RFLP
(RESTRICTION FRAGMENT LENGTH POLYMORPHISM)

PRESENTER: LIM LAY CHENG
RFLP Analysis

Polymorphic restriction site

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DNA (allele 1)

Add restriction enzyme

4 fragments

DNA (allele 2)

3 fragments

Estimated 10e5 RFLPs present in mammalian genome!
RFLP TECHNOLOGY

(1) Digest dsDNA with restriction enzyme

-Cuts dsDNA at specific recognition nucleotide sequences known as restriction sites.

-Each enzyme recognizes a certain Sequence, usually 4, 5, 6 or (rarely) 8 bases long.

-Examples of restriction enzymes Include: EcoRI, EcoRII, HindIII etc.
RE cut DNA molecules at define positions

<table>
<thead>
<tr>
<th>Endonuclease</th>
<th>DNA sequence</th>
<th>Cleavage products</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bam</em> HI</td>
<td>GGATCC</td>
<td>G</td>
</tr>
<tr>
<td></td>
<td>CCTAGG</td>
<td>GATCC</td>
</tr>
<tr>
<td><em>Eco</em> RI</td>
<td>GAATTC</td>
<td>G</td>
</tr>
<tr>
<td></td>
<td>CTTAAG</td>
<td>AATTC</td>
</tr>
<tr>
<td><em>Hind</em> III</td>
<td>AAGCTT</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>TTTCGAA</td>
<td>AGCTT</td>
</tr>
<tr>
<td><em>Hae</em> III</td>
<td>GGCC</td>
<td>G</td>
</tr>
<tr>
<td></td>
<td>CCGG</td>
<td>GG</td>
</tr>
<tr>
<td><em>Pst</em> I</td>
<td>CTGCAG</td>
<td>CTGCA</td>
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<tr>
<td></td>
<td>GACGTC</td>
<td>ACGTC</td>
</tr>
<tr>
<td><em>Sma</em> I</td>
<td>CCCGGG</td>
<td>CCC</td>
</tr>
<tr>
<td></td>
<td>GGGCCC</td>
<td>GGG</td>
</tr>
</tbody>
</table>

“Sticky” ends

Blunt ends
APPLY RESTRICTION ENZYME

-DNA sample & RE are put together in the tube

-The tube is shaken by rotation for DNA & RE to mix
**Water Bath**

-The tube is put on a plate floating on water at 37 degree C.

-It is left for 30 minutes.

-This is needed for the RE reaction to take place.
RFLP TECHNOLOGY

(2) Separate dsDNA fragments by agarose gel electrophoresis

The resulting DNA fragments are then separated by molecular size by agarose gel electrophoresis.
GEL VIEWING

- Gel can be stained and viewed under UV light.
- Each band reveals the presence of a population of DNA molecules of a specific size.
RFLP TECHNOLOGY

(3) Blotting

The DNA sticks to nylon or nitrocellulose membrane, creating an invisible image of DNA pattern on gel.
RFLP TECHNOLOGY

(4) Hybridization & Autoradiograph

-DNA probe in solution in plastic bag.

- Hybridization with radioactive probe.

- Detect labeled probes using X-ray film.
HOW IS IT USED?

- RFLPs have provided valuable information in many areas of biology including:
  - Systematic, genetic & ecology (genome mapping)
  - DNA fingerprinting (forensic crime investigation, paternity testing)
  - Screening human DNA for the presence of potentially deleterious genes
Example 1: Hereditary Disease Diagnostic

- Methylation
- Mutations destroy one restriction site
- Probe region
- Digest, separate
- Gel electrophoresis
- Southern blot
EXAMPLE 2: CRIME SCENE INVESTIGATION

Figure 5.15

Marker 1
Suspect A 2
Semen (clothing) 3
Suspect B 4
Marker 5
Vaginal swab 6
Victim 7
Control DNA 8
Marker 9
No DNA 10

Courtesy Lifecodes Corporation, Stamford, CT.
EXAMPLE 3: PATERNSITY TESTING

The mission of LabCorp DNA Identification Technology is to provide the most rapid, scientifically sound and cost-effective biological identification services focused on the needs of our customers.
LIMITATIONS OF RFLP

- Requires large amount of sample DNA
- Time consuming & labor intensive - take up to a month to complete
ALTERNATIVES

- PCR
- TRFLP – Terminal Restriction Length Polymorphism
THANK YOU