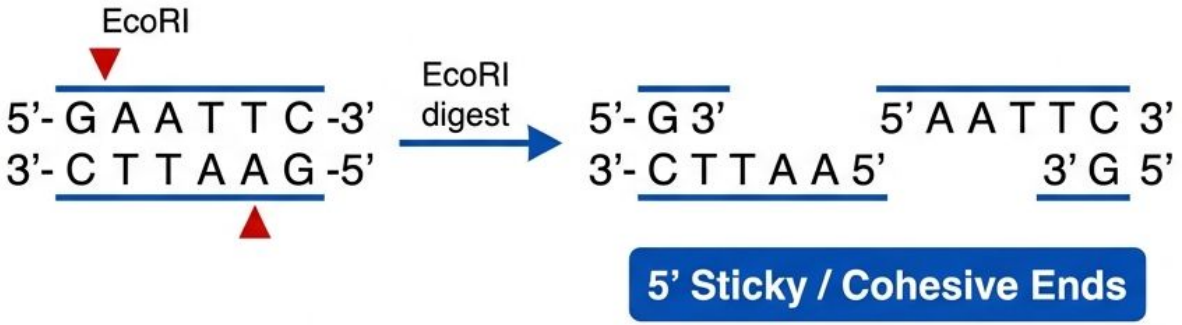
Restriction sites typically display Dyad Symmetry (Palindromes). They read the same 5′ → 3′ on both strands.



Frequency

- **4bp Cutter (*Alu*I):** AGCT (Frequent, ~256bp)
- **6bp Cutter (*Eco*RI):** GAATTC (Standard, ~4,096bp)
- **8bp Cutter (*Not*I):** GCGGCCGC (Rare, Genome Mapping)

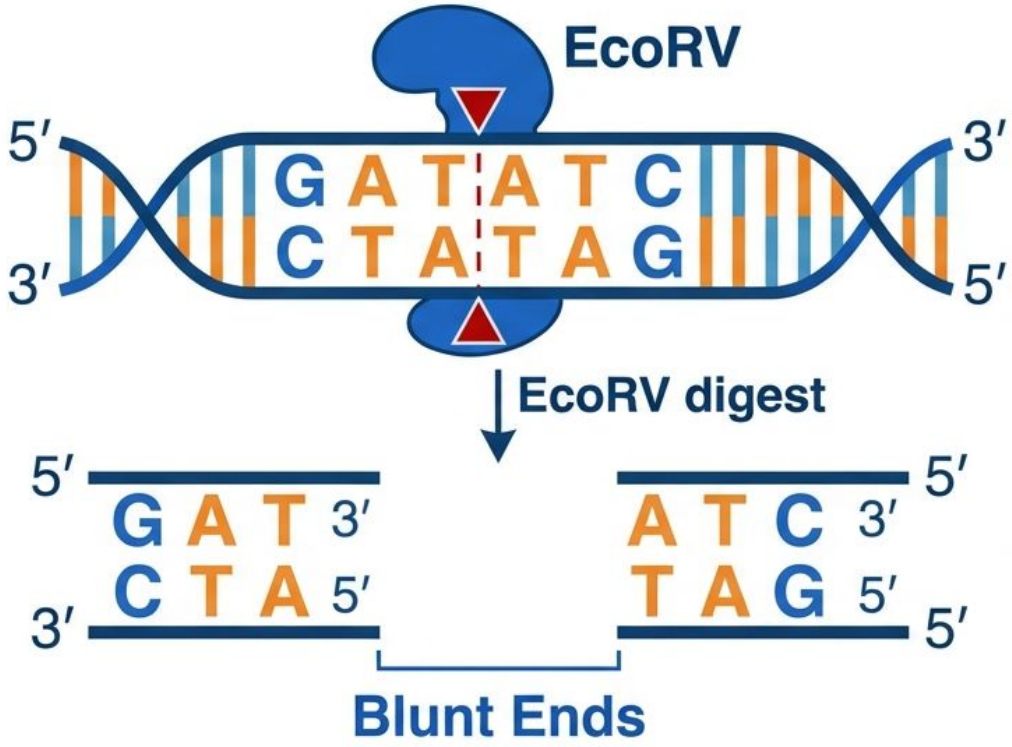
Cleavage Pattern 1: Sticky Ends



Mechanism: Staggered cleavage creates overhangs.

Benefit: Complementary overhangs hydrogen bond easily, increasing ligation efficiency.

Cleavage Pattern 2: Blunt Ends

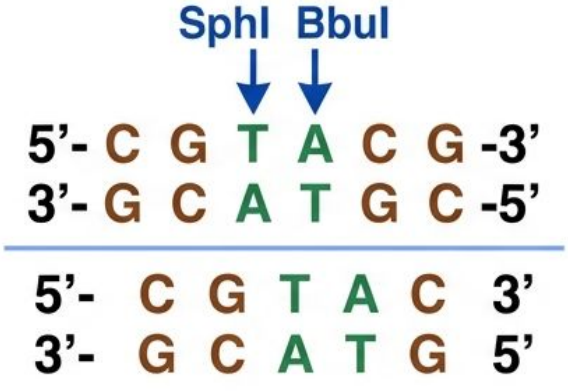


- **Mechanism:** Cleavage at the axis of symmetry.
- **Pros:** Universally compatible (Blunt-to-Blunt).
- **Cons:** Lower ligation efficiency (no hydrogen bonding to stabilize).

