TECHNIQUES IN MOLECULAR BIOLOGY:

DNA HYBRIDIZATION & CLONING

Course: Molecular Biology (BIOL333)

Instructor: Dr. Mahmoud A. Srour

Textbook:
Watson J, et al. (2014). Molecular Biology of the Gene, 7th ed. Chap 7

DNA hybridization

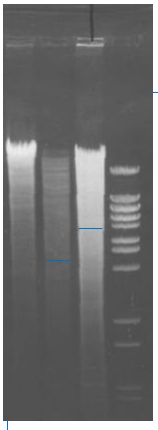
- Hybridization: the technique wherein renatured DNA is formed from separate single-stranded samples
- Applications> nucleic acid blotting
  - Southern blot hybridization
  - Northern blot hybridization

Southern Blot

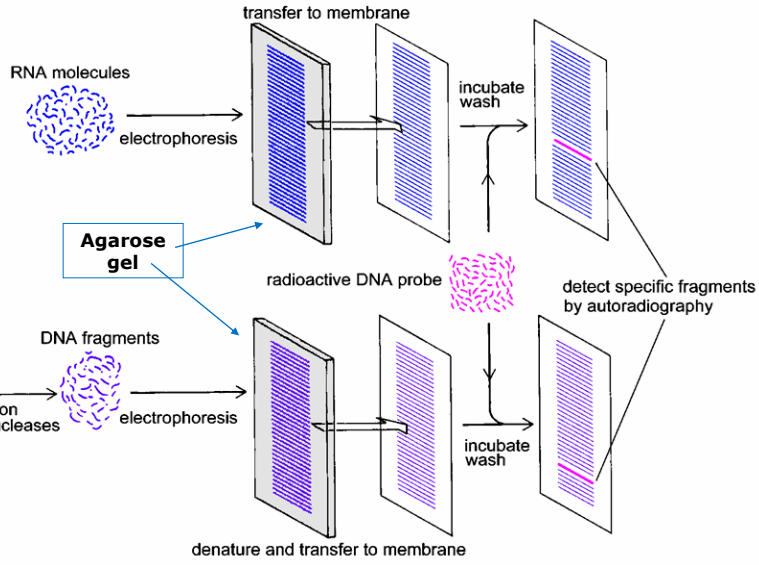
- Described by Dr. Edward Southern (1975)
- Used to identify particular DNA fragment
- **Method**
- Digest and electrophorese DNA on agarose gel
- dsDNA in gel is denatured using alkali (NaOH)
- Transfer from gel to positively charged membrane > "imprint" or "blot"
- Immobilize the DNA to membrane by UV-cross linking
- Detect with a labeled probe (complementary to a sequence within the gene of interest)> hybridization
- When X-ray film is exposed to hybridized membrane > autoradiogram

Northern Blot

- Used to identify particular RNA fragment
- RNA are short (typically <5 kb) are not digested
- Method is similar to Southern blot
- Applications: Study gene expression or quantify the mRNA level of a specific gene
- Can study one gene at a time



Southern & Northern Blots



DNA hybridization (animation)

- https://highered.mheducation.com/sites/9834092339/student_view0/chapter17/dna_probe__dna_hybridization_.html
- https://www.youtube.com/watch?v=yyLDwe_HXU0

