Recombinant DNA Technology

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Recombinant DNA technology is one of the recent advances in biotechnology, which was developed by two scientists named Boyer and Cohen in 1973.

Stanley N. Cohen (1935–) and Herbert Boyer (1936–) (bottom) constructed the first recombinant DNA using bacterial DNA and plasmids.

What is Recombinant DNA Technology?

- **Recombinant DNA technology is a technology which allows DNA to be produced via artificial means.**
- **The procedure has been used to change DNA in living organisms and may have even more practical uses in the future.**
- **It is an area of medical science that is just beginning to be researched in a concerted effort.**

What is Recombinant DNA Technology?

- Recombinant DNA technology works by taking DNA from two or more different sources and combining that DNA into a single molecule. That alone, however, will not do much.
- Recombinant DNA technology only becomes useful when that artificially-created DNA is reproduced. This is known as DNA cloning.

- Recombinant DNA technology begins with the isolation of a gene of interest (target gene). The target gene is then inserted into the plasmid or phage (vector) to form replicon.
- The replicon is then introduced into host cells to cloned and either express the protein or not.
- The cloned replicon is referred to as recombinant DNA. The procedure is called recombinant DNA technology. Cloning is necessary to produce numerous copies of the DNA since the initial supply is inadequate to insert into host cells.

What is Recombinant DNA Technology?

- Some other terms are also in common use to describe genetic engineering.
  - Genetic Engineering
  - Gene manipulation
  - Gene cloning (Molecular cloning)
  - Genetic modification

Cloning

- Cloning——In classical biology, a *clone* is a population of identical organisms derived from a single parental organism.
  - For example, the members of a colony of bacterial cells that arise from a single cell on a petri plate are clones. Molecular biology has borrowed the term to mean a collection of molecules or cells all identical to an original molecule or cell.

- Recombinant DNA technology——A series of procedures used to join together (recombine) DNA segments. A recombinant DNA molecule is constructed (recombined) from segments from 2 or more different DNA molecules. Under certain conditions, a recombinant DNA molecule can enter a cell and replicate there, autonomously (on its own) or after it has become integrated into a chromosome.

Steps of Recombinant DNA

1. Isolating (vector and target gene)
2. Cutting (Cleavage)
3. Joining (Ligation)
4. Transforming
5. Cloning
6. Selecting (Screening)

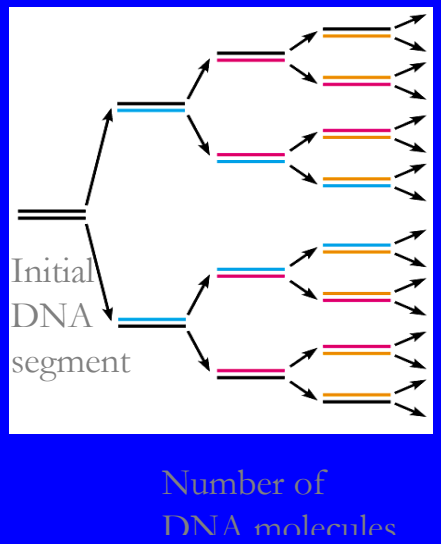
How to get a target genes?

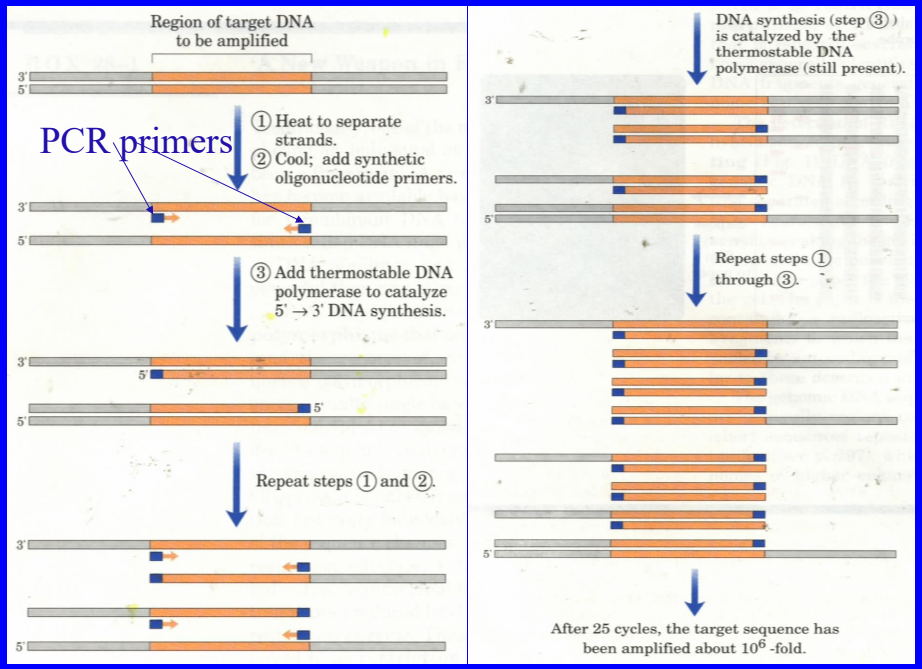
1. Genomic DNA
2. Artificial synthesis
3. PCR amplification
4. RT-PCR

