

Lesson Overview

15.2 Recombinant DNA

Finding Genes

In 1987, Douglas Prasher, a biologist at Woods Hole Oceanographic Institute in Massachusetts, wanted to find a specific gene in a jellyfish that codes for a molecule called green fluorescent protein, or GFP.

This natural protein, found in the jellyfish, absorbs energy from light and makes parts of the jellyfish glow.

Prasher thought that GFP from the jellyfish could be linked to a protein when it was being made in a cell, a bit like attaching a light bulb to that molecule.

Finding Genes

To find the GFP gene, Prasher compared part of the amino acid sequence of the GFP protein to a genetic code table and was able to predict a probable mRNA base sequence that would code for this sequence of amino acids.

Next, Prasher used a complementary base sequence to “attract” an mRNA that matched his prediction and would bind to that sequence by base pairing.

After screening a genetic “library” with thousands of different mRNA sequences from the jellyfish, he found one that bound perfectly.

Finding Genes

To find the actual gene that produced GFP, Prasher took a gel in which restriction fragments from the jellyfish genome had been separated and found that one of the fragments bound tightly to the mRNA. That fragment contained the actual gene for GFP.

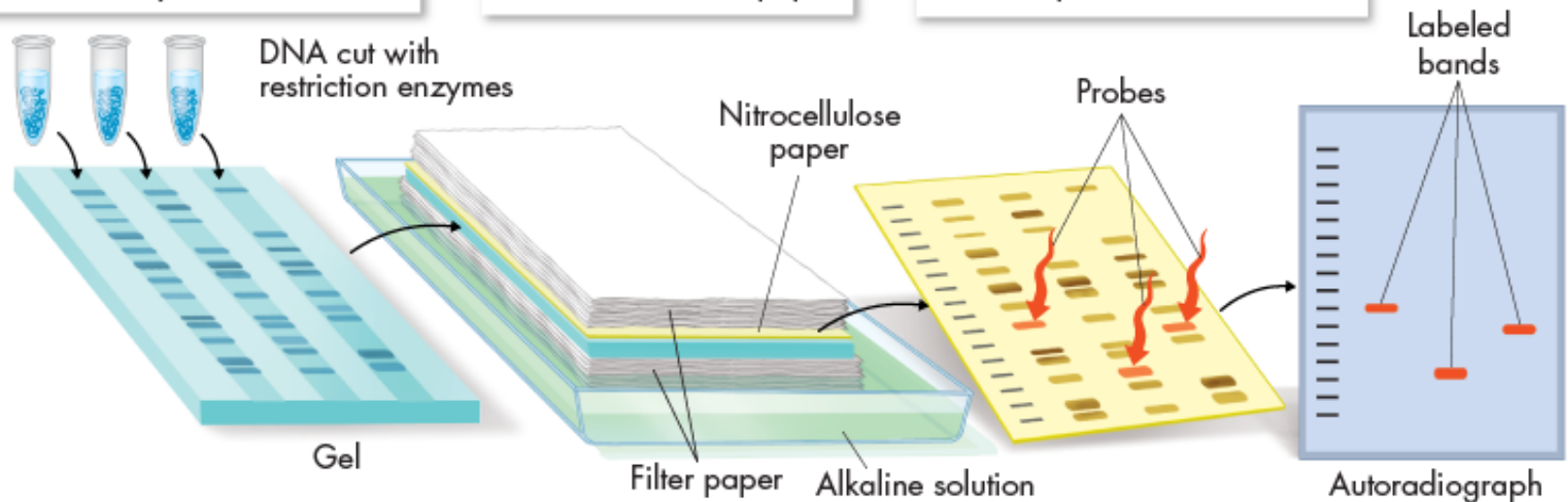
This method is called Southern blotting, after its inventor, Edwin Southern.

