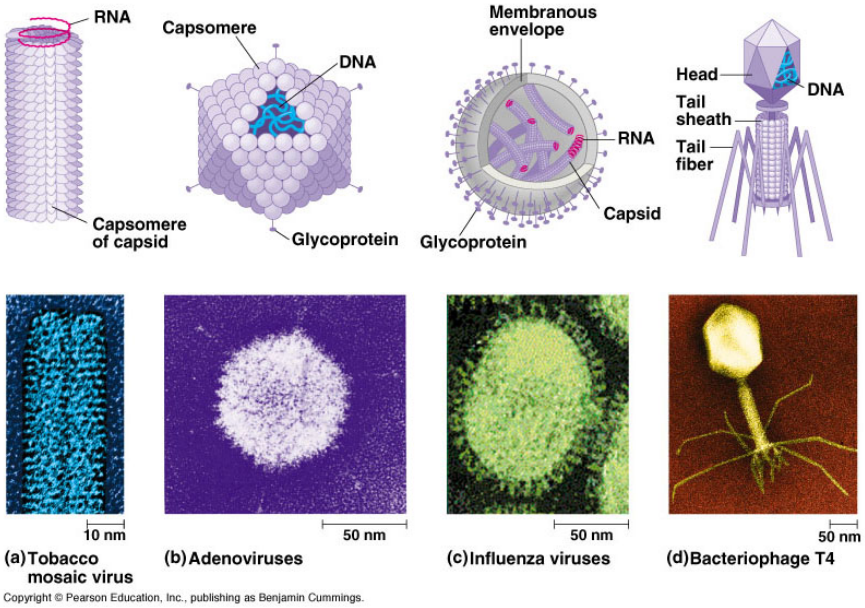
Viruses (Ch. 18)

- Structure
  - Not cells, not alive. genome, capsid, envelope
- Function
  - entry, replication, gene expression, self-assembly
  - Some assimilate into host genome
  - Origin as “runaway genes”

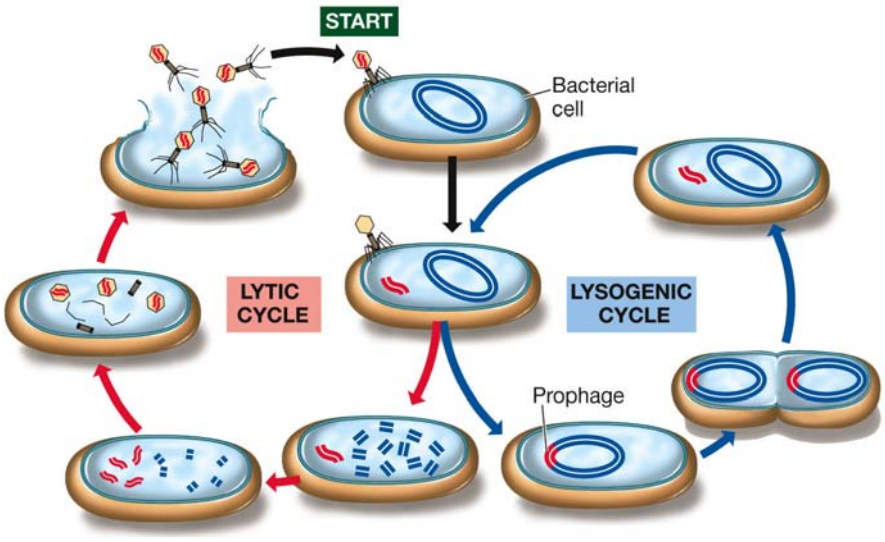
Some representative viruses



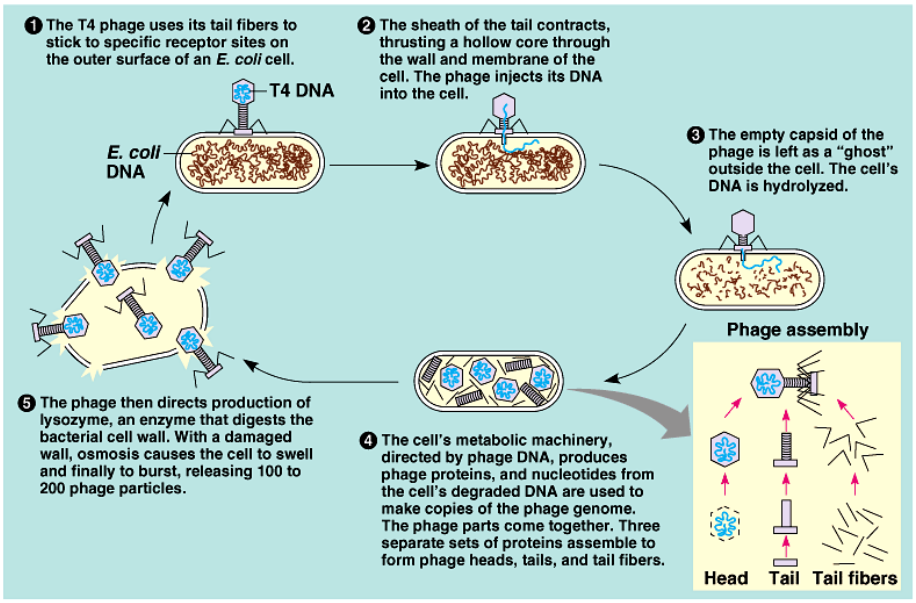
Even smaller- virioids

- Virioids: RNA with no capsid, and no structural (protein) genes
- Form siRNA's (small interfering RNA's) via dicer
- Some cause important plant diseases- highly contagious

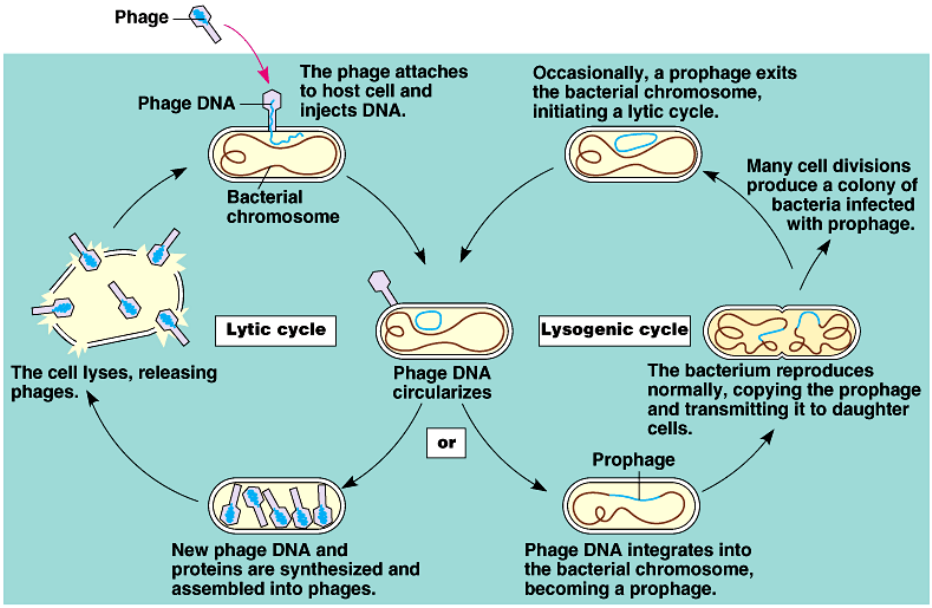
Life cycles of bacteriophages



The lytic cycle of phage T4



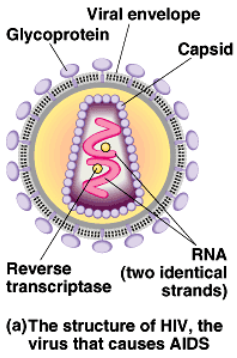
Lysogenic and lytic cycles of phage λ, a temperate phage



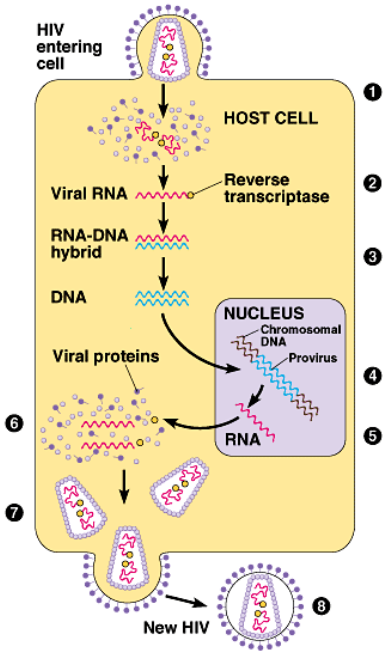
Retroviruses

- RNA genome
- Reverse transcriptase makes DNA using RNA as a pattern
- Includes Human Immunodeficiency Virus (HIV) which causes Acute Immunodeficiency Syndrome (AIDS)

