

## Lesson Overview

14.3 Studying the  
Human Genome

## Manipulating DNA

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- This difference makes DNA relatively easy to extract from cells and tissues.
- DNA molecules from most organisms are much too large to be analyzed, so they must first be cut into smaller pieces.

## Manipulating DNA

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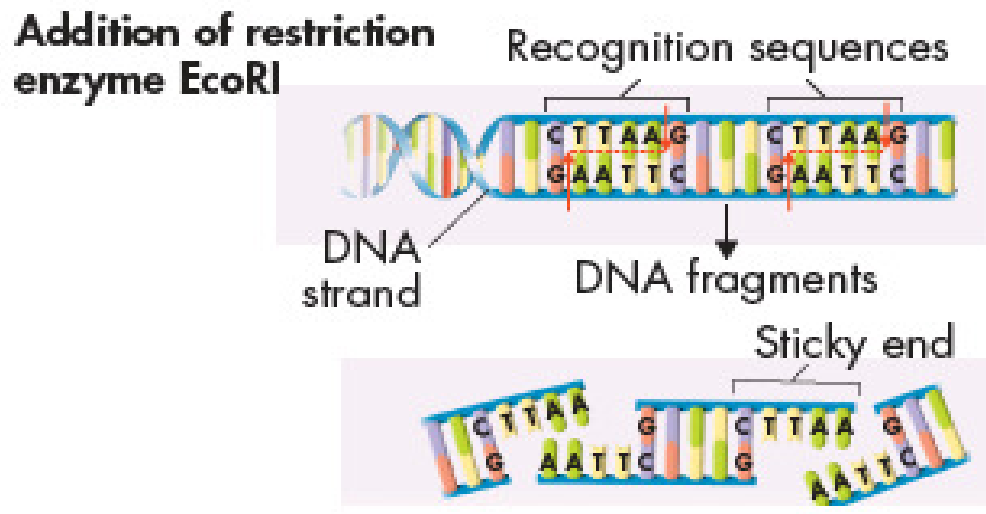
- Of the hundreds of known restriction enzymes, each cuts DNA at a different sequence of nucleotides.

## Manipulating DNA

For example, the *EcoRI* restriction enzyme recognizes the base sequence GAATTC.

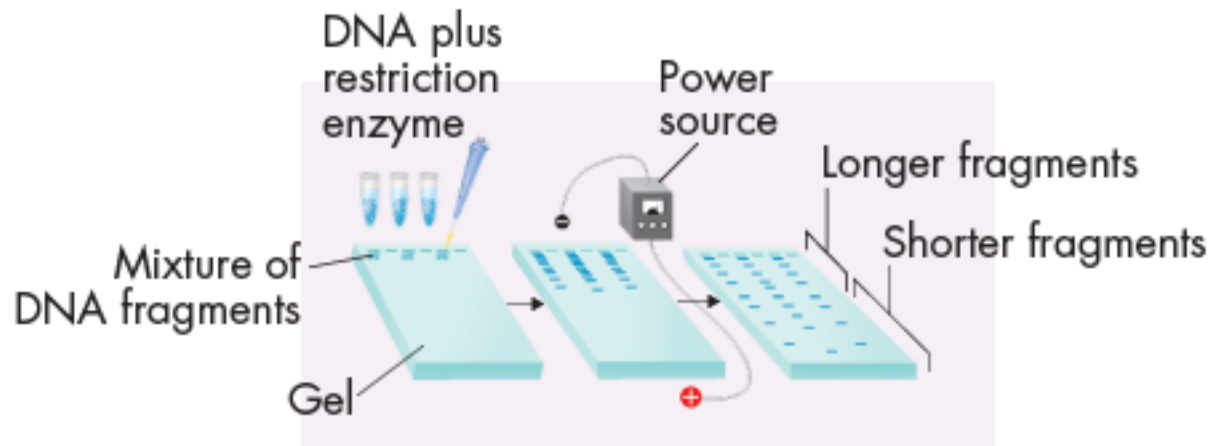
It cuts each strand between the G and A bases, leaving single-stranded overhangs, called sticky ends, with the sequence AATT.