Finding Genes- Southern Blot Analysis

1 Gel electrophoresis separates DNA fragments produced by restriction enzymes.

2 Bands on the gel are immobilized by blotting onto nitrocellulose paper.

3 Radioactive probes bind to fragments with complementary base sequences.



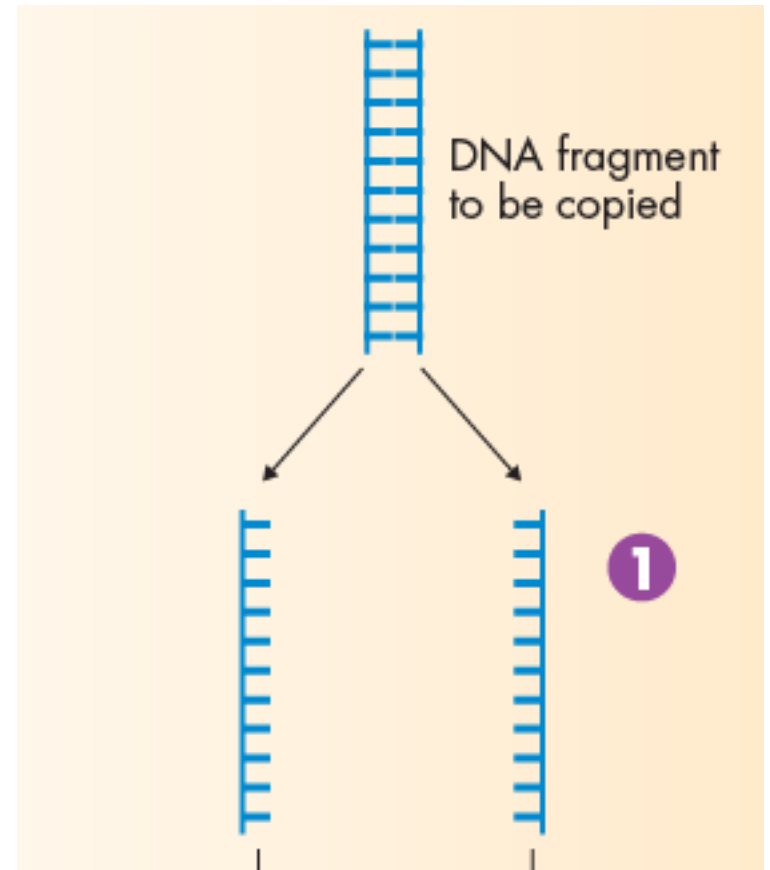
Finding Genes

Today it is often quicker and less expensive for scientists to search for genes in computer databases where the complete genomes of many organisms are available.

Polymerase Chain Reaction

Once biologists find a gene, a technique known as **polymerase chain reaction (PCR)** allows them to make many copies of it.

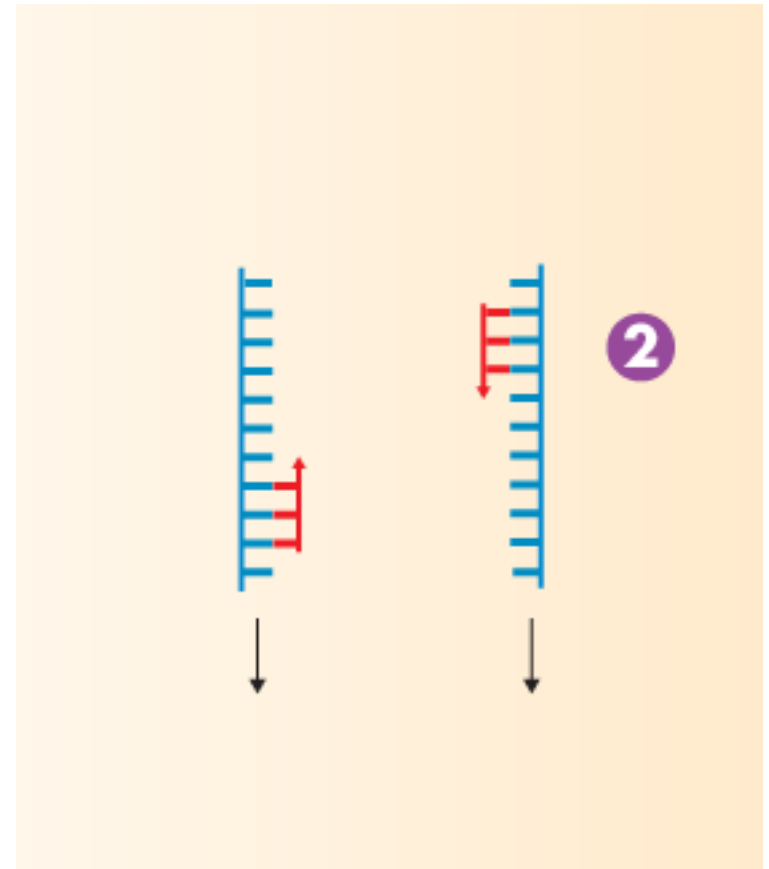
1. A piece of DNA is heated, which separates its two strands.



