Genomics & DNA sequences

- Satellite DNA: tandem repeats near centromere, heterochromatin
- Microsatellite: dinucleotide repeats
- VNTR: variable number of tandem repeats (mini-satellites)
- SINES (<500 bp, 500,000X in genome) and LINES (6400 bp, 100,000X), transposable

Eukaryotic organisms differ widely in the proportion of the genome consisting of repetitive DNA sequences and in the types of these sequences that are present. A eukaryotic genome typically consists of three components:

1. Unique, or single-copy, sequences. This is usually the major component and is typically from 30 to 75 percent of the chromosomal DNA in most organisms.
2. Highly repetitive sequences. This component constitutes from 5 to 45 percent of the genome. Some of these sequences are the satellite DNA referred to earlier. The sequences in this class are typically from 5 to 300 base pairs per repeat and are duplicated as many as $10^5$ times per genome.
3. Middle-repetitive sequences. This component is from 1 to 30 percent of a eukaryotic genome and includes sequences that are repeated from a few times to $10^3$ times per genome.

These different components can be identified according to the number of bands that appear in Southern blots with the use of appropriate probes or by other methods.
Recombinant DNA

- RFLP
- Restriction enzymes & palindromes
- Probes
- Restriction mapping
- Use of Vectors
- Cloning with plasmid vectors
- Site directed mutagenesis
- Constructing DNA libraries
- PCR analysis & technique
- DNA sequencing
RFLP

Restriction enzymes & palindromes

Recognition site

5' G-A-A-T-T-C 3'
3' C-T-T-A-A-G 5'

Treatment with EcoRI

5' G 3'
3' C-T-T-A-A 5'

Complementary tails
Gels and Southern Blotting

1. DNA samples cut with restriction enzymes are loaded on agarose gel for electrophoresis
   Lane 1: Radioactive size markers
   Lane 2: DNA cut with restriction enzyme A
   Lane 3: DNA with restriction enzyme B

2. DNA is separated by electrophoresis but invisible to the naked eye

3. DNA-binding filter, paper towels, and weight are placed on gel; buffer passes upward through sponge by capillary action transferring DNA fragments to filter

1. A population of cloned DNA fragments is prepared

2. DNA fragments are cut with restriction enzymes

3. The restriction fragments are separated by gel electrophoresis

4. Theoretical models are constructed that are consistent with initial results

5. Models are tested against results of double enzyme digests

6. Conclusions: model 1 is correct

Model 1

<table>
<thead>
<tr>
<th>Model</th>
<th>HindIII</th>
<th>Sall</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.8</td>
<td>5.8</td>
</tr>
<tr>
<td>0</td>
<td>0.8</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Predicted fragments from digestion with HindIII and Sall: 0.8 kb, 0.8 kb, and 5.8 kb

Model 2

<table>
<thead>
<tr>
<th>Model</th>
<th>HindIII</th>
<th>Sall</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.8</td>
<td>5.0</td>
</tr>
<tr>
<td>0</td>
<td>0.8</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Predicted fragments from digestion with HindIII and Sall: 0.8 kb, 1.2 kb, and 5.0 kb

Fragments generated by cutting with HindIII and Sall are 0.8, 0.8, and 5.8 kb in length, indicating that model 1 is correct.
Probes
Detection using probes

**Sickle cell anemia**

DNA extracted from white blood cells

Codon 6

5’  
Region covered by ASO probes  
3’

DNA is spotted onto binding filters, hybridized with ASO probe

(a) Genotypes

<table>
<thead>
<tr>
<th></th>
<th>AA</th>
<th>AS</th>
<th>SS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (β^A) ASO: 5’ – CTCTCTGGAGAAGTCTGC – 3’</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(b) Genotypes

<table>
<thead>
<tr>
<th></th>
<th>AA</th>
<th>AS</th>
<th>SS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutant (β^S) ASO: 5’ – CTCTCTGGAGAAGTCTGC – 3’</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Cystic Fibrosis**

ASO for normal DNA sequence in region of Δ508 mutation in cystic fibrosis

5’ CACCAAGATGATTTTTTTCC-3’
Region deleted in Δ508

ASO for mutant DNA sequence in region around Δ508 deletion

5’ CACCAATGATTTTTTC-3’

**ASO** = allele specific oligonucleotides
Can detect single nucleotide change
Pedigree analysis

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Fragment sizes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homozygous for chromosome A (A/A)</td>
<td>3 kb, 7 kb</td>
</tr>
<tr>
<td>Heterozygous (A/B)</td>
<td>3 kb, 7 kb, 10 kb</td>
</tr>
<tr>
<td>Homozygous for chromosome B (B/B)</td>
<td>10 kb</td>
</tr>
</tbody>
</table>
Pedigree analysis

Allele A = 5 kb
Allele B = 2 kb
On different chromosomes

III-1 = received A from father
And B from mother

III-2 received A from mother
And B from father

III-3 received B from both
Parents

Taken together mutant allele
With B,
Use of Vectors

- Use of Vectors
- Plasmids
Plasmid inserts

**FIGURE 15.8** A Petri plate showing the growth of host cells after uptake of recombinant plasmids. The medium on the plate contains a compound called X-gal. DNA inserts in the pUC vector disrupt the gene responsible for the formation of blue colonies. Cells in blue colonies do not carry any cloned DNA inserts, whereas white colonies contain vectors carrying DNA inserts.
Bacteriophage & Yeast Artificial chromosomes
Constructing DNA libraries