Southern Blots

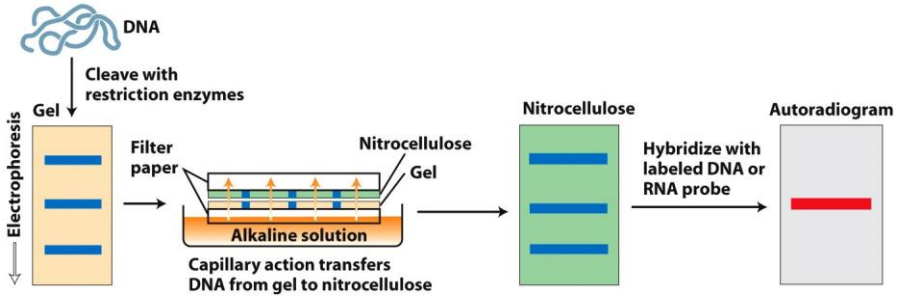


Figure 5-26
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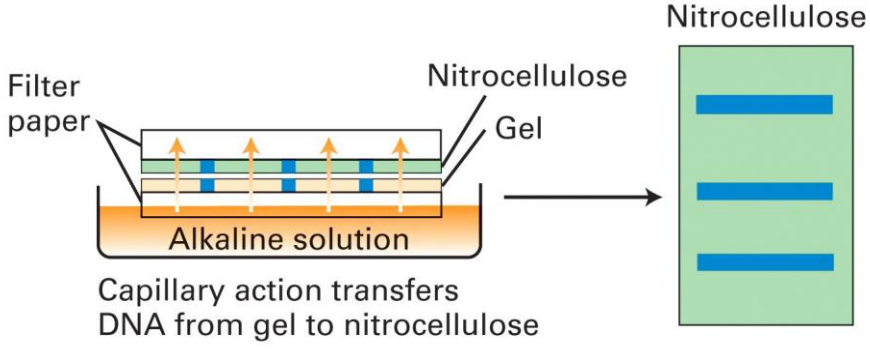
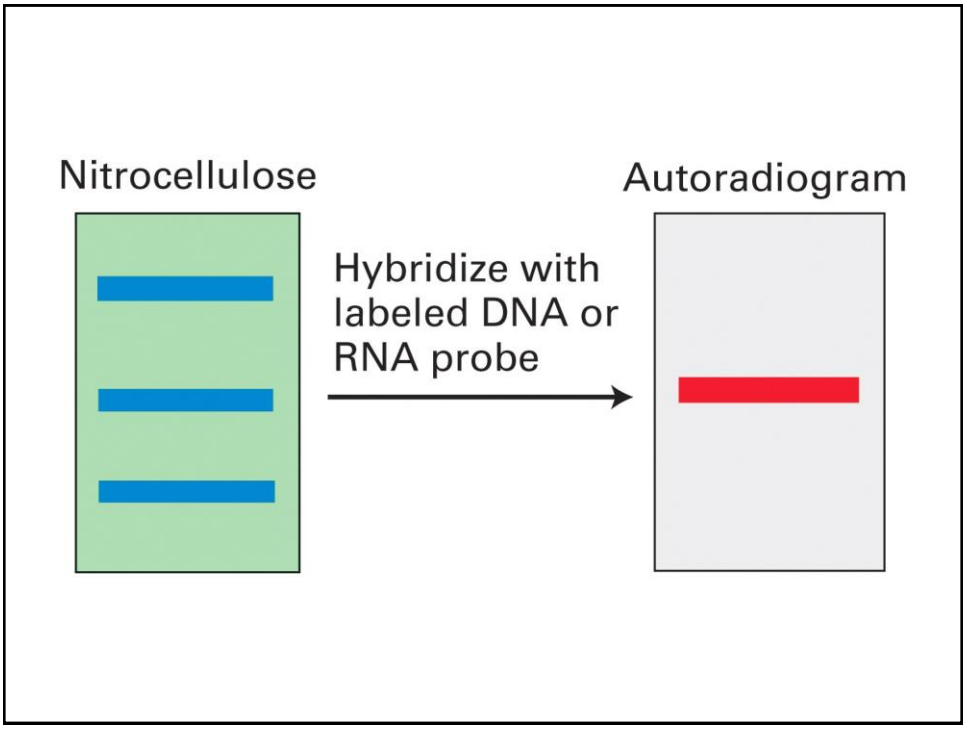
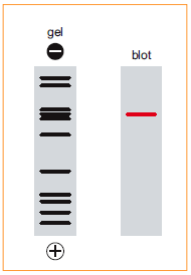
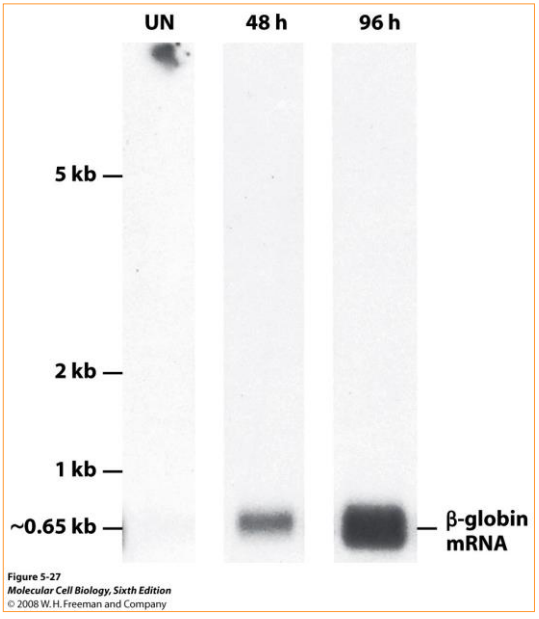