Polymerase Chain Reaction

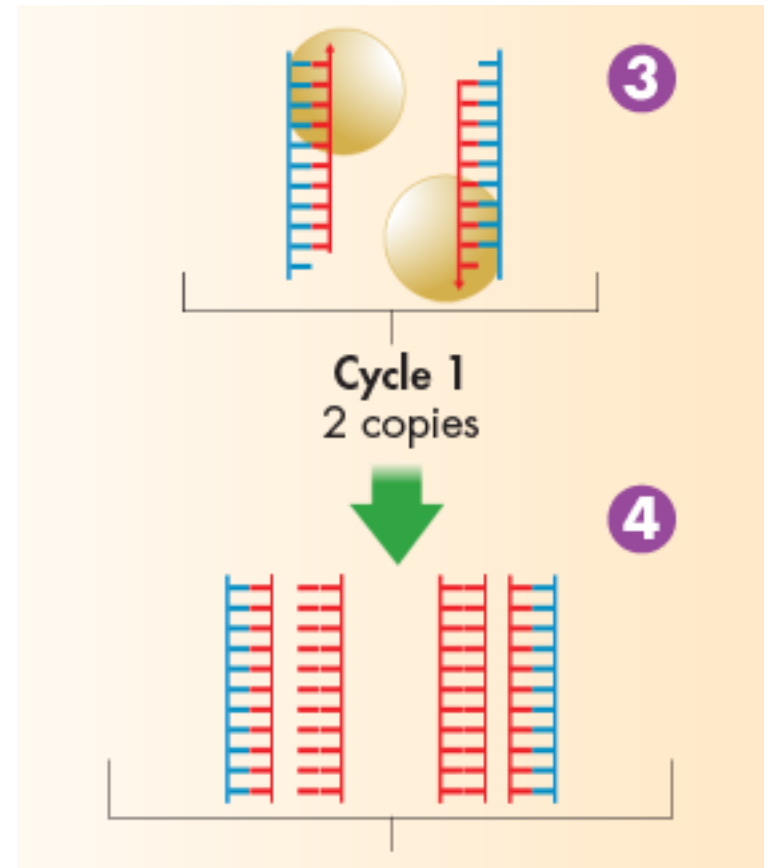
2. At each end of the original piece of DNA, a biologist adds a short piece of DNA that complements a portion of the sequence.

These short pieces are known as primers because they prepare, or prime, a place for DNA polymerase to start working.



Polymerase Chain Reaction

3. DNA polymerase copies the region between the primers. These copies then serve as templates to make more copies.
4. In this way, just a few dozen cycles of replication can produce billions of copies of the DNA between the primers.



Changing DNA



How is recombinant DNA used?



Recombinant-DNA technology—joining together DNA from two or more sources—makes it possible to change the genetic composition of living organisms.

Changing DNA

Scientists began wondering if it might be possible to change the DNA of a living cell, and many of them realized this had already been accomplished decades earlier in Frederick Griffith's bacterial transformation experiments.

During transformation, a cell takes in DNA from outside the cell, and that added DNA becomes a component of the cell's own genome.

Griffith's extract of heat-killed bacteria contained DNA fragments, which were taken up by live bacteria, transforming the live bacteria and changing their characteristics.

Combining DNA Fragments

Today, scientists can produce custom-built DNA molecules in the lab and then insert those molecules—along with the genes they carry—into living cells.