**Constructing DNA Libraries**

The production of cDNA from mRNA. Because many subcellular mRNAs have a poly(A) tail of variable length (A), at their 3' end, a short poly(dT) oligonucleotide can be annealed to this tail. The poly-dT acts as a primer for the enzyme reverse transcriptase, which uses the mRNA as a template to synthesize a complementary DNA strand. A characteristic hairpin loop formed as synthesis is terminated on the template. The poly-A tail is removed by alkaline treatment, and the cDNA is then digested with restriction enzymes. The 5' end of the resulting cDNA molecules is then labeled, either by nick translation or by filling in the 3' overhang created by the enzymatic digestion. The labeled cDNA molecules can be cloned into a suitable vector or used as a probe for library screening.

**Initial adjacent sequence (B)**

- **Subclone fragment, use to probe for overlapping clone**
  - **Subclone, re-probe**
    - **C**
      - **Subclone, re-probe**
        - **D**
          - **Subclone, re-probe**
            - **E**
              - **Target gene cloned**
                - **Region of chromosome**
                  - **Gene to be cloned**
Site directed mutagenesis

1. Determine gene sequence
   a. Original gene sequence

2. Isolate one strand
   b. Single strand

3. Add synthetic oligonucleotide
   c. Noncomplementary triplet

4. Strands hybridize
   d. Synthetic duplex

5. Semiconservative replication occurs (new synthesis)
   e. Original nucleotide sequence
   f. Mutant protein

FIGURE 14.22 Site-directed mutagenesis. A single strand of DNA from a gene of interest is isolated. This is hybridized with a synthetic oligonucleotide containing a triplet altered so as to encode an amino acid of choice. Following semiconservative replication, a different complementary base pair is present in one of the new duplexes. Upon transcription and translation, a mutant protein, “designed” in the laboratory, will be produced.

FIGURE 14.19 Flow diagram illustrating the principle of site-directed mutagenesis. A single strand of DNA from a gene of interest is isolated. This is hybridized with a synthetic oligonucleotide containing a triplet altered so as to encode an amino acid of choice. The partial duplex is completed under the direction of DNA polymerase and DNA ligase. Following semiconservative replication, a different complementary base pair is present in one of the new duplexes. Upon transcription and translation, a mutant protein, “designed” in the laboratory, will be produced.
PCR analysis & technique

**Cycle 1**
- DNA to be amplified

**Step 1**
- Denature DNA

**Step 2**
- Anneal primers

**Step 3**
- Extend primers
  *(Product of first cycle is two new DNA molecules)*

**Cycle 2**
- Denature and anneal new primers
Figure 12.32  Forensic use of DNA fingerprinting. Southern blot of DNA from victim (V) and defendant (D) in crime. Jeans and shirt refer to blood samples taken from the defendant. The pattern clearly matches the victim's blood, not the defendant's own blood. All of the other lanes of the blot contain controls and size standards. The probability that the blood stains were not from the victim was estimated at one in thirty-three billion, more than the number of people on earth. However, these probabilities are controversial, depending on statistical assumptions about variability in racial and ethnic subpopulations. (Courtesy of Cellmark Diagnostics, Germantown, Maryland.)
DNA sequencing

1. Primer is bound to template strand
   5' Primer 3'
   3' GATCTGTAC 5'
   Template strand

2. Reaction ingredients added
   - DNA polymerase
   - dATP
   - dCTP
   - dGTP
   - dTTP
   - small amount of ddATP dideoxynucleotide

3. Primer extension
   - Cannot form a phosphodiester bond with next incoming dNTP

4. Newly synthesized strands recovered, loaded on gel in "A" lane
   - Chain termination when a ddATP is inserted
DNA sequencing

3. Primer extension
   Chain termination
   Product recovery

4. Electrophoresis,
   imaging, data
   analysis
DNA sequence—Fluorescent dyes
# Gene Therapy

## TABLE 20-1: New Vectors for Gene Therapy

<table>
<thead>
<tr>
<th>Vector</th>
<th>Cell Targets</th>
<th>Capacity</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus</td>
<td>Lung, respiratory tract</td>
<td>7.5 kb</td>
<td>Efficient transfection</td>
<td>Strong immune response</td>
</tr>
<tr>
<td>Adeno-associated virus</td>
<td>Fibroblasts, T cells, others</td>
<td>4.5 kb</td>
<td>Transfects many cell types</td>
<td>Small insert size</td>
</tr>
<tr>
<td>Retroviruses</td>
<td>Proliferating cells</td>
<td>6 kb</td>
<td>Promoted expression</td>
<td>Low transfection efficiency</td>
</tr>
<tr>
<td>Lentiviruses</td>
<td>Strom cells, proliferating cells</td>
<td>8 kb</td>
<td>Efficient transfection</td>
<td>Related to HIV</td>
</tr>
</tbody>
</table>

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**Diagram:**
- **Bacterium** carrying plasmid with cloned normal human ADA gene
- Genetically disabled retrovirus
- **T cells** isolated from SCID patient
- Retrovirus infects blood cells, transfers ADA gene to cells
- Genetically altered cells are reimplanted, produce ADA
- Cells are grown in culture to ensure ADA gene is active
- Cloned ADA gene is incorporated into virus