Muscular Dystrophy
A Case Study of Positional Cloning

- Described by Benjamin Duchenne (1868)
- X-linked recessive disease causing severe muscular degeneration.
- 100% penetrance
- \( X^dY \) – affected male
- Frequency in males = 1/3600

Becker Muscular Dystrophy

- X-linked recessive
- Symptoms identical to DMD but much slower onset
- Maps to the same location on the X chromosome as DMD

Pedigree of Duchenne Muscular Dystrophy

Positional Cloning of Duchenne Muscular Dystrophy

A Case Study

- Genetic Mapping – based on recombination frequency (expressed as cM)
- Physical Mapping – actual molecular distance in nucleotide base pairs (expressed as bp, kb, or mb)
Approaches to Gene Cloning

- **Traditional** – based on the knowledge of the normal gene product
- **Positional** – cloning with no prior knowledge of the mutant gene product (protein) or its function.

Traditional Approaches to Gene Cloning

- Isolation of gene specific mRNA to make a cDNA probe.
- Degenerate oligonucleotide probes based on amino acid sequence.
- Expression cloning (e.g., antibody detection of the expressed protein by in a cloning vector).

Positional Cloning

Some Notable Examples of Genes Isolated by Positional Cloning

- Duchenne Muscular Dystrophy
- Fragile X Syndrome
- Cystic Fibrosis
- Huntington Disease
- Retinoblastoma
- Neurofibromatosis

A Great Web Site

- Go to: [http://www.dnalc.org/resources/BiologyAnimationLibrary.htm](http://www.dnalc.org/resources/BiologyAnimationLibrary.htm)
- Download Shockwave from this page.
- Click Play or down the files for later.
- Learn PCR, Southern Blotting, Sequencing, DNA forensics, etc.!!

Sidebar – Do you have a good understanding of the following?

- Southern Blot
- Northern Blot
- Radioactive labeling of DNA
- Plaque hybridization
- Colony hybridization
- Polymerase Chain Reaction (PCR)
- Nucleotide sequencing
Positional Cloning - Step 1

Genetic disease
Map to a chromosome site
Retrieve genomic clones that cover the mapped region
Identify and analyze exons
Isolate a cDNA
Isolate genomic clones
Characterize the normal gene
Mutation detection assays

Genetic disease
Map to a chromosome site

Cloning of the DMD Gene Using Deletion Analysis

- Patient B.B.
  - Rare cytological detectable deletion
  - Width of Xp21 significantly reduced
  - Multiple genetic disorders
    - DMD
    - Chronic granulomatous disease (CGC)
    - Retinitis pigmentosa
    - Deletion 10 mb in length

Normal X

Patient B.B.

Subtractive Hybridization

\[ G - (G-10) = 10 \]

Normal Denatured Genomic DNA
Patient B.B. Denatured DNA (excess)
Normal DNA Sequences Absent in B.B.

Outcome of Subtractive Hybridization

- Short DNA sequences in a plasmid vector
- Represent less than 1% of the genetic material within the deletion
- Which pERT clones map to the DMD gene?
Which pERT Clones (if any) Are Located Within the DMD Gene?

- Tested a panel of 57 DMD patients.
- 8 of 9 clones hybridized with all DMD patients.
- One pERT clone (pERT87) was absent in 5 DMD patients.
- Possible that these patients have microdeletions within the DMD gene in the sequence represented by pERT87.

Positional Cloning - Step 2

Map to a chromosome site

Retrieve genomic clones

pERT 87 Was Used to Screen a lambda-phage Genomic Library

Alignment of lambda-phage Clones by Restrictions Maps

R = EcoRI
K = KpnI

Chromosome walking

Genomic Contig After Three Rounds of Walking

Total Length of Cloned DNA
Genomic Contig After Nine Rounds of Bidirectional Walking

- pERT87 (200 bp)
- DXS164 (220 kb)

Genetic disease
- Map to a chromosome site
- Retrieve genomic clones that cover the mapped region
- Identify and analyze exons
- Isolate a cDNA
- Isolate a genomic clone
- Characterize the normal gene
- Mutation detection assays

Step 3 - Identification of Exons
- Retrieve genomic clones that cover the mapped region
- Identify and analyze exons

Screening for Coding Regions
- Sequence conservation
- Exon trapping
- Identification of CpG islands
- Screen cDNA libraries

Exon Trapping

Exon Trapping (continued)
**Zoo Blot (Inter-species Southern Blot)**

* Blot for the NF2 gene

**Results of Exon Trapping Using Small Subclones of DSX164**

![Diagram of exons and subclones](image)

**Step 4**

Identify and analyze exons

Isolate a cDNA

**Preparation of a cDNA Library**

1. Cell lysis
2. mRNA extraction
3. cDNA synthesis
4. Gel purification
5. Digestion of added vector
6. Ligation

**Preparation of a cDNA Library (continued)**

**Step 5 – Isolation of Full Genomic Sequence**

- Use cDNAs in Northern blots to determine the length of transcripts.
- Use cDNAs to screen genomic libraries for additional functional components of the gene.
**Step 6 – Characterization of the Normal Gene**

- Identify the positions of exons
- Identify promoter, 5’ and 3’ UT exons
- Sequence exons
- Predict amino acid sequence
- Attempt to predict function of gene product

**Properties of the Normal DMD Gene**

- Huge! – covers 2 mb of DNA
- 16 kb mRNA
- Over 75 exons
- Very long introns
- At least 8 promoters – 4 preceding the coding region and 4 in introns
- Expression seen in muscle, heart and brain (but a very low levels)

- Predicted protein length of about 3700 amino acids in its full length form
- Protein called **dystrophin** – 4 functional domains
- Exact function still unclear

**One More Step**

Proof that Mutations in the Cloned Genes Causes the Disease

- Genetic disease
  - Map to a chromosome site
  -Retrieve genomic clones that cover the mapped region
  - Identify and analyze exons
  - Isolate a cDNA
  - Isolate a genomic clones
  - Characterize the normal gene
  - Mutation detection assays