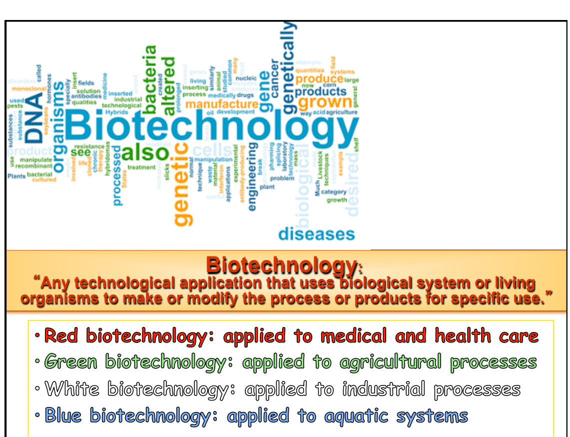


Biotechnology:
"Any technological application that uses biological system or living organisms to make or modify the process or products for specific use."

- Red biotechnology: applied to medical and health care
- Green biotechnology: applied to agricultural processes
- White biotechnology: applied to industrial processes
- Blue biotechnology: applied to aquatic systems

DNA TECHNOLOGY
TERMINOLOGY

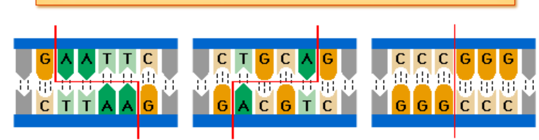
Similar terms — often used interchangeably

- Biotechnology: the manipulation of organisms to produce a product.
  - Fermentation, artificial breeding, pharmaceutical and nutritional supplements, and now…
- Genetic engineering: the direct manipulation of an organism's DNA.
- Recombinant DNA: insertion of DNA from one source into another.
- Transgenics: producing an organism with foreign DNA inserted into its genome.

DNA TECHNOLOGY
TOOLS & TECHNIQUES

- Restriction Digests
- RFLP — "genetic fingerprinting"
- Hybridizations & Molecular Probes
- Polymerase Chain Reaction (PCR)
- Recombinant DNA
- Gene Cloning
- Transgenics
- Gene Expression Analyses
- Genome Mapping & Sequencing

RESTRICTION ENZYMES

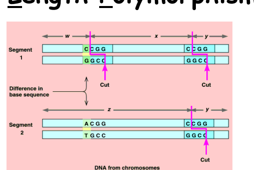


- Bacteria produce special enzymes to chop up viral DNA.
- Biotechnologist use these "**restriction enzymes**" to cut DNA in specific places (**restriction sites**).
- Many restriction enzymes cut the DNA polymer in a staggered pattern that produce "sticky" single-stranded ends to the DNA fragments.

RESTRICTION FRAGMENT ANALYSIS

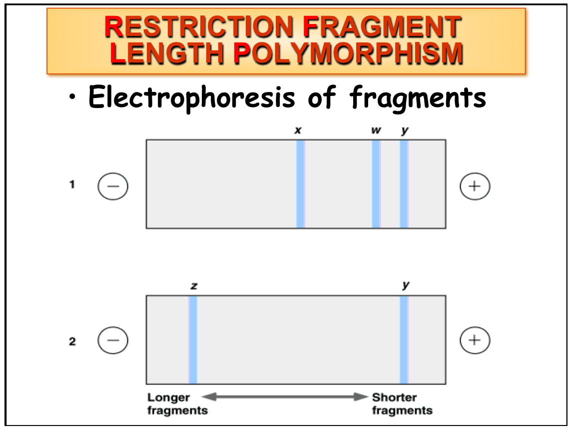
DNA Technology as a diagnostic tool

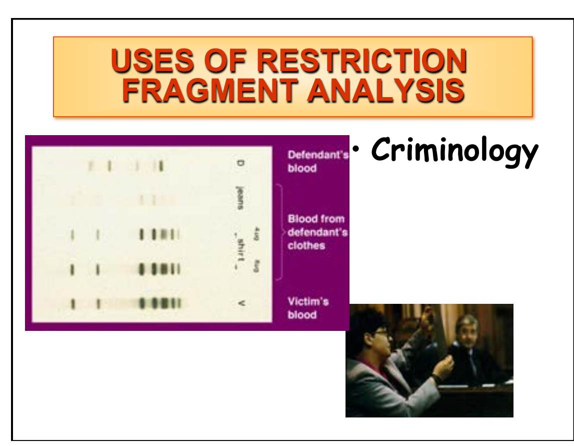
- Everyone's DNA is unique
- Closer the relationship the more similar the DNA
- Restriction Fragment Length Polymorphisms
  - RFLPs
  - "Ruff-lips"

