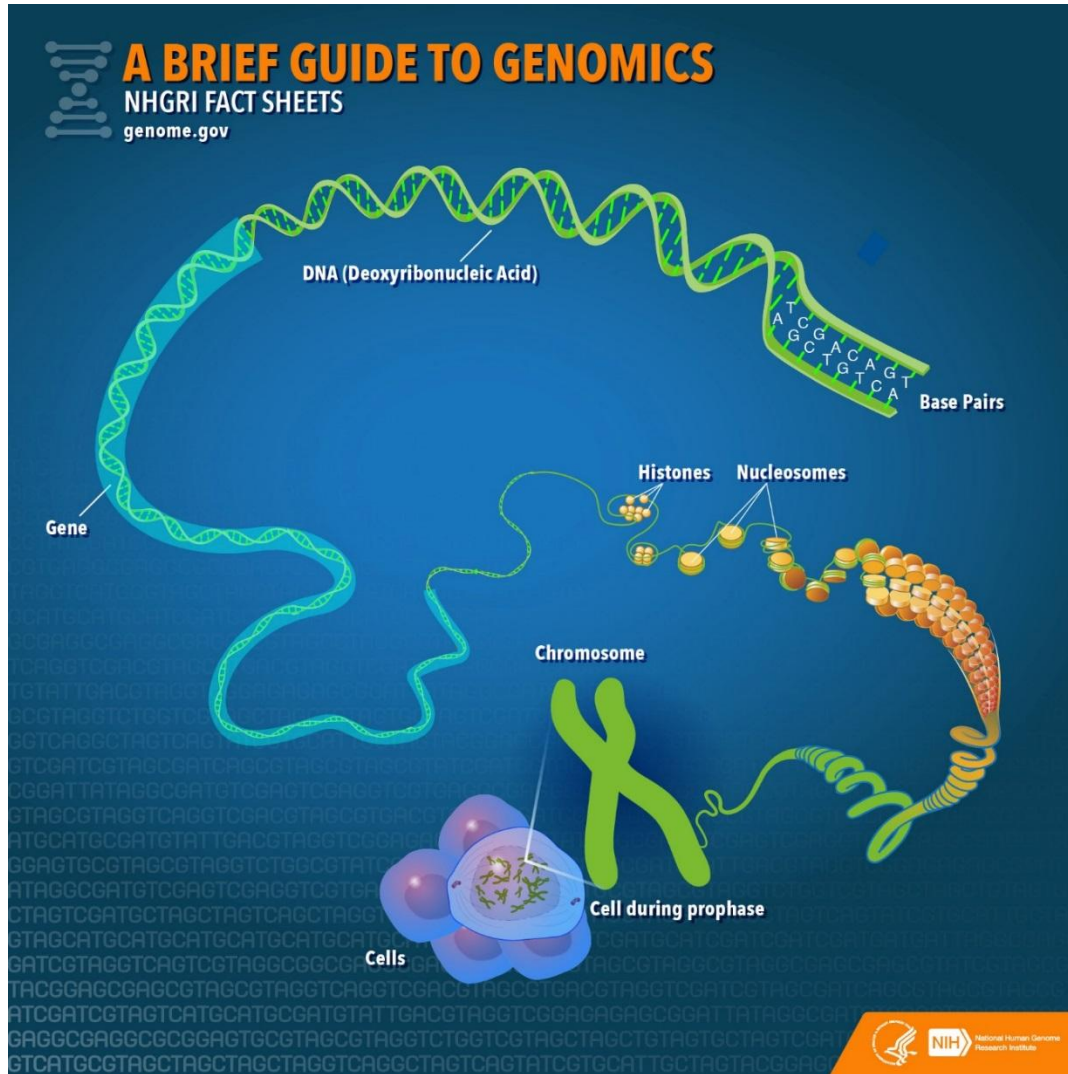


# **Identification of Gene Amplification Using Polymerase Chain Reaction (PCR)**

Cancer Biology  
2019

# PCR: Important Concepts

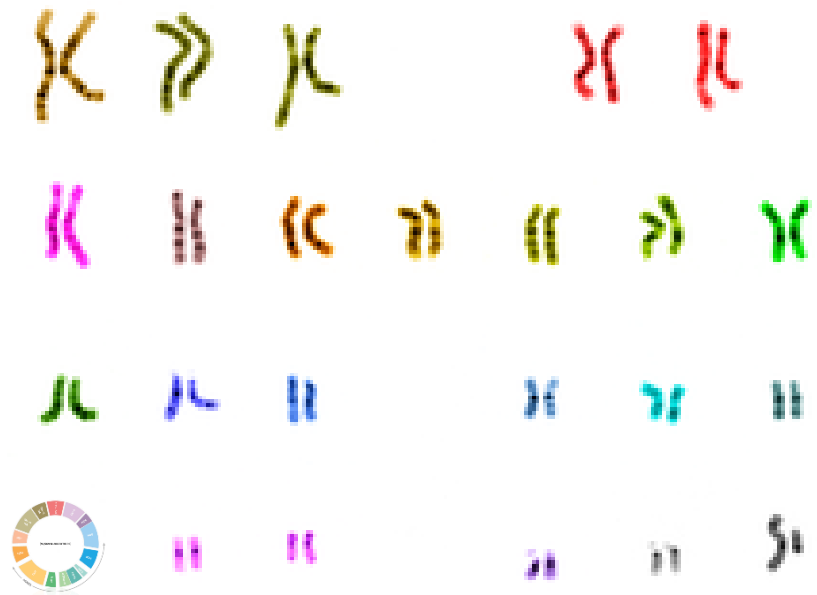


- **Nucleus** - Contains **DNA** - the blue print or instructions for all genetic information
- **Chromosomes** - much longer sequences of DNA that contain many genes
- **Genes** - sequence of DNA that tells the cell how to make a single **protein**

