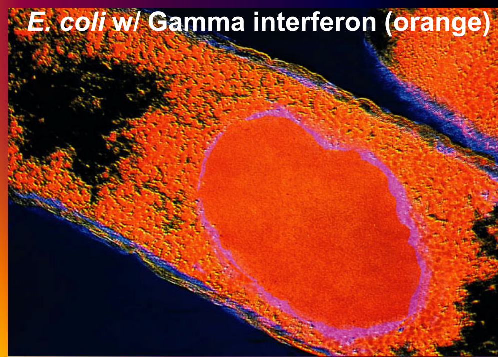
Chapter 9
Biotechnology & Recombinant DNA



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Microbiology Ch. 9

OBJECTIVES: Students should be able to: ……

1. Ch. 9: Diagram three methods of horizontal gene transfer in bacteria. In each case, what must happen for the exchanged DNA to be stably inherited?
2. Describe how Restriction Enzymes, Plasmids, and PCR can be used for molecular cloning and genetic engineering.
3. Distinguish between the general & technical uses of the term "biotechnology". What is **Recombinant DNA Technology/Genetic Engineering**?
4. Briefly (but including important details and methods), outline ***HOW to clone and identify a new gene***! *(NOT necessarily in that order!!)* ☺

- ***Objectives are your HOMEWORK between classes!!!***
  - ***DUE (w/ Study Guide questions) Wed./Thurs. at the start of Lab!!***

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Biotechnology and Recombinant DNA

- **Biotechnology:**
  - The use of microorganisms, cells, or cell components to make a product
  - Foods, antibiotics, vitamins, enzymes
- ***Recombinant* DNA Technology:**
  - Insertion or modification of genes to produce desired proteins from "foreign" sources

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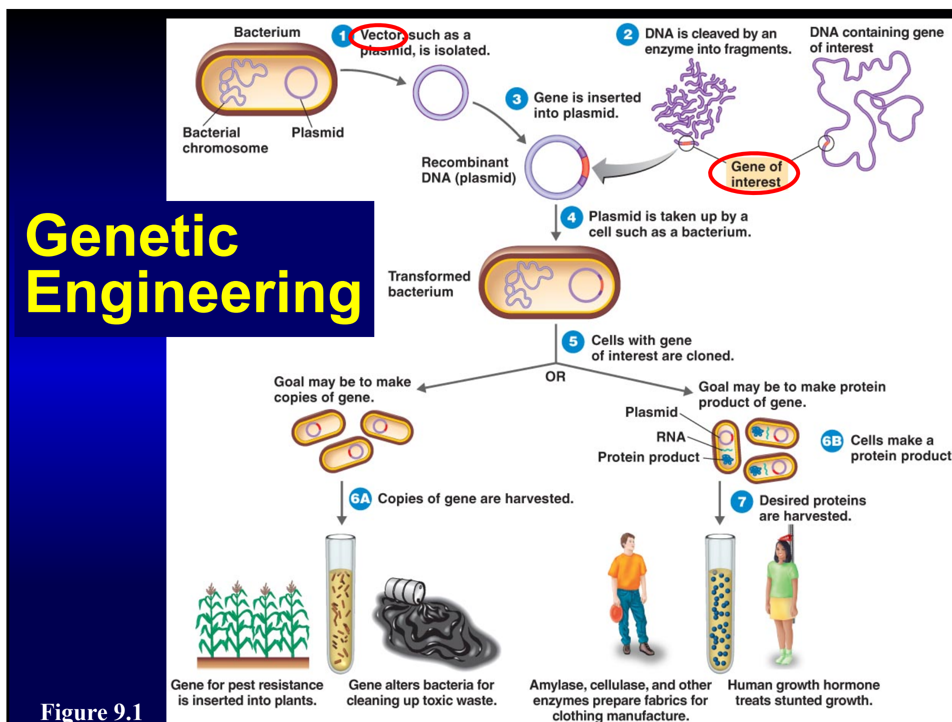

Figure 9.1

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Medical Products of Recombinant DNA Tech

