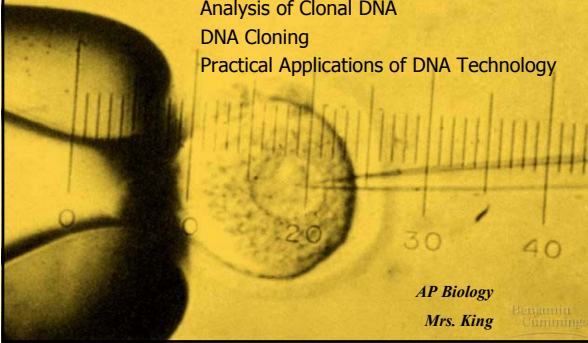


Ch 20

DNA Technology

- Analysis of Clonal DNA
- DNA Cloning
- Practical Applications of DNA Technology



DNA Cloning
Recombinant DNA

- Major tool-*restriction enzymes*
 - discovered in late 1960's
 - cut DNA at restriction site
 - highly specific
 - manufactured by bacteria
- Cloning vector* - carrier for moving DNA into a cell

Cloning

- producing many identical copies of a gene

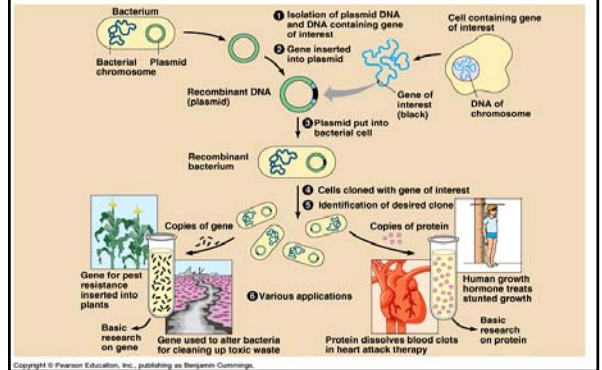
Recombinant DNA

- joining together of two fragments of DNA that are not normally joined together (e.g. joining together of eukaryotic DNA and prokaryotic DNA - usually in a cloning vector)

Cloning vector

- an agent such as a bacterial virus or plasmid into which foreign DNA can be inserted

Procedure for cloning a eukaryotic gene in a bacterial plasmid



Restriction Enzyme:
Source and Site of Action

Restriction Enzyme	Source	DNA Sequence Recognized	Ends of Cleaved Molecule
EcoRI	<i>Escherichia coli</i>	5'GAATTC 3CTTAAG	5'AATC - G G - CTTAA5'
BamHI	<i>Bacillus amyloliquefaciens</i>	5'GGATCC 3CCTAGG	5'GATC - G G - CCTAG5'
HindIII	<i>Haemophilus influenzae</i>	5'AAGCTT 3TTCGAA	5'AGCTT - A A - TTCGA5'
MspI	<i>Micrococcus species</i>	5'CCNAGG 3GGANTCC	5'CTNAGG - C C - GGANTC5'
TaqI	<i>Thermus aquaticus</i>	5TCGA 3'AGCT	5'CGA - T T - AGC5'
NcoI	<i>Nocardia otitidis</i>	5'GCGGCCGC 3CGCGGGG	5'GGCCGC - GC CG - CGCGG5'
AhaI*	<i>Arthrobacter luteus</i>	5'AGCT 3TCGA	5'AG - CT TC - GA5'

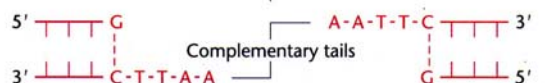
* = blunt ends

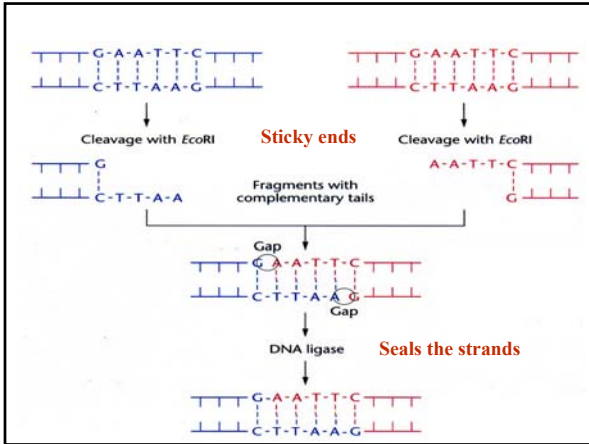
<http://www.people.virginia.edu/~rjb9u/restrenz.html>