Isoschizomers & Neoschizomers

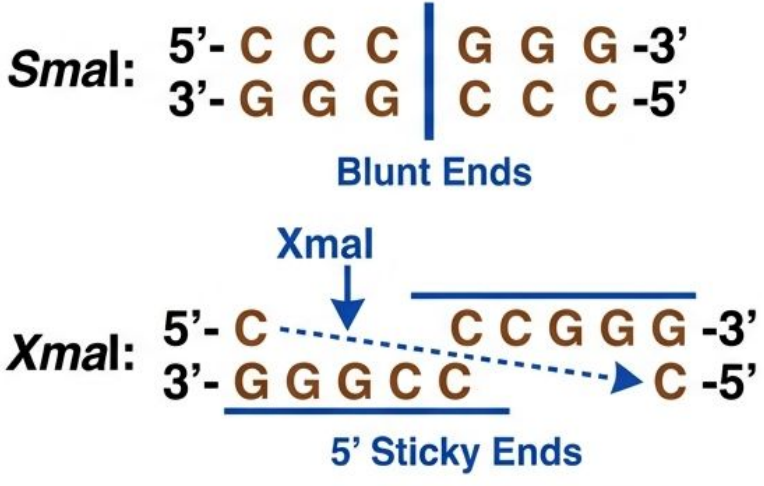
Isoschizomers (Same Site, Same Cut)

SphI and **BbuI**



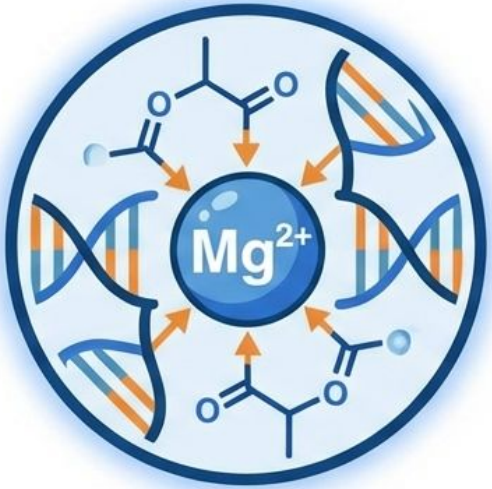
Neoschizomers (Same Site, Different Cut)

***Sma*I** vs ***Xma*I** (Target: CCCGGG)



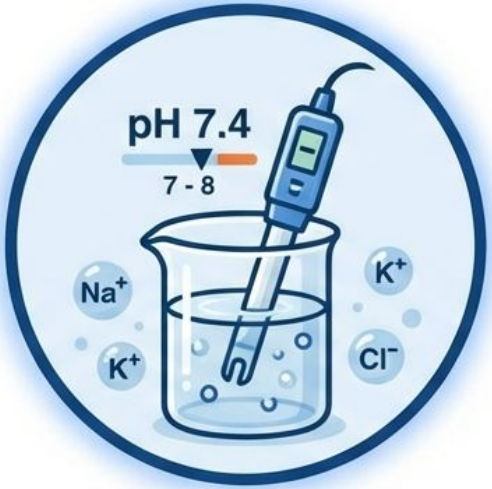
Neoschizomers allow researchers to generate different ends (sticky vs blunt) from the exact same DNA sequence.

Reaction Conditions



Cofactor

Magnesium (Mg^{2+}) is essential for Type II catalytic activity.



Buffer

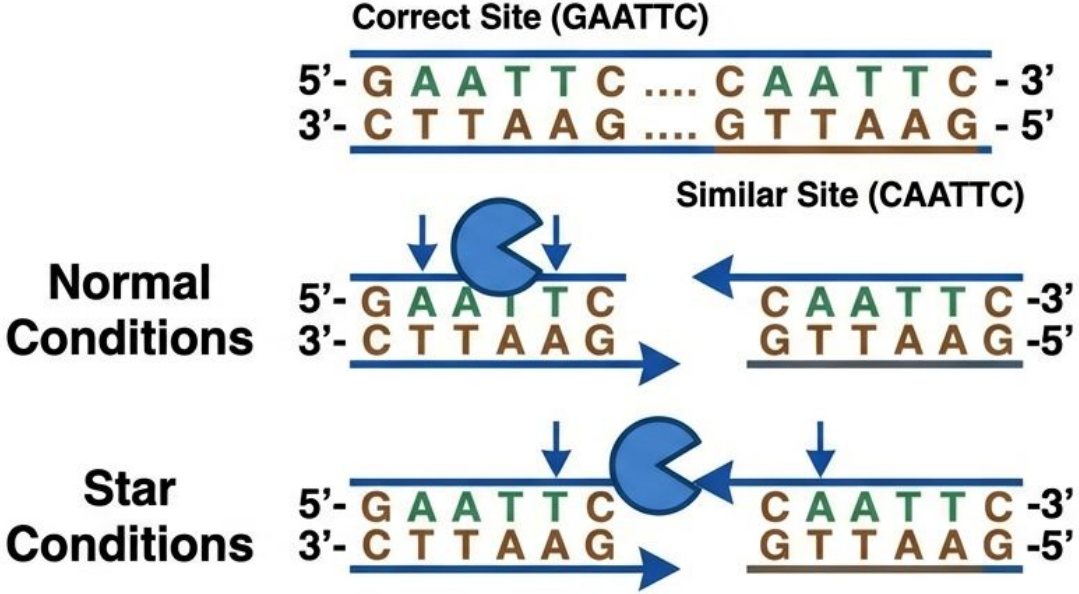
pH 7.0 - 8.0 and specific ionic strength (NaCl/KCl) required for specificity.

Temperature

37°C (Standard for *E. coli* enzymes). **Exception:** TaqI requires **65°C.**

Star Activity

Star Activity: When restriction enzymes lose specificity and cleave sequences *similar* but *not identical* to their recognition site.

