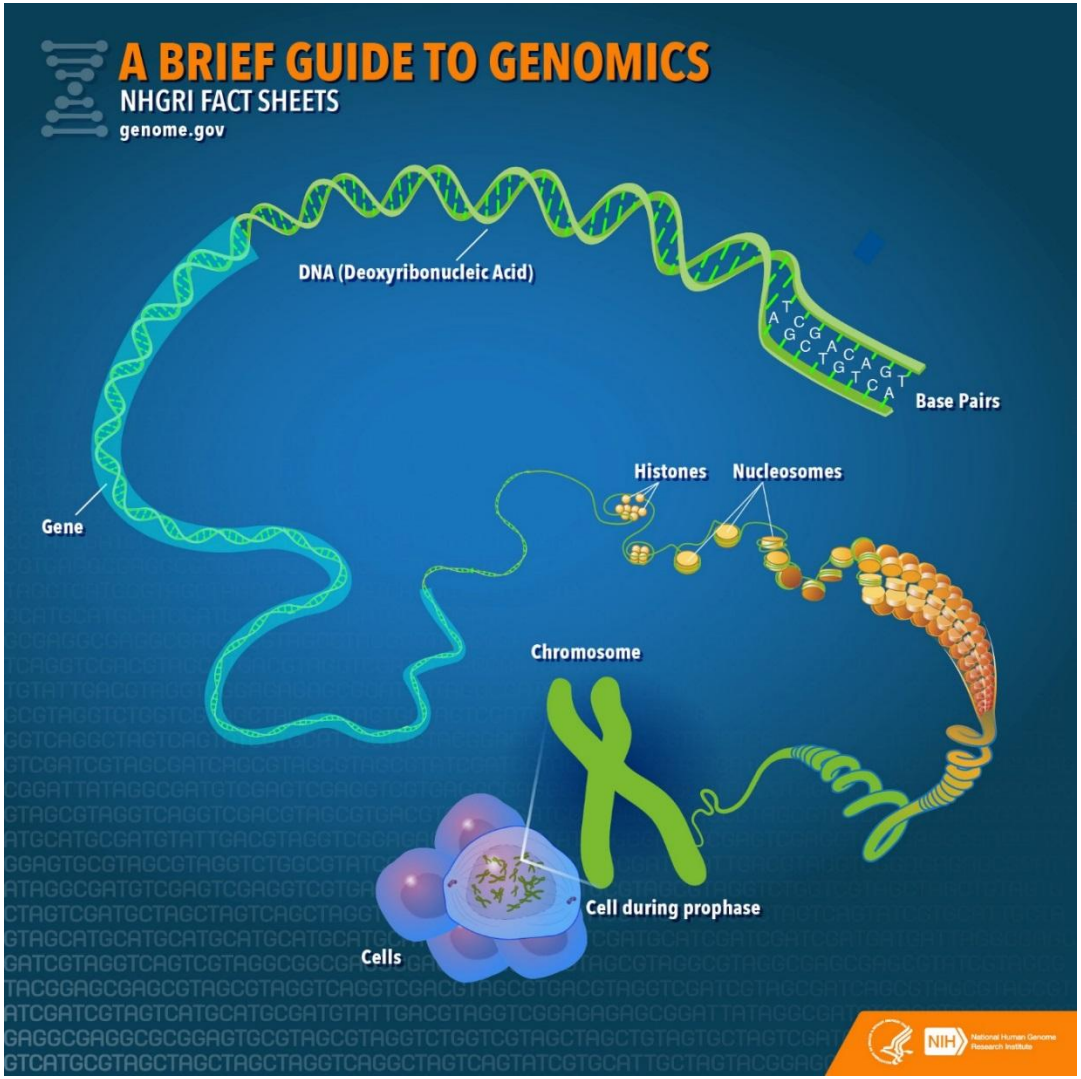
Identification of a Pathogenic Agent Using Polymerase Chain Reaction (PCR)

Influenza Outbreak Investigation

PCR: Important Concepts

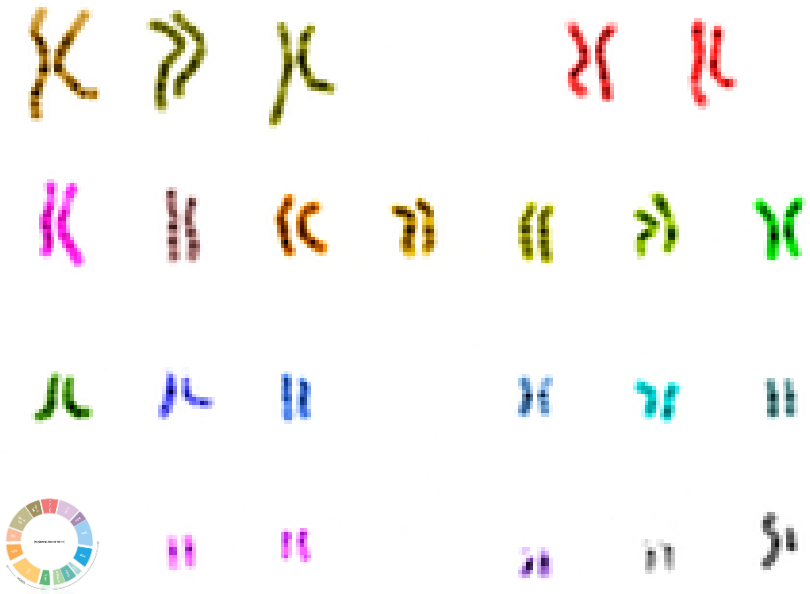


- Nucleus - Contains ***DNA*** - the blue print or instructions for all genetic information
- Chromosomes - much longer sequences of DNA that contain many genes
- Genes - sequence of DNA that tells the cell how to make a single ***protein***

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

What is a Genome?

