Tools for Molecular Biology

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Cancer Center, Room 235
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Lecture outline

- Restriction enzymes
- Recombinant DNA
- Gel electrophoresis
- Nucleic acid hybridization / Southern blot
- Polymerase chain reaction (PCR)
- Microsatellite analysis
- Sequencing

Restriction enzymes cleave DNA at specific sequences

Palindrome
Restriction enzymes cleave DNA at specific sequences

- **Blunt cut**
- **Sticky ends**

Restriction enzymes produce DNA fragments that can be easily rejoined

Creating recombinant DNA
Insertion of a DNA fragment into a bacterial plasmid with DNA ligase

Amplification of a specific DNA sequence

Recombinant DNA and medicine

<table>
<thead>
<tr>
<th>Product</th>
<th>Main use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human growth hormone (IGF1)</td>
<td>Treats diabetic animals</td>
</tr>
<tr>
<td>Human growth hormone (GH)</td>
<td>Treats dwarfism</td>
</tr>
<tr>
<td>Insulin-like growth factor (IGF)</td>
<td>Helps with growth and development</td>
</tr>
<tr>
<td>Human growth hormone (IGF2)</td>
<td>Increases muscle mass</td>
</tr>
<tr>
<td>Growth hormone (GH)</td>
<td>Increases muscle mass</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>Improves bone density</td>
</tr>
<tr>
<td>Testosterone</td>
<td>Improves muscle mass</td>
</tr>
<tr>
<td>Estrogen</td>
<td>Improves bone density</td>
</tr>
<tr>
<td>Insulin</td>
<td>Regulates blood sugar levels</td>
</tr>
<tr>
<td>Glucagon</td>
<td>Regulates blood sugar levels</td>
</tr>
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<td>Insulin-like growth factor (IGF1)</td>
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<tr>
<td>Insulin-like growth factor (IGF2)</td>
<td>Helps with growth and development</td>
</tr>
<tr>
<td>SAA (sialoadhesin)</td>
<td>Regulates blood sugar levels</td>
</tr>
<tr>
<td>TNF (tumor necrosis factor)</td>
<td>Causes cell death</td>
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<td>Causes cell death</td>
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Gel electrophoresis separates DNA molecules based on size

Complementary nucleic acid strands can bind each other by a process called hybridization
Southern blotting can detect a specific DNA molecule within a complex mixture.

- Pro – Glu – Glu –
- CCT –
- GAG –
- Mst II site – Pro –
- Val – Glu –
- CCT–G
- TG–GAG –
- Mst II

1.1 kb
1.3 kb

Type of hemoglobin

<table>
<thead>
<tr>
<th></th>
<th>β-1-globin gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal</td>
<td>βS</td>
</tr>
<tr>
<td>Adult</td>
<td>βA</td>
</tr>
</tbody>
</table>

- A
- S

Probe

β-hemoglobin gene

Mother Father Child Fetus

(AS) (AS) (SS) (AA) Genotype

Detection of Sickle-cell disease by RFLP analysis
In situ hybridization can locate specific genes on chromosomes

FISH: Fluorescent in situ hybridization

Amplification of the Her2 gene in breast cancer

Polymerase Chain Reaction (PCR)
**Diagnosis of Hemophilia by PCR**

**Design**
- 10 bp
- Sequencing analysis
- Digestion site
- Segments

**Pedigree Analysis**
- O

**Results**

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
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**Microsatellite analysis by PCR**

**Table:**
- Variants
- Description
- Analysis
- Site
- Controls

**Figure:**
- Diagram of microsatellite analysis with variant sites and analysis results.

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- PCR Movie
- Microsatellite analysis by PCR
- Diagnosis of Hemophilia by PCR
Synthesizing DNA copies (cDNA) of RNA

Reverse Transcription-PCR for diagnosis of HIV infection

Microarrays can measure the expression of many genes at the same time
Oncotype Dx for breast cancer

16 cancer-related genes

<table>
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<tr>
<th>Estrogen</th>
<th>Proliferation</th>
<th>Her2</th>
<th>Invasion</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER, PR, PGR</td>
<td>ERBB2, HER2</td>
<td>ERBB2</td>
<td>ERBB2, HER2, SHH, FGFR4</td>
<td>CCNB1</td>
</tr>
</tbody>
</table>

5 reference genes (controls)

- GUSB
- GAPDH
- ACTB
- B2M
- HPRT1

Sequencing DNA

Note: results give you the percentage of differentially expressed genes at the single nucleotide level.
DNA sequencing detects a 3-bp deletion in the CFTR gene in cystic fibrosis.

Next Generation Sequencing
Illumina HiSeq platform – “sequence by synthesis”
Next Generation Sequencing
Illumina HiSeq platform – “sequence by synthesis”

Traditional sequencing versus NGS

The Human Genome

- 2000-2003 first human genome sequenced
- 13 years
- $2.7 billion

- 2007
- 4 months
- $1.5 million

Illumina (NGS)
- 8 days
- $10,000

MinION (Oxford Nanopore Technologies)
- less than $1000 genome
- 15 minutes