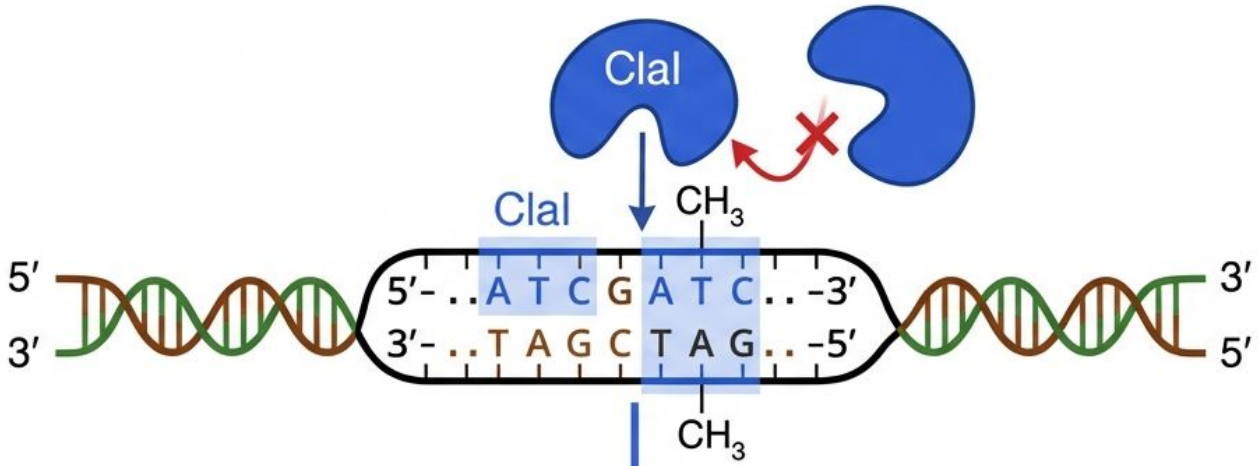


Causes

- High Glycerol (>5%)
- High Enzyme Concentration (>100 U/µg)
- Low Ionic Strength
- Prolonged Incubation time

Methylation Sensitivity

Standard *E. coli* strains express Dam and Dcm methylases. If a restriction site overlaps with a methylation site, the enzyme may be blocked.



Steric Hindrance: Methylation prevents enzyme binding.

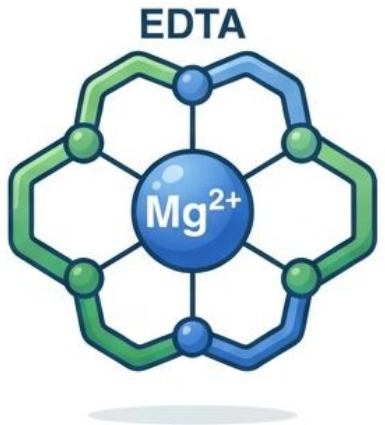
Solution: Use *dam-/dcm-* competent cells to propagate DNA for these sensitive enzymes.

Thermal Inactivation

Heat (The Standard)

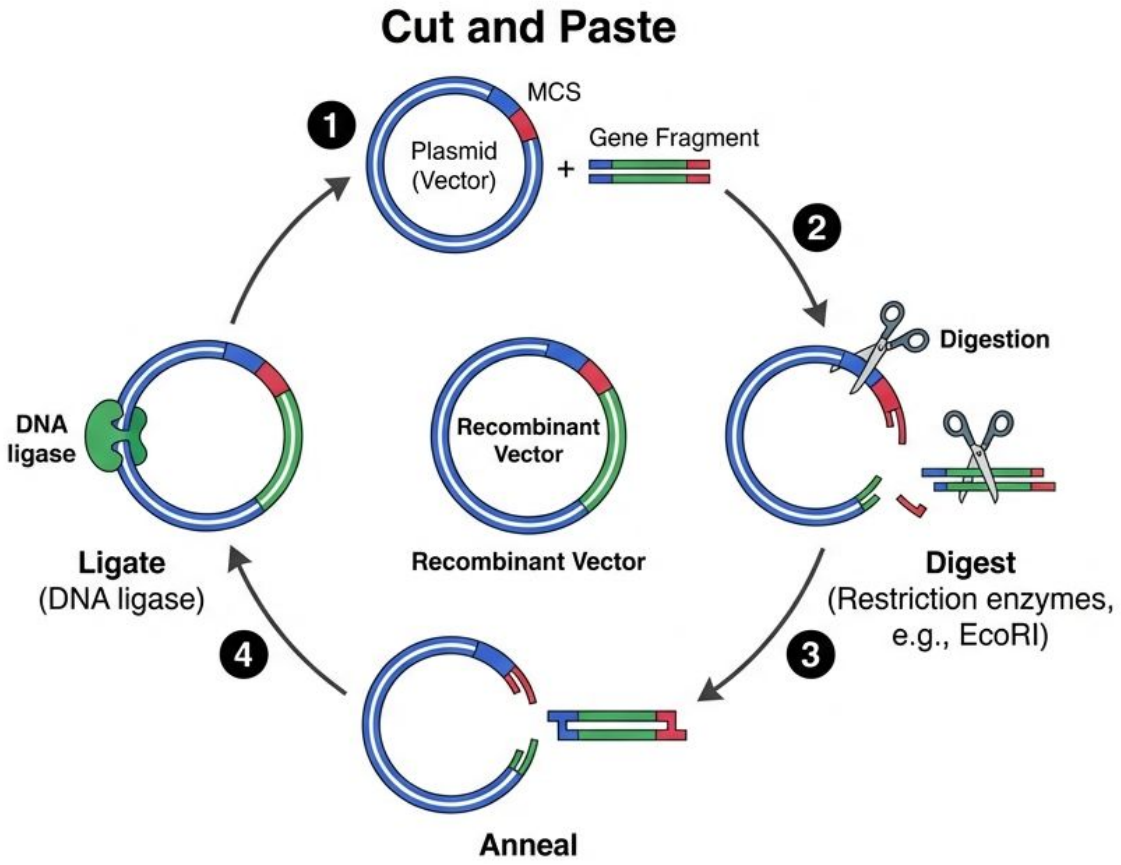
Heat Inactivation: 65°C for 20 minutes denatures most enzymes (e.g., EcoRI). Essential before ligation.