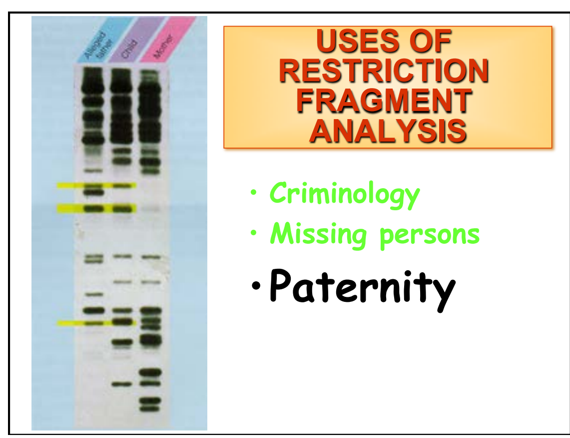


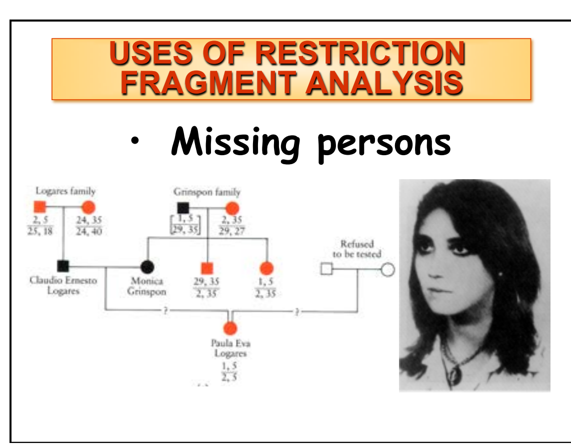
RESTRICTION DIGEST → RESTRICTION FRAGMENTS

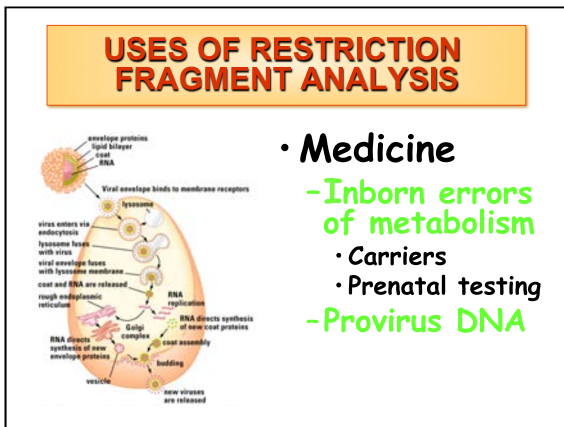
w x y
Segment 1
CCGG CCGG
GGCC GGCC
Cut Cut
Difference in base sequence
z y
Segment 2
ACGG CCGG
TGCC GGCC
Cut
DNA from chromosomes

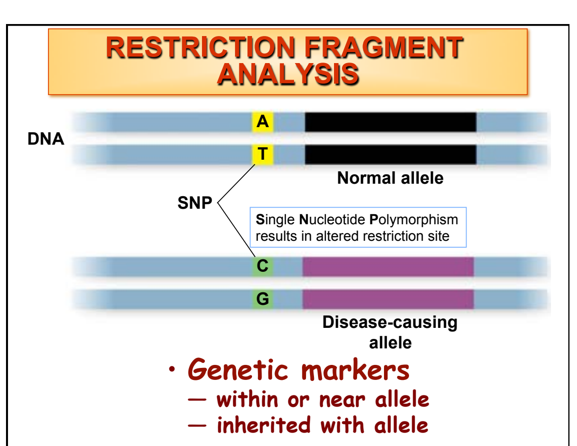

RESTRICTION FRAGMENT LENGTH POLYMORPHISM
• Electrophoresis of fragments
x
w
y
1
z
y
2
Longer fragments
Shorter fragments

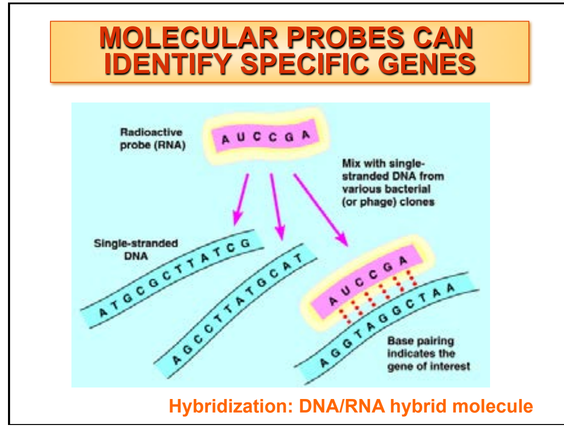

USES OF RESTRICTION FRAGMENT ANALYSIS
• Criminology
Defendant's blood
Blood from defendant's clothes
Victim's blood

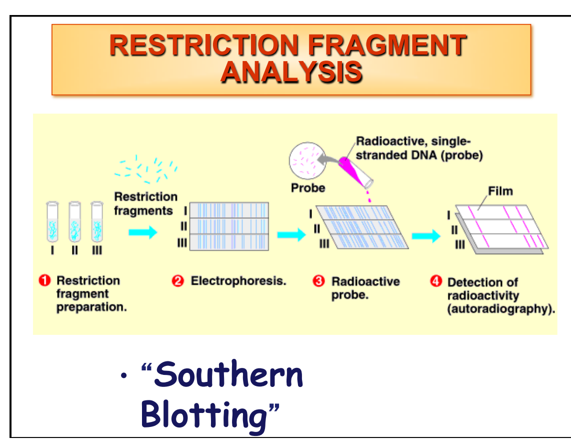

USES OF RESTRICTION FRAGMENT ANALYSIS
• Criminology
• Missing persons
• Paternity
Alleged father
Child
Mother

