Topic 6 – Making Recombinant DNA

Recombinant DNA – fragment of DNA composed of sequences originating from at least two different sources

Genetic transformation - introduction and expression of foreign DNA in a living organism

DNA sequencing

- DNA sequencing is the process of determining the exact sequence of base pairs for a particular DNA fragment or molecule

- It is done to help make recombinant DNA or to transform an organism

- Human Genome project
  - The human genome consists of approximately 30 000 genes
  - Constructing the genome map involved using gene mapping and DNA sequencing technology
  - Was finished in June 2000
  - Goal of the project was to use the information obtained to help treat and diagnose genetic disorders

    http://www.youtube.com/watch?v=XuUpnAz5y1g

Recombinant DNA

- Recombinant DNA can be used to transfer the genes from one organism into another
  - This would allow one organism to express the trait of another organism
  - This creates transgenic organisms or genetically modified organisms (GMO's)
    - http://www.cbc.ca/news/background/genetics_modification/
  - Ex. http://www.iptv.org/exploremore/ge/what/insulin.cfm
  - Ex. Round-up Ready Canola
    - A gene for resistance to the herbicide was put into the canola DNA
    - Allows farmers to kill weeds using Roundup (a herbicide) and it doesn't kill the canola
  - Golden Rice
    - High in vitamin-A because genes from other plants that make vitamin A were inserted in the rice DNA

How is Recombinant DNA made?

- **Restriction enzymes** are enzymes that cut DNA at a specific site
  - Allows genes to be cut out of the DNA of an organism containing a trait of interest (ex. herbicide resistance)
  - Restriction enzymes are found and isolated from bacteria species
  - Different restriction enzymes cut at different sites of the DNA
  - The enzyme binds at a **recognition site** and makes the cut in both strands of the DNA

Table 2 Restriction Enzymes and Their Recognition Sites

Microorganism of origin	Enzyme	Recognition site	After restriction enzyme digestion
Escherichia coli	*Eco*RI	5′-GAATTC-3′ 3′-CTTAAG-5′	5′-G AATTC-3′ 3′-CTTAA G-5′
Serratia marcescens	*Sma*I	5′-CCCGGG-3′ 3′-GGGCCC-5′	5′-GGG CCC-3′ 3′-CCC GGG-5′
Arthrobacter luteus	*Alu*I	5′-AGCT-3′ 3′-TCGA-5′	5′-AG CT-3′ 3′-TC GA-5′
Streptomyces albus	*Sal*I	5′-GTCGAC-3′ 3′-CAGCTG-5′	5′-G TCGAC-3′ 3′-CAGCT G-5′
Haemophilus parainfluenzae	*Hin*dIII	5′-AAGCTT-3′ 3′-TTCGAA-5′	5′-A AGCTT-3′ 3′-TTCGA A-5′

- **Sticky ends** are created by the restriction enzymes
  - the overhanging ends on a single strand of the DNA at the cut site are called sticky ends

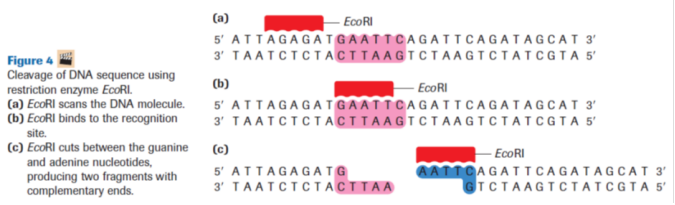
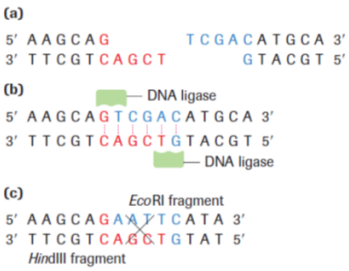


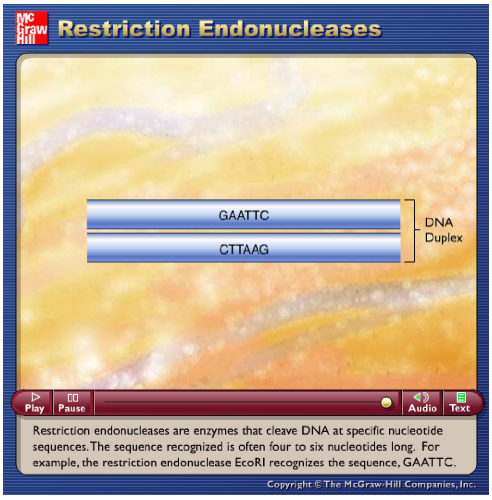
Figure 4
Cleavage of DNA sequence using restriction enzyme *Eco*RI.
(a) *Eco*RI scans the DNA molecule.
(b) *Eco*RI binds to the recognition site.
(c) *Eco*RI cuts between the guanine and adenine nucleotides, producing two fragments with complementary ends.