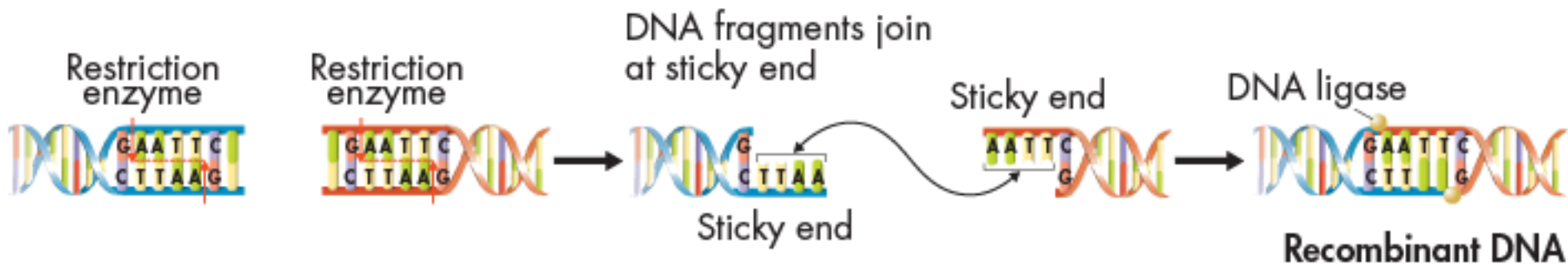
Machines known as DNA synthesizers are used to produce short pieces of DNA, up to several hundred bases in length.

These synthetic sequences can then be joined to natural sequences using DNA ligase or other enzymes that splice DNA together.

Combining DNA Fragments

A gene from one organism can be attached to the DNA of another organism.

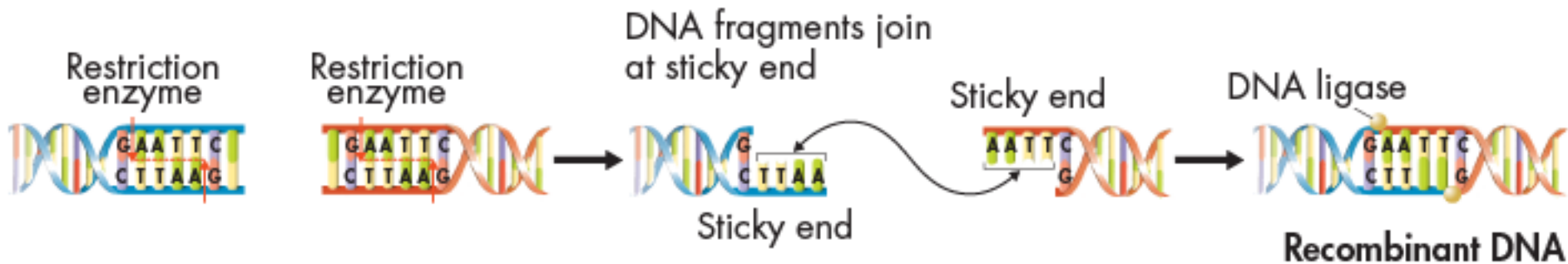
Restriction enzymes cut DNA at specific sequences, producing “sticky ends,” which are single-stranded overhangs of DNA.



Combining DNA Fragments

If two DNA molecules are cut with the same restriction enzyme, their sticky ends will bond to a DNA fragment that has the complementary base sequence. DNA ligase then joins the two fragments.

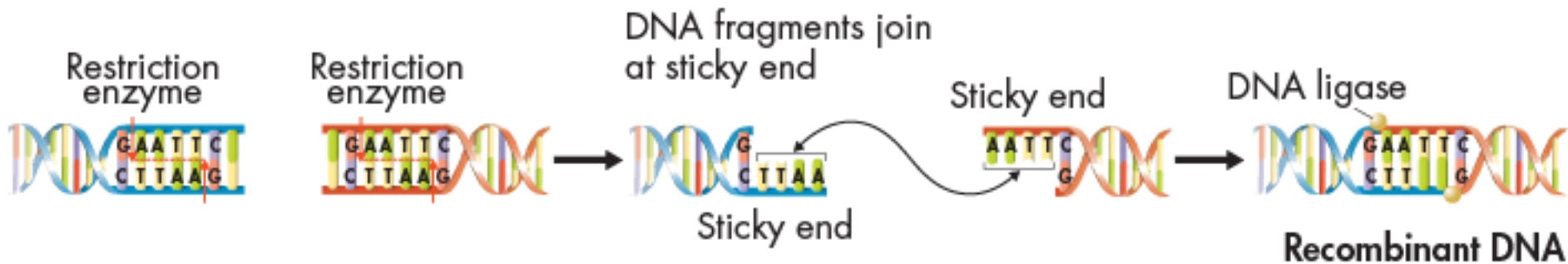
The resulting molecules are called **recombinant DNA**.