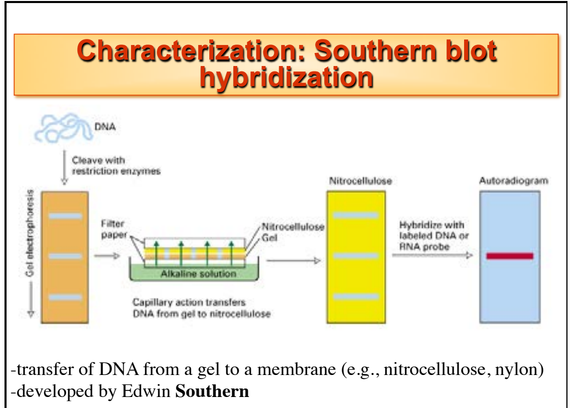

USES OF RESTRICTION FRAGMENT ANALYSIS
• Missing persons
Logares family
Grinspon family
Refused to be tested
Claudio Ernesto Logares
Monica Grinspon
Paula Eva Logares

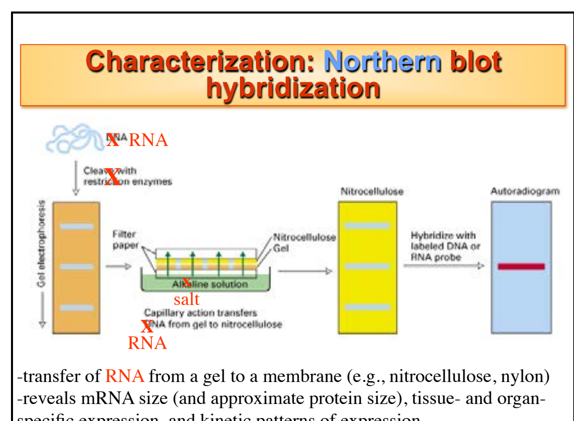

USES OF RESTRICTION FRAGMENT ANALYSIS
• Medicine
–Inborn errors of metabolism
• Carriers
• Prenatal testing
–Provirus DNA

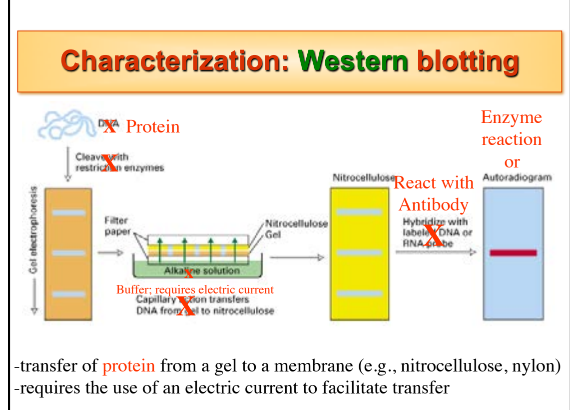

RESTRICTION FRAGMENT ANALYSIS
DNA
A
T
Normal allele
SNP
Single Nucleotide Polymorphism results in altered restriction site
C
G
Disease-causing allele
• Genetic markers
— within or near allele
— inherited with allele

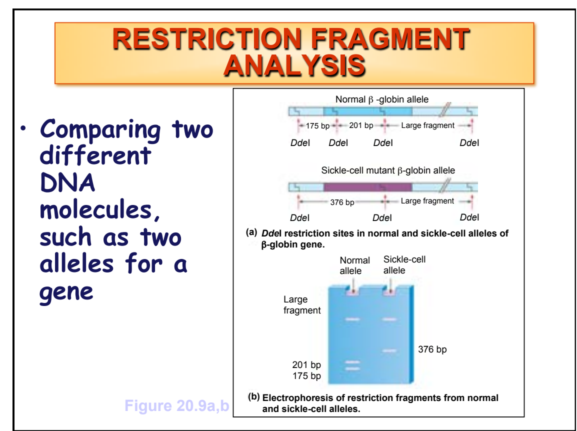

MOLECULAR PROBES CAN IDENTIFY SPECIFIC GENES
Radioactive probe (RNA)
AUCCGA
Mix with single-stranded DNA from various bacterial (or phage) clones
Single-stranded DNA
ATGCGCTTATCG
AGCCTTATGCAT
AGGTAGGCTAA
Base pairing indicates the gene of interest
Hybridization: DNA/RNA hybrid molecule

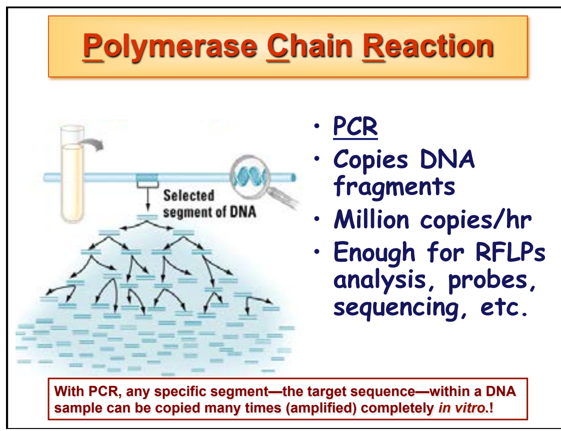

RESTRICTION FRAGMENT ANALYSIS
Radioactive, single-stranded DNA (probe)
Probe
Film
Restriction fragments
1 Restriction fragment preparation.
2 Electrophoresis.
3 Radioactive probe.
4 Detection of radioactivity (autoradiography).
• "Southern Blotting"