TABLE 9.1 **Some Pharmaceutical Products of Genetic Engineering**

Product	Comments
Alpha-interferon	Therapy for leukemia, melanoma, and hepatitis; produced by *E. coli* and *Saccharomyces cerevisiae* (yeast).
Antitrypsin	Assists emphysema patients; produced by genetically modified sheep.
Beta-interferon	Treatment for multiple sclerosis; produced by mammalian cell culture.
Bone morphogenic proteins	Induces new bone formation; useful in healing fractures and reconstructive surgery; produced by mammalian cell culture.
Colony-stimulating factor (CSF)	Counteracts effects of chemotherapy; improves resistance to infectious disease such as AIDS; treatment of leukemia; produced by *E. coli* and *S. cerevisiae*.
Epidermal growth factor (EGF)	Heals wounds, burns, ulcers; produced by *E. coli*.
Erythropoietin (EPO)	Treatment of anemia; produced by mammalian cell culture.
Factor VIII	Treatment of hemophilia; improves clotting; produced by mammalian cell culture.
Gamma-interferon	Treatment of chronic granulomatous disease; produced by *E. coli*.
Hepatitis B vaccine	Produced by *S. cerevisiae* that carries hepatitis-virus gene on a plasmid.
Influenza vaccine	Trial vaccine made from *E. coli* or *S. cerevisiae* carrying virus genes.
Interleukins	Regulate the immune system; possible treatment for cancer; produced by *E. coli*.
Monoclonal antibodies	Possible therapy for cancer and transplant rejection; used in diagnostic tests; produced by mammalian cell culture (from fusion of cancer cell and antibody-producing cell).

Table 9.1.1

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Selection & Mutation:

(methods for producing your desired/engineered gene product:)

1. **Selection:** Culture a naturally-occurring microbe that produces desired product
2. **Mutation:** Mutagens cause mutations that might result in a microbe with a desirable trait
3. **Site-directed mutagenesis:** purposefully change a specific DNA code to change a protein
4. ***Select and culture microbe with the desired mutation***

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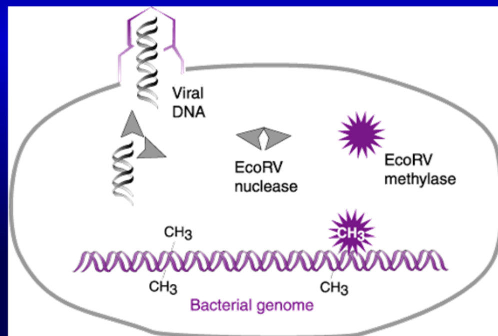
9.1) Restriction Enzymes

1. Cut specific sequences of DNA.
2. Destroy bacteriophage DNA in bacterial cells (natural function).
3. Recognize ***specific***, ***palindromic*** ***DNA sequences (4-8 bp).***
4. Cannot digest (host) DNA with methylated cytosines (methyl-C).



Hind-III: A/AGCTT
EcoR-I: G/AATTC
BamH-I: G/GATCC
Sal-I: G/TCGAC
Xba-I: T/CTAGA

MORE INFO:
http://www.dnatube.com/view_video2.php?viewkey=34dada217712d76f3d51

https://dnalc.cshl.edu/resources/animations/restriction.html
http://www.scq.ubc.ca/restriction-endonucleases-molecular-scissors-for-specifically-cutting-dna/

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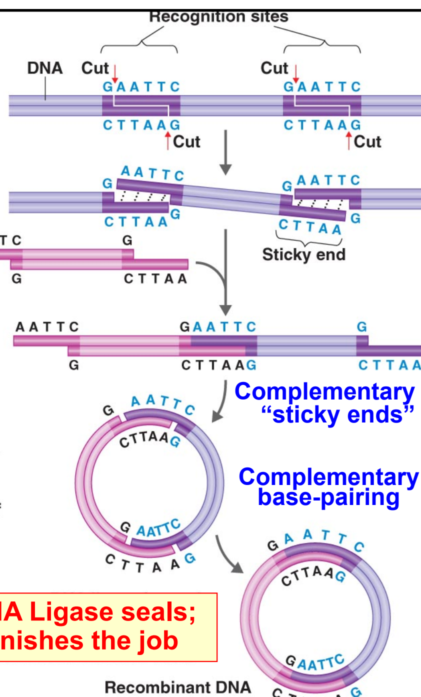
Figure 9.2

Hind-III: A/AGCTT
EcoR-I: G/AATTC
BamH-I: G/GATCC
Sal-I: G/TCGAC
Xba-I: T/CTAGA

1. Restriction enzyme cuts (red arrows) double-stranded DNA at its particular recognition sites, shown in blue.
2. These cuts produce a DNA fragment with two sticky ends.