Chelation (The Alternative)



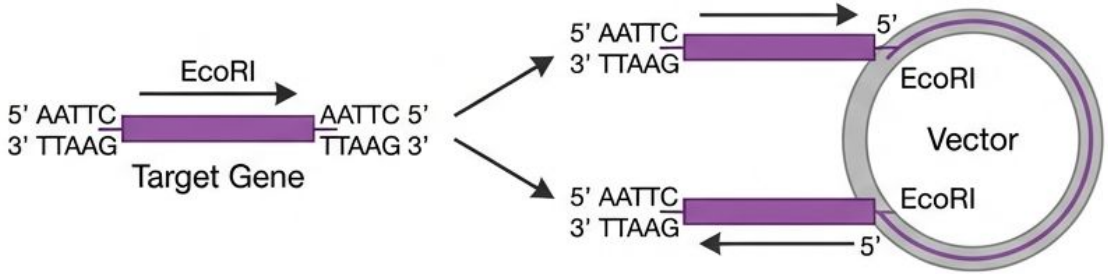
Chelation: For heat-resistant enzymes (e.g., BamHI, TaqI), add **EDTA**. EDTA binds Mg^{2+}, starving the enzyme of its cofactor.

Gene Cloning (Recombinant DNA)



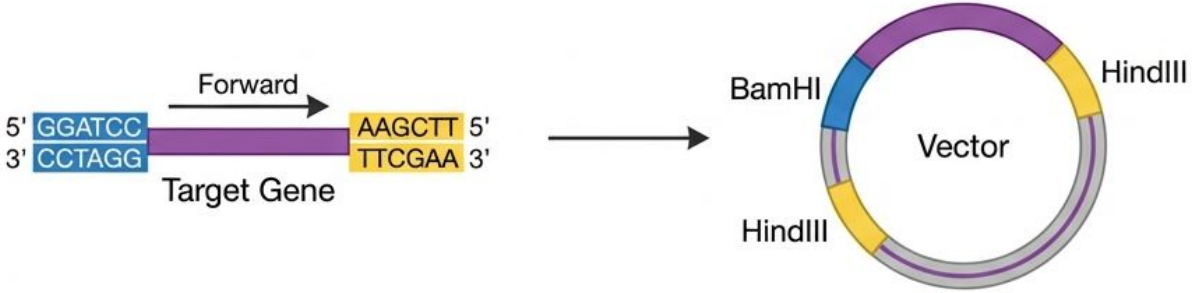
Directional Cloning

Single Enzyme (EcoRI)



50% Wrong Orientation

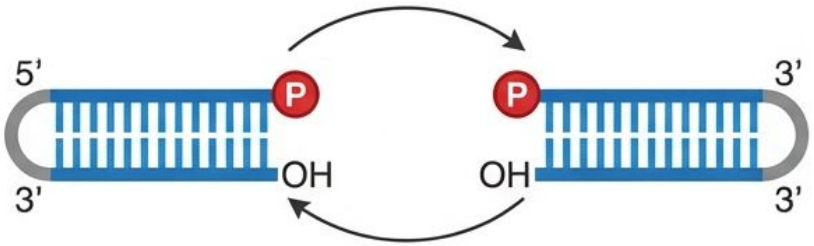
Double Enzyme (BamHI + HindIII)



100% Correct Orientation

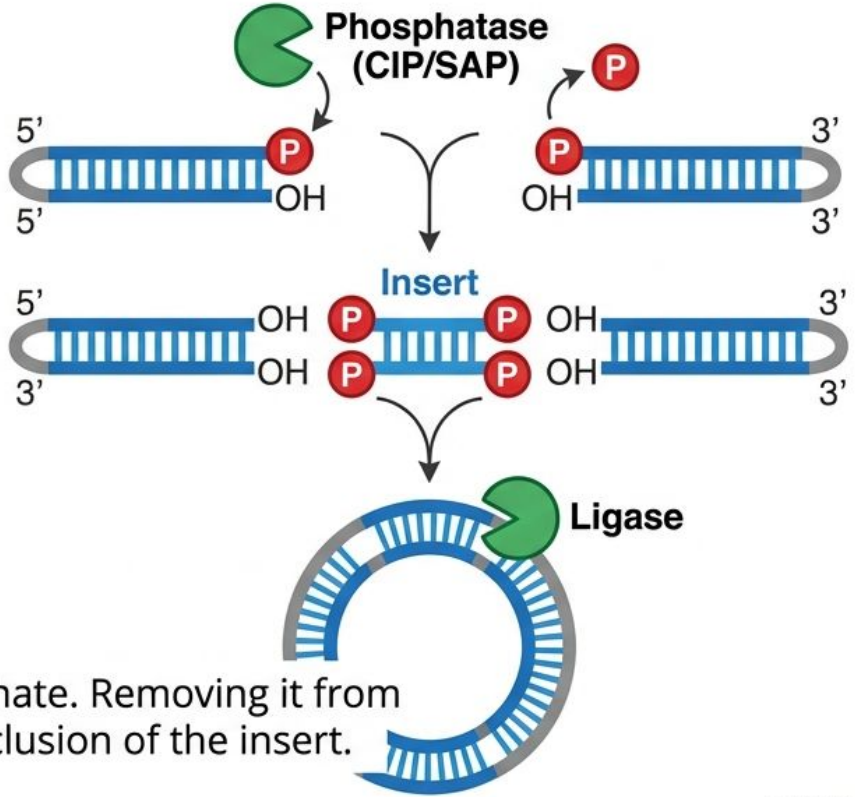
Preventing Self-Ligation

The Problem



Vectors tend to re-ligate to themselves (empty vector).