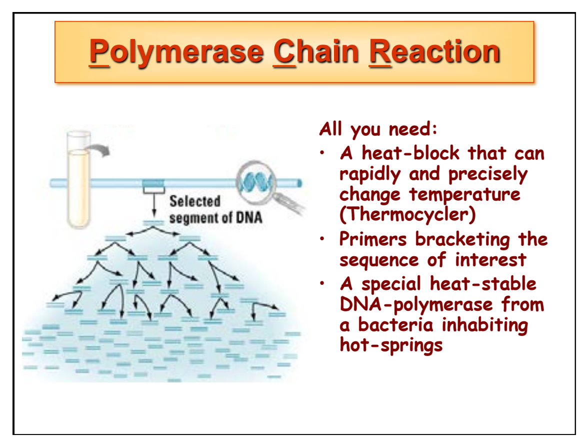

Characterization: Southern blot hybridization
DNA
Cleave with restriction enzymes
Gel electrophoresis
Filter paper
Nitrocellulose
Gel
Alkaline solution
Capillary action transfers DNA from gel to nitrocellulose
Nitrocellulose
Hybridize with labeled DNA or RNA probe
Autoradiogram
-transfer of DNA from a gel to a membrane (e.g., nitrocellulose, nylon)
-developed by Edwin Southern

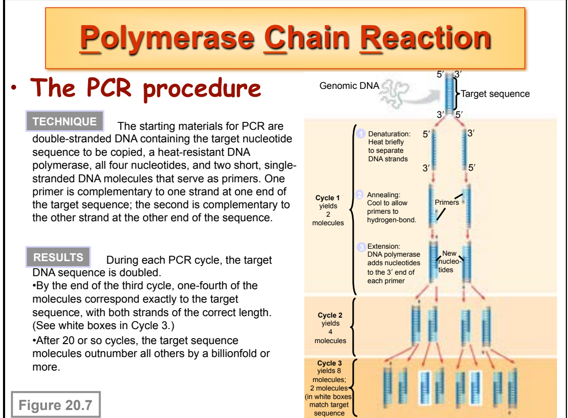

Characterization: Northern blot hybridization
RNA
Cleave with restriction enzymes
Gel electrophoresis
Filter paper
Nitrocellulose
Gel
Alkaline solution
salt
Capillary action transfers DNA from gel to nitrocellulose
RNA
Nitrocellulose
Hybridize with labeled DNA or RNA probe
Autoradiogram
-transfer of RNA from a gel to a membrane (e.g., nitrocellulose, nylon)
-reveals mRNA size (and approximate protein size), tissue- and organ-specific expression, and kinetic patterns of expression

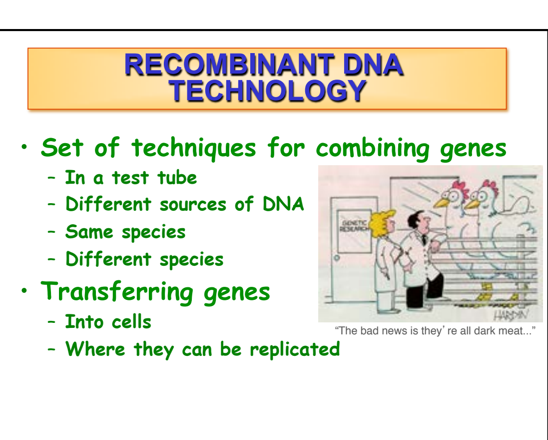

Characterization: Western blotting
Protein
Cleave with restriction enzymes
Gel electrophoresis
Filter paper
Nitrocellulose
Gel
Alkaline solution
Buffer; requires electric current
Capillary action transfers DNA from gel to nitrocellulose
Nitrocellulose
React with Antibody
Hybridize with labeled DNA or RNA probe
Enzyme reaction or
Autoradiogram
-transfer of protein from a gel to a membrane (e.g., nitrocellulose, nylon)
-requires the use of an electric current to facilitate transfer

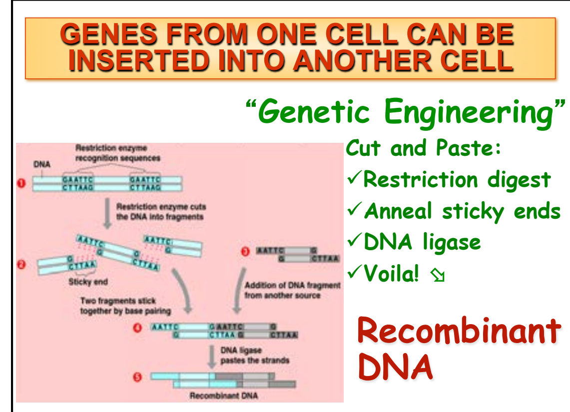

RESTRICTION FRAGMENT ANALYSIS
• Comparing two different DNA molecules, such as two alleles for a gene
Normal β-globin allele
175 bp
201 bp
Large fragment
DdeI
Sickle-cell mutant β-globin allele
376 bp
(a) DdeI restriction sites in normal and sickle-cell alleles of β-globin gene.
Normal allele
Sickle-cell allele
Large fragment
201 bp
175 bp
376 bp
(b) Electrophoresis of restriction fragments from normal and sickle-cell alleles.
Figure 20.9a,b

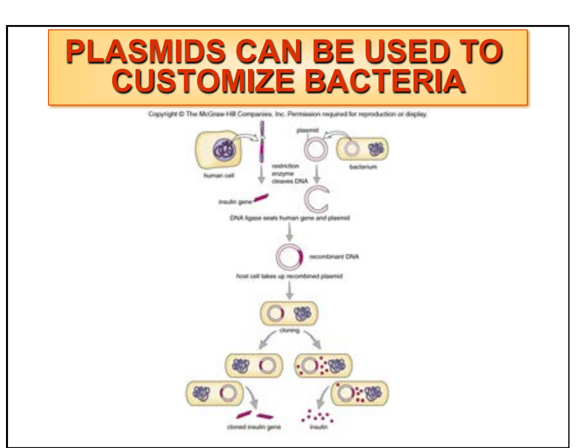

Polymerase Chain Reaction
Selected segment of DNA
PCR
Copies DNA fragments
Million copies/hr
Enough for RFLPs analysis, probes, sequencing, etc.
With PCR, any specific segment—the target sequence—within a DNA sample can be copied many times (amplified) completely in vitro.!