Combining DNA Fragments

Recombinant-DNA technology—joining together DNA from two or more sources—makes it possible to change the genetic composition of living organisms.

By manipulating DNA in this way, scientists can investigate the structure and functions of genes.



Plasmids and Genetic Markers

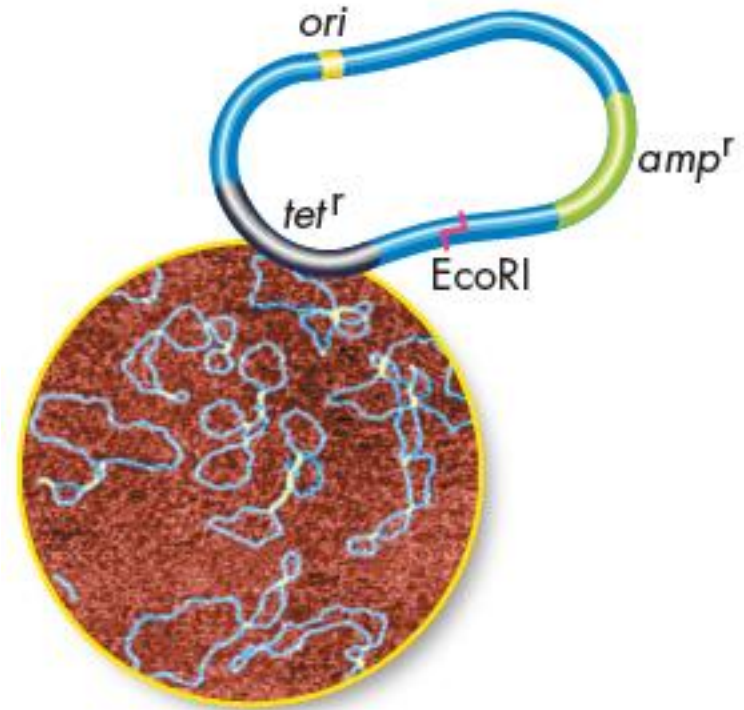
Scientists working with recombinant DNA soon discovered that many of the DNA molecules they tried to insert into host cells simply vanished because the cells often did not copy, or replicate, the added DNA.

Today scientists join recombinant DNA to another piece of DNA containing a replication “start” signal. This way, whenever the cell copies its own DNA, it copies the recombinant DNA too.

Plasmids and Genetic Markers

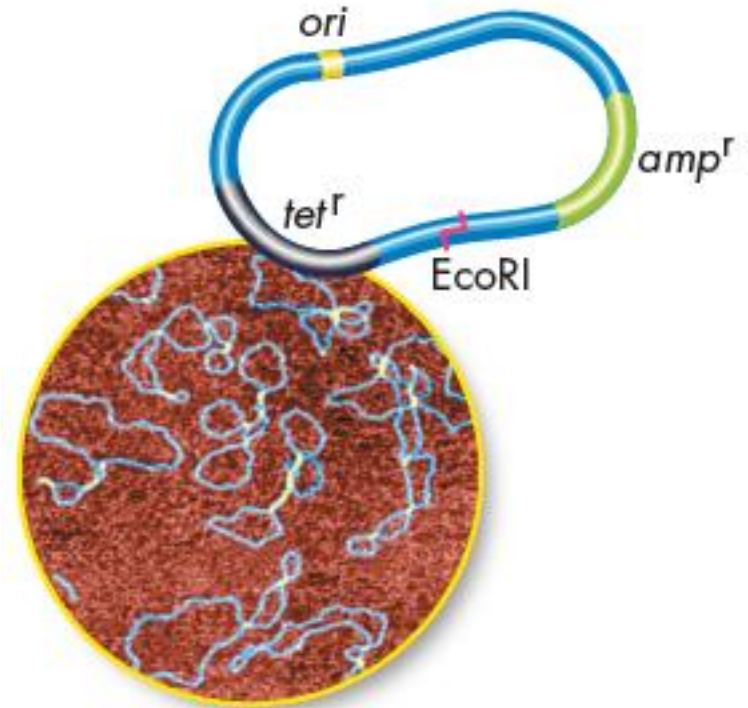
In addition to their own large chromosomes, some bacteria contain small circular DNA molecules known as **plasmids**.