Polymerase chain reaction (PCR)

- A technique called the polymerase chain reaction (PCR) has revolutionized recombinant DNA technology. It can amplify DNA from as little material as a single cell and from very old tissue such as that isolated from Egyptian mummies, a frozen mammoth, and insects trapped in ancient amber.

- **method is used to amplify DNA sequences**
- **The polymerase chain reaction (PCR) can quickly clone a small sample of DNA in a test tube**





RT-PCR

- **Reverse transcription polymerase chain reaction (RT-PCR) is a variant of polymerase chain reaction (PCR.**
- **In RT-PCR, however, an RNA strand is first reverse transcribed into its DNA complement (complementary DNA, or cDNA) using the enzyme reverse transcriptase, and the resulting cDNA is amplified using traditional PCR.**
  - **Template:RNA**
  - **Products: cDNA**

rDNA Technology

- Restriction Enzymes and DNA Ligase
- Plasmid Cloning Vectors
- Transformation of Bacteria
- Blotting Techniques

Restriction Enzymes

- Most significant advancement permitting rDNA manipulation
- Differ from other nucleases
  - recognize and cleave a specific DNA sequence (Type II restriction enzymes)

Restriction Enzymes

- Nomenclature
  - EcoRI
    - E = Escherichia — genus name
    - co = coli — species name
    - R = strain RY12 — strain or serotype
    - I = Roman numeral one = first enzyme
  - HinDIII
    - Haemophilus influenza serotype d 3rd enzyme

Restriction Enzymes

- Recognition sites
  - Generally 4, 6, or 8 bp in length
  - Most sites are palindromic
    - OTTO / HANNAH / REGAL LAGER
    - A MAN A PLAN A CANAL PANAMA
  - For REases - sequence reads the same in a 5'--->3' direction on each strand

Table 4.1 Recognition sequences of some restriction endonucleases

Enzyme	Recognition site	Type of cut end
*Eco*RI	G↓A—A—T—T—C C—T—T—A—A↑G	5′-phosphate extension
*Bam*HI	G↓G—A—T—C—C C—C—T—A—G↑G	5′-phosphate extension
*Pst*I	C—T—G—C—A↓G G↑A—C—G—T—C	3′-hydroxyl extension
*Sau*3AI	↓G—A—T—C C—T—A—G↑	5′-phosphate extension
*Pvu*II	C—A—G↓C—T—G G—T—C↑G—A—C	Blunt end
*Hpa*I	G—T—T↓A—A—C C—A—A↑T—T—G	Blunt end
*Hae*III	G—G↓C—C C—C↑G—G	Blunt end
*Not*I	G↓C—G—G—C—C—G—C C—G—C—C—G—G—C↑G	5′-phosphate extension