Polymerase Chain Reaction
Selected segment of DNA
All you need:
A heat-block that can rapidly and precisely change temperature (Thermocycler)
Primers bracketing the sequence of interest
A special heat-stable DNA-polymerase from a bacteria inhabiting hot-springs


Polymerase Chain Reaction
The PCR procedure
TECHNIQUE The starting materials for PCR are double-stranded DNA containing the target nucleotide sequence to be copied, a heat-resistant DNA polymerase, all four nucleotides, and two short, single-stranded DNA molecules that serve as primers. One primer is complementary to one strand at one end of the target sequence; the second is complementary to the other strand at the other end of the sequence.
RESULTS During each PCR cycle, the target DNA sequence is doubled.
•By the end of the third cycle, one-fourth of the molecules correspond exactly to the target sequence, with both strands of the correct length. (See white boxes in Cycle 3.)
•After 20 or so cycles, the target sequence molecules outnumber all others by a billionfold or more.
Figure 20.7
Genomic DNA
Target sequence
Denaturation: Heat briefly to separate DNA strands
Annealing: Cool to allow primers to hydrogen-bond.
Extension: DNA polymerase adds nucleotides to the 3' end of each primer
Primers
New nucleotides
Cycle 1 yields 2 molecules
Cycle 2 yields 4 molecules
Cycle 3 yields 8 molecules; 2 molecules (in white boxes) match target sequence


RECOMBINANT DNA TECHNOLOGY
Set of techniques for combining genes
In a test tube
Different sources of DNA
Same species
Different species
Transferring genes
Into cells
Where they can be replicated
"The bad news is they're all dark meat..."


GENES FROM ONE CELL CAN BE INSERTED INTO ANOTHER CELL
"Genetic Engineering"
Cut and Paste:
✓Restriction digest
✓Anneal sticky ends
✓DNA ligase
✓Voila!
Recombinant DNA
Restriction enzyme recognition sequences
DNA
Restriction enzyme cuts the DNA into fragments
Sticky end
Addition of DNA fragment from another source
Two fragments stick together by base pairing
DNA ligase pastes the strands
Recombinant DNA


PLASMIDS CAN BE USED TO CUSTOMIZE BACTERIA

Gene Cloning

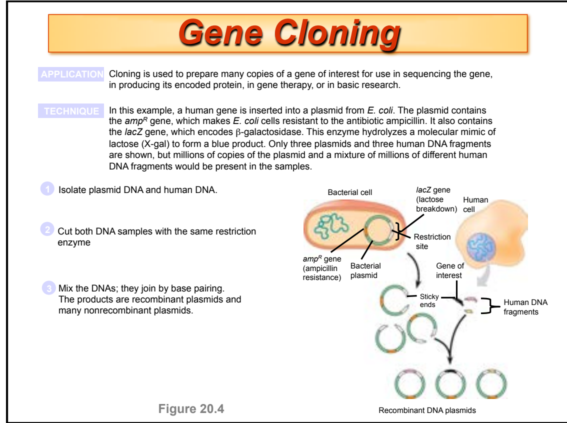
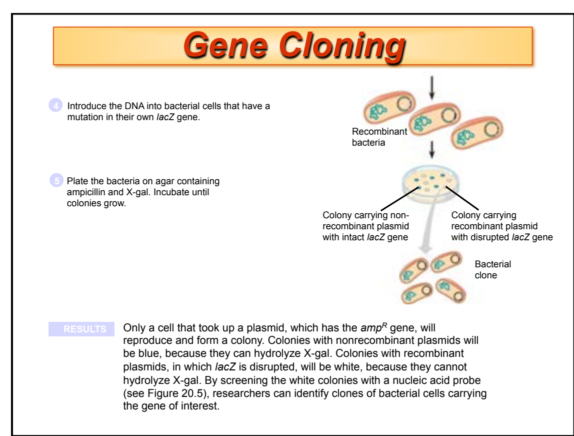


Figure 20.4

Gene Cloning



Identifying Clones Carrying a Gene of Interest

- A clone carrying the gene of interest
  - Can be identified with a radioactively labeled nucleic acid probe that has a sequence complementary to the gene, a process called nucleic acid hybridization
  - Same procedure as identifying bands on a Southern blot