The Solution

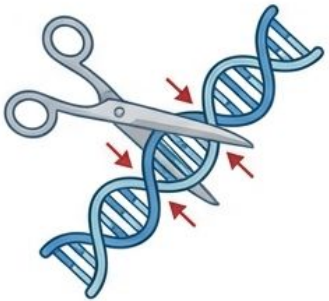


Ligase requires a 5' Phosphate. Removing it from the vector forces the inclusion of the insert.

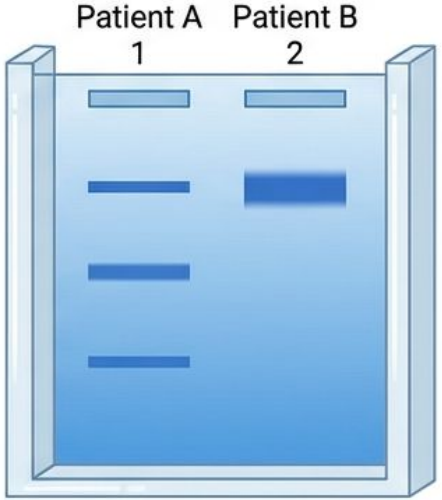
RFLP Analysis

Step 1:
DNA Extraction from two patients.

Step 2:
Digestion with Restriction Enzyme.



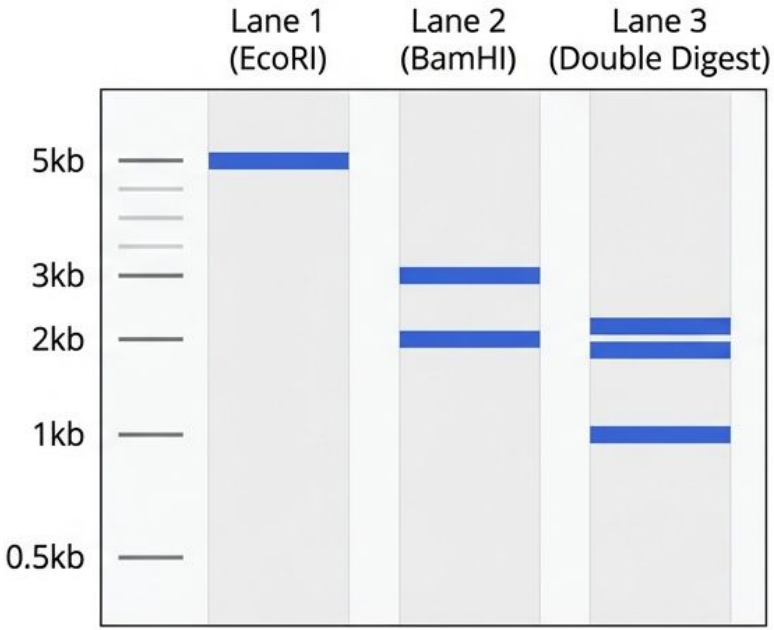
Step 3:
Electrophoresis.



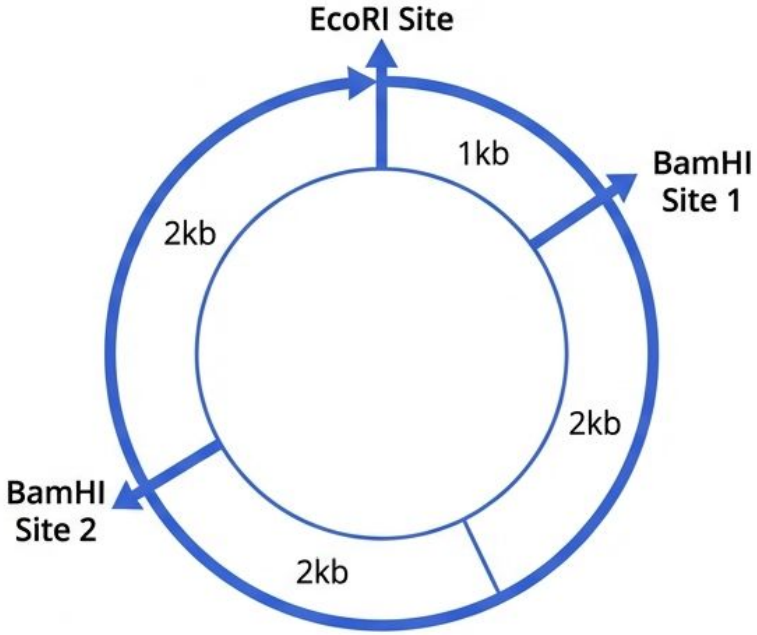
Differences in DNA sequence create different fragment lengths, visible as distinct banding patterns.

Restriction Mapping

The Gel Data



The Map Construction

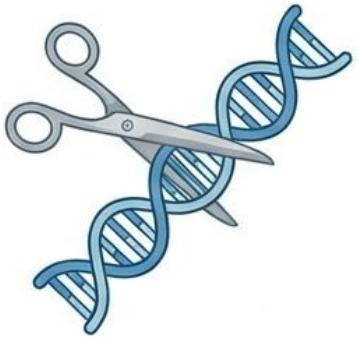


Logic puzzle visualization: Map construction based on fragment sizes and digest patterns.