HIV, a retrovirus

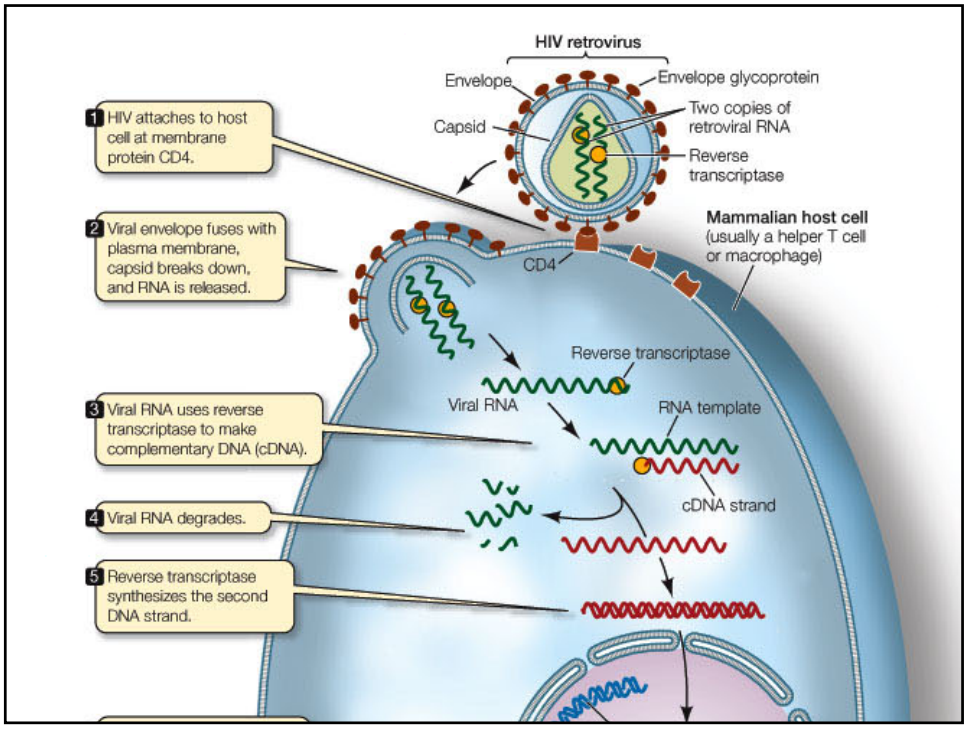


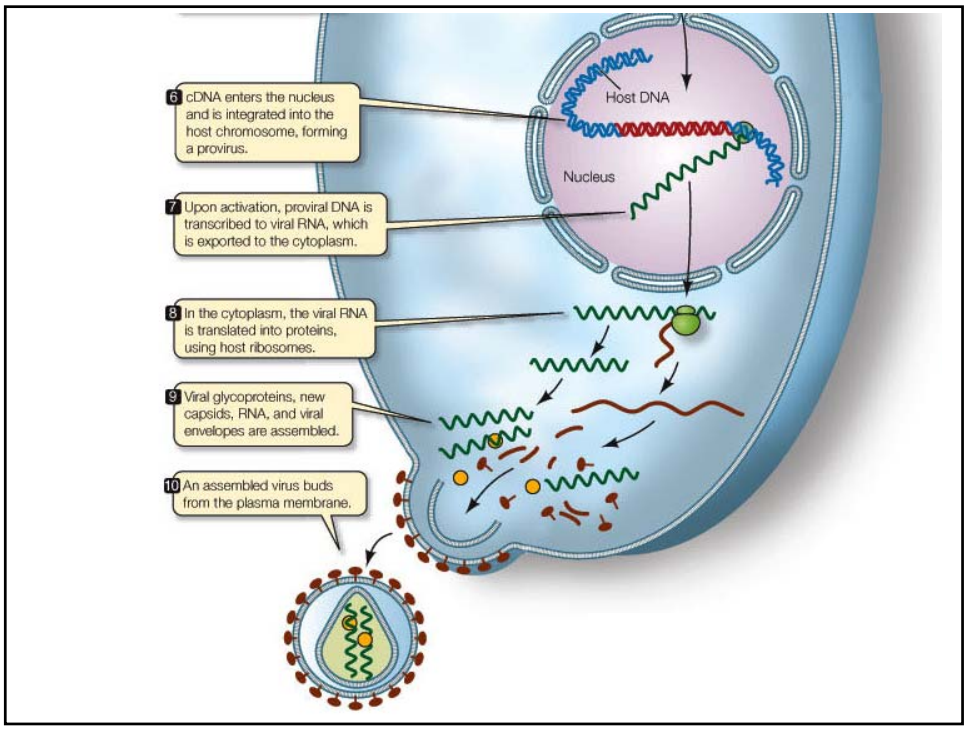
(a) The structure of HIV, the virus that causes AIDS



(b) The reproductive cycle of HIV

See Figure 18.4 Brooker


HIV retrovirus
Envelope
Envelope glycoprotein
Capsid
Two copies of retroviral RNA
Reverse transcriptase
1 HIV attaches to host cell at membrane protein CD4.
2 Viral envelope fuses with plasma membrane, capsid breaks down, and RNA is released.
Mammalian host cell (usually a helper T cell or macrophage)
CD4
Reverse transcriptase
Viral RNA
RNA template
3 Viral RNA uses reverse transcriptase to make complementary DNA (cDNA).
cDNA strand
4 Viral RNA degrades.
5 Reverse transcriptase synthesizes the second DNA strand.


6 cDNA enters the nucleus and is integrated into the host chromosome, forming a provirus.
Host DNA
Nucleus
7 Upon activation, proviral DNA is transcribed to viral RNA, which is exported to the cytoplasm.
8 In the cytoplasm, the viral RNA is translated into proteins, using host ribosomes.
9 Viral glycoproteins, new capsids, RNA, and viral envelopes are assembled.
10 An assembled virus buds from the plasma membrane.

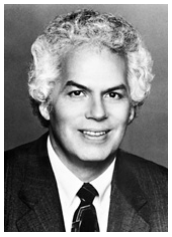
Viral diseases of man

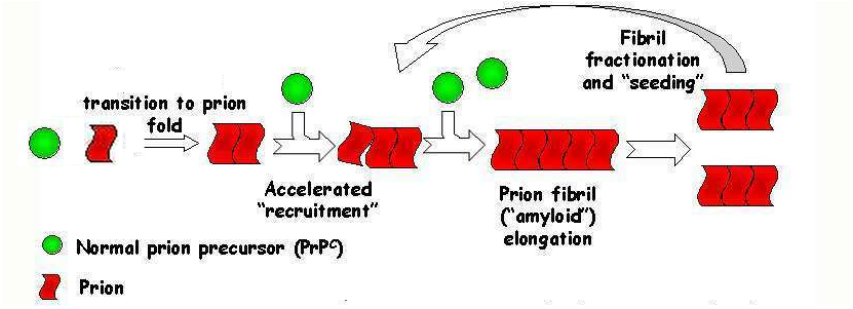
- AIDS, herpes, influenza, colds, polio, mumps, measles, smallpox, SARS, ebola, hantavirus, others
- Severity depends on the cells affected
  - cold virus – nasal epithelia
  - polio virus- motor neurons
  - HIV virus - helper T-cells

- Viral genes can make bacteria toxic (e.g. diptheria, scarlet fever, botulism)
- Some viruses cause cancer
- Can't use antibiotics against virus (why)?
- Vaccination- exposure to inactivated virus to sensitize immune system.