Nucleic acid probe hybridization

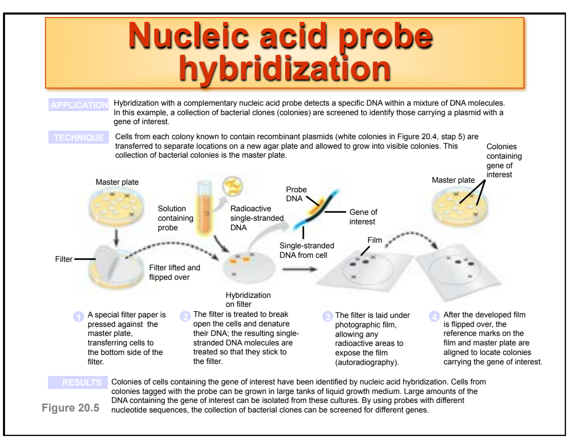
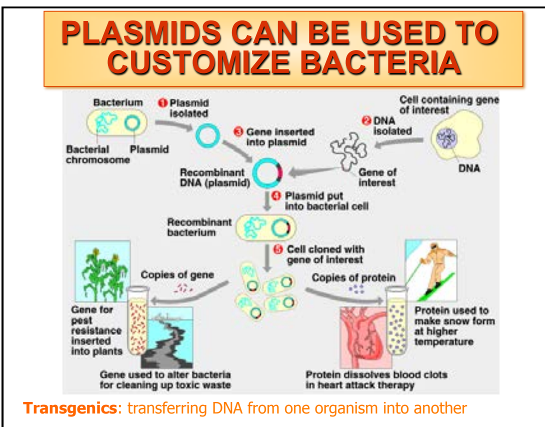


Figure 20.5

PLASMIDS CAN BE USED TO CUSTOMIZE BACTERIA



Transgenics: transferring DNA from one organism into another

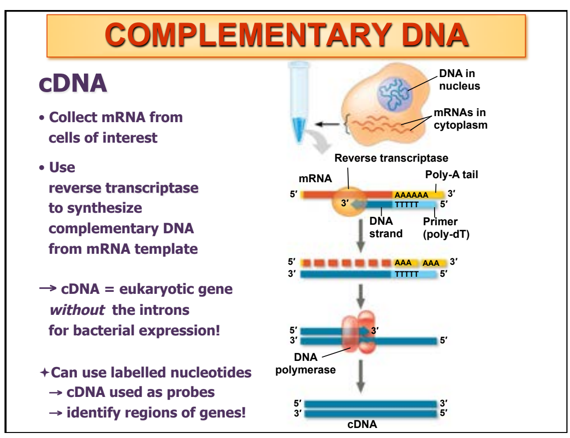
Can Bacteria Express Eukaryotic Genes?

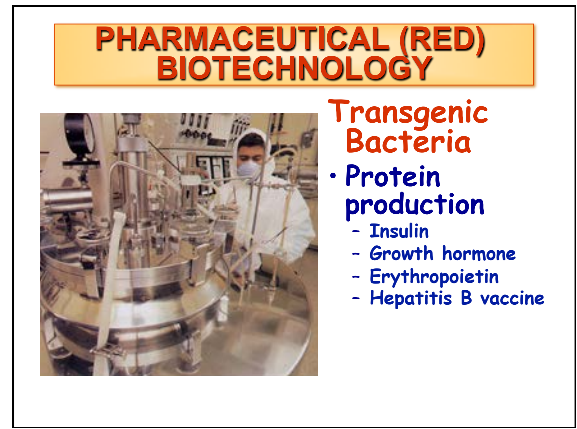
Some problems:

- **Since <2% of eukaryote DNA carries genetic information, how do you know which parts have genes?**
- **Since bacteria do not do post-transcriptional modification, how can they express eukaryotic genes?**

Answer:

cDNA (complementary DNA)