The sticky ends can bond, or stick, to a DNA fragment with the complementary base sequence.



## Manipulating DNA

Once DNA has been cut by restriction enzymes, scientists can use a technique known as gel electrophoresis to separate and analyze the differently sized fragments.

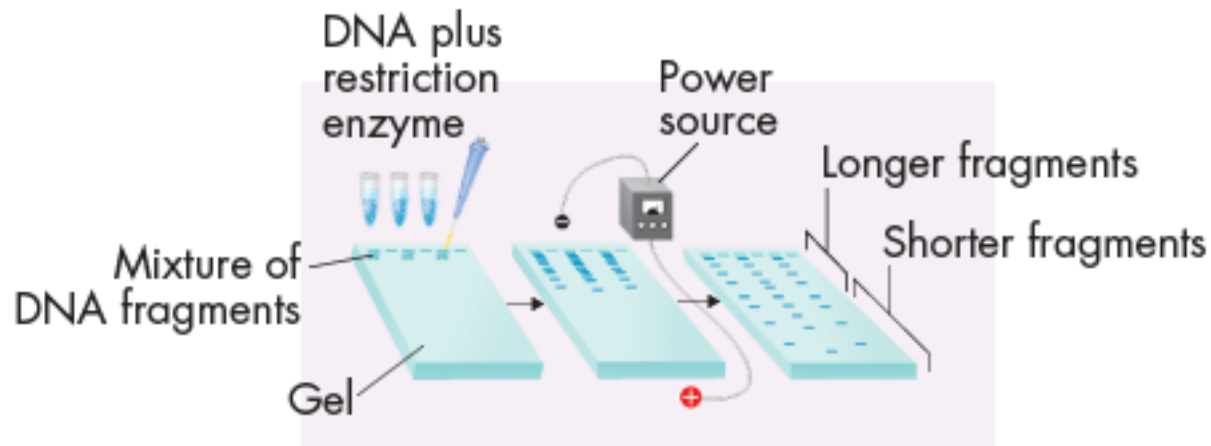




## Manipulating DNA

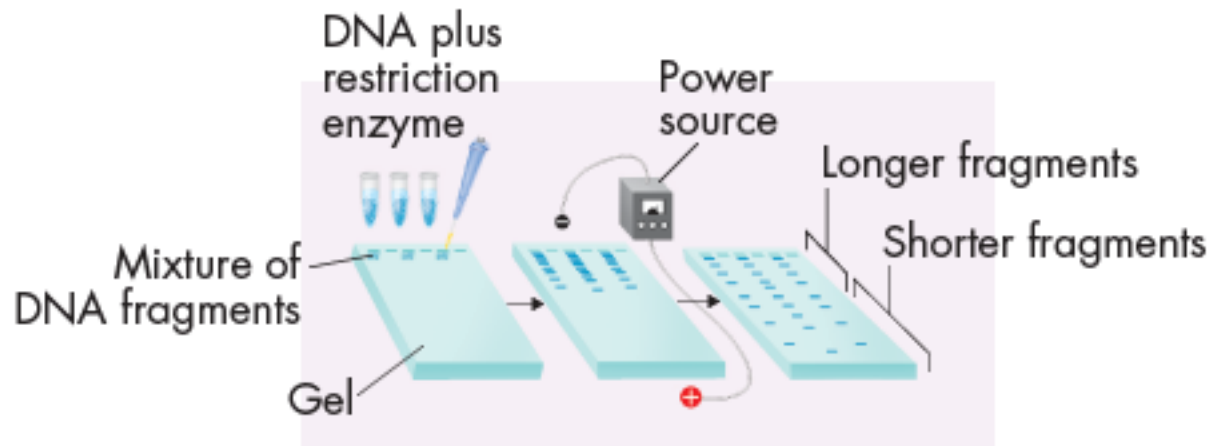
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- A mixture of DNA fragments is placed at one end of a porous gel.