- ***Genome***
  - all the genetic material of an organism
  - includes both the genes (the coding regions) and the noncoding DNA, and DNA of the mitochondria and chloroplasts.
- **<u>Human</u> genome**
  - 23 pairs of chromosomes plus the DNA in the mitochondria

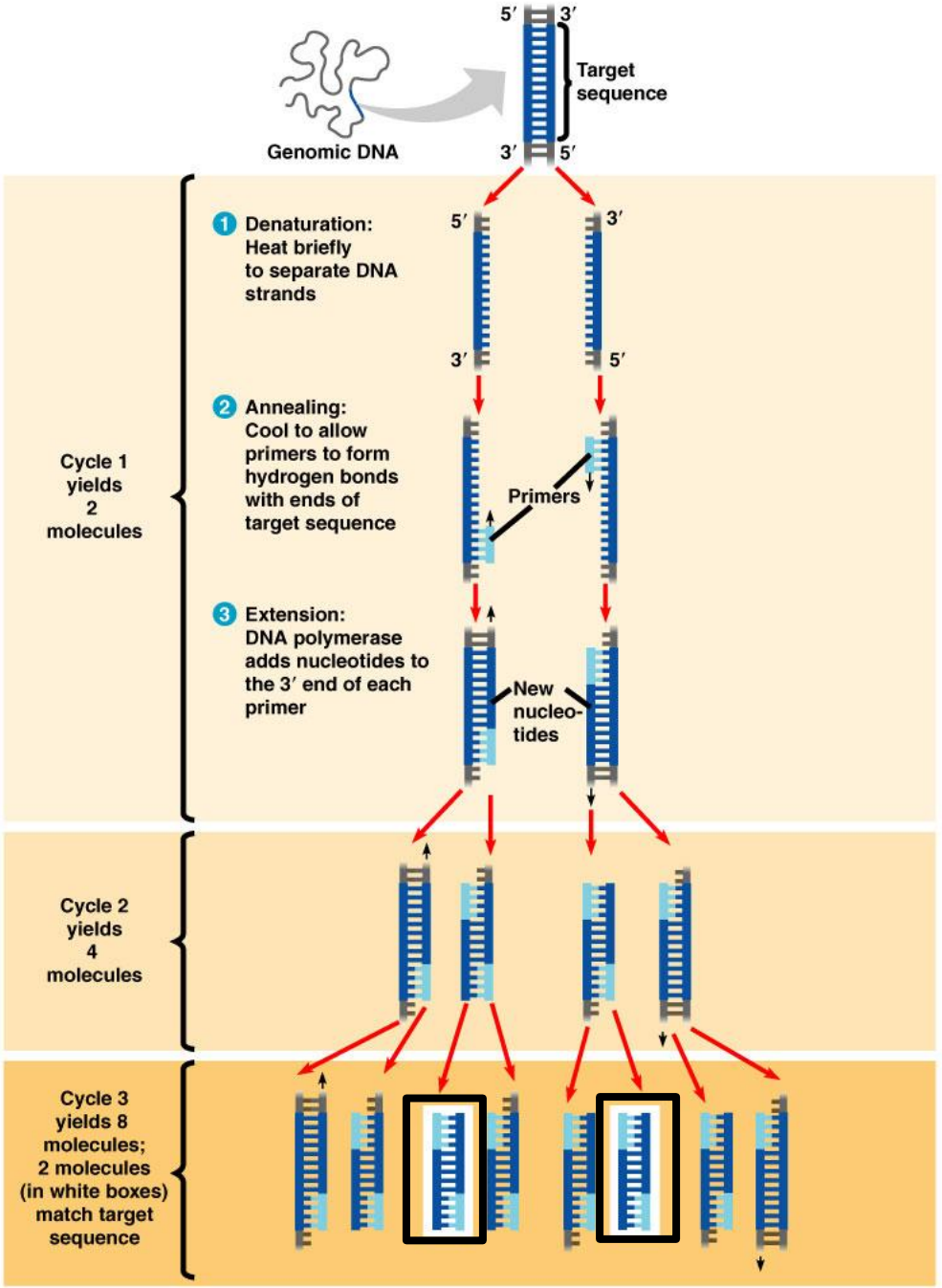


https://en.wikipedia.org/wiki/Genome

https://genographic.nationalgeographic.com/genetics-overview/

Polymerase Chain Reaction (PCR)

- Major Breakthrough in the early 1980s by 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 (GMOs)
  - Genetic Testing