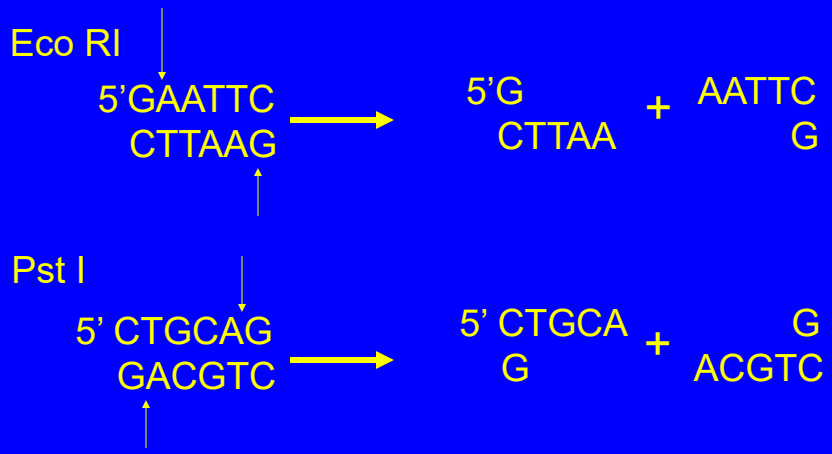
Restriction Enzymes

- EcoRI
  5’ GAATTC 3’
  3’ CTTAAG 5’
- Hind III
  5’ AAGCTT 3’
  3’ TTCGAA 5’

Restriction Enzymes

- Cleave DNA to generate different “ends”
  - Staggered cut
    - 5’ extension
    - 3’ extension
  - Blunt end

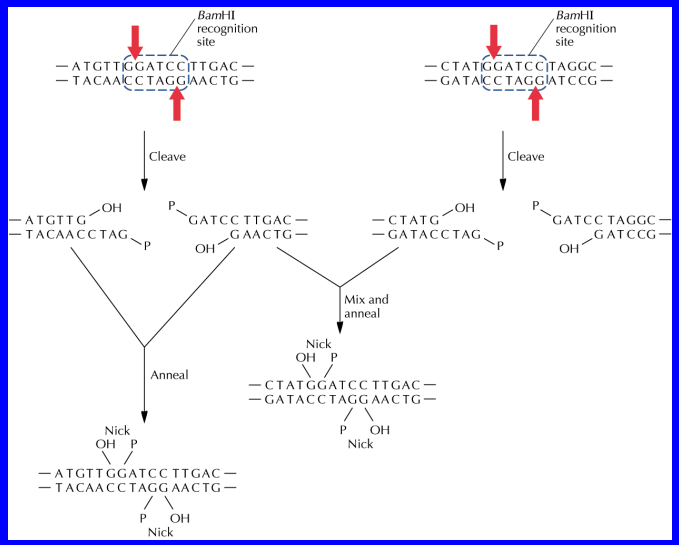
Staggered Cut / 5’ or 3’ Extension



Restriction Enzymes in DNA Cloning

- How are REases used ?
  - Ends are “sticky”
  - Complementary
  - Any two DNAs cut with same enzyme can stick together through complementary base pairing

Annealing sticky ends



rDNA Technology

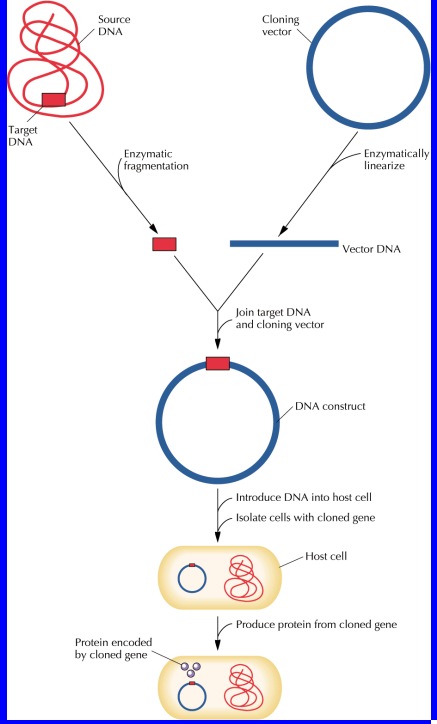
- Restriction Enzymes and DNA Ligase
- Plasmid Cloning Vectors
- Transformation of Bacteria
- Creating and Screening Genomic Libraries
- cDNA Library Construction
- Vectors for Cloning Large Pieces of DNA
- Blotting Techniques