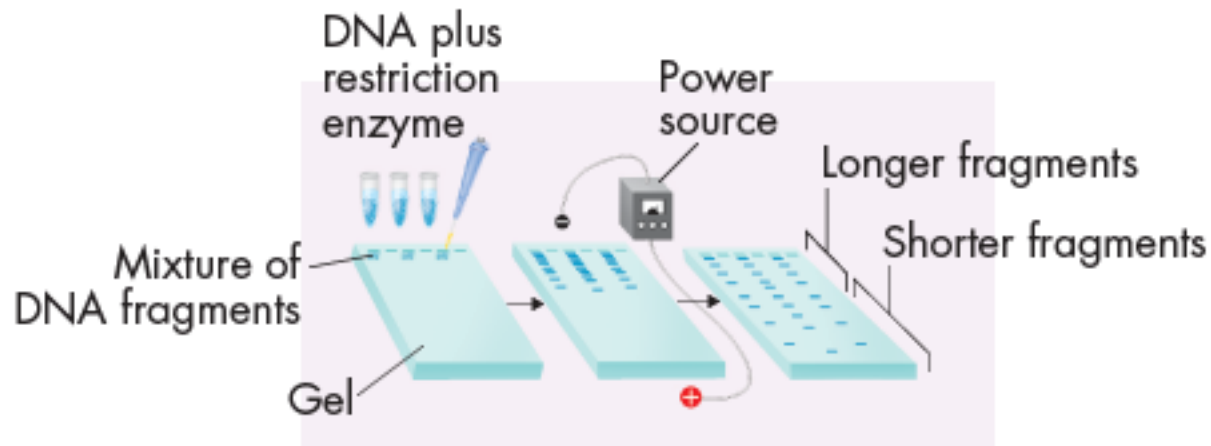
## Manipulating DNA

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- When an electric voltage is applied to the gel, DNA molecules (which are negatively charged) move toward the positive end of the gel.
- The smaller the DNA fragment, the faster and farther it moves.

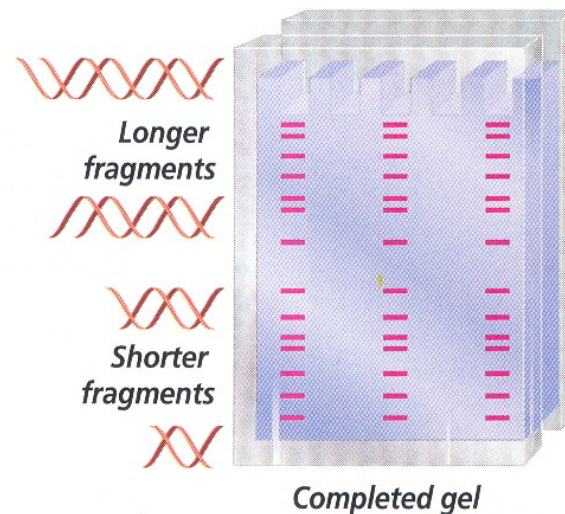


## Manipulating DNA

The result is a pattern of bands based on fragment size.

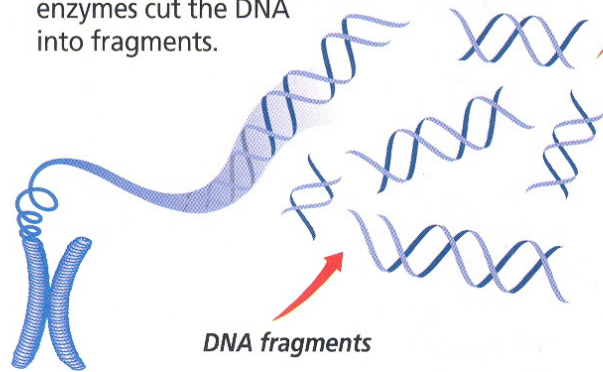
Specific stains that bind to DNA make these bands visible.