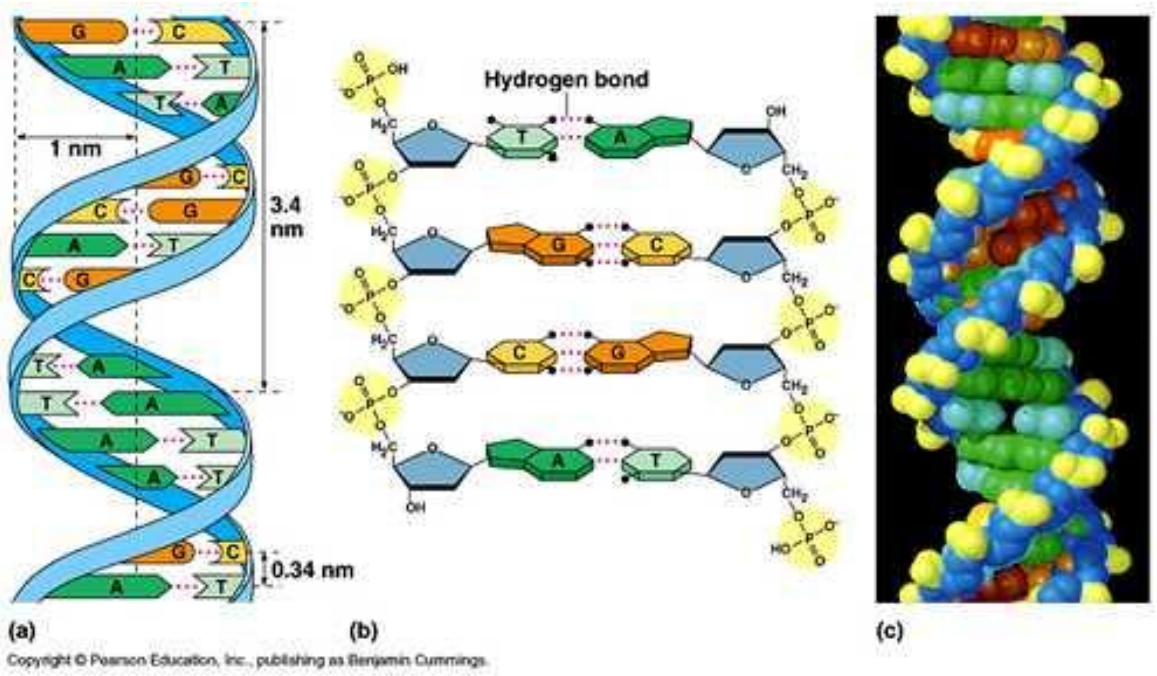
PCR Concepts: Chemical Nature of DNA

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

Primers Determine DNA to be Copied

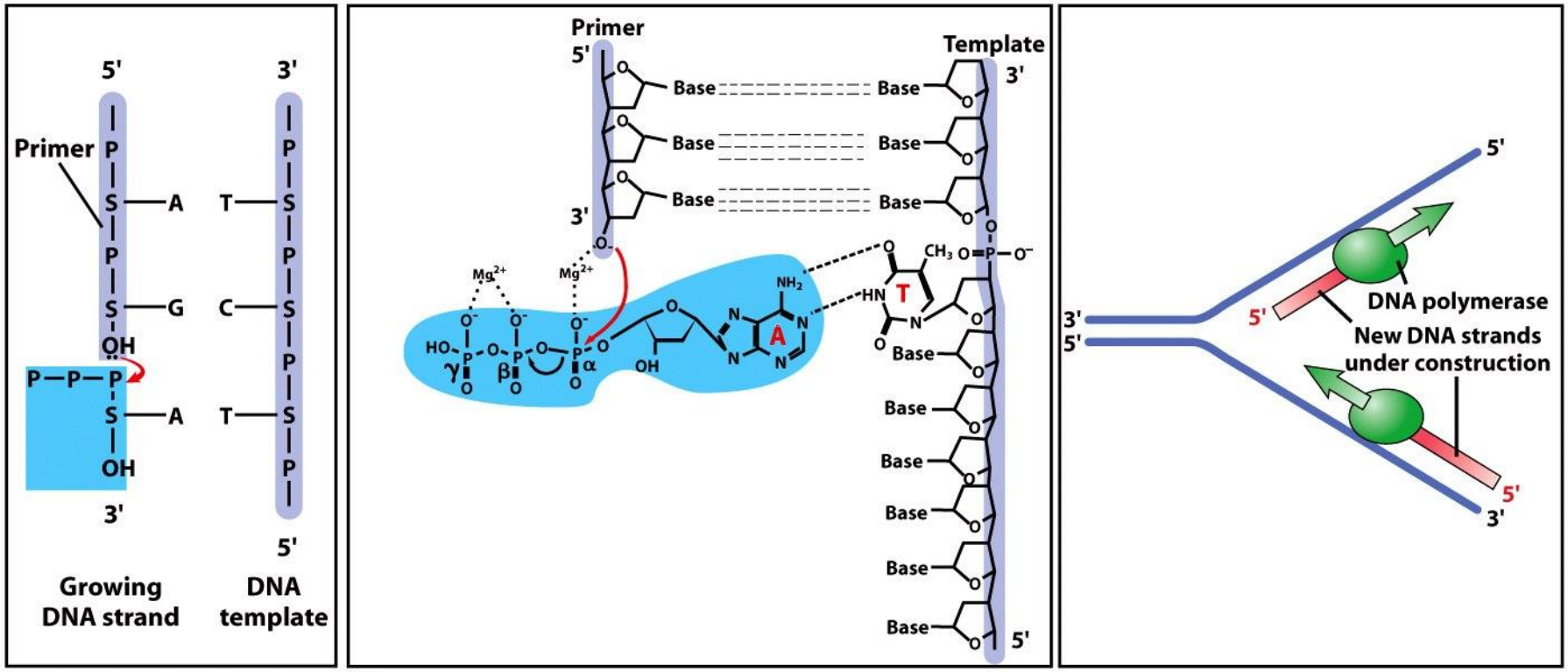
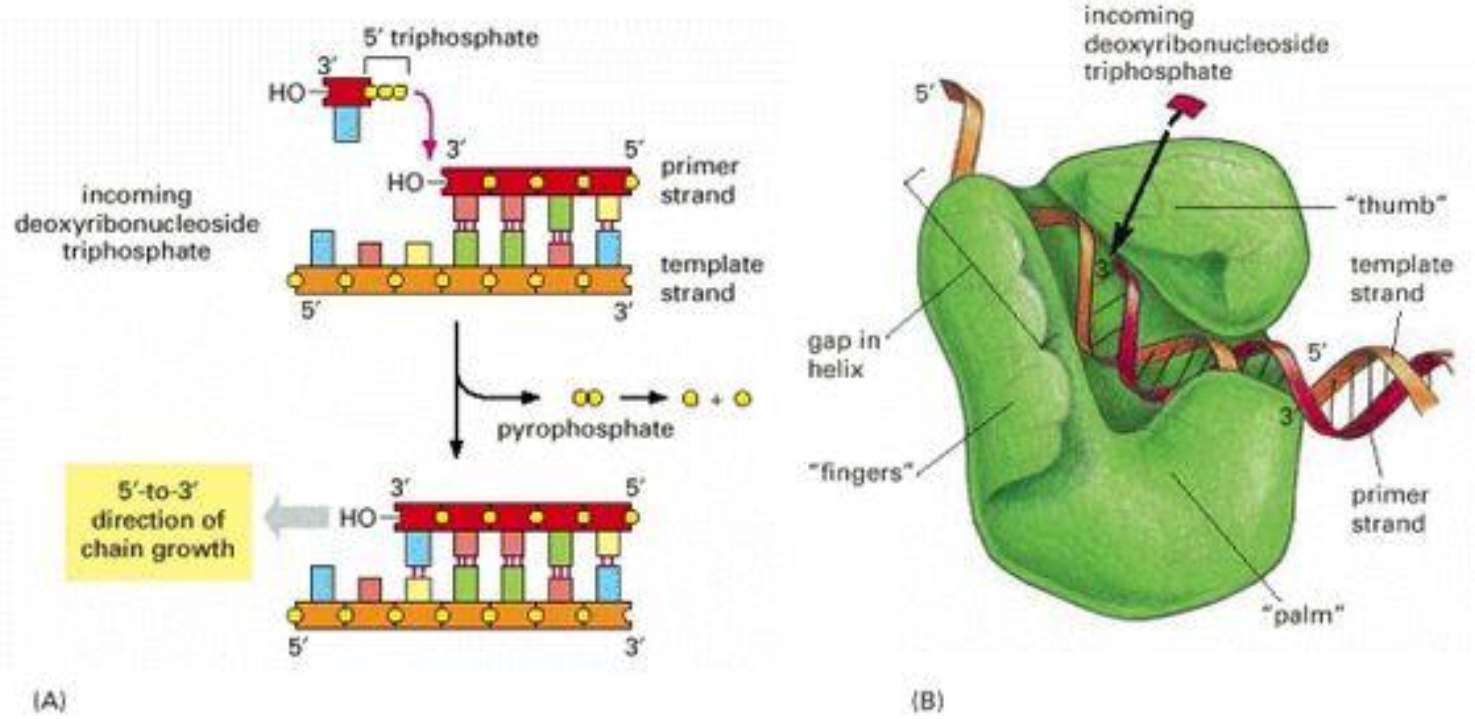


Figure 13-8 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

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



https://www.pinterest.com/pin/549228117026609182/

What Do You Need to Perform PCR?

PCR Ingredients

PCR Tube with 2X OneTaq

1.7 ml Microfuge Tube

1. DNA "template" — *Your DNA sample*
2. DNA 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*
6. 2X One Taq Master Mix also contains — *Gel Dye and glycerol*

Will *any* DNA polymerase work for PCR?