Recognition site



Treatment with EcoRI

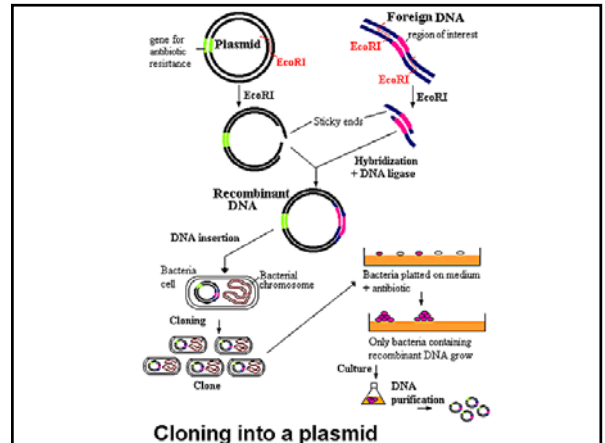
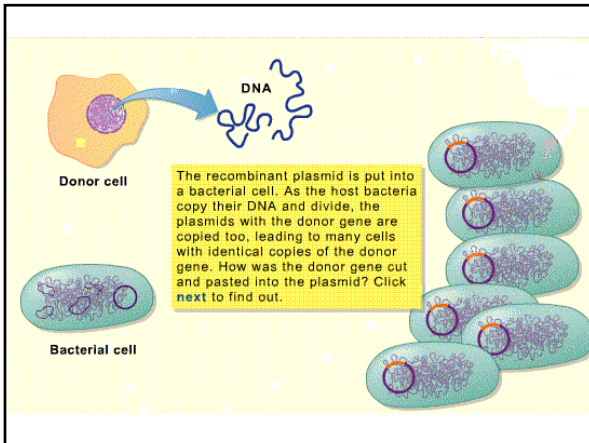




Cut and Paste DNA

Restriction enzyme name	Recognition sequence
BamHI	G GATCC CCTAG G
EcoRI	G AATTC CTTAA G
HindIII	A AGCTT TTCGA A

AGCTAAGCTTAGCT Gene X GCTTAACGCGAATTCCT
TCGATTGCAATCGA CGAATTGCGCTTAAGGA



Getting an eukaryotic gene to function in a prokaryote

- Basic problem: introns
- Solution: production of cDNA
 - artificially produced DNA that is made by using reverse transcriptase on a mRNA

CELL NUCLEUS

DNA of eukaryotic gene: Exon Intron Exon Intron Exon

1 Transcription → RNA primary transcript

2 RNA splicing (removes introns) → mRNA

TEST TUBE

3 Isolation of mRNA from cell and addition of reverse transcriptase; synthesis of DNA strand

4 Degradation of RNA

5 Synthesis of second DNA strand

cDNA of gene (no introns)

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DNA Libraries

- DNA cut with restriction enzymes and inserted into plasmids, inserted into bacteria, and stored
- whole genome is maintained
- cDNA libraries eliminate everything except the genes

(a) Plasmid library

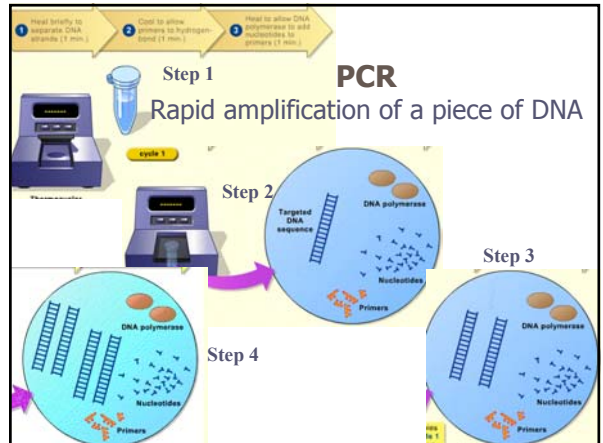
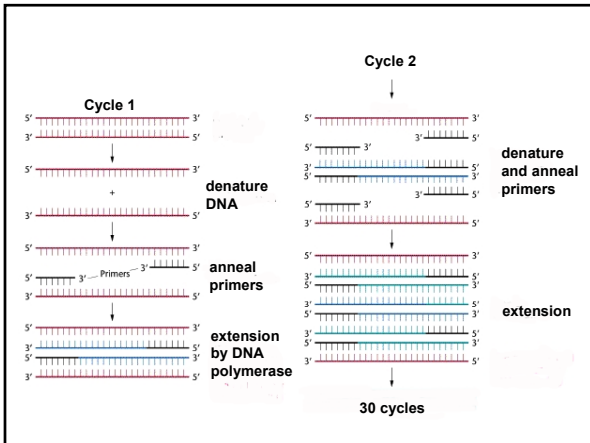
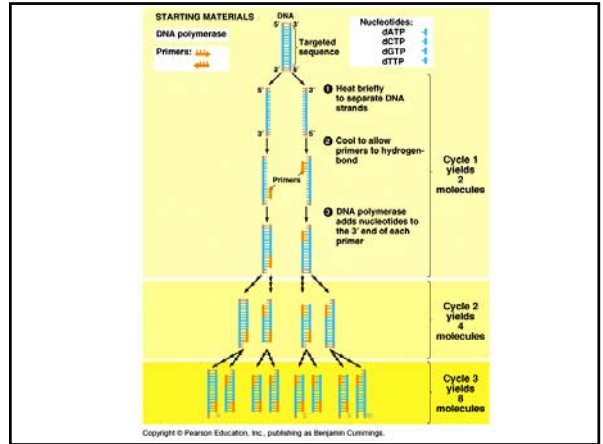
(b) Phage library