DNA cut with same RE

3. When two such fragments of DNA cut by the same restriction enzyme come together, they can join by base pairing.
4. The joined fragments will usually form either a linear molecule or a circular one, as shown here for a plasmid. Other combinations of fragments can also occur.
5. **DNA Ligase seals; finishes the job**



http://www.dnai.org/b/index.html
--> Techniques → Cutting and Pasting

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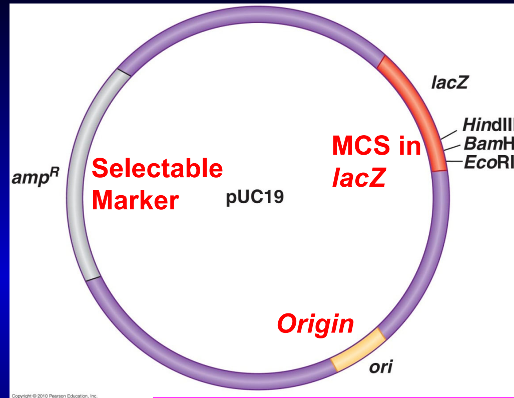
9.2) Vectors

- Carry new DNA to a desired cell.
- *"Shuttle vectors"* can replicate in several different species.
- Plasmids and viruses can be used as vectors.

Figure 9.3



1. Selectable marker to ID recombinant bacteria
2. *Origin* of Replication
3. Multiple-Cloning Site
4. Disruptable gene to screen for recombinant plasmids

http://highered.mcgraw-hill.com/sites/0072556781/student_view0/chapter14/animation_quiz_1.html
http://www.sumanasinc.com/webcontent/animations/content/plasmidcloning.html

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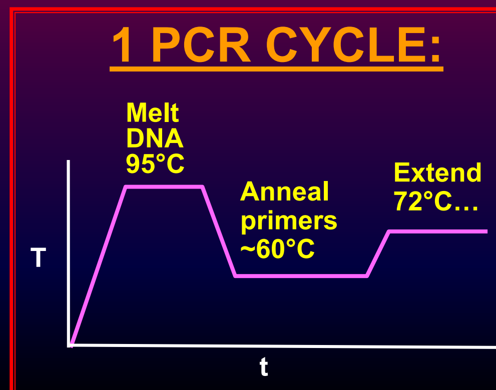
9.3) Polymerase Chain Reaction (PCR)

- To make multiple copies of a piece of DNA enzymatically.
  - dNTPs, Dpol (__), template DNA, ***specific DNA primers***

Used to:

1. Clone DNA for recombination
2. Amplify DNA to detectable levels.
   - Detection / identification!!
3. Sequence DNA.
4. Diagnose genetic disease.
5. **Detect pathogens.**

1 PCR CYCLE:



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PCR

Figure 9.4.1

Target DNA

First cycle

1. Incubate target DNA at 94°C for 1 minute to separate the strands.
2. Add primers, nucleotides (deoxynucleotides), and DNA polymerase.
3. Primers attach to single-stranded DNA during incubation at 60°C for 1 minute.
4. Incubate at 72°C for 1 minute; during this time, two copies of target DNA are formed.

dCTP
dATP
dTTP
Primer
dGTP
DNA polymerase

Thermostable DNA Polymerase (*Taq*)

- ***Thermus aquaticus*** = thermophilic bacterium in Yellowstone hot springs; ***Taq Dpol does not denature under thermocyling conditions***

http://www.dnai.org/b/index.html → manipulations → tech. → ampl.

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PCR

Thermus aquaticus is an aerobic heterotrophic eubacterium found in hot springs, an ideal environment for an organism whose optimal growth temperature is between 50° and 80°C and whose ideal pH lies between 7.5 and 8. ***T. aquaticus*** was first discovered in Yellowstone National Park (U.S.A.) in 1965 and has since been isolated in thermal environments worldwide.