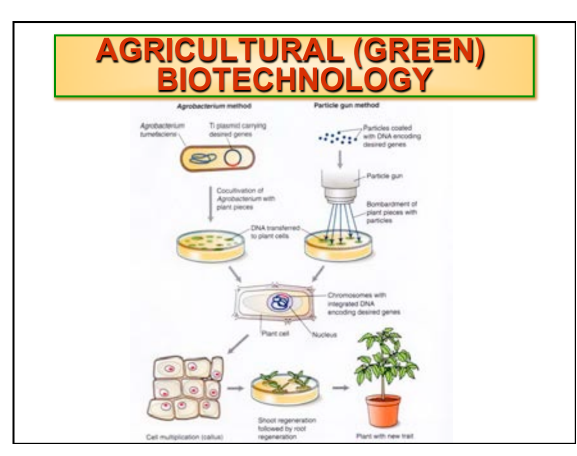


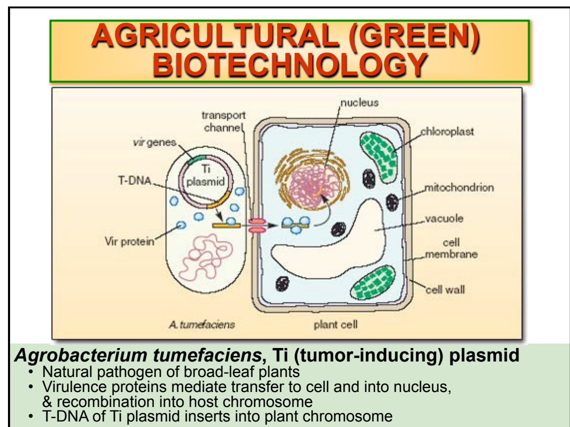


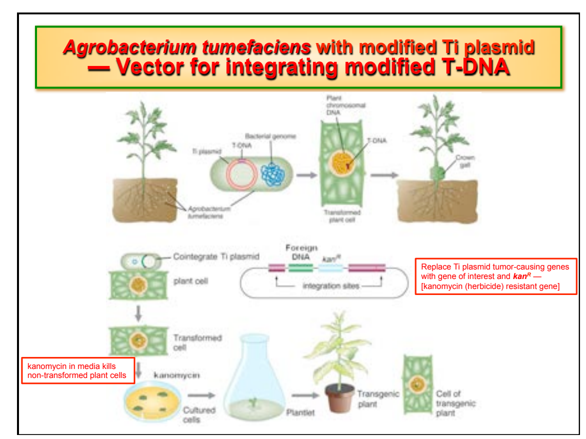
TRANSFORMING EUKARYOTES WITH RECOMBINANT DNA

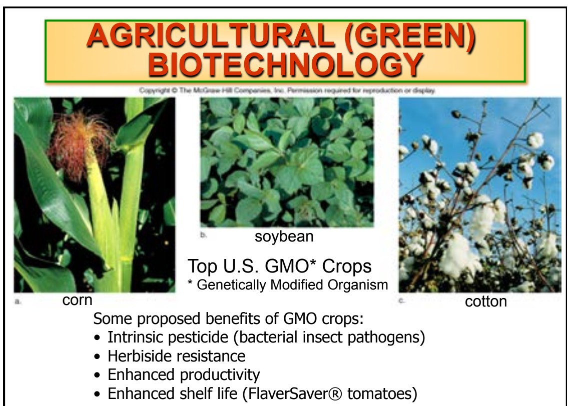
Transgenic Plants, Fungi, & Animals

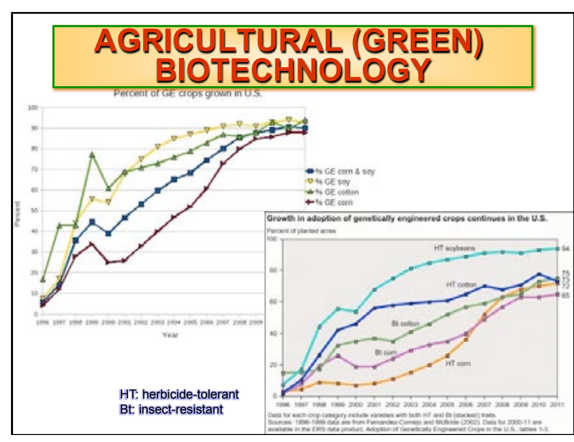
- ***Agrobacterium*** **Ti plasmid**
  - Natural pathogen of broad-leaf plants
  - Ti plasmid inserts into plant chromosome
- **Microparticle accelerator "gene gun"**
  - DNA fragments coated onto gold or tungsten particles
  - Particle blasted by gas pressure burst through tissue, leaving trail of DNA residue in cells
- **Microfiber "gene whiskers"**
  - DNA fragments coated onto microscopic needles
  - Needles and cells suspended and shaken; impaled cells take up DNA from needles
- **Electroporation**
  - Rapid electrical pulses induce cellular pores to open allowing small fragments of DNA to enter

