Lecture( )

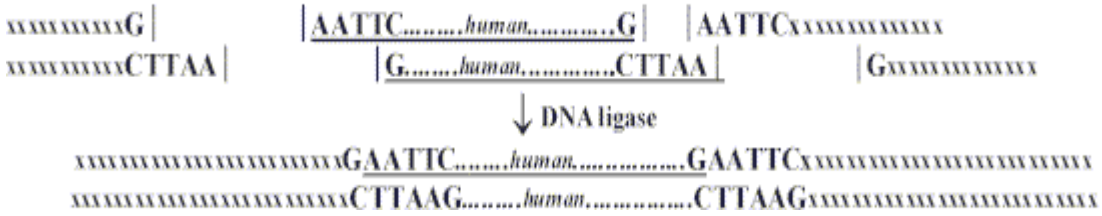
Genetic Engineering

Gene Cloning

1. *Restriction enzymes*: Hundreds of protective bacterial enzymes hydrolyze specific palindromic sequences of invading DNA (i.e. viruses or plasmids).

Enzymes	Target sequences	"Sticky end" products	
EcoRI:	↓ GAATTC CTTAAG ↑	G CTTAA	AATTC G
BamHI:	↓ GGATCC CCTAGG ↑	G CCTAG	GATCC G
HindIII	↓ AAGCTT TTCGAA ↑	A TTCGA	AGCTT A

2. *Formation of recombinant DNA.* Two foreign DNA's (i.e. human and bacterial) hydrolyzed by the *same* restriction enzyme have complementary, *sticky ends* which form hybrid, *recombinant DNA molecules* sealed by *DNA ligase.*



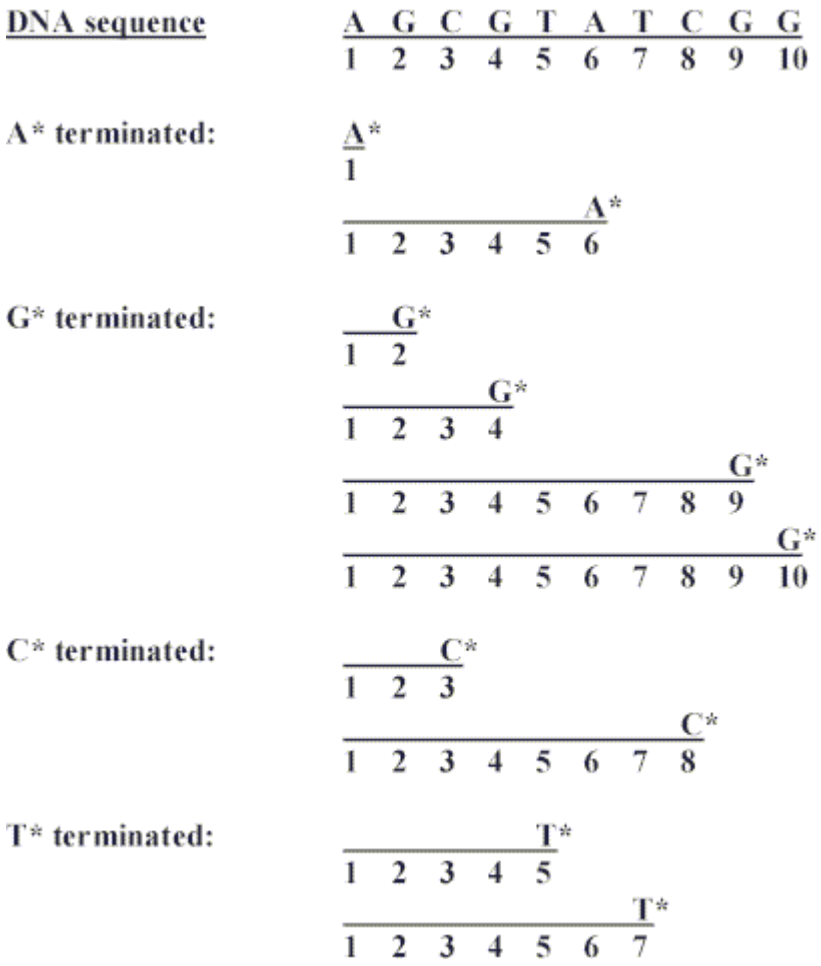
3. Expressing cloned DNA in bacteria and yeast.