Joining DNA to a plasmid, and then using the recombinant plasmid to transform bacteria, results in the replication of the newly added DNA along with the rest of the cell's genome.



Plasmids and Genetic Markers

Plasmids used for genetic engineering typically contain a replication start signal, called the origin of replication (*ori*), and a restriction enzyme cutting site, such as *EcoRI*.



Plasmids and Genetic Markers

Plasmids are also found in yeasts, which are single-celled eukaryotes that can be transformed with recombinant DNA as well.

Biologists working with yeasts can construct artificial chromosomes containing centromeres, telomeres, and replication start sites.

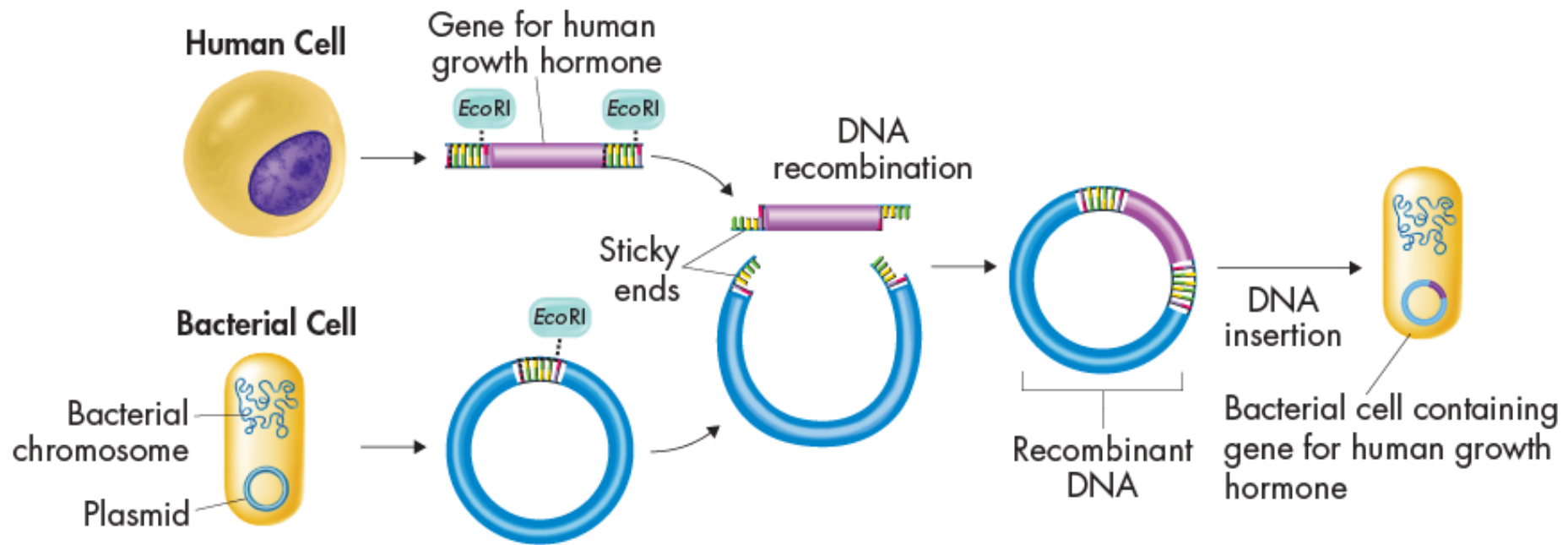
These artificial chromosomes greatly simplify the process of introducing recombinant DNA into the yeast genome.

