

Biotechnology and Recombinant DNA (Chapter 9)

Lecture Materials

for

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Primary Source for figures and content:

Tortora, G.J. *Microbiology An Introduction* 8th, 9th, 10th ed. San Francisco: Pearson Benjamin Cummings, 2004, 2007, 2010.

Biotechnology = use of microbes, cells, or cell components to make a product

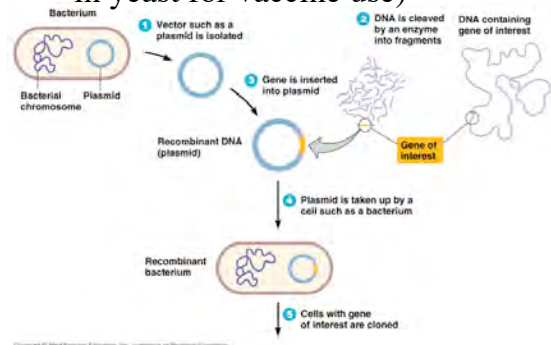
Genetic engineering = inserting genes into cells, this involves:

Recombinant DNA technology = the techniques for making recombinant DNA

Recombinant DNA (rDNA) = DNA assembled in the lab that contains the desired genes

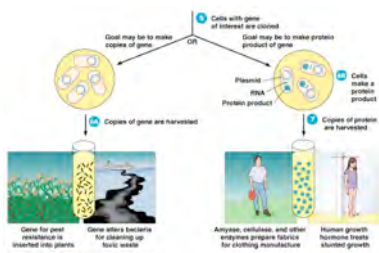
(e.g. insulin to express in bacteria for disease therapy)

(e.g. viral envelope genes for expression in yeast for vaccine use)



Cloning = extracting or copying a gene of interest from its genomic source and putting it in an expression vector. Steps:

1. Obtain the gene (PCR, restriction digest)
2. Ligate it into a vector (vector = carrier piece of DNA)
3. Transform the new recombinant DNA into bacteria/cells
4. Grow up a population of transformed cells that contain the DNA (cells = clones)
5. A. Harvest large quantities of the DNA for use in other cells (cloning, gene therapy, etc), or more commonly:
B. Harvest the gene product (protein) from the clones (therapeutics, vaccines)



Tools for Genetic Engineering

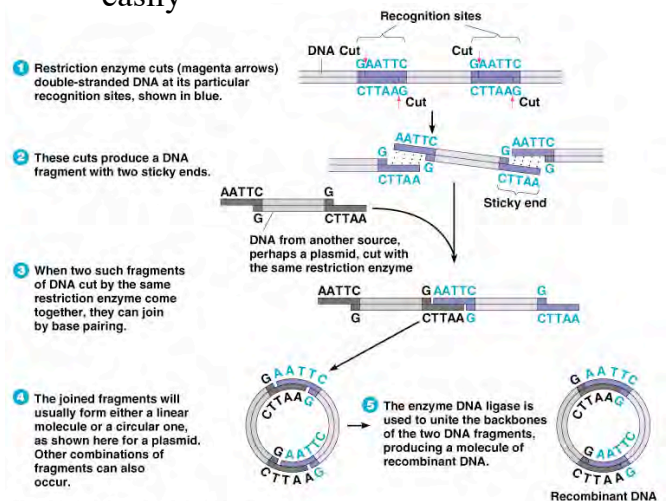
1. Restriction enzymes

-DNA cutting enzymes produced by bacteria

-Cut DNA at specific base sequences

-for cloning, use 4, 6, or 8 base cutters that leave “sticky ends”

sticky ends = single stranded overhangs of DNA that will base pair and re-ligate easily



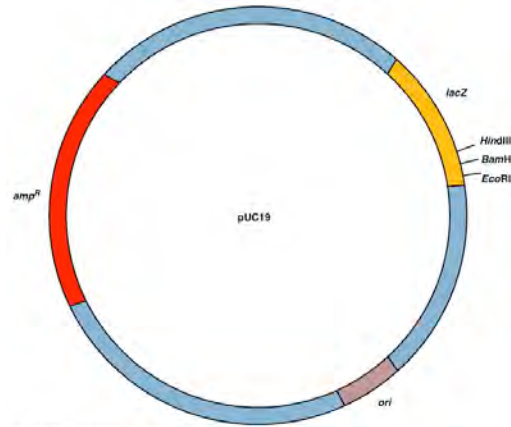
- hundreds of restriction enzymes are known
- each cuts a unique sequence of DNA
- easy to find one that cuts your target DNA and cloning vector