Northern blotting
> autoradiogram

Southern blot > autoradiogram





Nucleic acid Probes

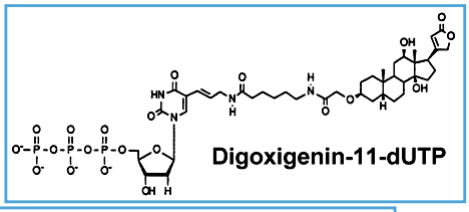
- Previously cloned genes
- Synthetic oligonucleotides or DNA/RNA fragment (complementary to part or all of target sequence)
- Radioactive labeled (^{32}P-dNTP)
- Nonradioactive labeled
  - DiG-dUTP
  - Biotin-dUTP

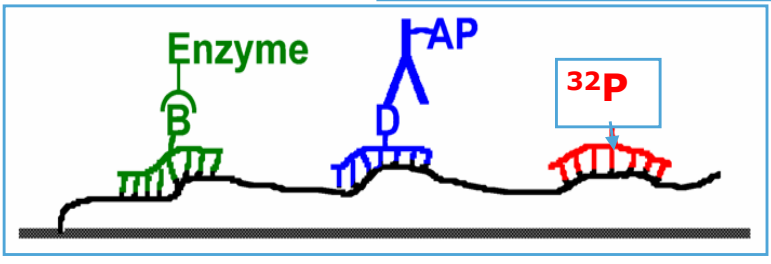
Nucleic acid Probes

- **The two basic methods for labeling probes are:**
- End labeling using T4 polynucleotide kinase:
  - adds the γ-phosphate from ^{32}P-ATP to the 5'-OH group
- Incorporation of labeled nucleotides into probe via PCR:
  - labeled nucleotides are usually nucleotides modified with either a radioactive atoms or fluorescent moiety.
  - Radioactive nucleotides typically have ^{32}P incorporated in the α–phosphate of one of the 4 dNTPs

Labeled DNA Probes

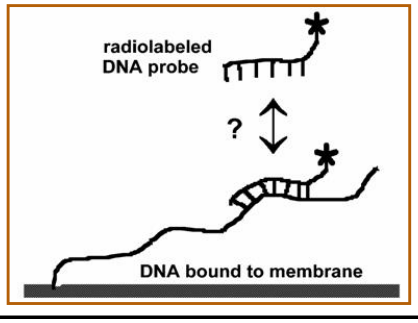
- Enzyme-Linked Probes
  - Biotin /streptavidin
  - Digoxigenin-UTP/antibody
  - Radioactive ^{32}P