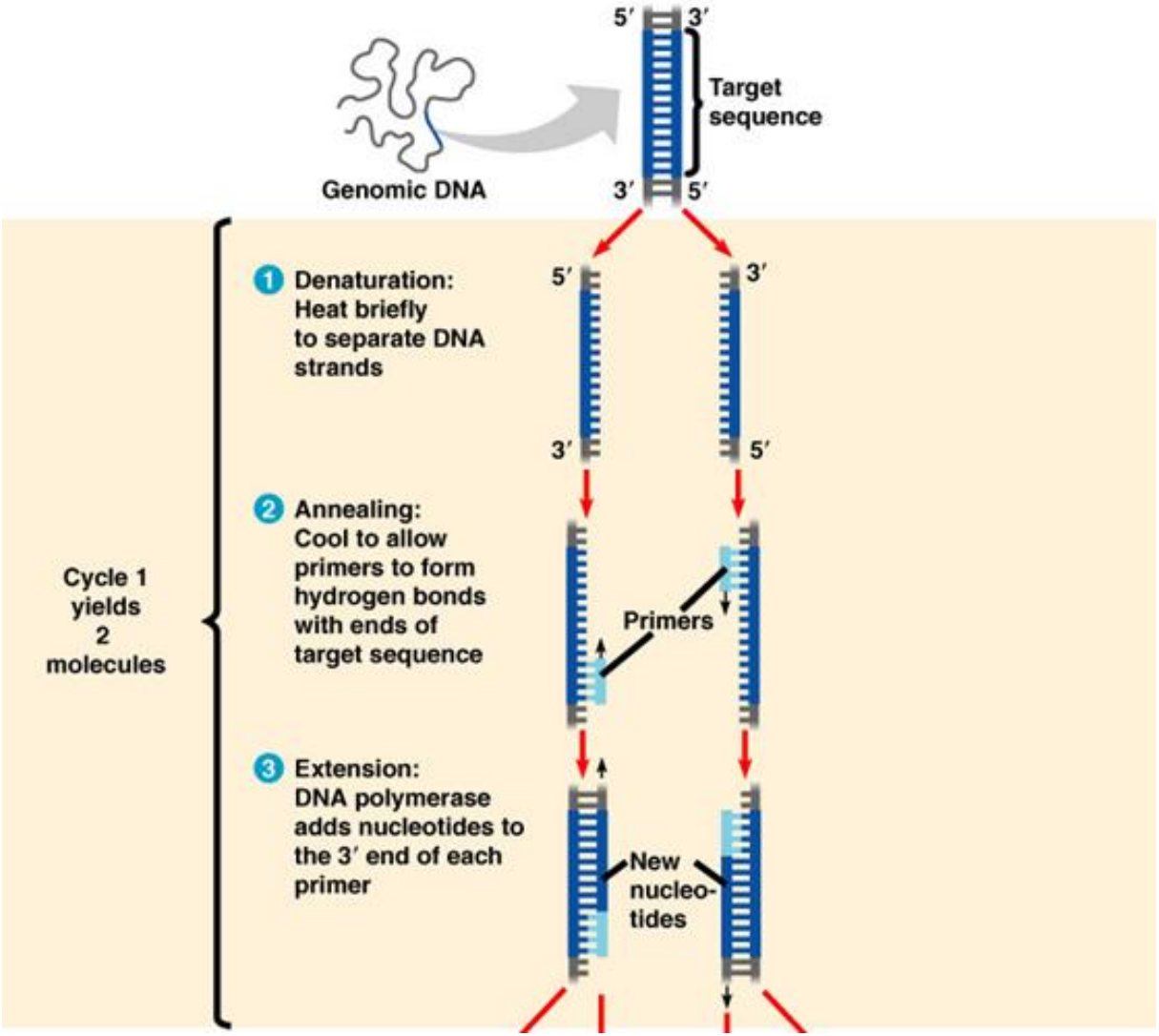
Breakthrough: *Taq* Polymerase Was the Key

- *Taq* DNA polymerase was isolated from the bacterium *Thermus aquaticus.*
- *Taq* polymerase is stable at the high temperatures (~95°C) used for denaturing DNA.