Prions: infective proteins (not viri)

- transmissible spongiform encephalopathies
- Zombies?
- Stanley Prusiner





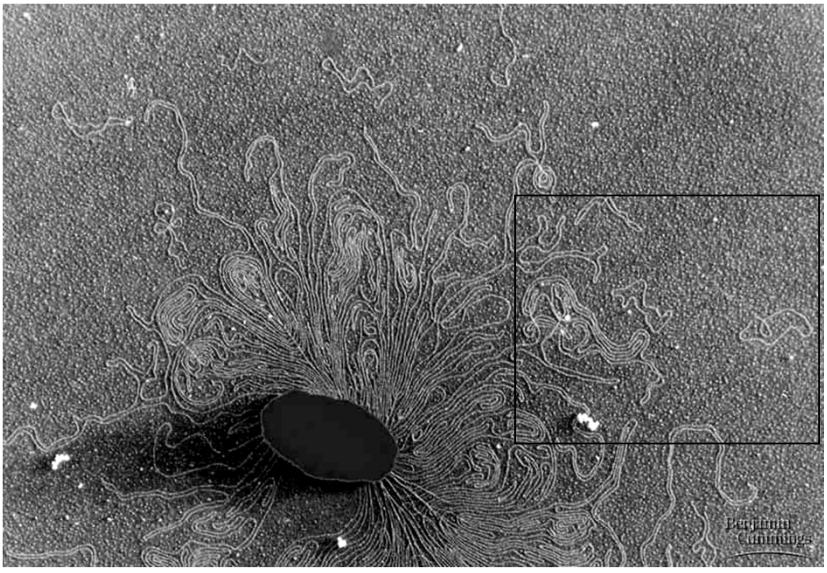
How did viruses evolve?

- Many viruses can become part of host chromosome- “prophage” or “provirus”
- may have originated from mobile genetic elements – basically, genes that can move between cells or between chromosomes
- These elements may have evolved because they facilitate genetic recombination

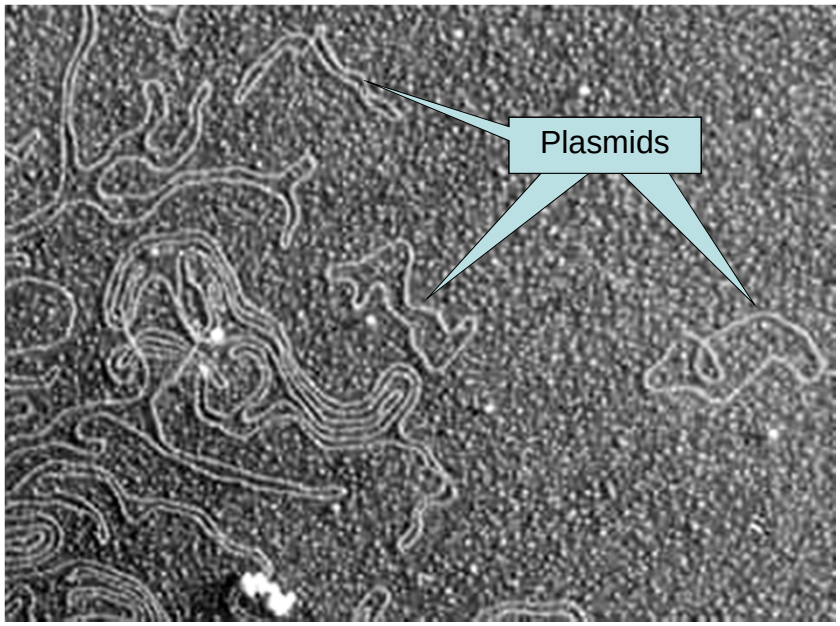
Genetic recombination

- Creates new combinations of alleles
- Eukaryotes use meiotic sex
- Prokaryotes have other ways to exchange and recombine genes:
- plasmids, transformation, transduction, conjugation, transposons

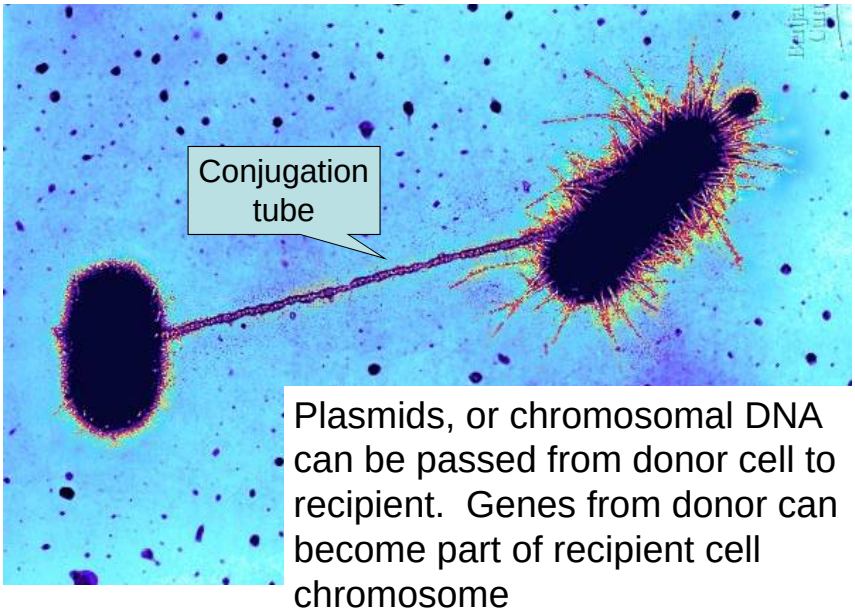
Bacterium releasing DNA with plasmids



Bacterium releasing DNA with plasmids

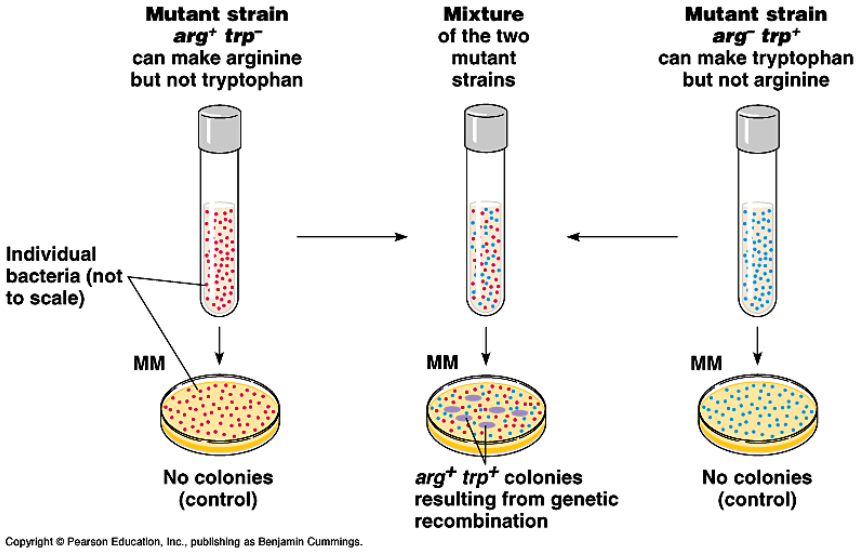


Bacterial conjugation



Plasmids, or chromosomal DNA can be passed from donor cell to recipient. Genes from donor can become part of recipient cell chromosome

Detecting genetic recombination in bacteria
(compare with Brooker 18.15)



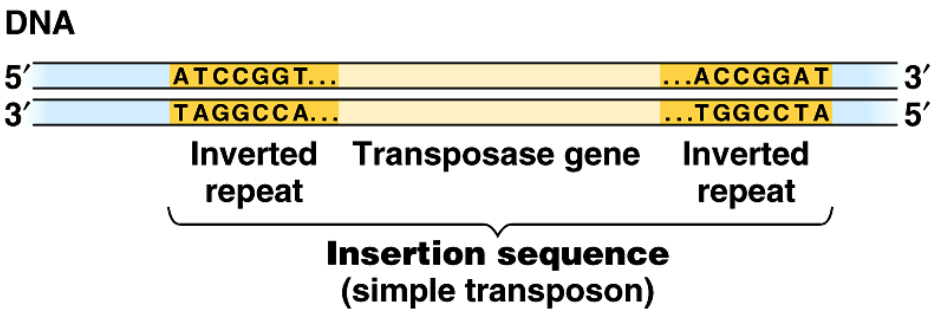
R-plasmids

- Antibiotic resistance plasmids carry from 1-10 different antibiotic resistance genes
- Evolution caused by use of antibiotics in medicine, livestock
- How could several resistance genes end up together in one plasmid?