Summary & Evolution: The Next Generation

Restriction Enzymes

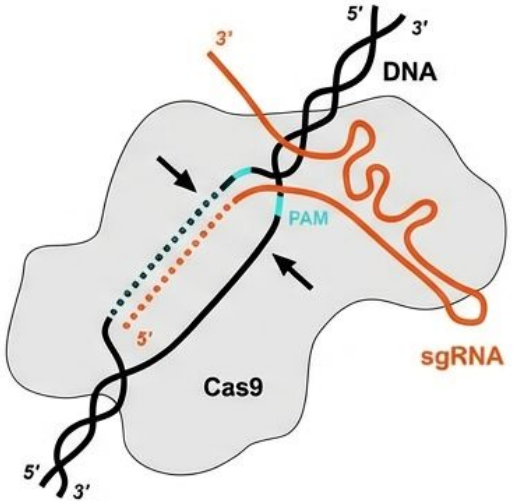
- Fixed recognition (4-8bp).
- Dependent on natural palindromes.
- The Foundation of Cloning.



Molecular Scissors

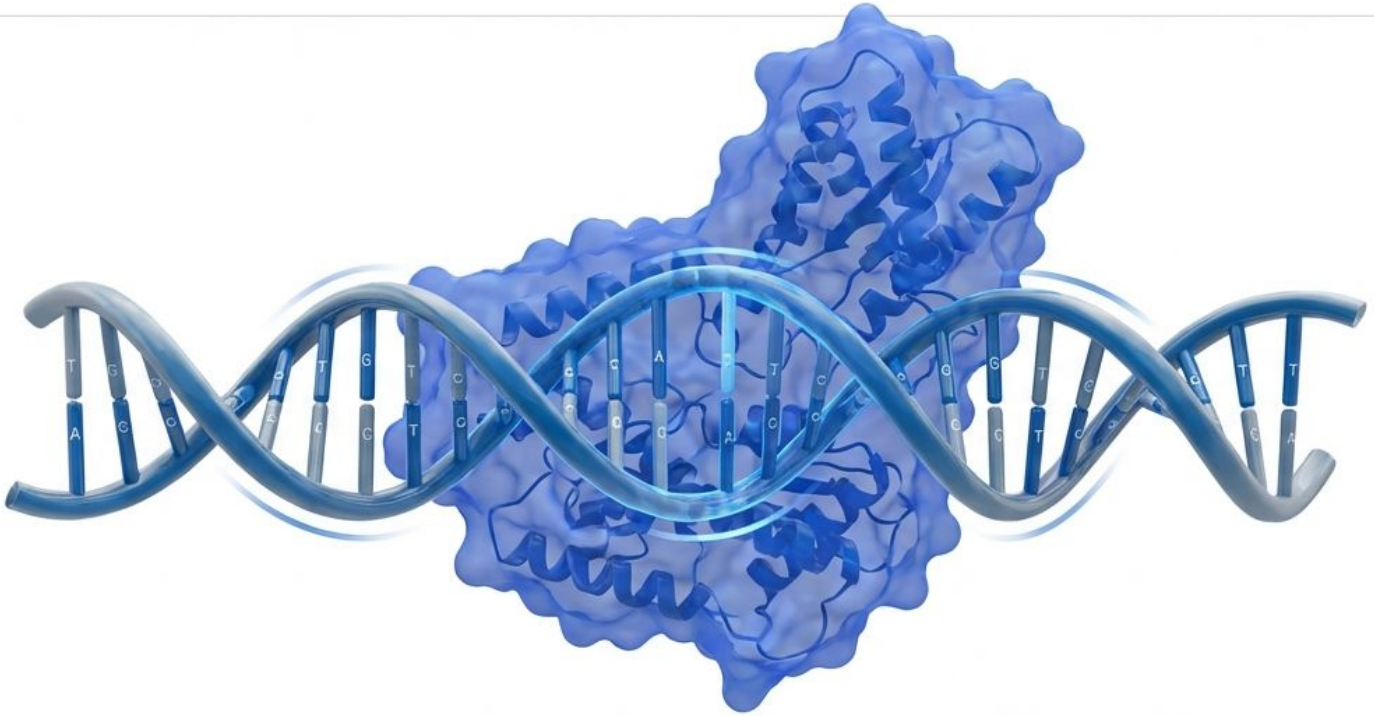
CRISPR-Cas9

- Programmable targeting (Guide RNA).
- High Specificity (~20bp).
- Genome Editing in living cells.



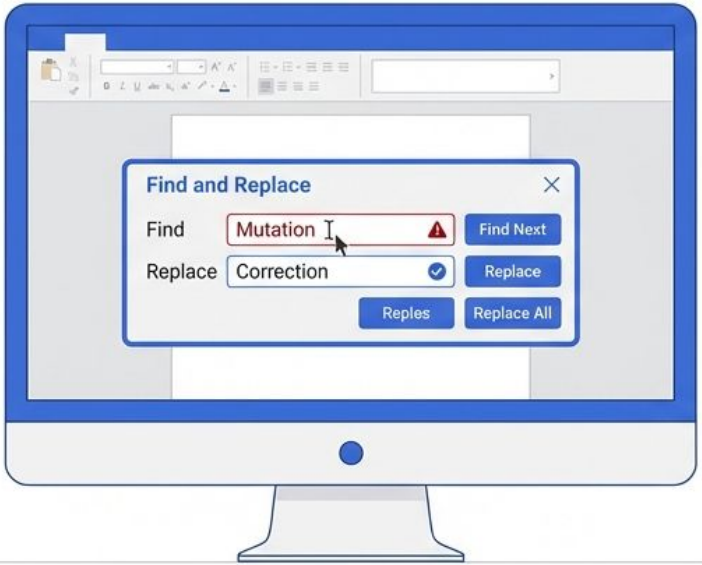
CRISPR-Cas9: The Genetic Scissors

A Revolution in Genome Editing



The power to rewrite the code of life.