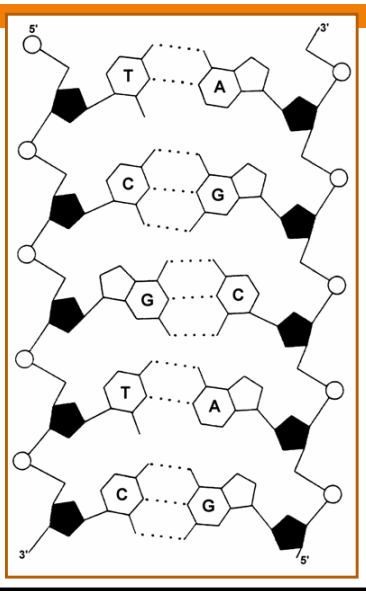




Factors Affecting Hybridization

- Temperature
- Ionic strength
- Probe length
- Probe mismatch
- % GC





Other forms of hybridization

- **Dot/Slot Blot**
  - DNA samples are spotted directly on the membrane and No electrophoresis is needed
- **Fluorescent in situ hybridization (FISH)**
  - combine with microscopy to localize cells expressing gene

DNA Cloning

- DNA cloning: the ability to construct recombinant DNA molecules and maintain them in cells
- A variety of techniques, often referred to as *recombinant DNA technology* are used in DNA cloning
- Isolation of a large amount of a single pure DNA molecule facilitates analysis of that DNA molecule.

DNA Cloning

Vector + Insert DNA

↓

Recombinant DNA

↓

Replication (Amplification) of Recombinant DNA within host cells

↓

Isolation*, sequencing, and manipulation of purified DNA fragment

Definition of terms used in cloning

- **Recombinant DNA:** any DNA molecule composed of sequences derived from different sources
- **Vector:** autonomously replicating genetic element used to carry an insert DNA (or cDNA fragment) into host cell for the purpose of gene cloning
- The most common host used is *E. coli* (genetically modified)
- **cDNA:** DNA molecule copied from an mRNA by reverse transcription

Recombinant vectors:

- Recombinant vectors: autonomously-replicating DNA used to 'carry' and amplify foreign DNA within host cells
- ***E. coli* plasmids**
- Phage lambda
- Cosmids (phage lamda + plasmid)

- BAC- bacterial artificial chromosomes
- YAC- yeast artificial chromosomes

Bacterial plasmids

- Occur naturally in bacteria (plasmids also present in single-cell eukaryotes, e.g, yeast)
- Circular dsDNA
- Autonomous replication
- Extra-chromosomal elements
- 1-200 kb size range
- Present in multiple copies per cell
- Transmitted during conjugation
- Exist in parasitic or symbiotic relationship with host cell (e.g, Antibiotic resistance)

Class activity?

- Why bacterial genomes and plasmids exist in a circular configuration??

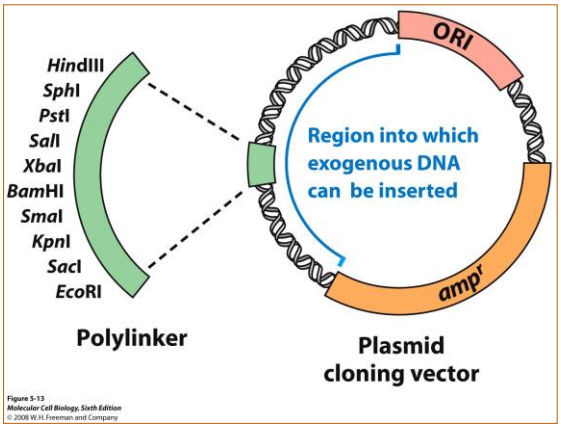
Engineered / Recombinant plasmids

- **Basic elements of recombinant plasmids:**
- Small in size >1.2-3 kb
- Origin of replication, specific DNA sequences of 50-100bp> (Ori)
- Selection marker (antibiotic resistance gene)
- Polylinker/ multiple cloning site