2. Vectors

vectors = small pieces of DNA used for cloning

- must be self replicating or integrate into the genome
- must be small enough to be manipulated in vitro
- must have a selectable marker so transformed cells can be isolated (e.g. antibiotic resistance gene)
- must have restriction enzyme cutting sites to clone genes into (e.g. a MCR: multiple cloning region)

- most vectors are plasmids (self replicating circles of DNA)



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Shuttle vector = plasmid capable of functioning in multiple species (needs multiple selection markers, different promoters, etc)

- some are based on viral DNA and are used to integrate permanently into the genome of the transformed cells (e.g. Retroviruses, Adenoviruses, Herpesviruses)

3. Polymerase Chain Reaction (PCR)

- used to amplify/copy DNA
- can turn a few copies of DNA into billions in a few hours (exponential multiplication)

DNA synthesis in a tube requires:

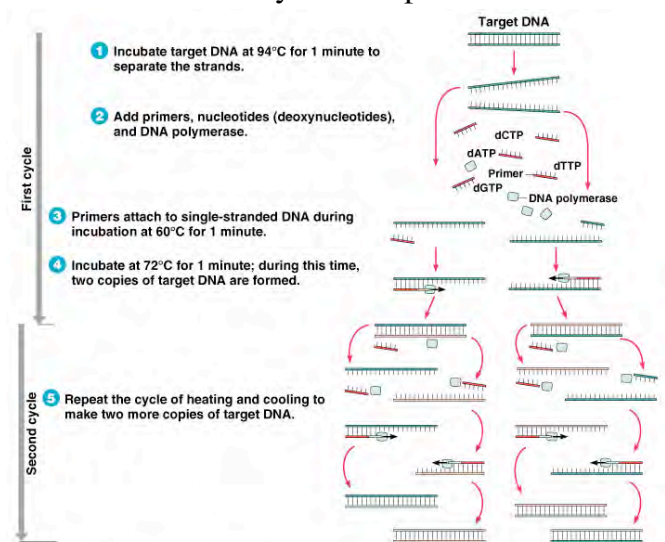
1. template DNA (gene you want to copy)
2. primers specific for the template
3. free nucleotides (dNTPs)
4. DNA polymerase

Replication process:

1. Denature: reaction is heated to 94-95°C to separate the ds DNA into ss templates
2. Anneal: temperature is lowered to 50-60°C to allow primers to complementary base pair to the ss template DNA
3. Extend: temperature is raised to 72°C to allow DNA polymerase to synthesize complementary strands

Steps repeated 20-30 times

- each new DNA can serve as template in the next round/cycle of replication:



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- 1 template x 30 rounds = 1.1×10^9 molecules
- PCR only good for DNA ~4kb or less (a gene, not whole genome)

-PCR used for:

1. cloning a gene out of an organism to express in a vector
2. to detect low levels of an infectious agent
3. to make more DNA from a limited sample for further analysis

Techniques for Genetic Engineering

1. Obtaining DNA of interest

A. Prokaryotes

-either cut out of genome with restriction enzymes or

-PCR copies from the genome

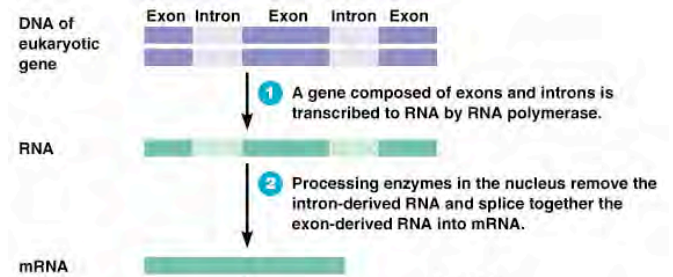
B. Eukaryotes

-get by reverse transcription:

-eukaryotic genes have introns between the coding exons

-during transcription both introns and exons are copied into RNA

-exons are then spiced together to form mRNA for translation



-prokaryotes cannot splice: eukaryotic genes must have introns removed before expression in bacteria

-yeast are eukaryotes and can splice, but often splice human genes incorrectly, thus it is best to splice genes before expression in yeast as well

Reverse transcription:

-reverse transcriptase (enzyme from retrovirus) is used to make a DNA copy from a spliced mRNA