Researchers can remove individual restriction fragments from the gel and study them further.

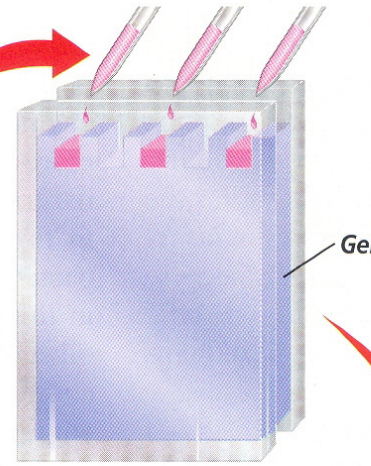


### Overview of Gel Electrophoresis

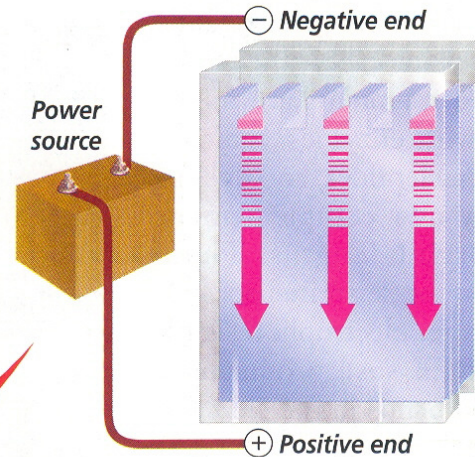
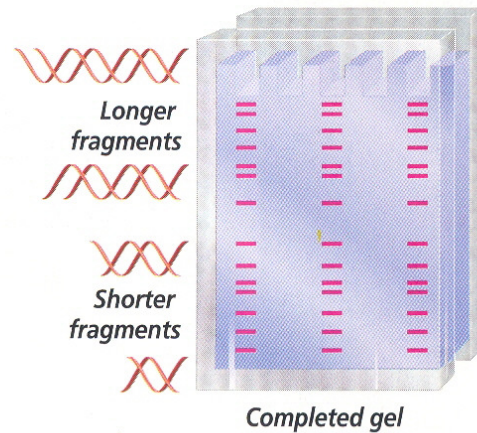
- 1 Restriction enzymes** Either one or several restriction enzymes is added to a sample of DNA. The enzymes cut the DNA into fragments.



- 2 The gel** A gel, with a consistency similar to gelatin, is formed so that small wells are left at one end. Into these wells, small amounts of the DNA sample are placed.



- 4 The fragments move** The negatively charged DNA fragments travel toward the positive end. The smaller the fragment, the faster it moves through the gel. Fragments that are the farthest from the well are the smallest.



- 3 The electrical field** The gel is placed in a solution, and an electrical field is set up so that one end of the gel is positive and the other end is negative.

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- PCR is used to make millions of copies of a particular DNA sequence.

Scientists use PCR to amplify sections of DNA that scientists have identified as being highly variable amongst individuals.

## Manipulating DNA

### Polymerase Chain Reaction (PCR) Process...

- Step One = Heat the DNA (this separates the two strands)
- Step Two = As the DNA cools, primers bind to the DNA strands
- Step Three = DNA polymerase starts copying the region between the primers
- Step Four = The process is repeated to make additional copies of the gene sequence



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Typically, the DNA from the desired gene is inserted into a bacterial plasmid.