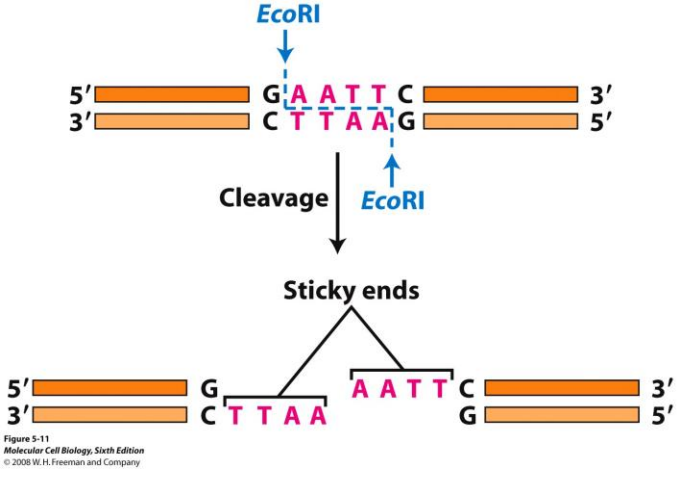
- Most vectors are derived from pUC or pBR322
- Cloning capacity ~ up to 10kb (also up to 20kb or larger is possible, but is difficult)

Basic components of an *E. coli* "cloning plasmid" (Vector DNA)

- Basic elements of a cloning vector:
  1. Origin of replication
  2. Selectable marker, usually an antibiotic resistance gene
  3. Polylinker, with one or more unique sites for REs



Cleavage of DNA by EcoRI



Ligation of restriction fragments with complementary sticky ends.

Ligation of sticky ends is more efficient than blunt ends.