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



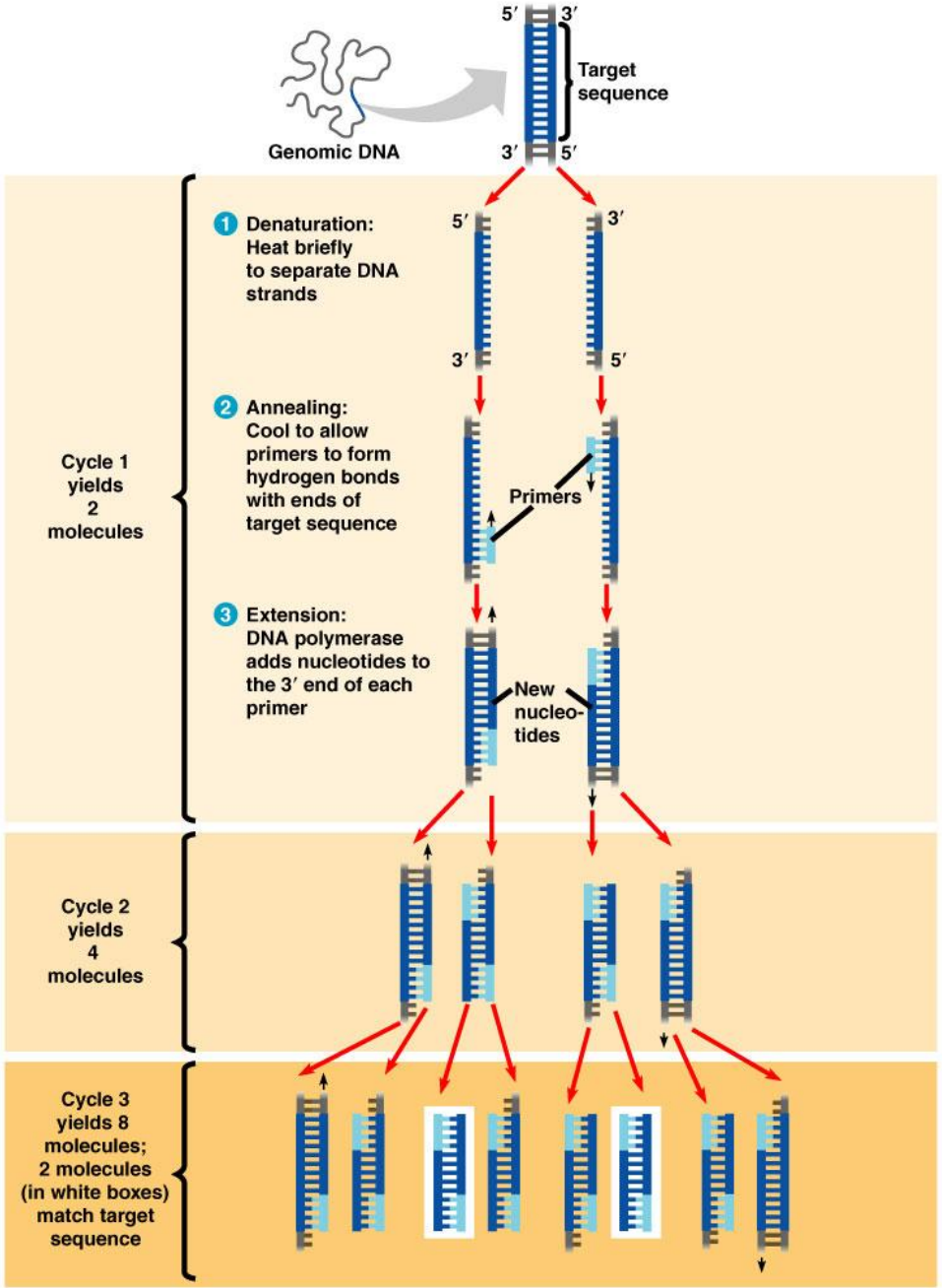
http://www.dnai.org/b/index.html

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

Number of PCR Cycles (n)	Copies of DNA (2n)
0	1
1	2
2	4
3	8
4	16
5	32
6	64
7	128
8	256
9	512
10	1024
20	1,048,576
30	1,072,741,824

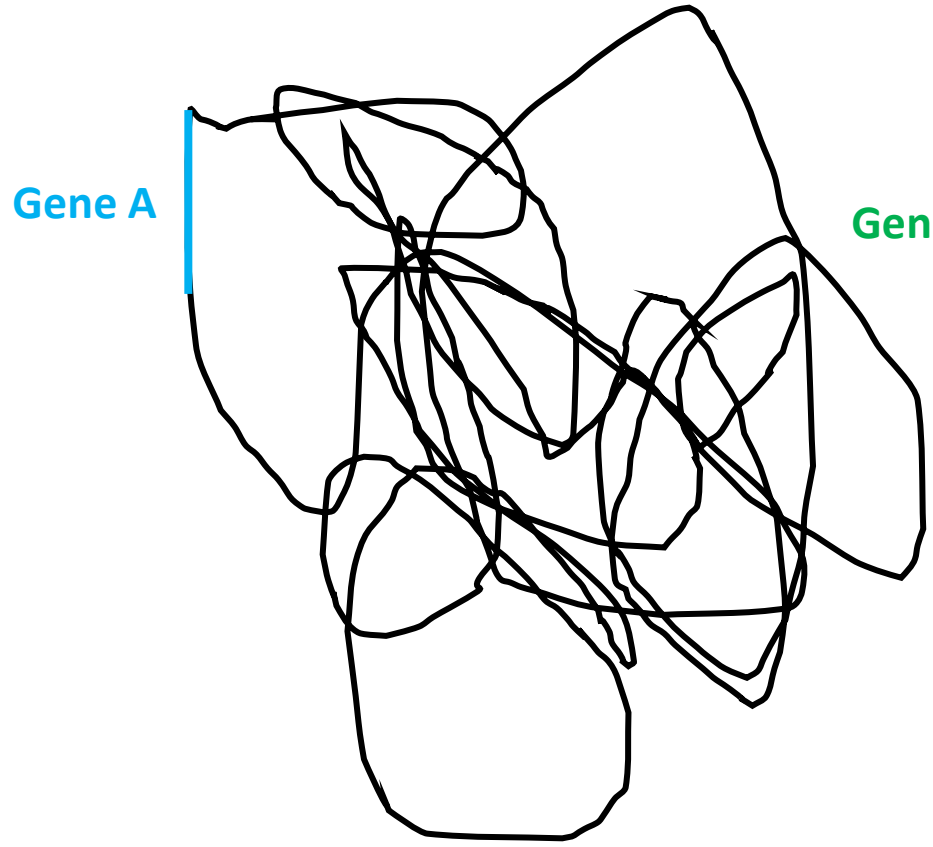
https://www.youtube.com/watch?v=iQsu3Kz9NYo