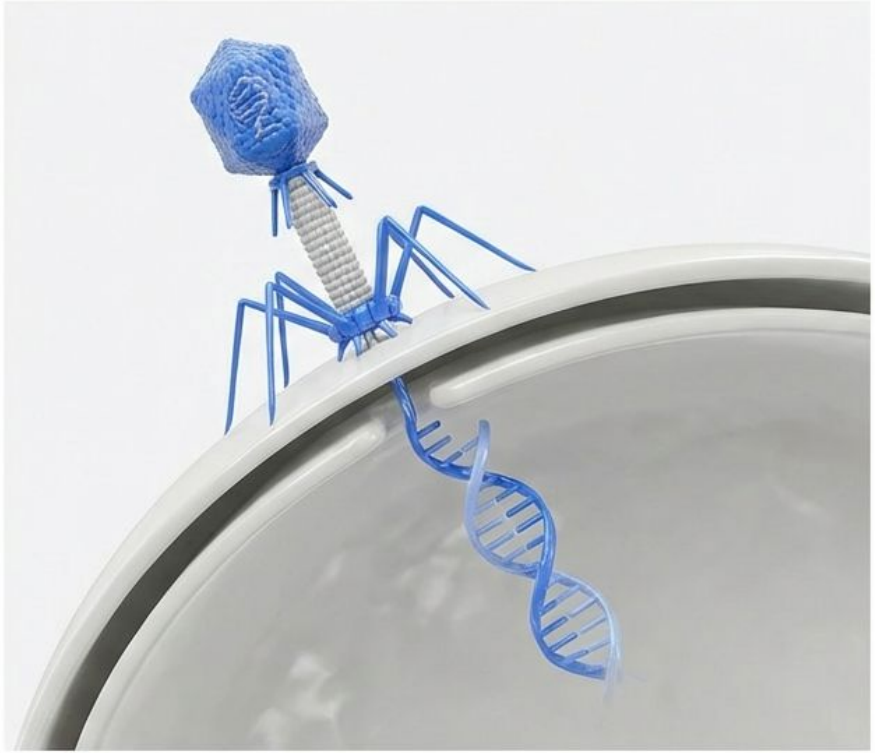
The ‘Find and Replace’ for DNA



Imagine the genome as a document with 3 billion letters.

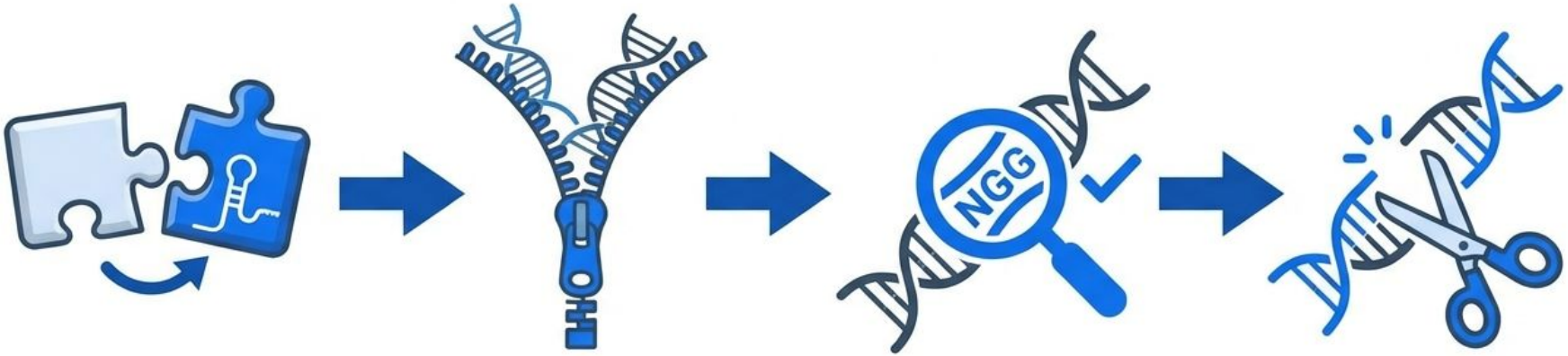
- **The Problem:** A ‘typo’ (mutation) causes disease.
- **The Solution:** CRISPR acts like the Ctrl+F tool.
- **The Function:** It searches for a specific sequence and edits it with precision.

A Bacterial Shield



- **Origin:** CRISPR was not invented in a lab; it was discovered in nature.
- **The War:** It is an adaptive immune system found in bacteria and archaea.
- **The Enemy:** Bacteriophages (viruses that attack bacteria).
- **The Defense:** Bacteria use CRISPR to ‘remember’ and destroy viral invaders.

