### Cystic Fibrosis and Gene Therapy

**Lecture Notes**

**Biol 100 – K. Marr**

- **Topics for this Lecture**
  - Gene Therapy as a treatment for Cystic Fibrosis
- **Reading assignments in Essential Biology**
  - Chapter 12: DNA Technology:
    - Restriction Enzymes (p. 225)
    - Gel Electrophoresis (p. 229)
    - PCR (Polymerase Chain Reaction) p. 228
    - DNA Fingerprinting (pp. 227-230)
    - Human Gene Therapy (pp. 235-236)

### What is the hope for people with cystic fibrosis?

#### Do you want to have a healthy child?

1. **Screen potential carriers of CF**
   - E.g. Use DNA Probe
2. **Screen for CF gene in embryo**
   - Only implant embryos without CF allele

#### Do you want a cure for yourself?

3. **Gene Therapy**
   - Use a viral vector to insert the normal CFTR gene into the lungs cells of people with CF
   - Somatic vs. Germ line gene therapy

### How to use a DNA Probe to Screen for the CF Gene

1. Isolate DNA from patient
2. Heat to separate DNA strands
3. Add labeled probe that has complementary base sequence to mutant gene
4. Add restriction enzymes (cuts DNA into fragments) and separate by gel electrophoresis

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**Separation of DNA fragments by Gel electrophoresis**

| a. | DNA and dye are loaded in a well on a gel, and an electric field is placed across the gel. |
| b. | DNA fragments move through the gel; shorter fragments move faster than longer fragments. |
| c. | Place photographic film over gel to detect DNA labeled with the probe |

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**Optional Reading**

- Cystic Fibrosis Foundation: Gene Therapy and CF
  - [http://www.cff.org/about_cf/gene_therapy_and_cf.cfm](http://www.cff.org/about_cf/gene_therapy_and_cf.cfm)
- Center for Gene Therapy and other Genetic Diseases
  - [http://genetherapy.genetics.uiowa.edu/](http://genetherapy.genetics.uiowa.edu/)
- Cystic Fibrosis Research Directions
- See "Lecture Related Resources and Enrichment" at the class website

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**Separation of DNA fragments by Gel electrophoresis**

- Smaller fragments move faster than larger fragments through the porous gel.
- Use photographic film to locate DNA fragments bound with radioactive probe

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![Gel electrophoresis diagram](image)
What’s Necessary for Gene Therapy to Work?

1. **Identify the defective gene**
   - e.g. CFTR gene discovered in 1989

2. **Use PCR to make copies of good gene**
   - PCR = polymerase chain reaction

3. **Get good gene into the right cells (need vector)**
   - Use a viral vector

4. **Get the cells to transcribe and translate the good gene**
   - Must make the right amount of protein at the right time and get it to the right place

Two Kinds of Gene Therapy

Replace defective gene in.....

1. **Body cells:**
   - Permanent cure for individual

2. **Egg cell:**
   - Permanent cure for future generations
   - Banned by most countries!! Why?
     - Can’t control where gene inserts. Possible Consequences:
       - Abortive or defective embryos! Why?

   → Could cause cancer! Why?

The polymerase chain reaction (PCR)—another view

1. Heat briefly to 94°C to break hydrogen bonds and strands separate
2. Cool to 55°C to allow primers to hydrogen bond
3. Taq DNA polymerase adds nucleotides to 3' end of each primer

Cycle 1 produces 2 DNA molecules
Cycle 2 produces 4 DNA molecules
Cycle 3 produces 8 DNA molecules

PCR—what’s needed

1. DNA—only a tiny amount is needed!
2. Heat stable DNA polymerase (e.g. *taq* DNA polymerase)
3. DNA primers that bind just outside the DNA to clone
4. DNA nucleotides
5. Thermocycler or water baths at 94°C, 55°C and 72°C

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**DNA fingerprints from a murder case**

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Common Vectors used in Gene Therapy

1. Retroviruses (RNA viruses)
2. Adenoviruses (DNA viruses)
3. Liposomes
4. Naked DNA

1. Modified Retroviruses (RNA viruses) (1 of 2)

Advantages
- Good at
  - Used with partial success treating Gaucher’s disease
  - Successfully cured 4 babies of S.C.I.D.S. in early 2000
    - Severe Combined Immunodeficiency Syndrome (Bubble Baby)

Disadvantages
1. Inserts genes randomly. Possible Consequences?
2. Usually needs an actively dividing host cell
  - Therefore, not used for Cystic Fibrosis
3. Modified virus may

Use of Gene Therapy to modify blood stem cells
e.g. S.C.I.D.S. and Gaucher Disease

2. Liposomes

- hollow sphere surrounded by a lipid bilayer
- Place gene of interest inside
- Clinical trials underway with the CFTR gene

Advantages
- Clinical trials underway with the CFTR gene

Disadvantages

3. Modified Adenoviruses—a DNA viruses

Advantages
- Most adenoviruses don’t cause
- Clinical trials are underway with the CFTR gene

Disadvantages
- Inefficient at
4. Naked DNA

Advantages
- No threat

Disadvantages
- Very inefficient at

Injecting DNA into a Cell

Problems Doing Gene therapy (1 of 2)
Inefficient gene delivery — not suitable for all genetic diseases

1. Most effective if
   - Only to correct a few cells with the gene
   - E.g. Blood stem cells: SCIDS and Gaucher Disease

2. Less effective or Ineffective if
   - Brain cells (Tay-Sacs disease, Huntington’s disease)
   - Cystic Fibrosis

Problems Doing Gene therapy (2 of 2)

4. Insertion of Gene isn’t always permanent
   - E.g. Gaucher Disease: temporary cure until GCase gene “popped” out of chromosome

5. Insertion of gene into genome could disrupt other genes.
   - Possible consequences?

6. Some viruses elicit immune response or may cause disease
   - E.g. Jesse Gelsinger died in 1999