(*n*: 10 → 1,024; 20 → 1,048,576; 30 → 1,073,741,824)

Second cycle

5. Repeat the cycle of heating and cooling to make two more copies of target DNA.

Figure 9.4.2

Similar to microbial reproduction:

- ***the number of DNA molecules generated from 1 starting double-helix is 2^n, where n= number of PCR cycles.***
- ***More than 1 starting DNA (#) = # x 2^n.***

http://www.sumanasinc.com/webcontent/anisamples/molecularbiology/pcr.html
http://highered.mcgraw-hill.com/sites/0072437316/student_view0/chapter16/animations.html#

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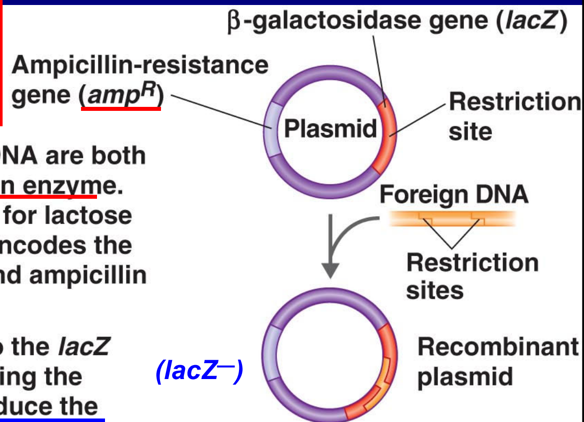
9.4) Genetic Engineering: A. Molecular Cloning

1. Selectable marker to ID recombinant bacteria
2. Origin of Replication
3. Multiple-cloning site
4. Disruptable gene to screen for recombinant plasmids



1. Plasmid DNA and foreign DNA are both cut with the same restriction enzyme. The plasmid has the genes for lactose hydrolysis (the *lacZ* gene encodes the enzyme β-galactosidase) and ampicillin resistance.
2. Foreign DNA will insert into the *lacZ* gene. The bacterium receiving the plasmid vector will not produce the enzyme β-galactosidase if foreign DNA has been inserted into the plasmid.

Figure 9.11.1

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(A.) Molecular Cloning (cont'd)

3. The recombinant plasmid is introduced into a bacterium, which becomes ampicillin resistant.

http://www.dnalc.org/resources/animations/transformation2.html
http://www.dnalc.org/resources/animations/transformation1.html

4. All treated bacteria are spread on a nutrient agar plate containing ampicillin and a β-galactosidase substrate and incubated. The β-galactosidase substrate is called X-gal.
5. Only bacteria that picked up the plasmid will grow in the presence of ampicillin. Bacteria that hydrolyze X-gal produce galactose and an indigo compound. The indigo turns the colonies blue. Bacteria that cannot hydrolyze X-gal produce white colonies.

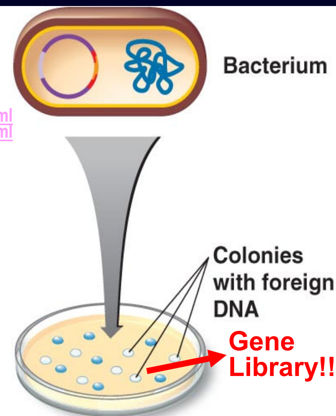

Fig. 9.11.2

1. Bacterial cells that have taken up plasmid grow into colonies on Amp.
2. White colonies on X-Gal have plasmid with *lacZ* gene disrupted by recombinant insert! (*lacZ*⁻) – have the foreign DNA!
   - …. Selective & Differential media!!! …. → want amp^R & $lacZ^-$!!!!

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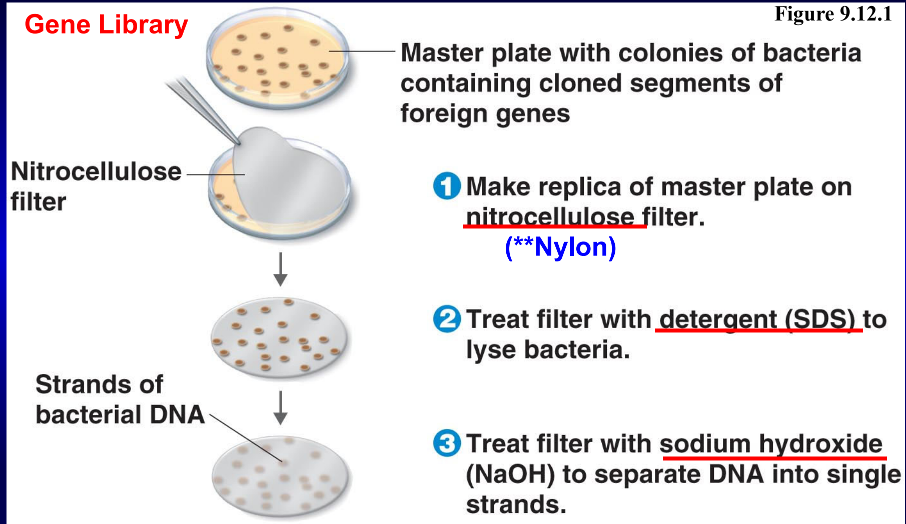