# 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.

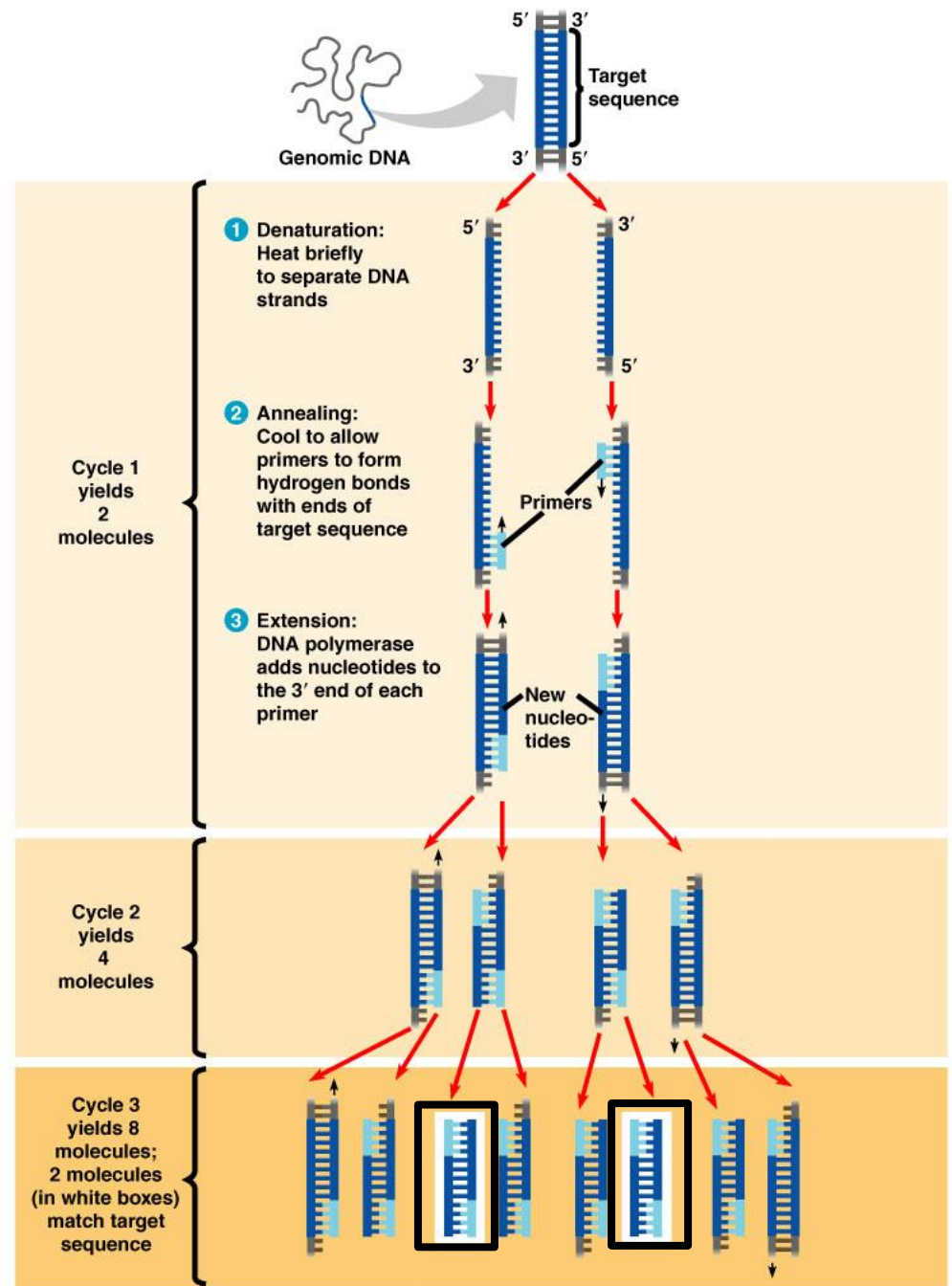


- **Human genome**

- 23 pairs of chromosomes plus the DNA in the mitochondria

# 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



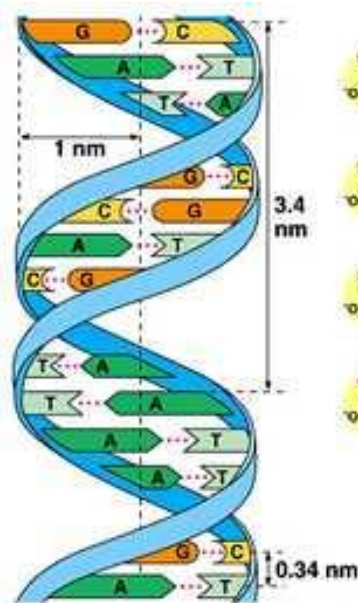
# PCR Concepts: Chemical Nature of DNA

DNA is double stranded.