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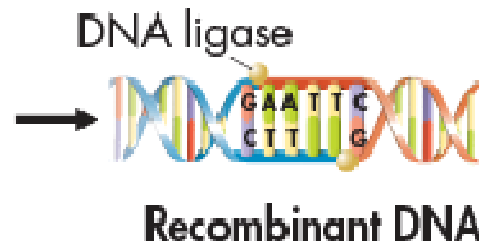
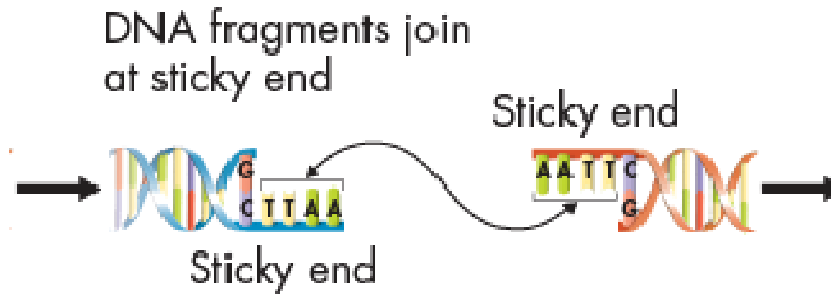
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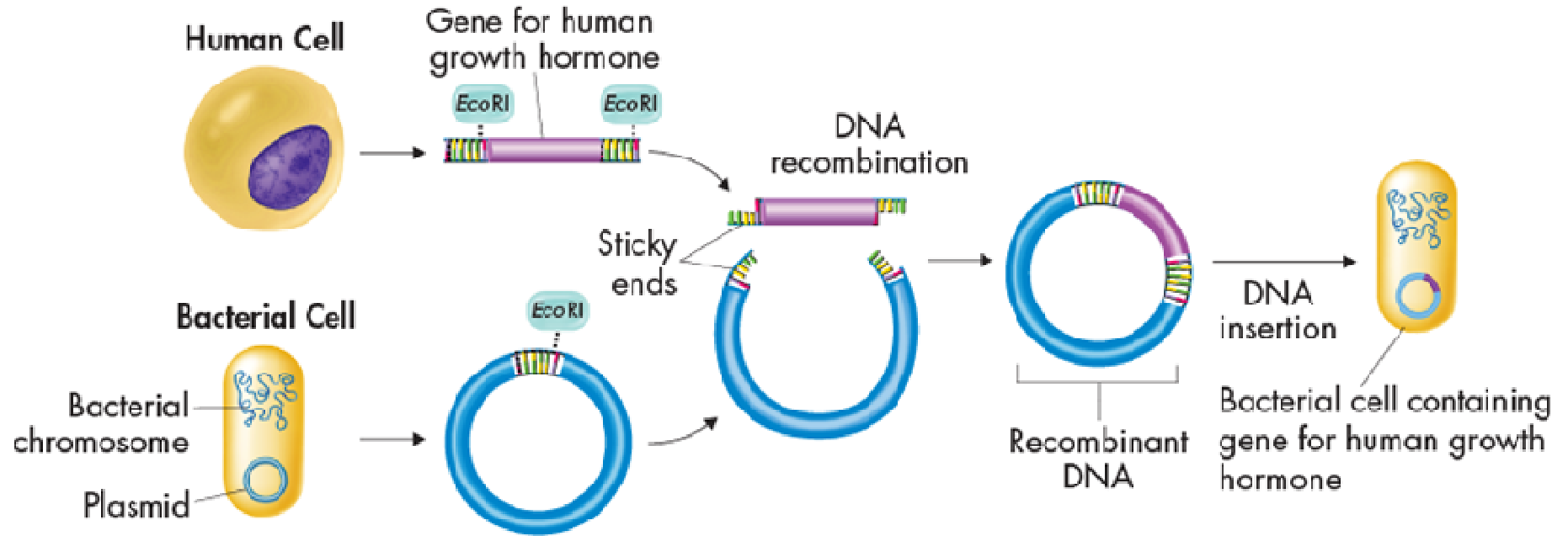
- The bacterial cell will then create the protein that the desired gene codes for.

# Manipulating DNA



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## Recombinant DNA Technology – Plasmid DNA Transformation



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