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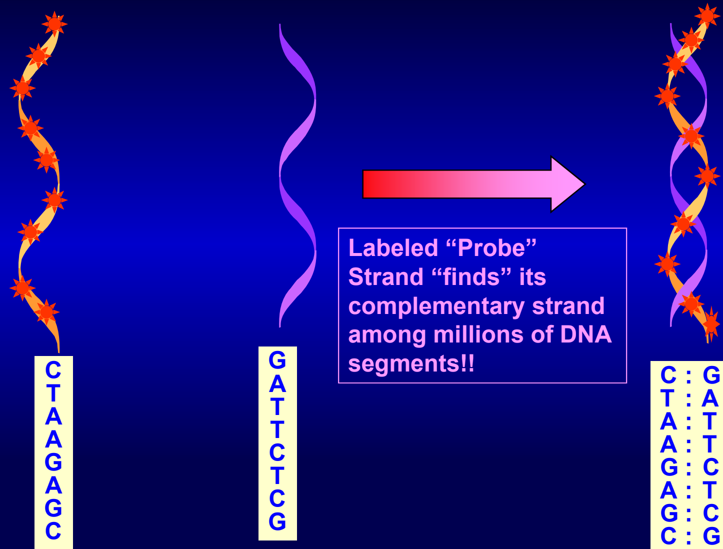

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Making a Gene Product

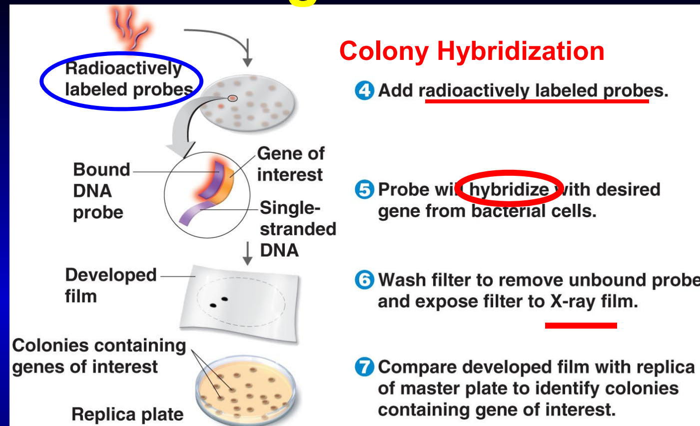

Figure 9.12.2

- ***Probe*** = labeled single strand of DNA or RNA;
  - "seeks out" its complementary sequence among target nucleic acids
  - binds by complementary base-pairing and "tags" where related sequences are found.
- ***HYBRIDIZATION*** = complementary base-pairing between very similar DNA strands from two different sources.
  - (eg: a synthetic probe, and target bacterial chromosomal DNA)

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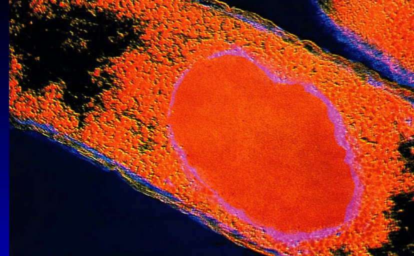
E. coli



- **"Model Organism"**

1. Used because it is easily grown and its genomics are known
2. Cells must be lysed to get engineered product
3. Need to eliminate endotoxin (lipid A; LPS) from products

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C. Genetic Engineering Using *Agrobacterium*

Crown Gall

Figure 9.18

Agrobacterium tumefaciens bacterium

Restriction cleavage site

Ti plasmid

T-DNA

1. The plasmid is removed from the bacterium, and the T-DNA is cut by a restriction enzyme.
2. Foreign DNA is cut by the same enzyme.
3. The foreign DNA is inserted into the T-DNA of the plasmid.

Recombinant Ti plasmid

4. The plasmid is reinserted into a bacterium.
5. The bacterium is used to insert the T-DNA carrying the foreign gene into the chromosome of a plant cell.

Inserted T-DNA carrying foreign gene

6. The plant cells are grown in culture.
7. A plant is generated from a cell clone. All of its cells carry the foreign gene and may express it as a new trait.

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Safety Issues and Ethics

1. Avoid accidental release
2. Genetically modified crops must be safe for consumption and for the environment
3. Who will have access to an individual's genetic information?

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