Detecting a Pathogen in Serum

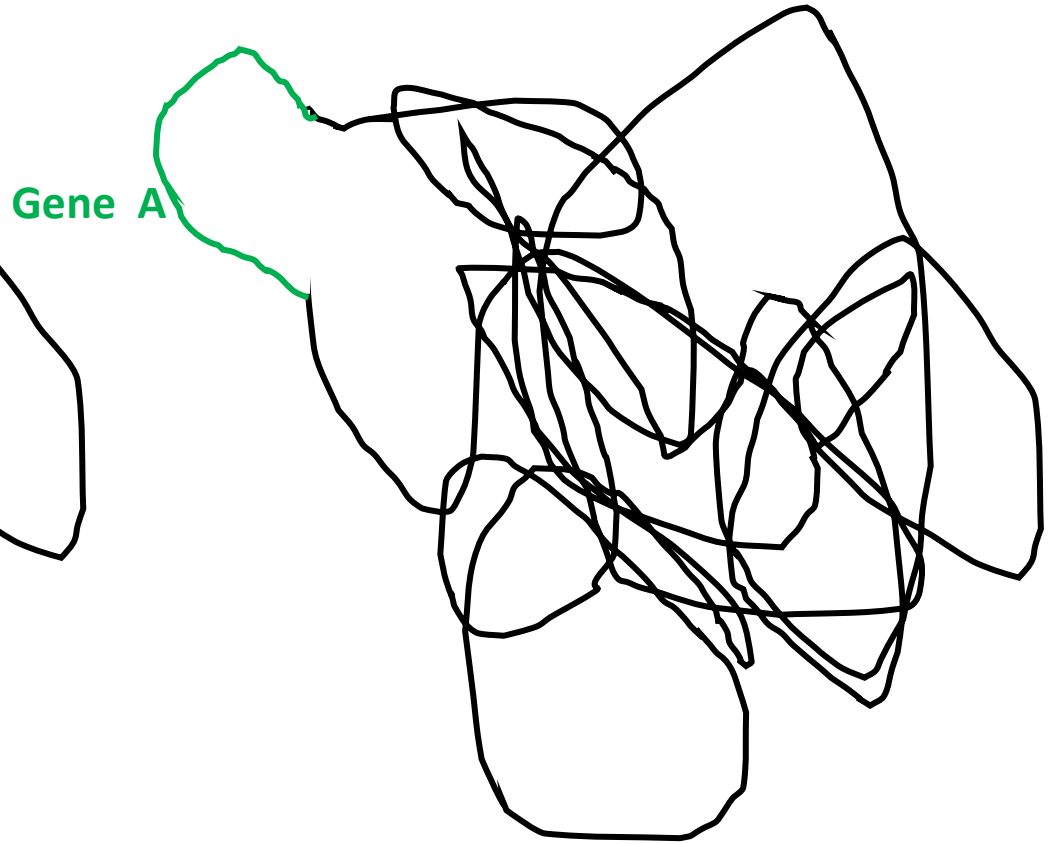
- Viruses are too small to see under a microscope
- Often there are too few of them to detect by ELISA
- **Polymerase Chain Reaction (PCR)** is simple, rapid, specific, and more sensitive than other detection methods
- PCR is an *in vitro* (test tube) method that ***amplifies*** (makes many copies of) specific target sequences in nucleic acids
- Powerful tool to detect and identify organisms, from microbes to humans

The Genome of a Pathogen is Relatively Large

Focus on specific regions of the genome (1 or 2 genes)