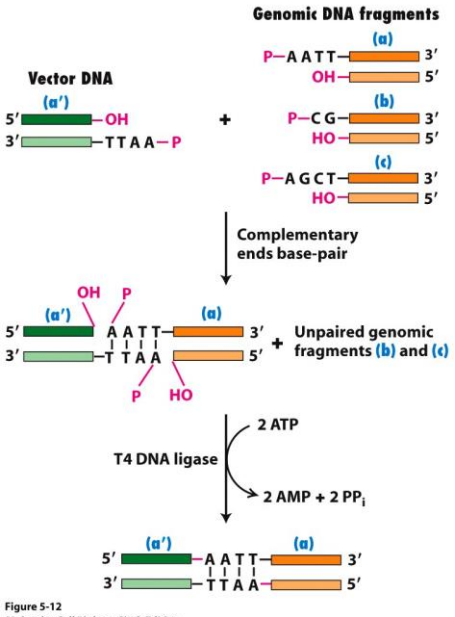


Figure 5-12
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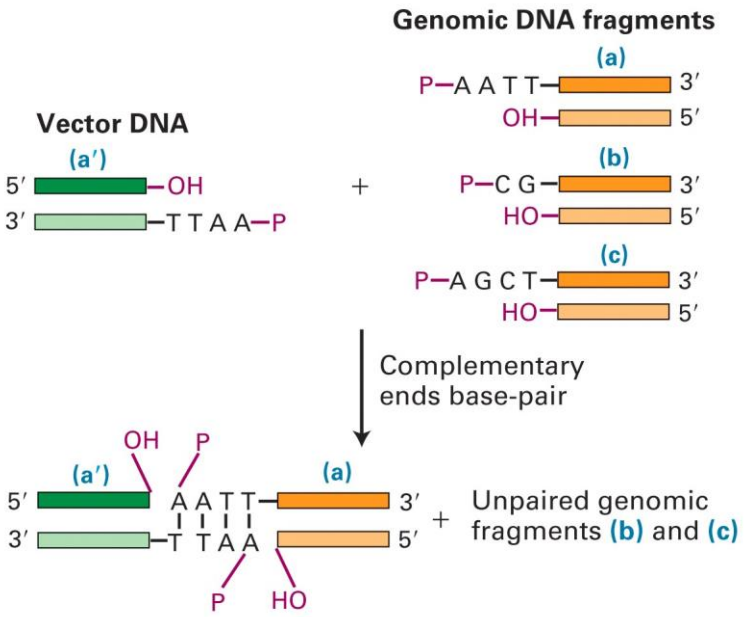
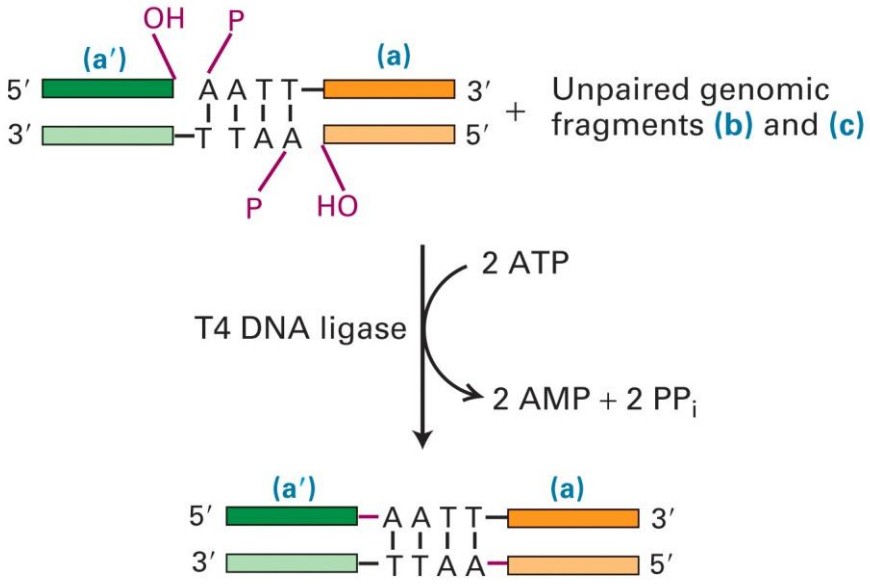
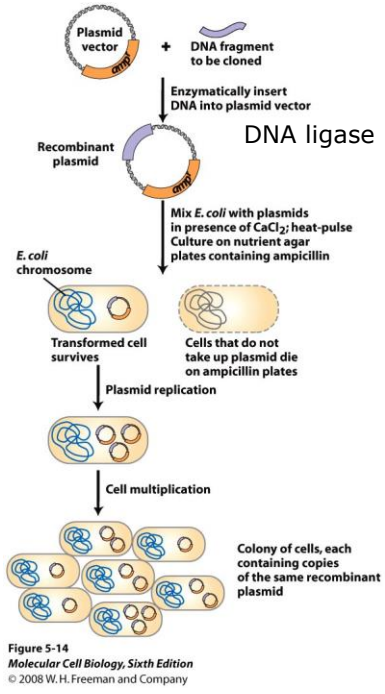




