[The Genetic Code] DNA/RNA/Protein

**What is a Gene?**
- A region of DNA that controls a discrete hereditary characteristic, usually corresponding to a single protein.

**What is RNA?**
- The sequence of a strand of mRNA is based on the sequence of a complementary strand of DNA. Template for protein synthesis.

[The Genetic Code] DNA

**DNA (deoxyribonucleic acid)**
- Long polymer chain composed of four types of subunit
- Four subunit: Adenine (A), Cytosine (C), Guanine (G), Thymine (T)
- Double Helix
- Complementary base pair: A-T, G-C
- 25,000 genes in Human, 3 billion base pairs

[The Genetic Code] Transcription/Translation

**Transcription**
- DNA converts to a single stranded mRNA

**Translation**
- Sequence of mRNA translated to Protein
- These proteins perform various functions in and out of the cell

**The Genetic Code inside DNA determines the sequence of amino acids that make up protein**
Molecular Cell Biology

- http://www.whfreeman.com/lodish/
- http://bcs.whfreeman.com/lodish5e/
- Chapter 1 and Chapter 4

[The Genetic Code] Other Resources

- http://www.biology.iupui.edu/biocourses/N100/2k3ch13dogma.html

[Microarray] Microarray Technology

- (DNA) Microarray
  - also commonly known as gene chip, DNA chip, or biochip
  - a collection of microscopic DNA spots attached to a solid surface, such as glass, plastic or silicon chip forming an array for the purpose of expression profiling, monitoring expression levels for thousands of genes simultaneously.
- Used for genetic sequence analysis
  - New targets for drugs or other therapeutic intervention
  - Diagnostic markers for certain disease

- http://www.reactionbiology.com
[Microarray] DNA Microarray Manufacturing Methods

- **Photolithography**
  - Pioneered by Affymetrix Inc. (Largest market share)
  - Utilizing photolithography to pattern specific DNA arrays
  - Light-activated areas allow a single nucleotide to chemically couple to the substrate
  - Advantages
    - Precise
    - Small spot size
    - Control
  - Disadvantages
    - Lower Yield
    - Cost
  - Current GeneChips have 20-µm features and contain up to 400,000 different probes/chip (2001)

- **Mechanical Printing**
  - Spotting of pre-made sequence
  - Inkjet (Agilent Technologies Inc.)
  - Soft lithography & µCP (micro contact printing)
  - Advantages
    - Cheap
    - Longer Chains
  - Disadvantages
    - Less Specificity
    - Lower Density

**Photolithography Diagram**

**Mechanical Printing Diagram**

[Microarray] Case Study: Microarray Plate Technology

- **Affymatrix GeneChip Microarrays**
  - Presentation 1: An Overview of the Manufacturing of GeneChip® Microarrays
  - Presentation 2: The Structure, Function, and Applications of GeneChip® Microarrays

- **Spotting of Microarrays**
  - Movie 1: Microarray Video

[Microarray] DNA Hybridization

- **Strategy is in selective attachment of molecules for screening**
  - Use robot to spot many “target” molecules (DNA or protein).
  - Add second (unknown) sample.
  - Some molecules may bind to complementary molecules.
  - Wash.
  - Add fluorescent label that only attaches to bound molecules. Look for fluorescent locations.
  - Large number of spots = high throughput screen.

**DNA Hybridization Diagram**
**[Microarray] Protein Microarray**

- Implementation of a variety of protein-protein, protein-drug, and protein-small molecule interactions
- Implementation of a variety of sandwich assays
  - Detection can be colorimetric using secondary antibodies labeled with AP, HRP, biotinylated secondary antibodies that bind labeled streptavidin or direct fluorescent labeling.
- Implementation of reverse phase microarrays
- Implementation of reverse phase microarrays
- Implementation of synthetic proteins, peptides, and engineered proteins to detect the presence of proteins in complex samples

**[Microarray] Antigen/Antibody Binding**

- **Sandwich Assay**
  1. Plate is coated with a capture antibody
  2. Sample is added, and any antigen present binds to capture antibody
  3. Detecting antibody is added, and binds to antigen
  4. Enzyme-linked secondary antibody is added, and binds to detecting antibody
  5. Substrate is added, and is converted by enzyme to detectable form

http://www.proteinmicroarrays.com

http://en.wikipedia.org/wiki/ELISA