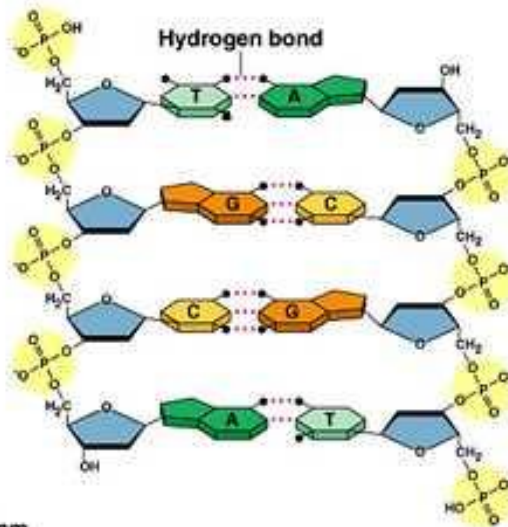
Each strand is the complement of the other

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

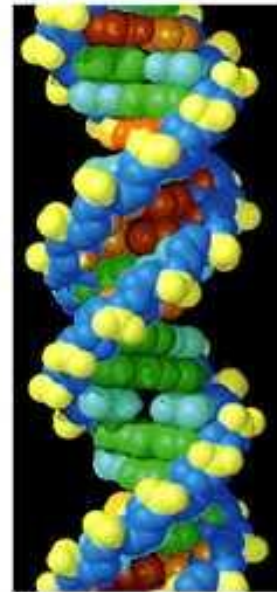
Double Helix



(a)  
Copyright © Pearson Education, Inc., publishing as Benjamin Cummings.



(b)



(c)