DNA cloning in a plasmid vector permits amplification of a DNA fragment



Plasmid preparation ←

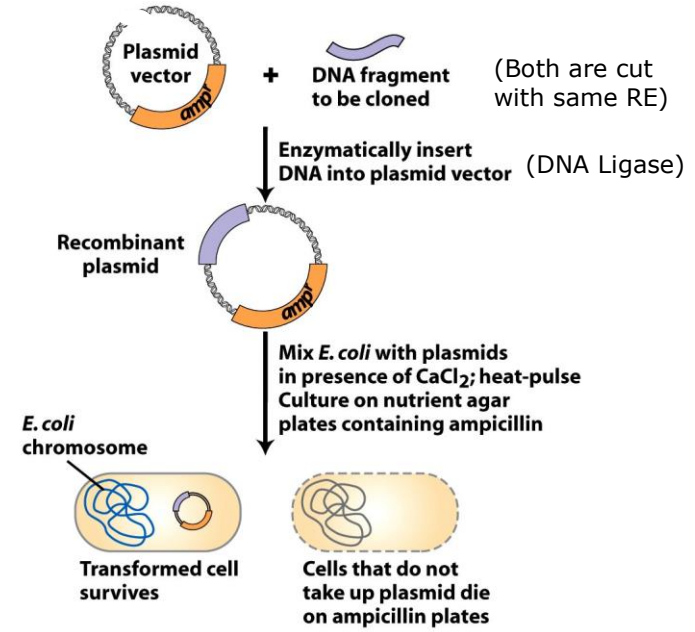


Figure 5-14 part 1
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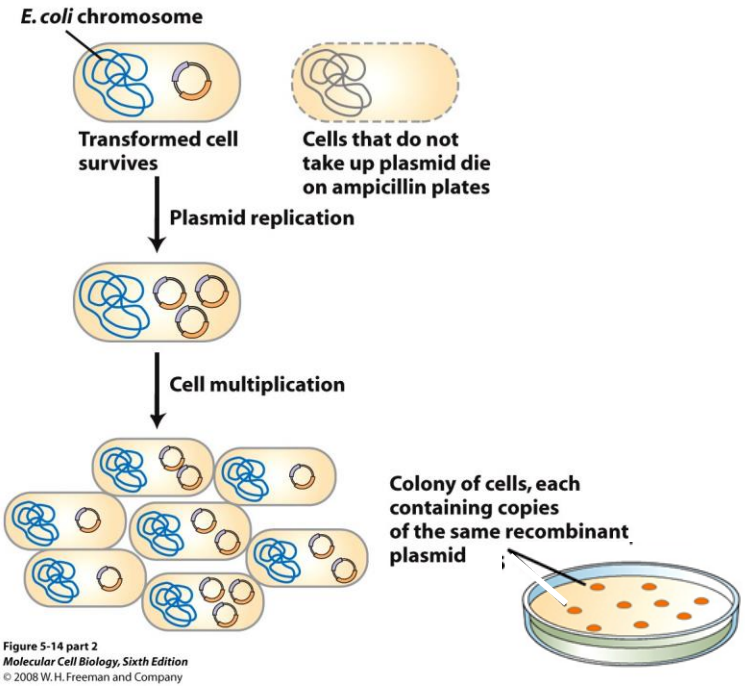

Figure 5-14 part 2
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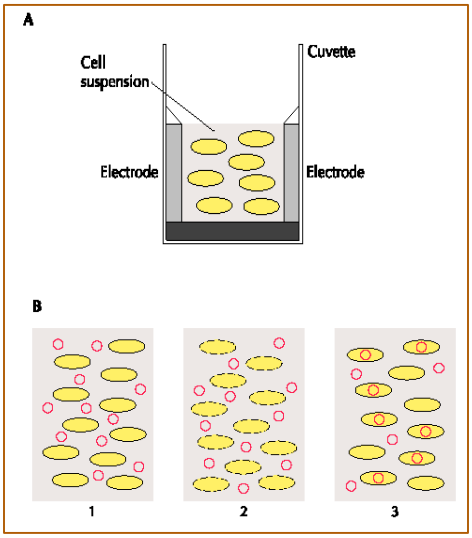
Genetic Transformation of Prokaryotes

- Transformation: the process by which a host organism can take up DNA from its environment

- **Two methods are commonly used:**

1. Chemical transformation
   - Usually involves $CaCl_2$ and heat shock
2. Electroporation
   - Electric field mediated membrane permeabilization
   - 10-100 times more efficient that chemical approach

Electroporation



- Cells suspended in DNA solution in cuvette between two electrodes
- High voltage electric field pulses administered
- DNA migrates through HVEF induced openings in cells

Recombinant vectors:

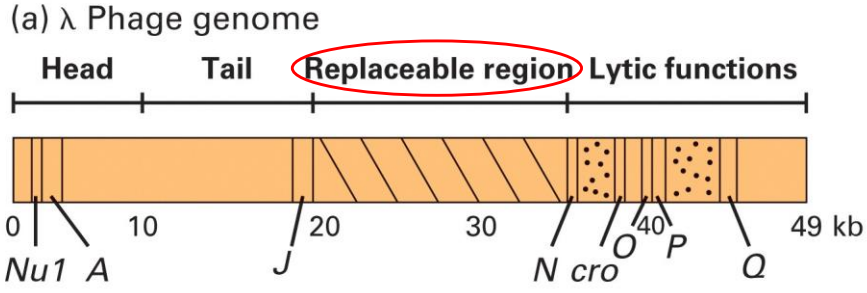
- Recombinant vectors: autonomously-replicating DNA used to 'carry' and amplify foreign DNA within host cells
- *E. coli* plasmids
- **Phage lambda**
- Cosmids (phage lamda + plasmid)