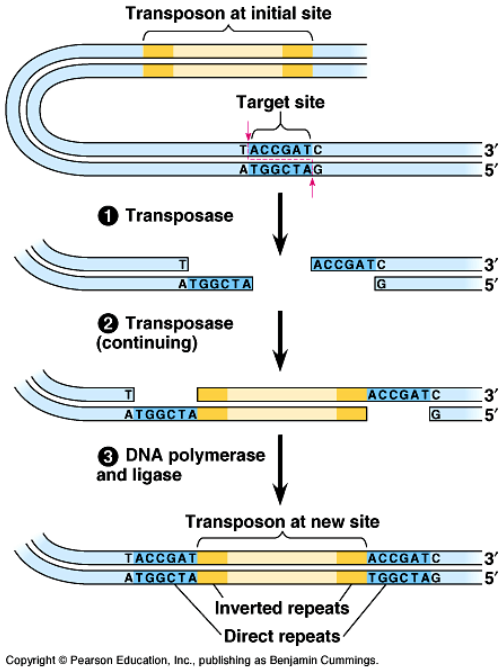
Transposons (Chap 21)

- Genetic elements that can move
- Occur in both prokaryotes and eukaryotes
- Simplest form is insertion sequence that inserts randomly, causes mutation

Insertion sequences ("transposable elements") the simplest transposons

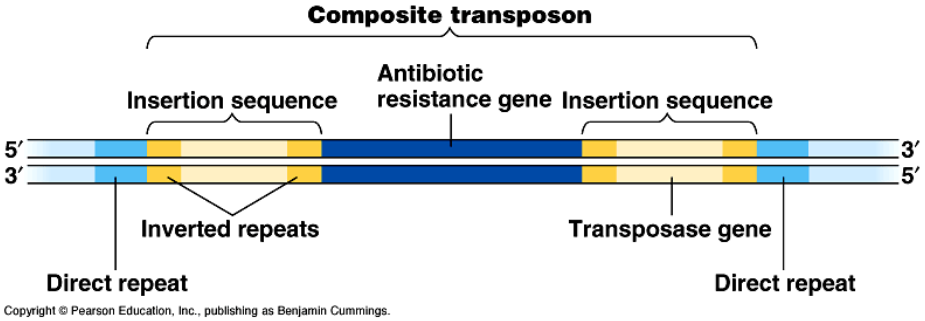


Insertion of a transposon and creation of direct repeats



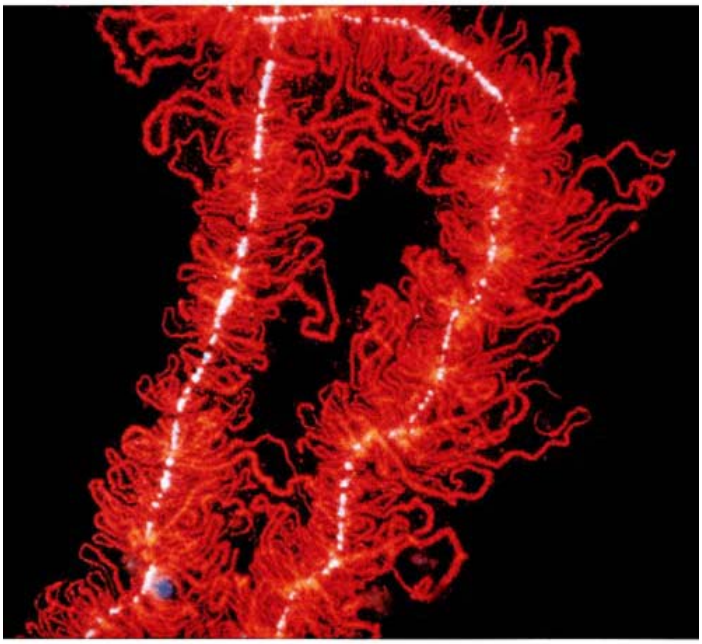
- Transposons (jumping genes") were discovered by Barbara McClintock via mutations in corn embryos that cause color patterns
- Transposition causes mutation by interrupting genes
- Any given transposon doesn't jump often – it may become a permanent part of the genome.
- Eukaryote genomes are littered with them (half or more of DNA)

A composite transposon with an antibiotic resistance gene



Complex transposon = two insertion sequences bracket & move other genes, can alter position (linkage) of genes

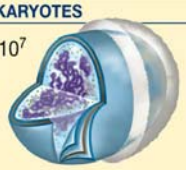
Chromatin in a developing salamander ovum

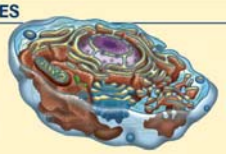


Eukaryote genomes (Chap 21)

A Comparison of Prokaryotic and Eukaryotic Genes and Genomes

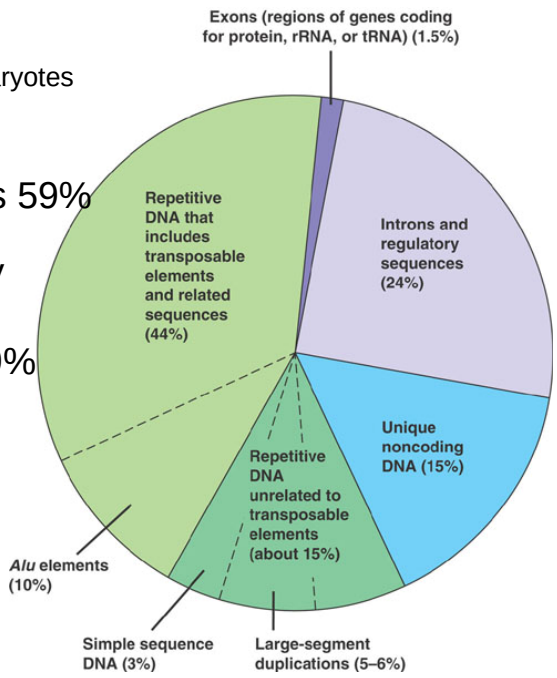
CHARACTERISTIC	PROKARYOTES	EUKARYOTES
Genome size (base pairs)	10^4–10^7	10^8–10^{11}
Repeated sequences	Few	Many
Noncoding DNA within coding sequences	Rare	Common
Transcription and translation separated in cell	No	Yes
DNA segregated within a nucleus	No	Yes
DNA bound to proteins	Some	Extensive
Promoters	Yes	Yes
Enhancers/silencers	Rare	Common
Capping and tailing of mRNA	No	Yes
RNA splicing required (spliceosomes)	Rare	Common
Number of chromosomes in genome	One	Many





LOTS of DNA in eukaryotes mostly non-coding

- Repetitive elements 59%
- Introns & regulatory elements, other noncoding DNA 39%
- Structural genes only ~1.5% of DNA



Repetitive DNA: 59% of genome

- Satellite DNA micro (1-3 bp) & mini (10-40 bp) tandem repeats (includes telomeres, centromeres)
- Transposon related (SINEs & LINEs) including Alu elements
- Moderately repetitive DNA (large sequences, including genes for ribosomes, tRNAs)
- Pseudogenes

Alu elements (10-11% of genome)

- a very abundant class of short interspersed repetitive DNA, similar to the gene for RNA of the signal recognition particle that binds ribosomes to ER
- 300 bp over & over...11% of human genome
- *Naming: cut by* restriction enzyme Alu-1 *Arthrobacter luteus*.
- *Significance as genetic markers in forensics, phylogenetics*

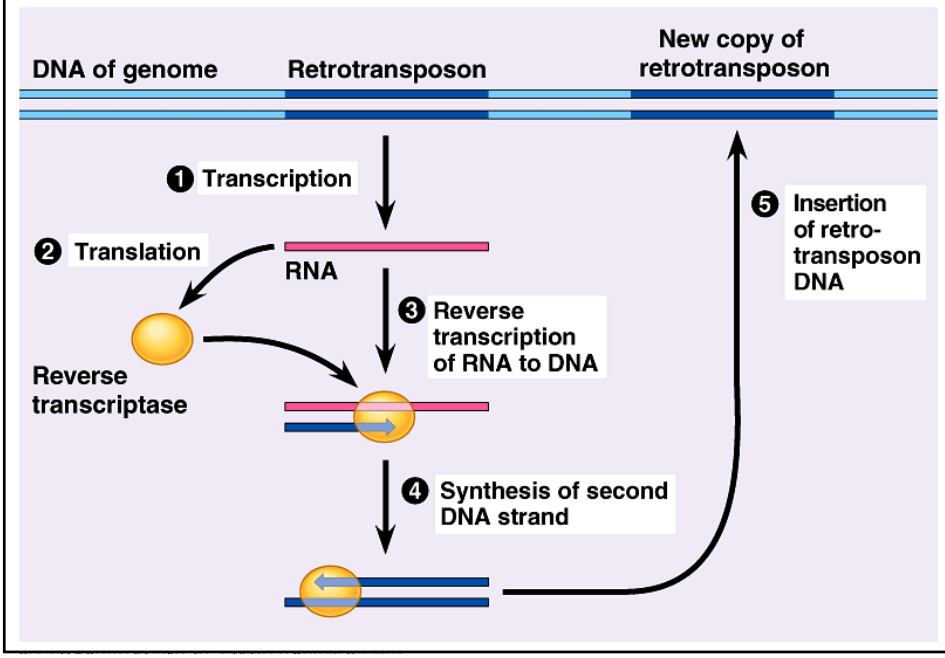
Retrotransposons

How did human genome end up with 1.5 million Alu elements?