Plasmids and Genetic Markers

Bacteria can be transformed using recombinant plasmids.

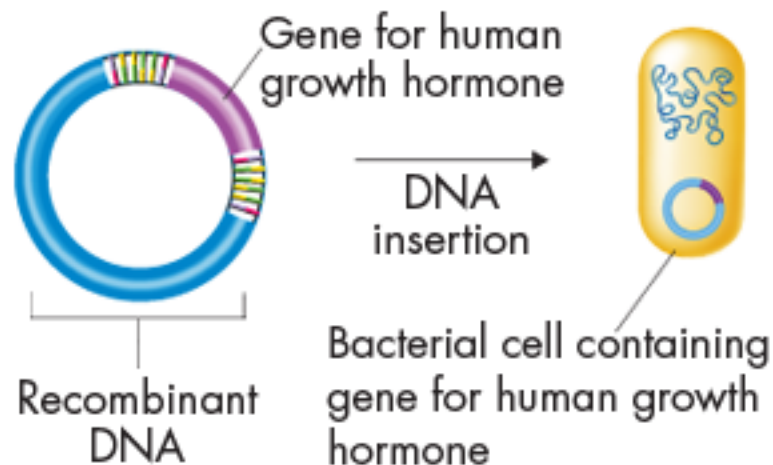
Scientists can insert a piece of DNA into a plasmid if both the plasmid and the target DNA have been cut by the same restriction enzymes to create sticky ends.

Plasmid DNA Transformation Using Human Growth Hormone



Plasmids and Genetic Markers

The new combination of genes is then returned to a bacterial cell, which replicates the recombinant DNA over and over again and produces human growth hormone.

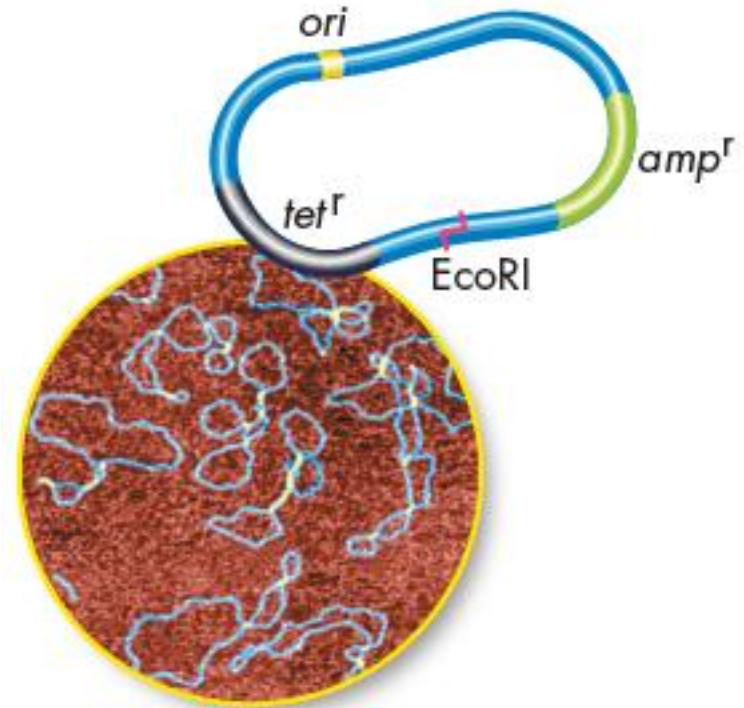


Plasmids and Genetic Markers

The recombinant plasmid has a genetic marker, such as a gene for antibiotic resistance. A **genetic marker** is a gene that makes it possible to distinguish bacteria that carry the plasmid from those that don't.

This plasmid contains the antibiotic resistance genes *tet^r* and *amp^r*.

After transformation, the bacteria culture is treated with an antibiotic. Only those cells that have been transformed survive, because only they carry the resistance gene.



Transgenic Organisms



How can genes from one organism be inserted into another organism?



Transgenic organisms can be produced by the insertion of recombinant DNA into the genome of a host organism.

Transgenic Organisms

The universal nature of the genetic code makes it possible to construct organisms that are **transgenic**, containing genes from other species.

Transgenic organisms can be produced by the insertion of recombinant DNA into the genome of a host organism.