- BAC- bacterial artificial chromosomes
- YAC- yeast artificial chromosomes

Phage Lambda (λ phage)

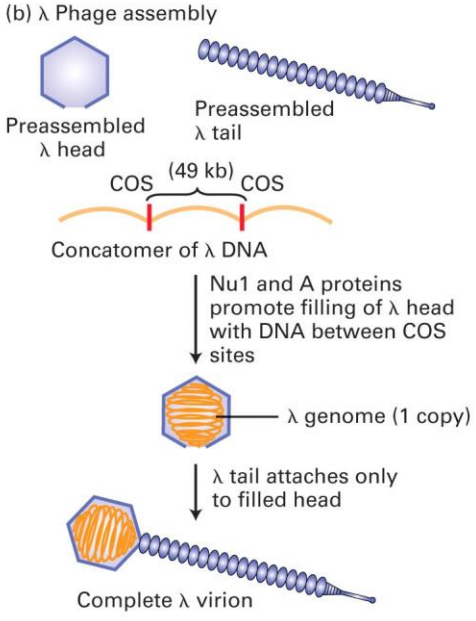
- Infection of E. coli by λ phage is 1000-fold more efficient than plasmid transformation
- Many more clones of phage can be grown on a single plate
- Infection and lysis of cells> ~100 phages /cell>plaques
- Can be assembled *in vitro*
- Cloning capacity ~ 25 kb

Bacteriophage Lambda genome and packaging of phage DNA



Some regions of the genome can be replaced by exogenous DNA (diagonal lines) or deleted (dotted) without affecting the ability of phage to infect host cells and assemble new virions.

Bacteriophage Lambda: *in vitro* assembly



Recombinant vectors:

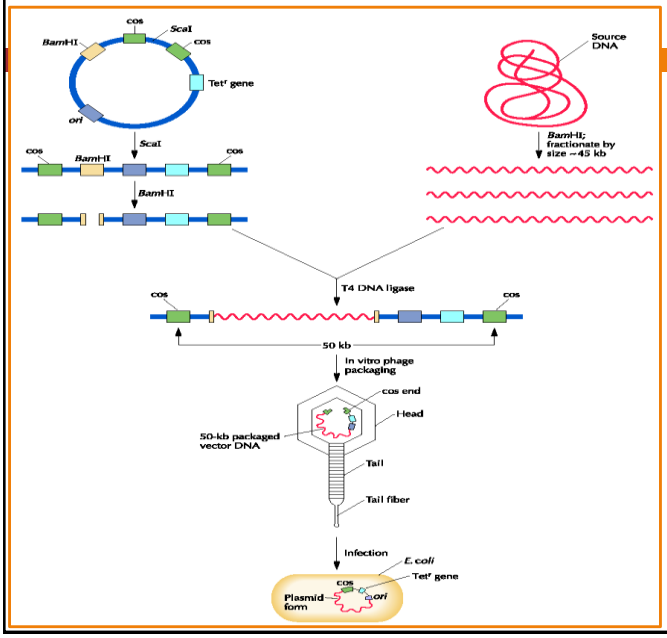
- Recombinant vectors: autonomously-replicating DNA used to 'carry' and amplify foreign DNA within host cells
- *E. coli* plasmids
- Phage lambda
- **Cosmids (phage lamda + plasmid)**

- BAC- bacterial artificial chromosomes
- YAC- yeast artificial chromosomes

Cosmids

- Combine the properties of plasmids and λ phage
- Cloning capacity 35-45 kb
- Common cosmids: pLFR-5 (6kb): a plasmid with 2 cos sites
- Cosmids based on P1 phage (115 kb) can carry up to 85kb insert

Cosmid Cloning System



λ-cos sites inserted into a small plasmid

Target DNA ligated between two cosmid DNA molecules

Recombinant DNA packaged and *E. coli* Infected as before

Can clone DNAs up to 45 kb