-the intron-free DNA is called cDNA

-cDNA can be used for cloning in prokaryotes

Process: RT-PCR

1. collect spliced mRNA from human cells

2. Add:

-primers complementary to the ends of the mRNA sequence to be copied

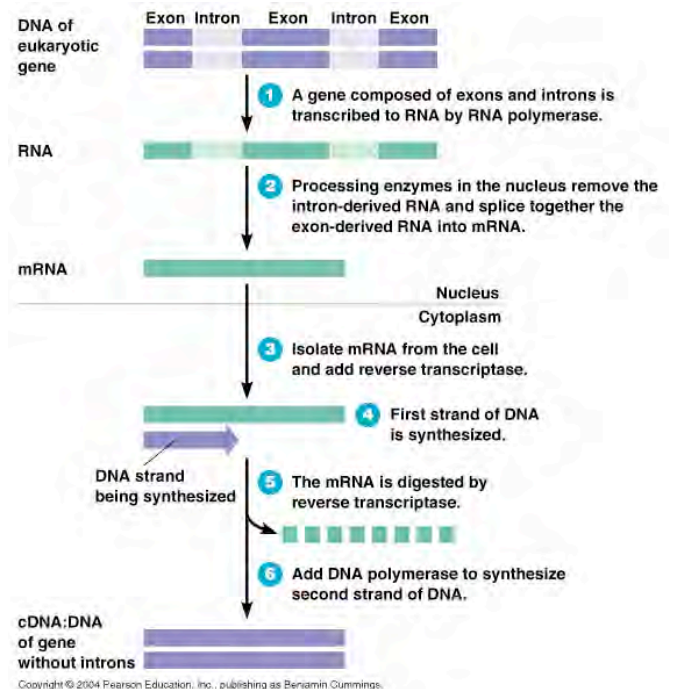
-enzyme:

reverse transcriptase: binds primers and synthesizes DNA copy

-dNTPs

3. Incubate 37°C: ssRNA → ssDNA

4. Add DNA polymerase, run PCR cycles to make many ds copies of the DNA = cDNA



2. Inserting foreign DNA into cells = Transformation

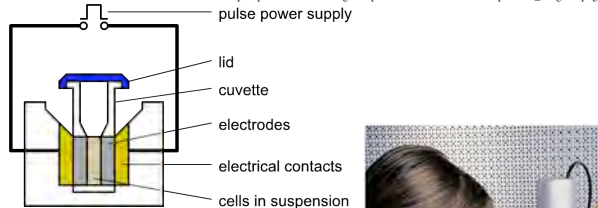
-in order to be transformed a cell must first be made competent to take up foreign DNA:

A. Chemicals

-CaCl₂ makes pores in cell membrane

B. Electroporation

-electric shock forms temporary holes in membrane



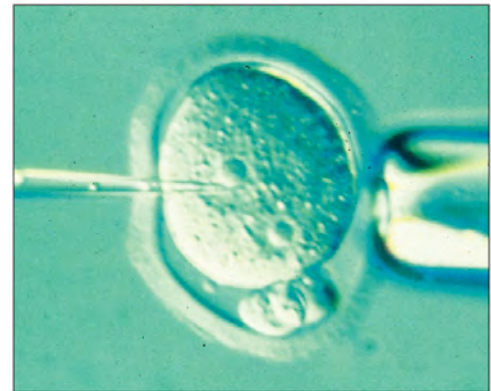
C. Gene gun

-microscopic particles of gold are coated with the DNA and shot with a burst of helium into cells



D. Microinjection

-use tiny needle to inject DNA into the cell



-the inserted DNA, regardless of method, must be on a self replicating plasmid or integrated into the host genome
-if not it will be degraded and lost from the cell (no transformed cells, no clones)

3. Selecting a clone

-few ligation events produce desired result (your gene in a vector)

-transformation is very low frequency event

-few cells will be transformed with desired vector

-need to select those that 1) have vector 2) with your gene

Blue/White Screening:

-plasmid contains Amp^R so that all bacteria transformed with vector will survive on ampicillin (non-transformed cells die)