Vectors- Cloning Vehicles

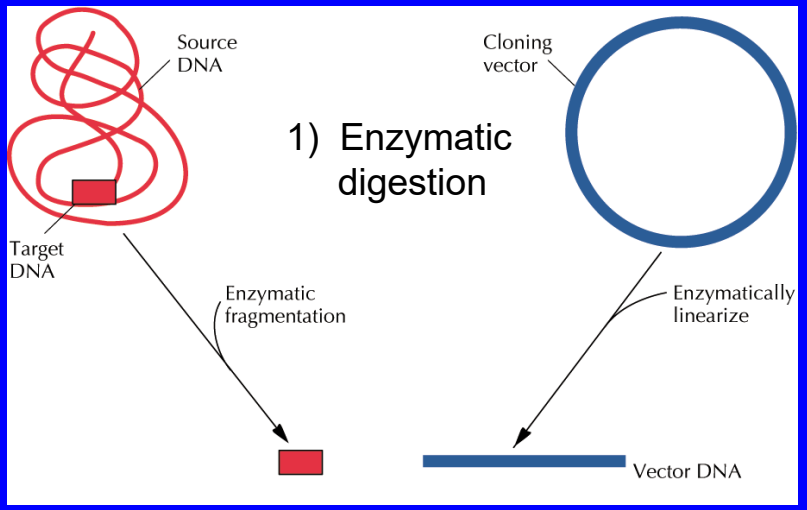
- **Cloning vectors can be plasmids, bacteriophage, viruses, or even small artificial chromosomes. Most vectors contain sequences that allow them to be replicated autonomously within a compatible host cell, whereas a minority carry sequences that facilitate integration into the host genome.**

- **All cloning vectors have in common at least one unique cloning site, a sequence that can be cut by a restriction endonuclease to allow site-specific insertion of foreign DNA. The most useful vectors have several restriction sites grouped together in a multiple cloning site (MCS) called a polylinker.**

Recombinant DNA Cloning Procedure



Recombinant DNA Cloning Procedure



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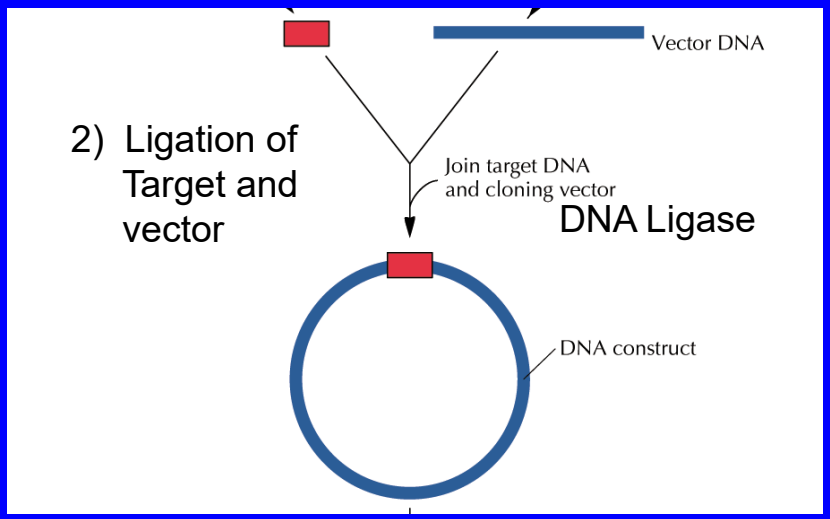
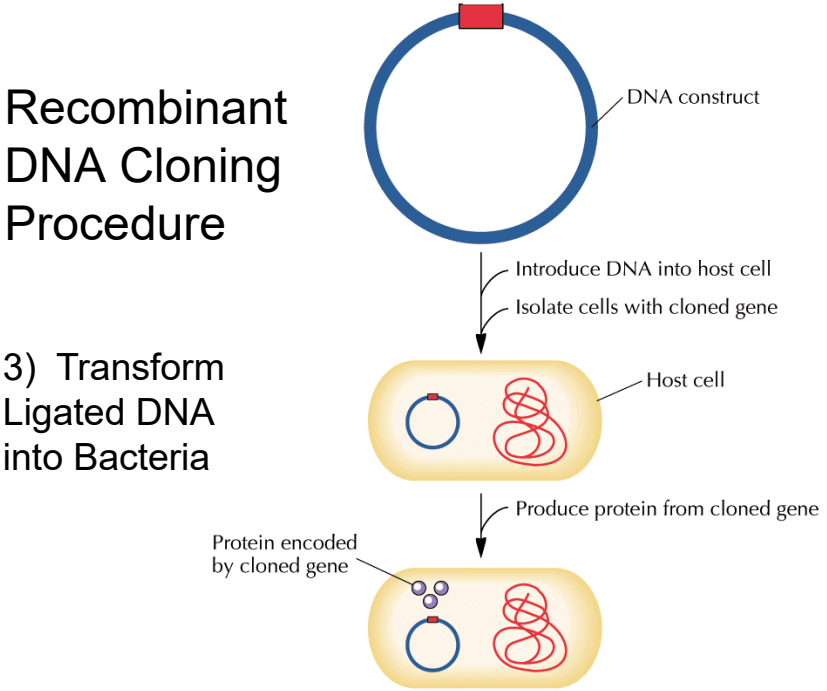