Genetic elements are replicated and moved by retrotransposition.

Retrotransposons ("copy and paste" transposons) are similar to retroviruses

Retrotransposon movement



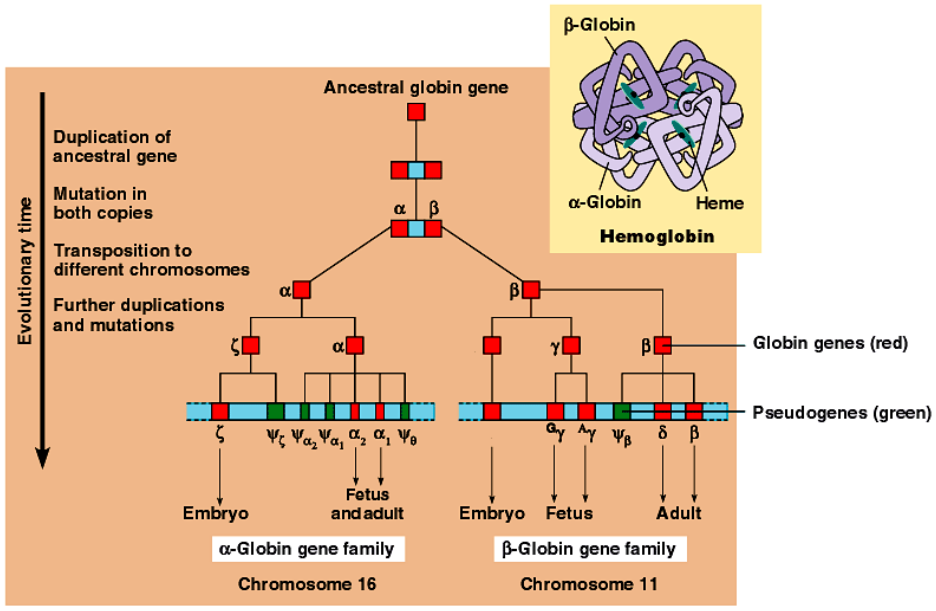
Repetitive DNA (59%)

- Simple sequence (satellite) DNA (3%)
  - Multiple, tandem copies of short sequences
  - Why “satellite”? AT vs GC density
  - Telomeres & centromeres
  - Significance in forensics, phylogenetics

Gene duplication & gene families

- Many protein coding genes have also undergone replication in genome
- Pseudogenes- recognizably homologous with functional genes but not transcribed.
- multigene families, e.g. globin gene families.
- The genome is an untidy scene, littered with clues to evolutionary history

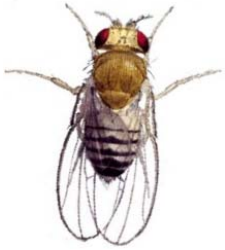
The evolution of human α-globin and β-globin gene families



What about the coding genes? (1.5%)

Functions of protein-coding genes in *Drosophila* *(sums to 80%)*

13,449 genes
18,941 mRNAs



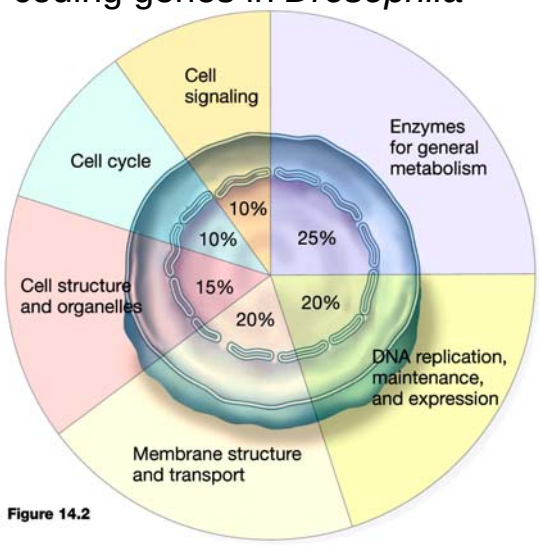


Figure 14.2

DNA and Biotechnology (Ch. 20)

- Biotechnology: methods for investigating and manipulating DNA in research, medicine, agriculture, criminal law, industry
- Genomics: study of genomes, including mapping, sequencing and gene function.
  - Structural genomics
  - Functional genomics
  - *Comparative genomics*
  - Bioinformatics

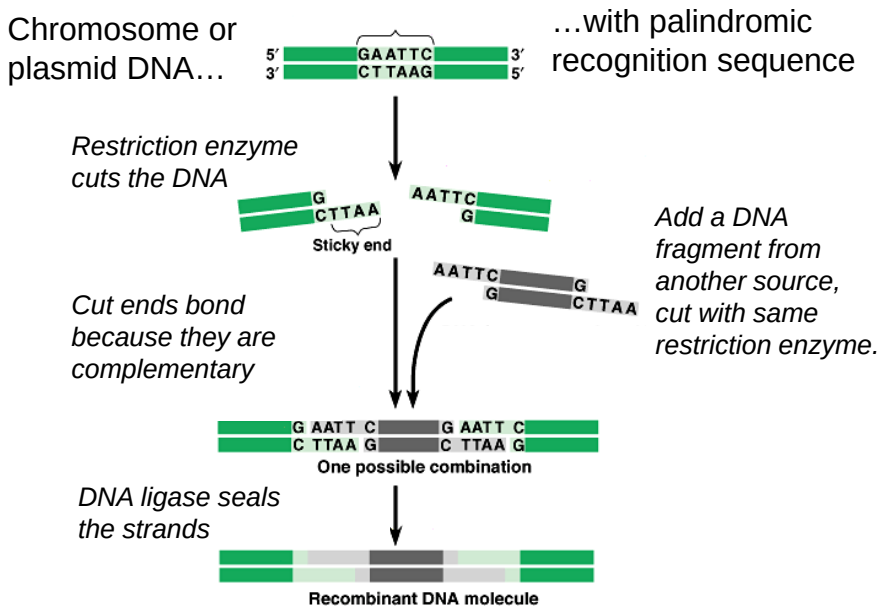
Recombinant DNA overview

- genes from two different sources are artificially combined, often in a bacterial plasmid or yeast chromosome
- recombinant DNA put into bacteria, yeast, or other easily cultured cells
- cells multiply and therefore produce more copies of the gene ("cloning" the gene)
- cells manufacture the gene product (protein)

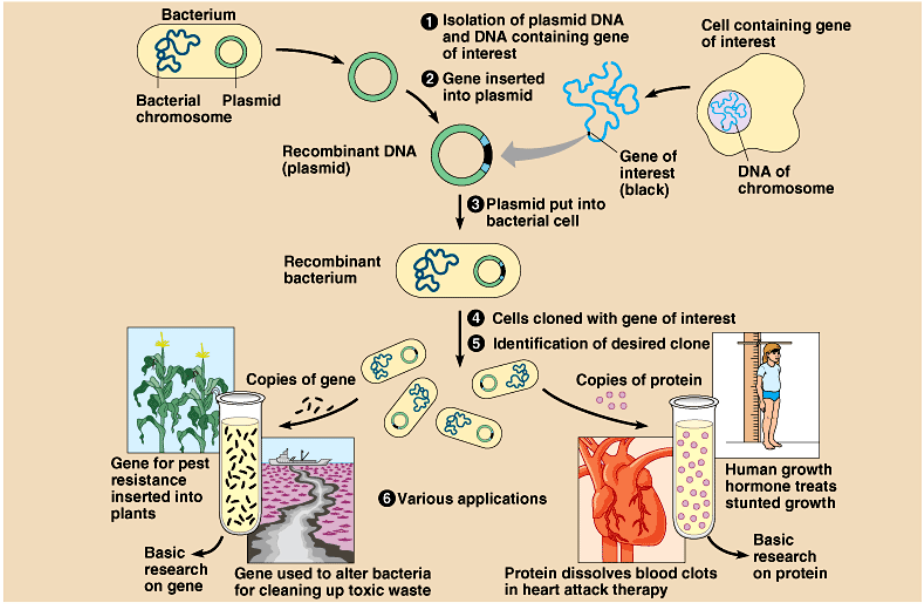
Using restriction enzymes to make recombinant DNA