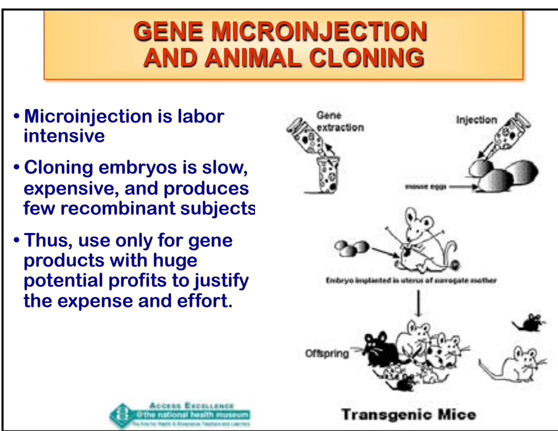








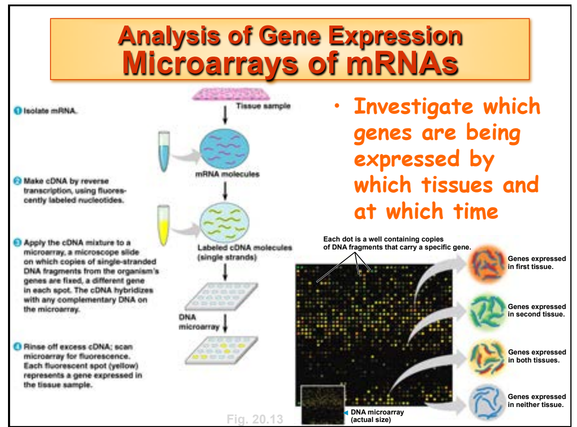


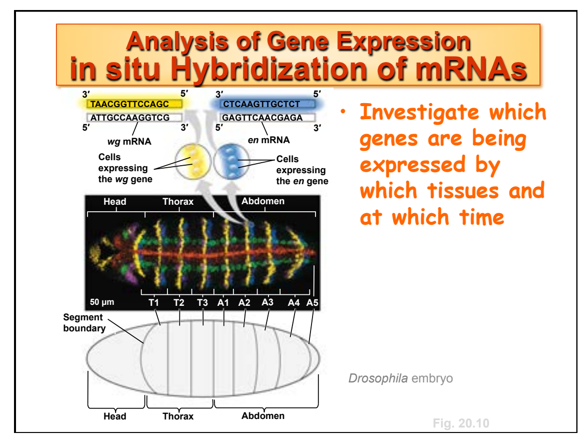


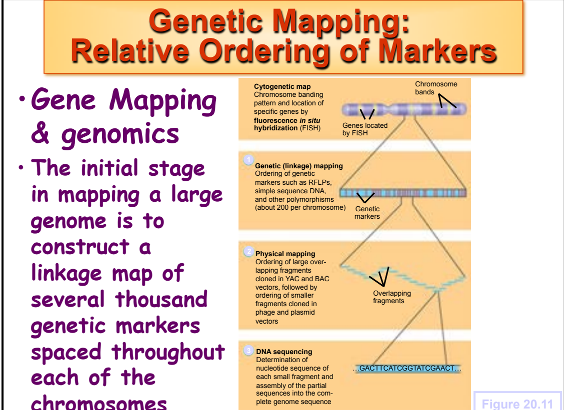
The latest hot tool!
CRISPR & CAS

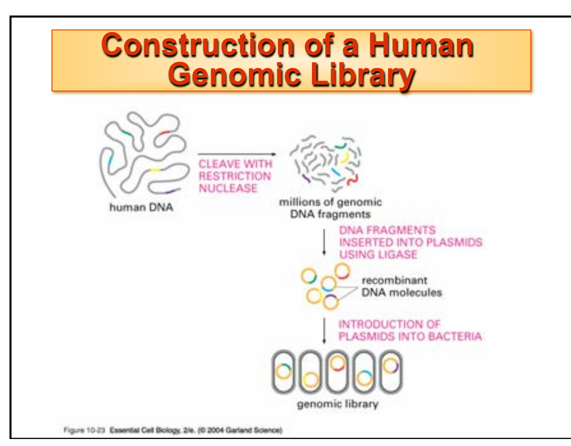
- *CRISPR*: "clustered regularly interspaced short palindromic repeats"
  - Repeating DNA sequences flanking unique proviral DNA.
  - Proviral DNA transcribed as "guideRNA" (gRNA or crRNA)
- CAS: CRISPR-associated proteins
  - Bind to gRNA
  - Use "target sequence" of gRNA to specifically bind/cut new invading viral DNA
- *NOW*, if we replace the gRNA on CAS with an RNA sequence of our choice, we can cut DNA at any specific site!
  - 20-nt target sequence much more specific than 4–8-nt sequence of restriction sites.
  - Customizable!

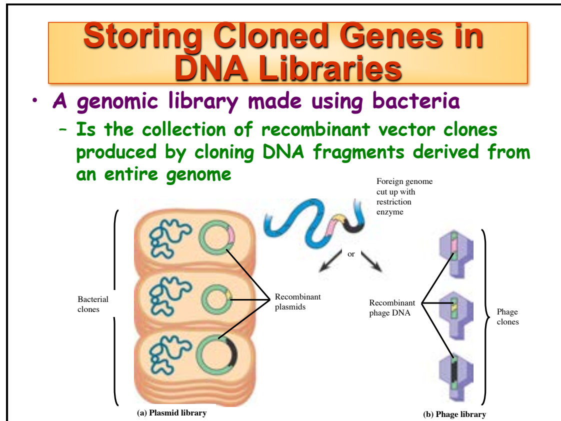
https://www.youtube.com/watch?t=141&v=2pp17E4E-O8

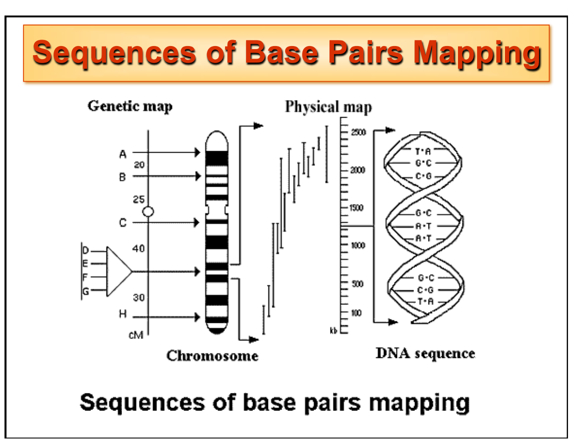

Analysis of Gene Expression
Microarrays of mRNAs
Investigate which genes are being expressed by which tissues and at which time
Fig. 20.13


Analysis of Gene Expression
in situ Hybridization of mRNAs
Investigate which genes are being expressed by which tissues and at which time
Drosophila embryo
Fig. 20.10


Genetic Mapping:
Relative Ordering of Markers
Gene Mapping & genomics
The initial stage in mapping a large genome is to construct a linkage map of several thousand genetic markers spaced throughout each of the chromosomes
Figure 20.11


Construction of a Human Genomic Library
human DNA
CLEAVE WITH RESTRICTION NUCLEASE
millions of genomic DNA fragments
DNA FRAGMENTS INSERTED INTO PLASMIDS USING LIGASE
recombinant DNA molecules
INTRODUCTION OF PLASMIDS INTO BACTERIA
genomic library


Storing Cloned Genes in DNA Libraries
A genomic library made using bacteria
Is the collection of recombinant vector clones produced by cloning DNA fragments derived from an entire genome
Foreign genome cut up with restriction enzyme
Bacterial clones
Recombinant plasmids
Recombinant phage DNA
Phage clones
(a) Plasmid library
(b) Phage library


Sequences of Base Pairs Mapping
Genetic map
Physical map
Chromosome
DNA sequence
Sequences of base pairs mapping