Recombinant DNA Cloning Procedure



Plasmid Cloning Vectors

- Recombinant DNA needs to be replicated in bacterial cell
- Self-replicating piece of DNA
  - termed cloning vehicle
  - can be plasmid or phage

Plasmid Cloning Vectors

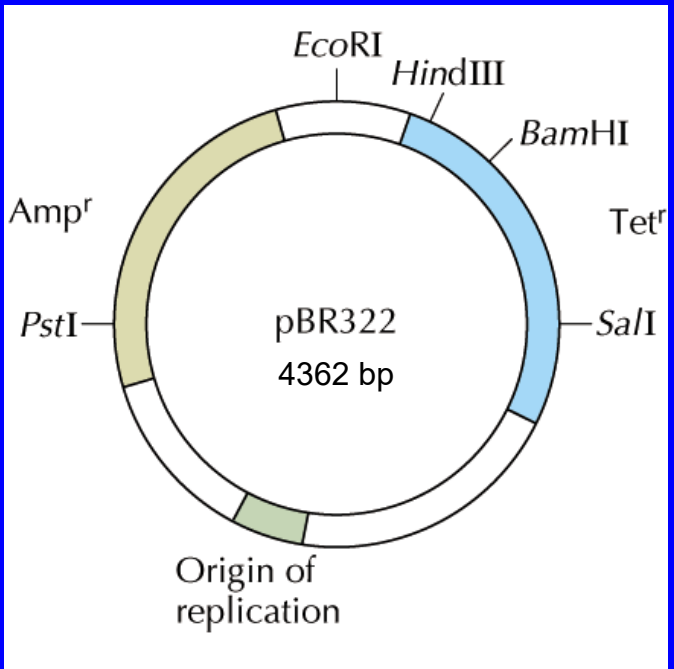
- Small circular piece of DNA
- Exists separate from chromosome
- Derived from naturally occurring plasmids

- High copy number = 700 copies / cell
- Low copy number = 1-4 copies / cell

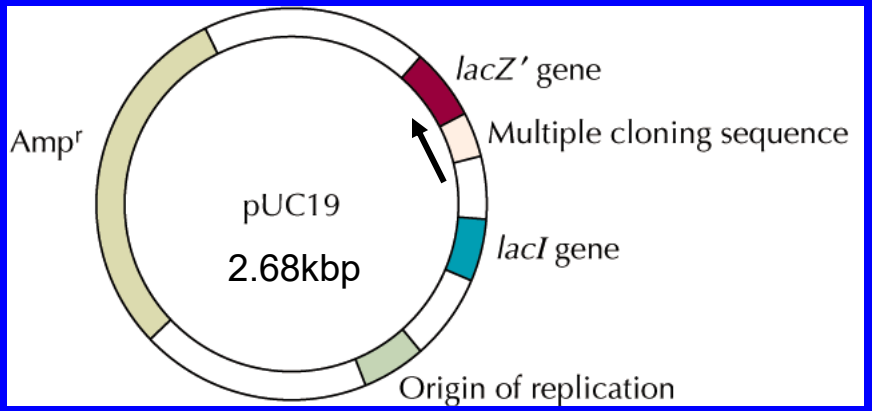
Plasmid Cloning Vectors

- Derived from naturally occurring plasmids
- Altered features
  - small size (removal of non-essential DNA)
    - higher transformation efficiency
  - unique restriction enzyme sites
  - one or more selectable markers
  - origin of replication (retained from original plasmid)
  - other features: promoters, etc.