- Restriction enzymes cut DNA at particular palindromic recognition sequences.
- “sticky ends” of fragments can combine due to complementarity
- mix DNA fragments from two sources cut with same restriction enzyme
- complete annealing of recombinant DNA with ligase

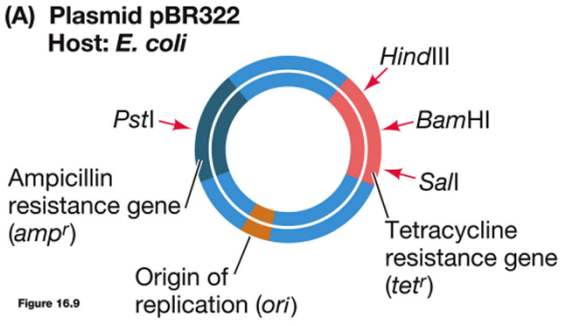
Using a restriction enzyme to make recombinant DNA



Using recombinant plasmids in biotechnology

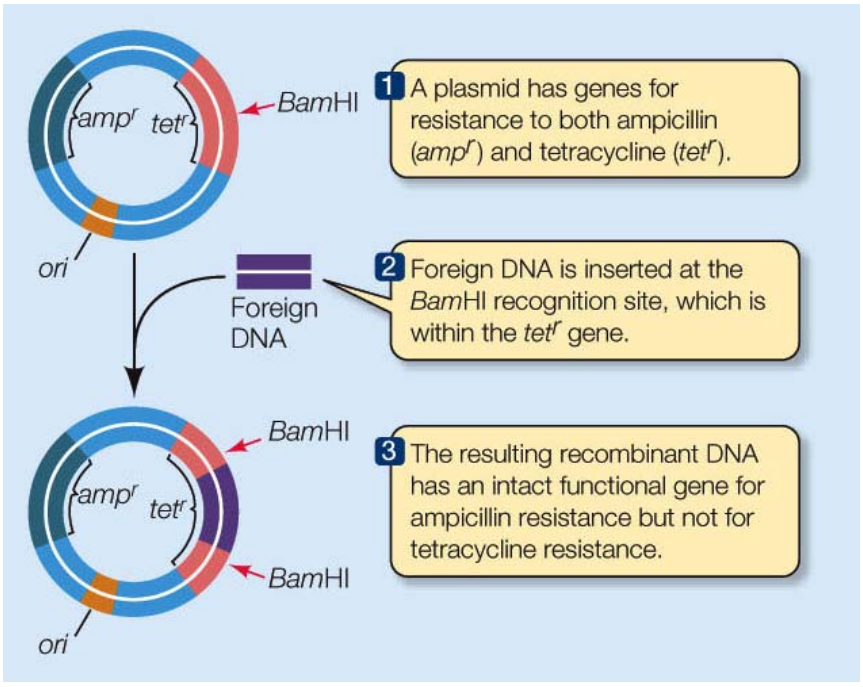


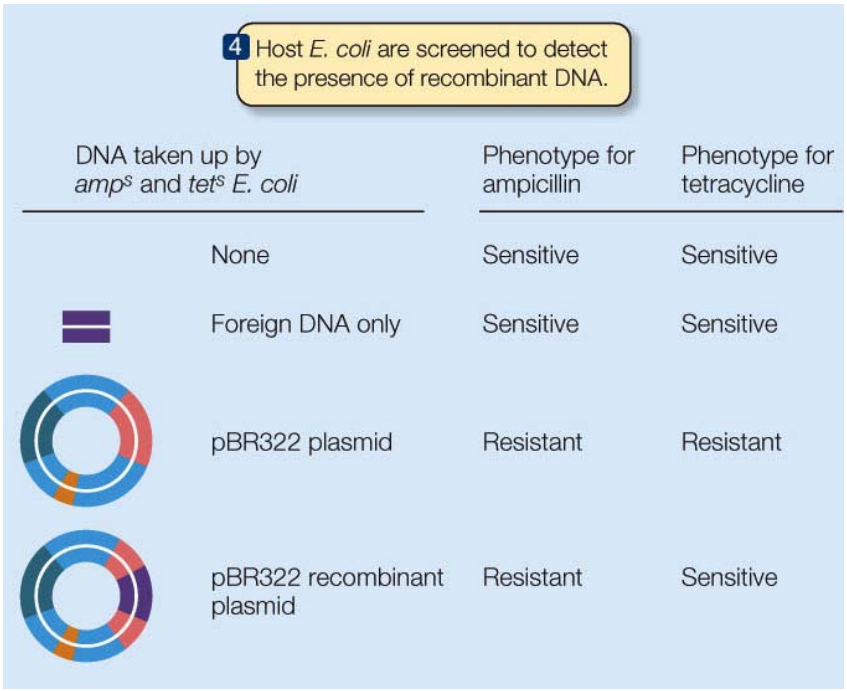
Identifying cells with recombinant plasmids



This plasmid has a couple of “reporter genes” that confer antibiotic resistance.