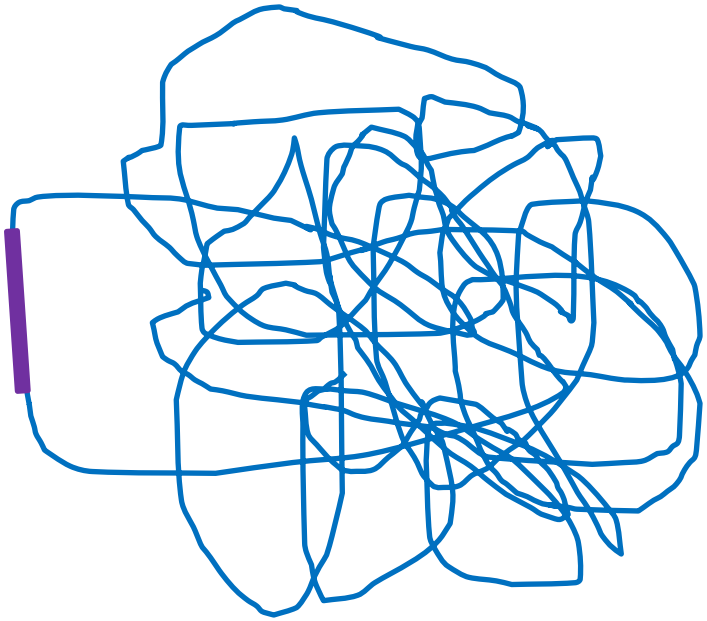
Viral Strain 1.1



Viral Strain 1.2

The Genome of a Pathogen is Relatively Large

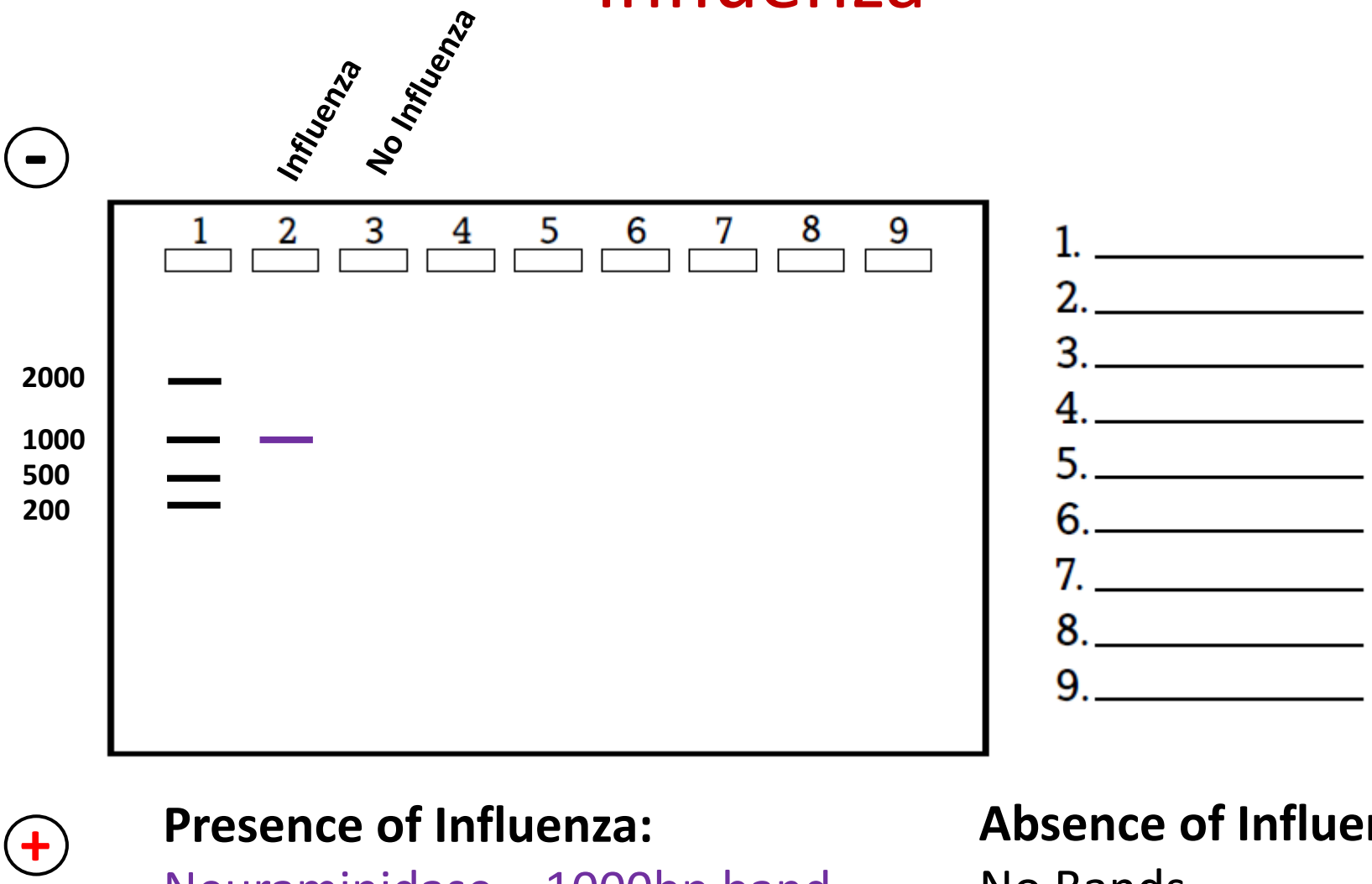
Focus on specific regions of the genome (1 or 2 genes)
The influenza ***neuraminidase*** gene is unique to influenza



Influenza genome

Other viral genome

Sample PCR Results to Detect Influenza



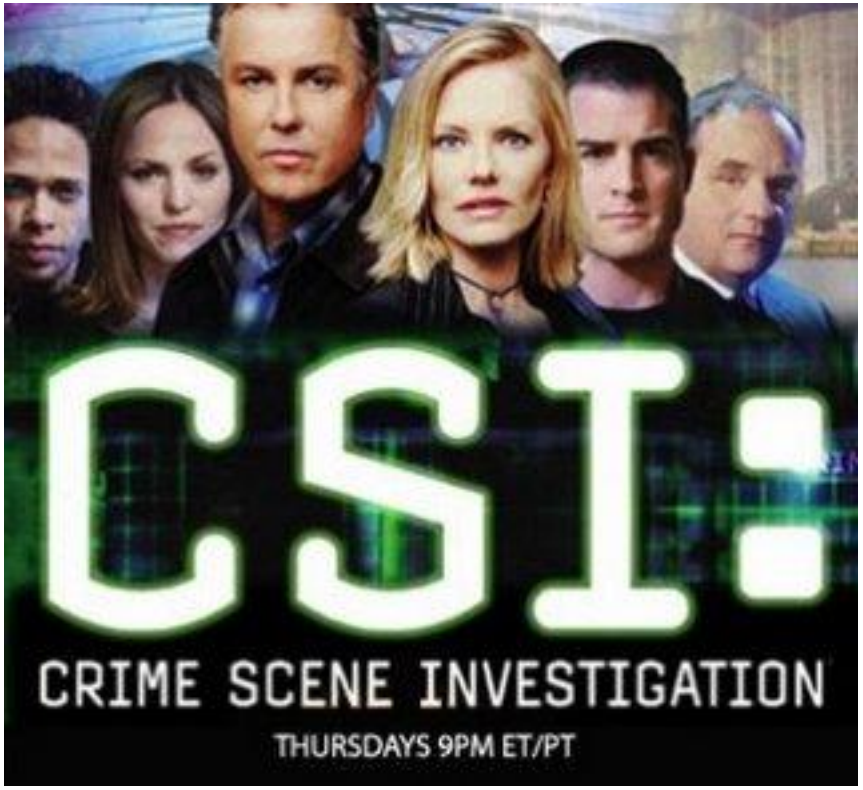
Presence of Influenza:

Neuraminidase = 1000bp band

Absence of Influenza:

No Bands

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