pBR322 old-style general purpose plasmid



pUC19



Multiple Cloning Sequence (Polylinker)

EcoRI KpnI BamHI Sal I

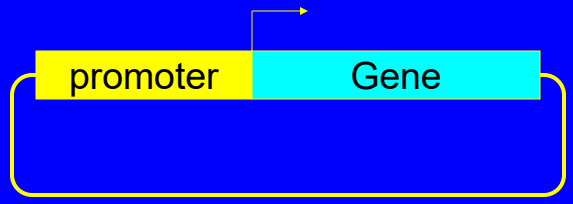
GAATTCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGAC

SacI SmaI XbaI

Part of MCS of pUC19 and other plasmids

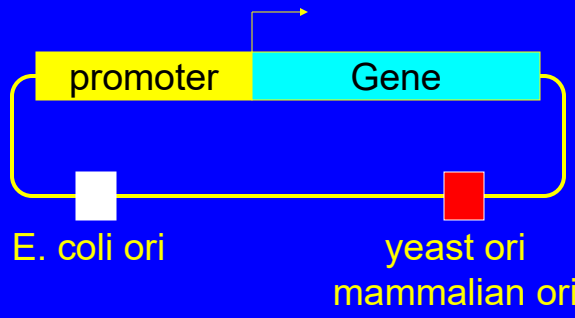
Plasmid Cloning Vehicles

Some plasmids (Expression Plasmids) have promoters upstream of cloning sites for expression of genetic info encoded by DNA fragment



Shuttle Plasmid Cloning Vehicles

Some plasmids (Shuttle Plasmids) have origins of replication for E. coli and another organism: yeast, mammalian cells or other bacteria



Transformation of Bacteria

- rDNA constructed in the lab must be introduced into “host” cell
- Cells must be able to take up DNA - “COMPETENT”
- Growing bacteria will produce lots of copies of the DNA

Transformation of Bacteria

- Two basic methods to produce competent bacteria (able to take up added DNA)
  - Chemical competent
  - Electroporation

Transformation of Bacteria

- Chemical competent
  - Divalent metal ion Ca^{++} , required
  - treat cells with ice-cold $CaCl_2$ solutions
  - Ca^{++} ions alter membrane so it is permeable to DNA

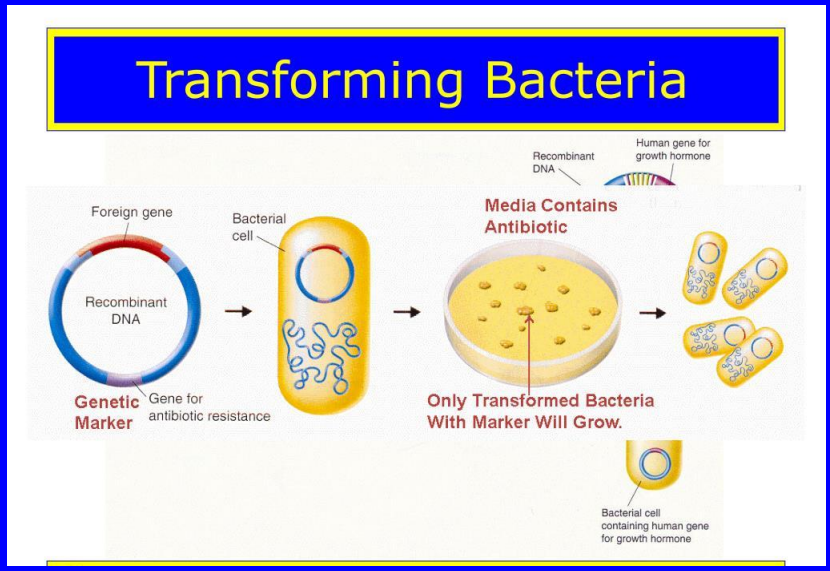
Transformation of Bacteria

- Electroporation
  - Cell/DNA mix given high voltage electric shock
  - 2.5kvolts, ~5msec
  - useful for high efficiency transformation
  - 10^9 transformants / µg of DNA

