PCR
Review: Important Concepts

Nucleus - Contains DNA - the blueprint for all genetic information

Chromosomes - much longer sequences of DNA that contain many genes

Genes - sequence of DNA that tells the cell how to make a single PROTEIN

https://www.youtube.com/watch?v=V9BZ3zx8b8I  (Bill Nye video)
What is a Genome?

A **genome** is the genetic material of an organism.

It consists of DNA (or RNA in RNA viruses). The **genome** includes both the genes (the coding regions) and the noncoding DNA, as well as the genetic material of the mitochondria and chloroplasts.

The **human genome** consists of the 23 pairs of chromosomes found in the cell nucleus plus the DNA found in the mitochondria.

https://en.wikipedia.org/wiki/Genome

https://genographic.nationalgeographic.com/genetics-overview/
Polymerase Chain Reaction - PCR

Major Breakthrough in the early 1980s
Kerry Mullis – 1993 Nobel Prize

Short stretches of DNA could be copied very quickly and easily – DNA synthesis in a tube

Applications
- Forensics (CSI)
- Evolutionary Relationships
- Cloning (Jurassic Park)
- Genetic Testing
PCR Concepts: Chemical Nature of DNA

DNA is double stranded.
Each strand is the complement of the other

A T G C C G A A T
T A C G G C T T A

Double Helix

Polymer of nucleotide Base pairs
Adenine (A) pairs with Thymine (T)
Cytosine (C) pairs with Guanine (G)

http://www.youtube.com/watch?v=qy8dk5iS1f0
PCR is DNA Replication – In a Test Tube

1. Base pair rules must be followed
2. New strands made in one direction 5’ to 3’
3. Instead of copying the entire genome, the primers direct the reaction
DNA Polymerase
What Do You Need to Set Up a PCR Reaction?
What Do You Need to Set up a PCR Reaction?

1. DNA Template: the DNA containing the region you wish to amplify

2. DNA polymerase: the enzyme that builds the complementary DNA strand (In Master Mix)

3. dNTPs: deoxy nucleotide triphosphates: the DNA building blocks – dATP, dCTP, dGTP, dTTP (In Master Mix)

4. Buffer: the correct salts, ions needed for the polymerase to function well (In Master Mix)

5. Primers: short single-stranded pieces of DNA that are complementary to either end (5’/3’) of the region of DNA that you want to amplify
PCR Ingredients

1. DNA “template”  
   Your purified DNA sample

2. Taq Polymerase  
   Heat-stable DNA polymerase

3. Deoxynucleotides (dNTPs)  
   Building blocks of DNA

4. Primers  
   Small pieces of DNA bind to your gene

5. Buffer and water  
   Maintain pH of reaction
Will *any* DNA polymerase work for PCR?
Taq DNA polymerase was isolated from the bacterium *Thermus aquaticus*.

*Taq* polymerase is stable at the high temperatures (~95°C) used for denaturing DNA.

Now researchers could add DNA polymerase once and it would work for 30 cycles.
PCR: First Cycle

3 Steps

1) **Denature** template DNA – 95 degrees

2) **Anneal** – Primer binds to complimentary site 45-72 degrees

3) **Extension** - Taq polymerase synthesizes new strand – 68-72 degrees

4) **Return** to denature and repeat 30X

http://www.dnai.org/b/index.html
More Cycles = More DNA

Each cycle DOUBLES the amount of target DNA

Cycle 3 is the first cycle where a double stranded molecule is produced that is the EXACT size of the target DNA

TARGET DNA IS DEFINED BY THE DISTANCE BETWEEN TWO PRIMERS
# The Power of PCR

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[https://www.youtube.com/watch?v=iQsu3Kz9NYo](https://www.youtube.com/watch?v=iQsu3Kz9NYo)
What is PCR and What it is Not?

http://www.youtube.com/watch?v=6iFDphWXjw4
Classic PCR Animations

1) http://www.dnalc.org/ddnalc/resources/pcr.html
2) http://www.youtube.com/watch?v=x5yPkxCLads
3) http://www.hhmi.org/biointeractive/polymerase-chain-reaction-pcr
4) https://www.youtube.com/watch?v=iQsu3Kz9NYo