-plasmid contains lacZ gene to code β-galactosidase

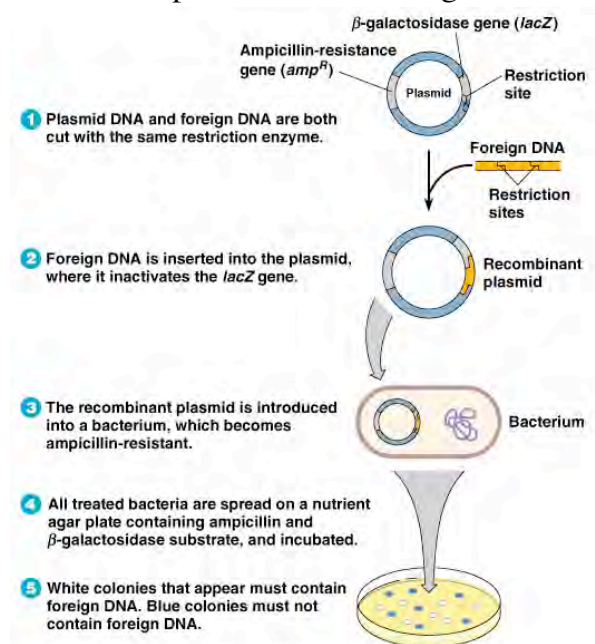
-β-gal enzyme hydrolyzes X gal substrate into a blue product

-your gene is cloned into middle of lacZ

-vectors that contain your gene in lacZ cannot make β-gal, no blue product is formed, colonies are white on X-gal

-vectors that re-ligate without your gene have intact lac Z, make β-gal, and colonies are blue on X-gal

-white colonies are selected and sequenced to confirm presence of correct gene



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4. Making your gene product

- usually inducible promoters are used to allow expression of gene independent of host cell gene expression
- e.g. Lac Operon: inducible promoter that can be turned on with IPTG
- level of expression can be controlled by concentration of inducer
- once expressed (transcription → translation), gene product is purified from host cells
- yeast are often used as host cell for protein products as they tend to secrete product into media where it is easily collected
- if cells do not secrete product they must be lysed (always if the vector DNA is the product)

Applications of Genetic Engineering/rDNA

- produce useful substances cost effectively
- obtain info about a gene or gene product for research, medicine or forensics
- alter characteristics of cells or organisms

1. Therapeutic applications

- Make hormones and enzymes to treat genetic diseases (e.g. insulin)
- Synthesize safe vaccines (e.g. subunit vaccines, DNA vaccines)
- Gene therapy: transform human cells with a normal gene to replace a mutated gene that is causing disease (use viral vectors for integration)

2. Scientific applications

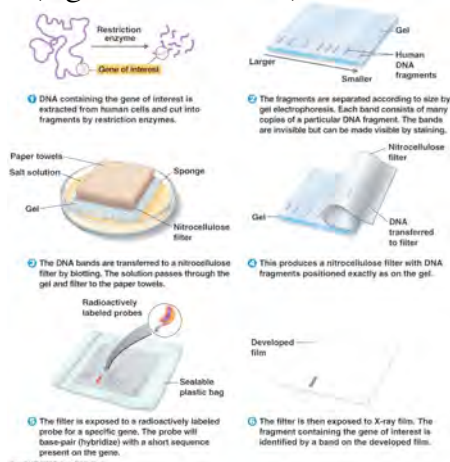
A. DNA sequencing:

- clone to get enough copies to sequence
- use sequence info to predict gene products and their function (human genome project)

Bioinformatics = the science of

understanding the function of genes through computer assisted analysis

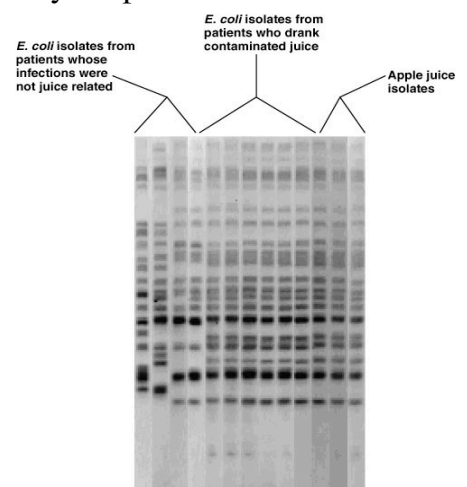
- identify mutations to do “genetic screening” on embryos (e.g. Southern Blot)



B. DNA fingerprinting / RFLP analysis

- PCR and cloning produces enough DNA from small samples to allow analysis
- cut with restriction enzymes
- each person's DNA slightly different in sequence
- each person produces unique pattern of restriction fragments: Restriction Fragment Length Polymorphism

- identify paternity, crime suspect, strain of pathogen



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3. Agricultural applications

A. transform plants for better qualities:

- faster growth
- better fruit production
- disease resistance
- growth in low nutrients
- bush instead of vine
- miniature plants

B. growth hormone for animals

- bigger faster meat production
- greater milk production