Like bacterial plasmids, the DNA molecules used for transformation of plant and animal cells contain genetic markers that help scientists identify which cells have been transformed.

Transgenic Organisms

Transgenic technology was perfected using mice in the 1980s.

Genetic engineers can now produce transgenic plants, animals, and microorganisms.

By examining the traits of a genetically modified organism, it is possible to learn about the function of the transferred gene.

Transgenic Plants

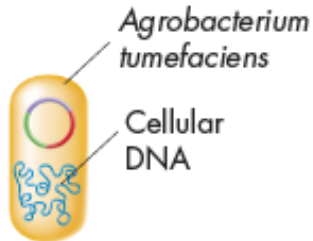
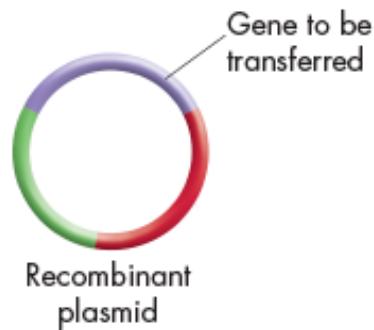
Many plant cells can be transformed using *Agrobacterium*.

In nature this bacterium inserts a small DNA plasmid that produces tumors in a plant's cells.

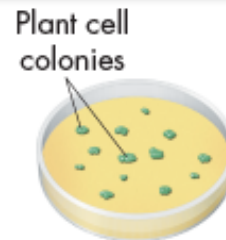
Scientists can deactivate the plasmid's tumor-producing gene and replace it with a piece of recombinant DNA. The recombinant plasmid can then be used to infect and transform plant cells.

The transformed cells can be cultured to produce adult plants.

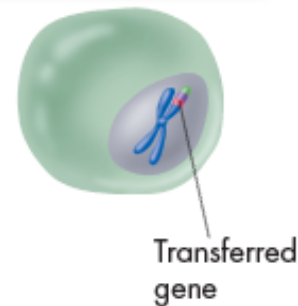
Transgenic Plants: Transforming a Plant with Agrobacterium



Transformed bacteria introduce plasmids into plant cells.



Inside a plant cell, *Agrobacterium* inserts part of its DNA into the host cell chromosome.



A complete plant is generated from the transformed cell.



Transgenic Plants

There are other ways to produce transgenic plants as well.

When their cell walls are removed, plant cells in culture will sometimes take up DNA on their own.

DNA can also be injected directly into some cells.

If transformation is successful, the recombinant DNA is integrated into one of the plant cell's chromosomes.

Transgenic Animals

Scientists can transform animal cells using some of the same techniques used for plant cells.

The egg cells of many animals are large enough that DNA can be injected directly into the nucleus.

Once the DNA is in the nucleus, enzymes that are normally responsible for DNA repair and recombination may help insert the foreign DNA into the chromosomes of the injected cell.

Transgenic Animals

Recently it has become possible to eliminate particular genes by constructing DNA molecules with two ends that will sometimes recombine with specific sequences in the host chromosome.

Once they recombine, the host gene normally found between those two sequences may be lost or specifically replaced with a new gene.

This kind of gene replacement has made it possible to pinpoint the specific functions of genes in many organisms, including mice.

Cloning

A **clone** is a member of a population of genetically identical cells produced from a single cell

The technique of cloning uses a single cell from an adult organism to grow an entirely new individual that is genetically identical to the organism from which the cell was taken.

Clones of animals were first produced in 1952 using amphibian tadpoles.

In 1997, Scottish scientist Ian Wilmut announced that he had produced a sheep, called Dolly, by cloning.

Cloning

Animal cloning uses a procedure called nuclear transplantation.

The process combines an egg cell with a donor nucleus to produce an embryo.

First, the nucleus of an unfertilized egg cell is removed.

Cloning

Next, the egg cell is fused with a donor cell that contains a nucleus, taken from an adult.

The resulting diploid egg develops into an embryo, which is then implanted in the uterine wall of a foster mother, where it develops until birth.

Cloned cows, pigs, mice, and even cats have since been produced using similar techniques.

Cloning Animals—Nuclear Transplantation