Restriction sites (red arrows) lie within the reporter genes

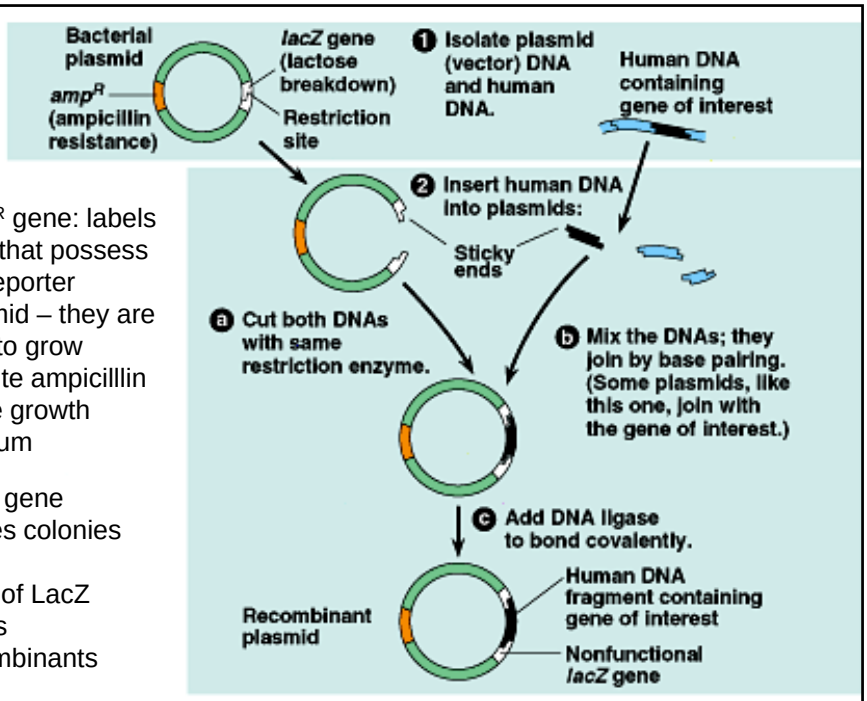


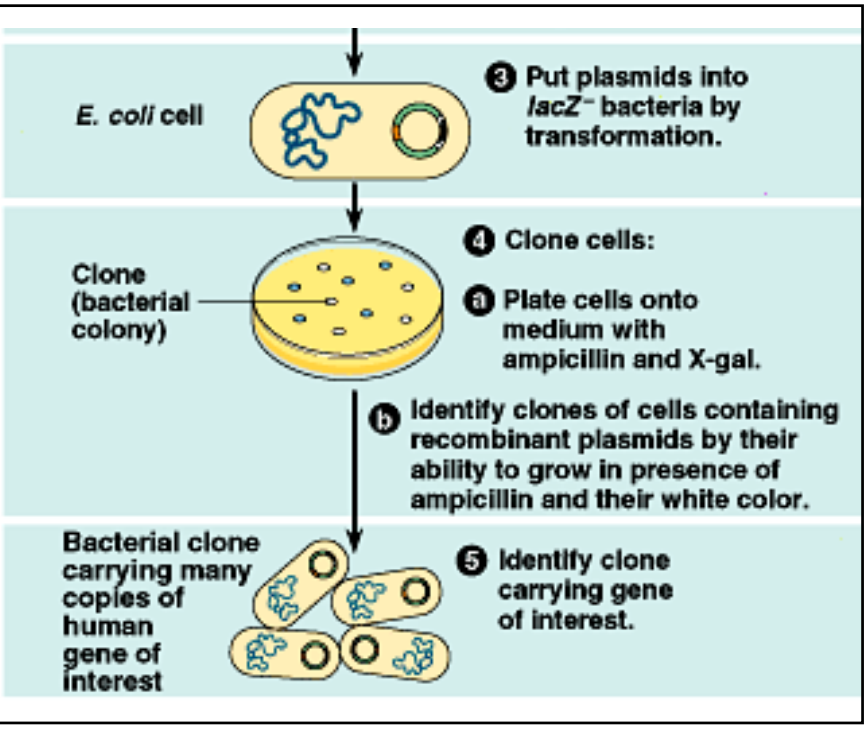


DNA taken up by *amp*^{s} and *tet*^{s} *E. coli*	Phenotype for ampicillin	Phenotype for tetracycline
None	Sensitive	Sensitive
Foreign DNA only	Sensitive	Sensitive
pBR322 plasmid	Resistant	Resistant
pBR322 recombinant plasmid	Resistant	Sensitive

Amp^R gene: labels cells that possess the reporter plasmid – they are able to grow despite ampicilllin in the growth medium

LacZ gene makes colonies blue.
Loss of LacZ labels recombinants





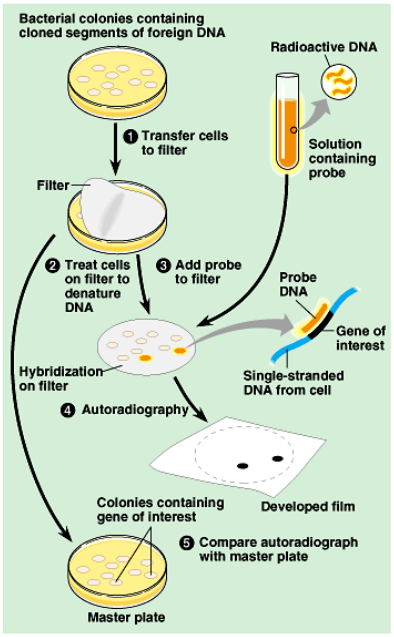
Ways to get the recombinant DNA into cells

- Bacterial plasmids
  - Transformation
  - transduction with virus
- Plant cells
  - Ti plasmid from *Agrobacter tumifaciens*
  - ballistic method
- Yeast
  - Yeast artificial chromosomes (YAC's)

Selection of recombinant cells with particular genes of interest

- recombinant methods are haphazard-
- They produce recombinant libraries – multiple clones carrying different parts of the DNA.
- Must identify clones with genes of interest
  - test enzyme activity
  - label with monoclonal antibodies
  - use labeled complementary DNA probe

Using a nucleic acid probe to identify a cloned gene



Many colonies, each containing a recombinant plasmid

Which colonies have gene of interest?

DNA probe is SS DNA complementary to part of the gene of interest

Fluorescent tag or radiolabel probe to ID the colonies that carry the gene

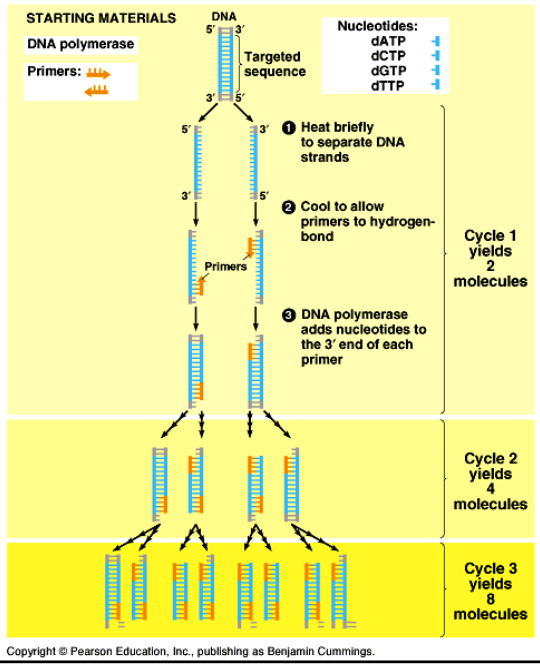
cDNA

- prokaryotes lack mRNA processing- can't remove introns from eukaryote transcripts
- cDNA (complementary DNA) is prepared using mRNA from source cells and reverse transcriptase
- cDNA joined to bacterial promoter genes before insertion into bacteria

PCR and DNA amplification

- multiple copies of DNA molecules are needed for sequencing, uses in forensic, diagnostic applications
- PCR (polymerase chain reaction) makes many copies of selected parts of the DNA *in vitro*
- Kary Mullis Nobel 1993

The polymerase chain reaction (PCR)



See 20.5 in text

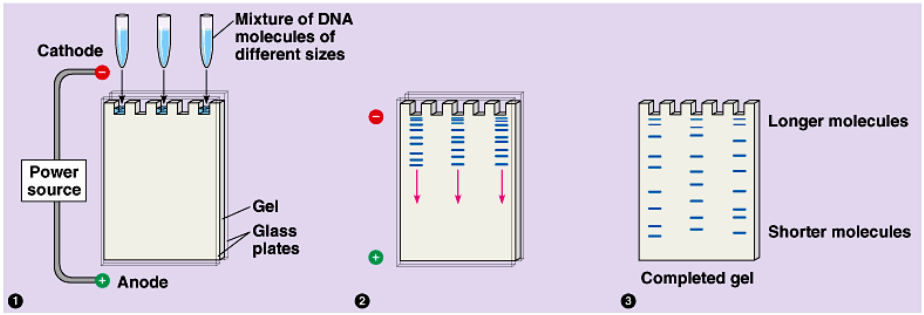
PCR animation link

Methods for analyzing DNA

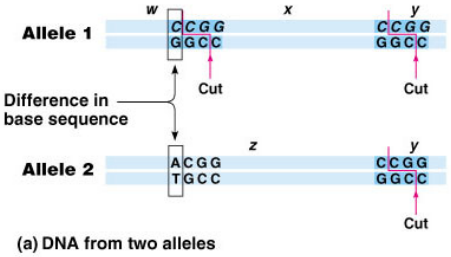
- Restriction fragment analysis
  - Gel electrophoresis
  - Visualization with ethidium bromide
  - Southern Blot using radiolabeled DNA probes
- Mapping
  - Linkage mapping
  - Physical mapping
- Sequencing

Gel electrophoresis

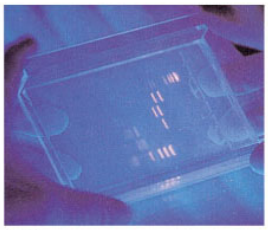
- Separates DNA molecules by size
- Movement through gel caused by electric field (DNA has net negative charge)