- To create recombinant DNA, pieces of DNA from two sources must be joined together

- An enzyme called **DNA ligase** puts two sticky ends together and binds them

Figure 6
DNA ligase is able to join complementary sticky ends produced by the same restriction enzyme via a condensation reaction.
(a) Complementary sticky ends produced by *Hin*dIII
(b) Hydrogen bonds form between complementary bases. DNA ligase creates bonds between nucleotides in the DNA backbones.
(c) If fragments are not complementary, then hydrogen bonds will not form.

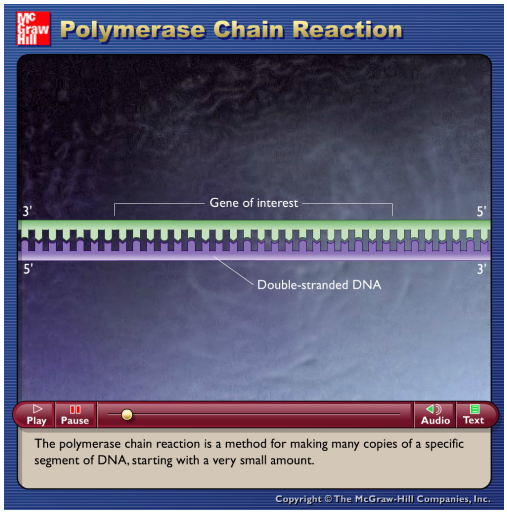


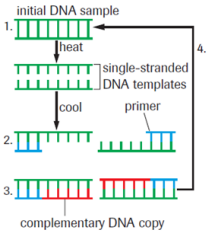


***Taq* DNA Polymerase and the Polymerase Chain Reaction**
http://highered.mcgraw-hill.com/sites/0072556781/student_view0/chapter14/animation_quiz_6.html

Polymerase chain reaction (PCR) - a technique for amplifying a DNA sequence by repeated cycles of strand separation and replication

- Allows the production of billions of pieces of DNA from small amounts of DNA
- Used in forensic analysis
- depends on the special property of Taq polymerase.
  - Taq DNA polymerase is found in the bacterium Thermos aquaticus, which lives at extremely high temperatures





four simple steps (**Figure 7**).

Figure 7
Steps in the PCR
1. The mixture is heated to a temperature high enough to break the hydrogen bonds in the double helix of the DNA and separate the strands. This forms single-stranded DNA templates.
2. The mixture is cooled, and the primers form hydrogen bonds with the DNA templates.
3. *Taq* polymerase synthesizes a new stand of DNA from the DNA template by complementary base pairing, starting at each primer.
4. The cycle of heating and cooling is repeated many times.

Transformation

- Using various enzymes, scientists can isolate DNA fragments containing a gene or genes.

- Multiple copies of the fragment can be prepared using PCR.

- The DNA fragment may also be joined (annealed) to other DNA fragments.

- **Transformation** is any process by which foreign DNA is incorporated into the genome of a cell
  - o An organism with foreign DNA in its genome is said to be **transgenic**

- Genes are often inserted into a bacteria DNA sequence
  - o Helps study gene expression, create and maintain a stock of a DNA fragment (gene), or to synthesize a useful gene product (insulin)

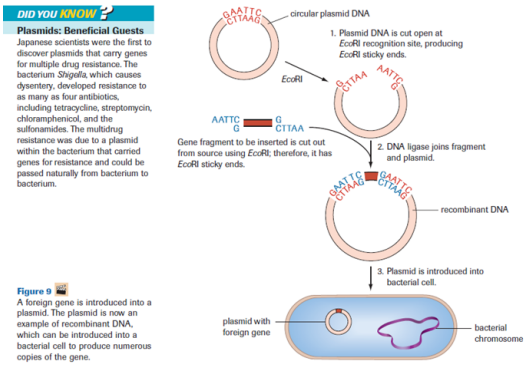


Figure 9
A foreign gene is introduced into a plasmid. The plasmid is now an example of recombinant DNA, which can be introduced into a bacterial cell to produce numerous copies of the gene.