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PCR

polymerase chain reaction

- ☞ DNA doubled in each cycle
 - starting with 1 molecule of DNA
 - 1 million copies after 20 cycles
- ☞ Key to procedure is thermostable DNA polymerase (Taq polymerase)
- ☞ Ability to amplify minute amounts of DNA valuable in many disciplines
 - basic and applied research
 - criminal forensic science
 - ecology
 - analysis of ancient DNA



PCR in 25 minutes?



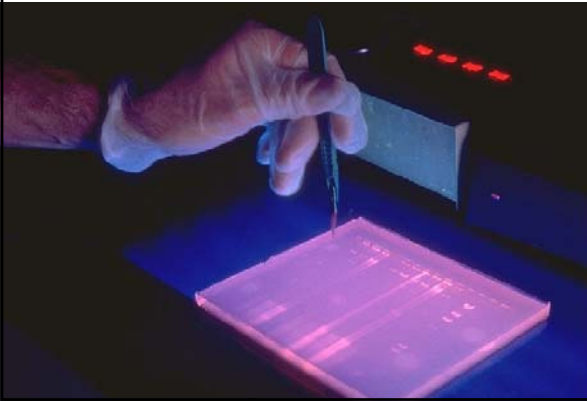
- ☞ The 9800 Fast PCR System is the first fully integrated solution delivering fast PCR performance in a standard 96-well format.
- ☞ The system reduces PCR reaction time from two hours to 25 minutes or less—advancing you quickly to the next step of your research.

http://marketing.appliedbiosystems.com/mk/get/9800_LANDING?isource=fr_E_Nature_Methods

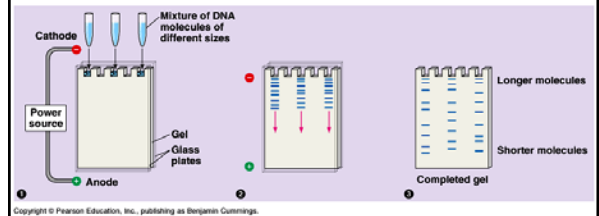
Gel Electrophoresis

- ☞ Produces DNA "fingerprints"
- ☞ Separates RFLP's by using an electrical field
- ☞ Can be used to identify some genetic diseases
- ☞ Presence of a particular gene
- ☞ Used as genetic markers

DNA band pattern



For linear DNA molecules, separation depends mainly on size (length of fragment) with longer fragments migrating less along the gel.



➤ Differences in DNA sequence on homologous chromosomes that produce different restriction fragment patterns are scattered abundantly throughout genomes, including the human genome.

➤ These **restriction fragment length polymorphisms (RFLPs)** can serve as a genetic marker for a particular location (locus) in the genome.

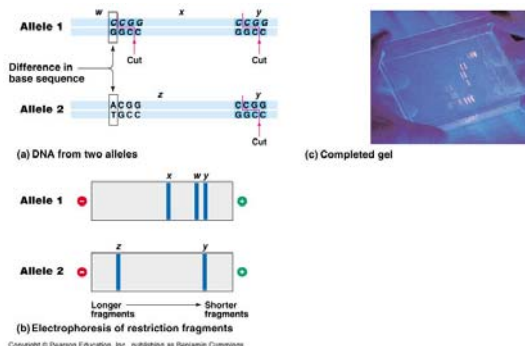
- A given RFLP marker frequently occurs in numerous variants in a population.
- RFLP markers inherited in a Mendelian fashion, they can serve as genetic markers for making linkage maps.

➤ Most of human diversity is in the form of **single nucleotide polymorphisms (SNPs)**, single base-pair variations.

- In humans, SNPs occur about once in 1,000 bases, meaning that any two humans are 99.9% identical.

➤ The locations of the human SNP sites will provide useful markers for studying human evolution and for identifying disease genes and genes that influence our susceptibility to diseases, toxins or drugs.

➤ Restriction fragment analysis is sensitive enough to distinguish between two alleles of a gene that differ by only base pair in a restriction site.



Practical Applications of DNA Technology

- Disease diagnosis
- Gene Therapy
- Pharmaceutical products
- Forensic uses
- Environmental applications
- Agricultural applications



Other Technologies

- ✔ Hybridization
- ✔ Human Genome Project
- ✔ Physical mapping
- ✔ Sequencing DNA
- ✔ Genome Analysis (DNA sequences, gene expression, and gene function)