Mechanism Summary: Step-by-Step



1. Bind
(Cas9 + sgRNA)

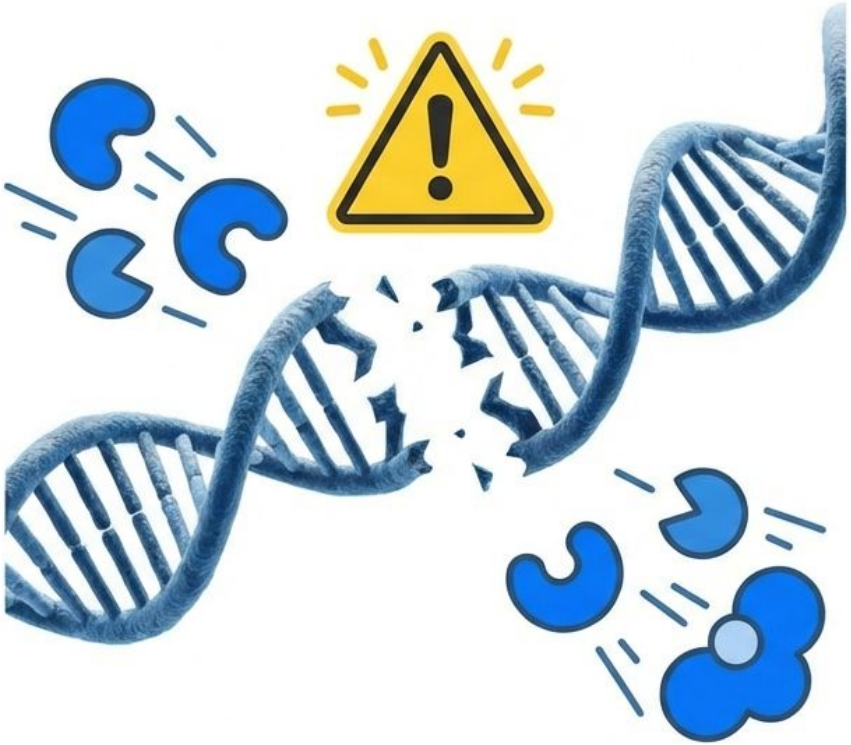
2. Unwind
(DNA Search)

3. Check
(PAM & Match)

4. Cut
(Double Strand Break)

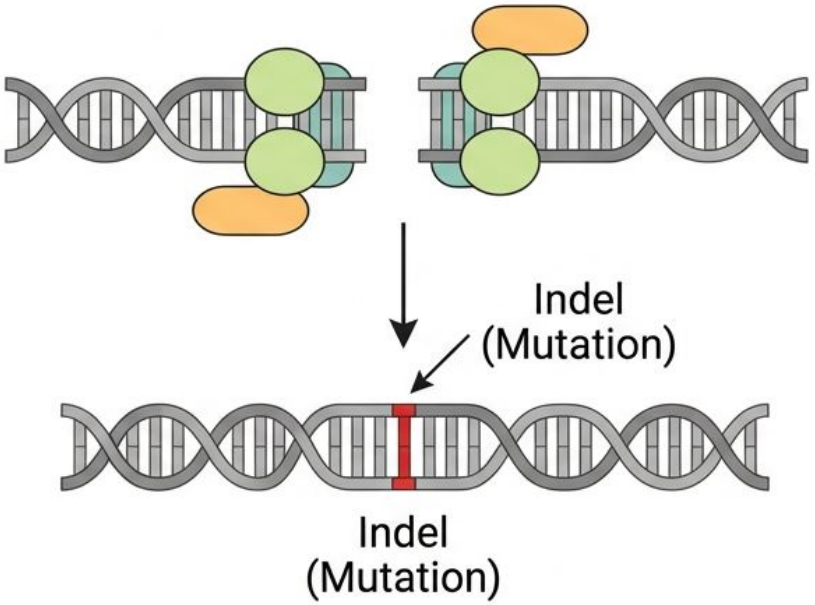
The complex scans the genome, verifies the address, and makes the cut.

The Cut: Cellular Panic Mode



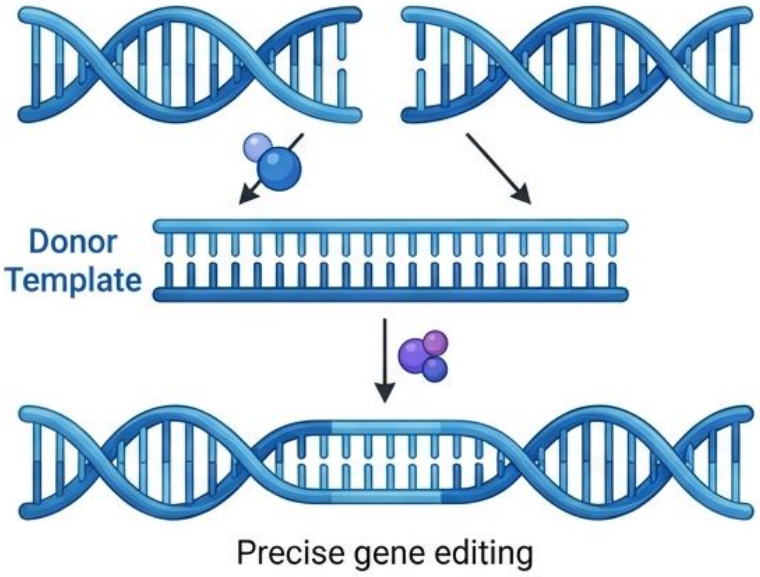
- **The Status:** Cas9 has created a Double-Strand Break (DSB).
- **The Crisis:** To the cell, this is a life-threatening emergency.
- **The Response:** Emergency Repair Crews (cellular enzymes) rush to fix the break.
- **The Outcome:** Depends entirely on HOW the cell fixes this break.

Repair Pathway 1: NHEJ (The Quick Fix)



- **Name:** Non-Homologous End Joining (NHEJ).
- **The Method:** Glue the ends back together immediately.
- **The Result:** Error-prone. Random letters are lost or added (Indels).
- **The Application:** Gene Knockout. The mutation breaks the gene's instructions, effectively turning it off.

Repair Pathway 2: HDR (The Precise Edit)



- **Name:** Homology-Directed Repair (HDR).
- **The Method:** Copy-Paste from a Template.
- **The Requirement:** Scientists inject a ‘Donor DNA’ template along with Cas9.
- **The Result:** Precise editing. The cell copies the correct sequence from the donor.
- **The Application:** Gene Knock-in. Fixing a typo or inserting a new gene.

THANKS FOR LISTENING