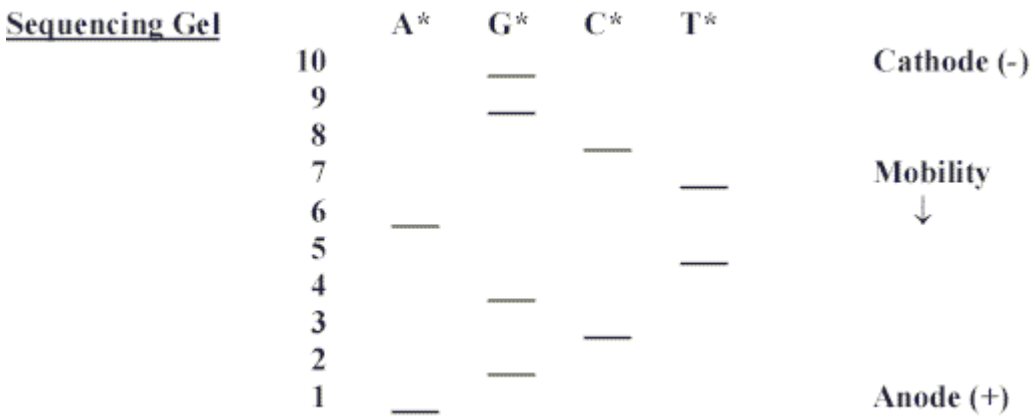
Bacteria and contain *plasmids,* small circular DNA's, which replicate independently of the chromosome. By making identical cuts in donor DNA containing genes of interest and specially designed plasmids, *recombinant DNA molecules* are formed. In bacteria or yeast *transformed* with plasmids, the foreign genes are *transcribed* and *translated.* Yeasts can *splice split, eukaryotic mRNA.*

4. D*etecting bacteria with recombinant plasmids producing proteins of interest.*

- **Transformed bacterial colonies are grown on agar plates.**
- **Bacterial colonies are transferred to a *replica, reference plate.*.**
- **The bacteria on the agar plate are lysed to release proteins *in situ*.**
- **The proteins are blotted on a nitrocellulose sheet.**
- **Radiolabeled antibodies specific for the protein are added to the lysed colonies. The plates are exposed to X-ray film to identify the transformed colony.**
- **The DNA is extracted from the cells on the reference plate for analysis.**

DNA Sequencing: Sanger base-specific, replication termination.





Note: The negatively charged, sequence fragments are separated by *size* by an electric current in a gel; the smaller the fragment the faster its mobility in the gel.

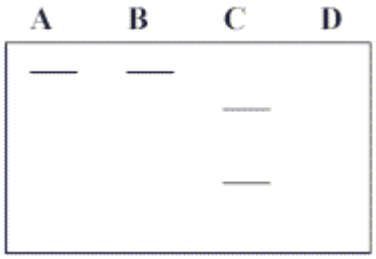
Restriction Fragment Length Polymorphisms (RFLP's)

The lengths of the DNA fragments produced by treating the DNA of different individuals with the same restriction enzymes are frequently different. These differences are referred to as *restriction fragment length polymorphisms*, (i.e. "riflips").

Detection of RFLP's by Blot Hybridization.

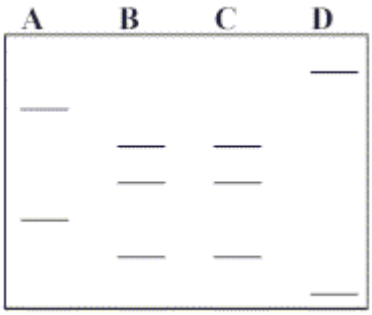
- **Cut DNA into *fragments* with a restriction enzyme.**
- **Separate the *fragments* in agarose gels using an electric current.**
- **Denature the *fragments* into single strands.**
- **Blot transfer the *fragments* from the gel to a nylon membrane.**
- **Incubate the *blot* with a radioactive *hybridization probe.***
- **The positions of the *fragments are d*etected as radioactive bands on X-ray film.**

Uses of RFLP's



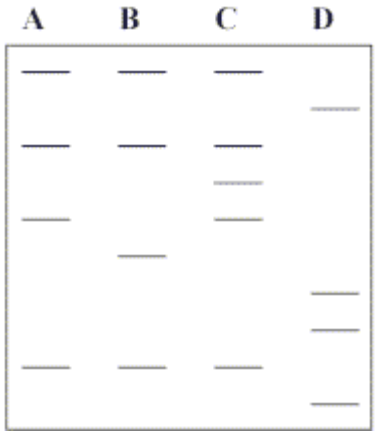
Diagnosis of Genetic diseases

A = Normal gene - Individual 1
B = Normal gene - Individual 2
C = Point mutation
D = Deletion



Forensic cases

A = Victim
B = Evidence
C = Guilty suspect
D = Innocent suspect



Relationship between species

A, B, C = Closely related
D = Less closely related