Transformation of Bacteria

- Both methods are very inefficient
  - only a few % of cells actually take up DNA
- How are the transformed cells selected?
  - antibiotic resistance gene on plasmid
  - ampicilin, tetracycline, chloramphenicol, etc.
  - transformed cells grow; non-transformed die

Transformation of Bacteria



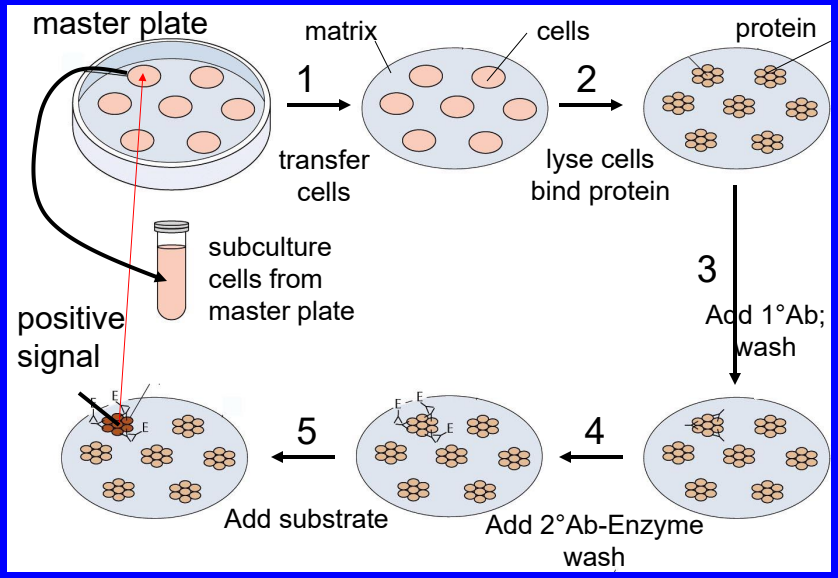
Blotting Techniques

- Several techniques for size fraction of nucleic acid fragments and proteins
  - Nucleic Acids
    - Agarose Gels
    - Polyacrylamide Gels - higher resolution
  - Proteins
    - SDS PAGE - denatured proteins

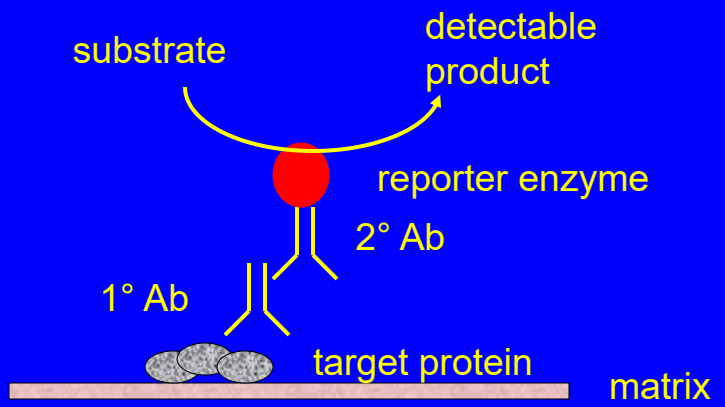
Blotting Techniques

- How can one fragment be detected in a complex mixture?
  - Transfer the macromolecule to a membrane
  - Detect with
    - complementary nucleic acid probe or
    - with an antibody to the specific protein

Antibody-based (Immunological) Screen



Antibody (Immunological) Screen



Blotting Techniques

Blot Type	Matrix	Molecule	Detection
Southern	agarose	DNA	nucleic acid
northern	agarose	RNA	nucleic acid
western	polyacrylamide	Protein	antibody

Blotting Techniques: Info Obtained

- Southern Blots
  - presence of fragment (gene)
  - # of fragments (approx. # of genes)
  - sizes of fragments
  - sequence similarity between target & probe

Blotting Techniques: Info Obtained

- Northern blots
  - presence of RNA in tissue
  - level of expression
  - size of mRNA
  - sequence similarity between target & probe

Blotting Techniques: Info Obtained

- Western blots
  - presence of protein in tissue
  - level of expression
  - size of protein