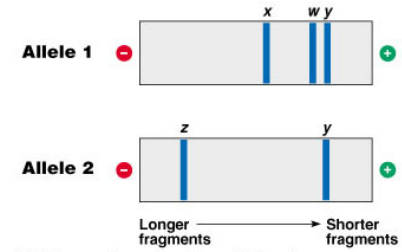
RFLP analysis



(a) DNA from two alleles



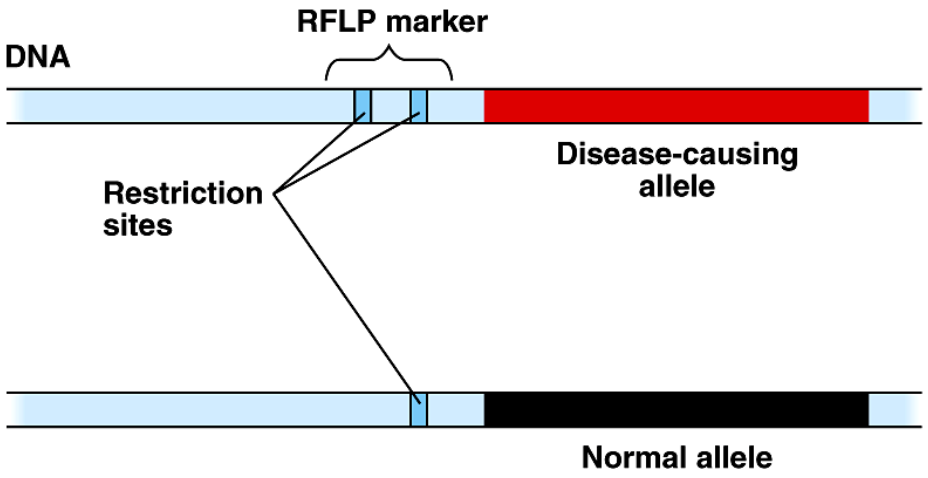
(c) Completed gel



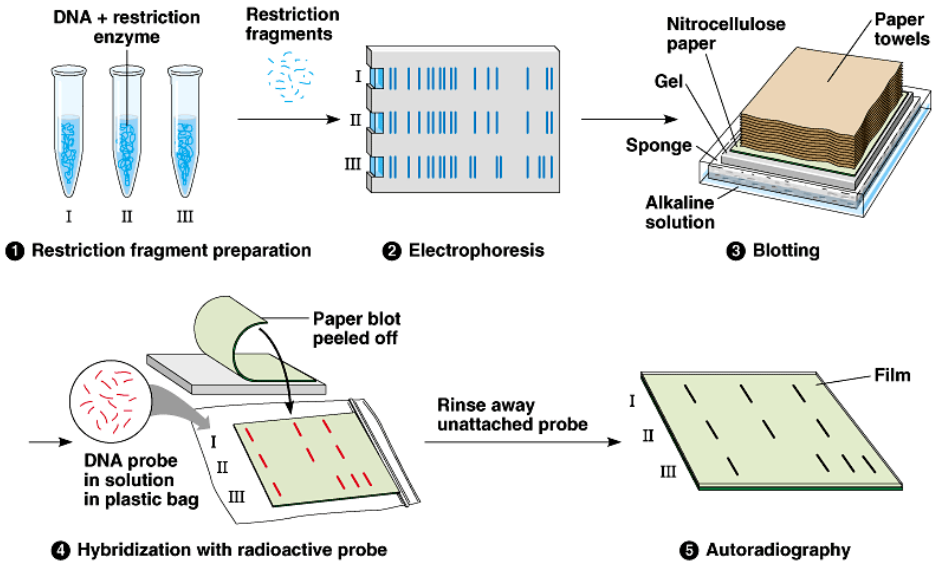
(b) Electrophoresis of restriction fragments

RFLP = Restriction fragment length polymorphism

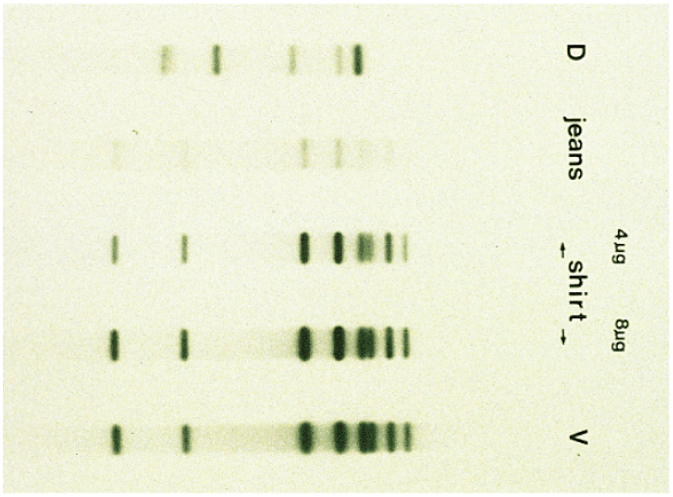
RFLP markers close to a gene



RFLP using Southern blot



DNA fingerprints from a murder case

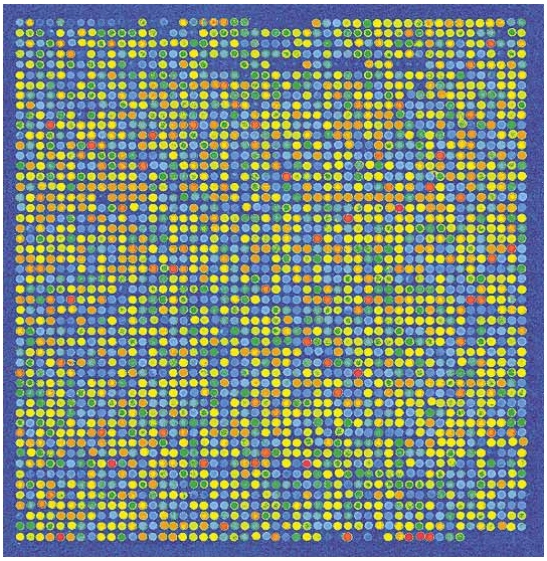


Defendant's blood

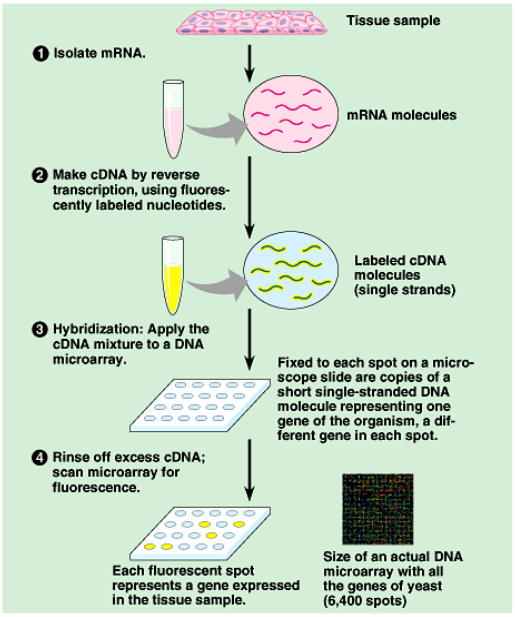
Blood from defendant's clothes

Victim's blood

"Gene chip" DNA microarray assay for gene expression



DNA microarray assay for gene expression

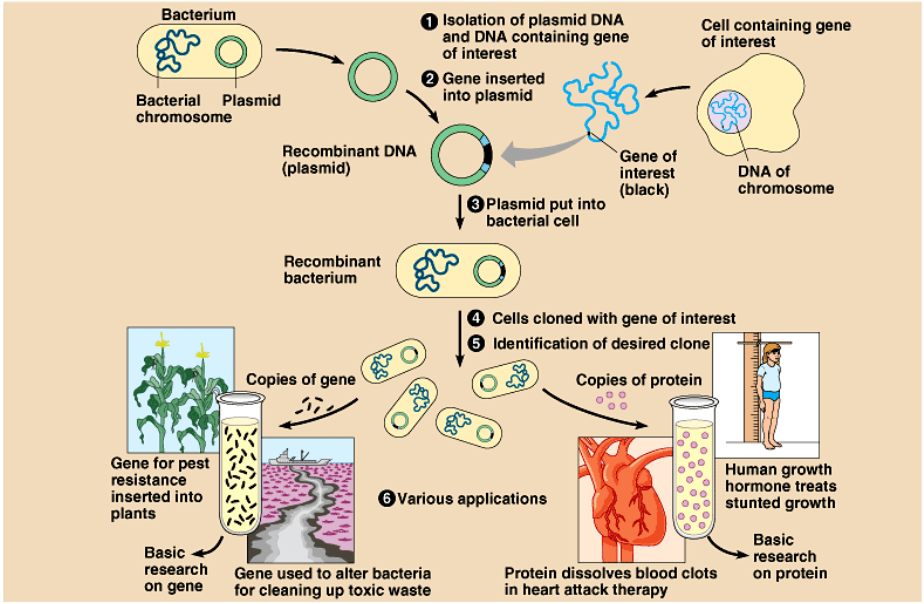


(a) Procedure using labeled cDNA prepared from a tissue sample

DNA technologies

- Diagnosis of diseases
- Forensic uses of DNA
- Genetic engineering
  - Transgenic microbes, plants & animals
  - Production of useful proteins
  - Gene therapy
- Phylogenetics

Using recombinant plasmids in biotechnology



"Golden" rice contrasted with ordinary rice

Concerns about GM organisms

Examples

- Bt corn – monarch butterflies
- Roundup-Ready soybeans – superweeds
- Golden rice
  http://en.wikipedia.org/wiki/Golden_rice