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

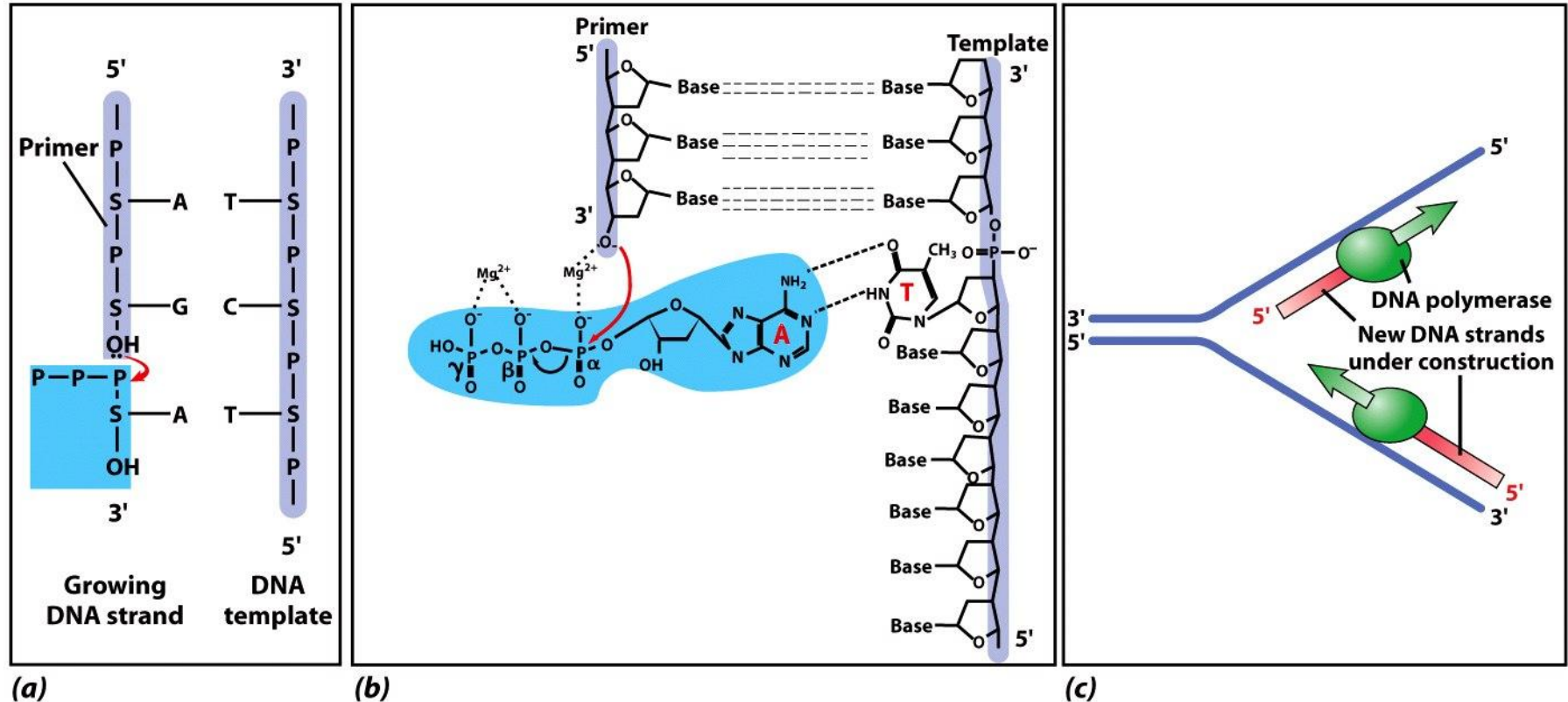
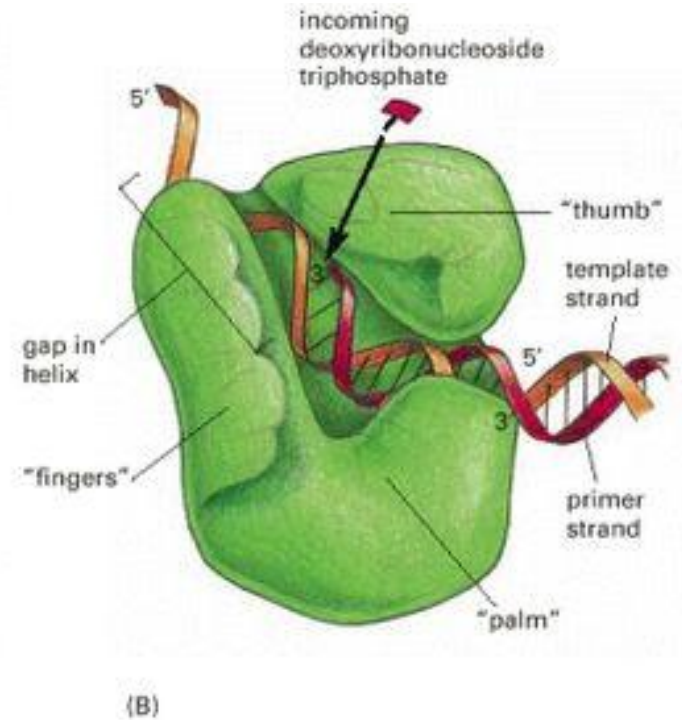
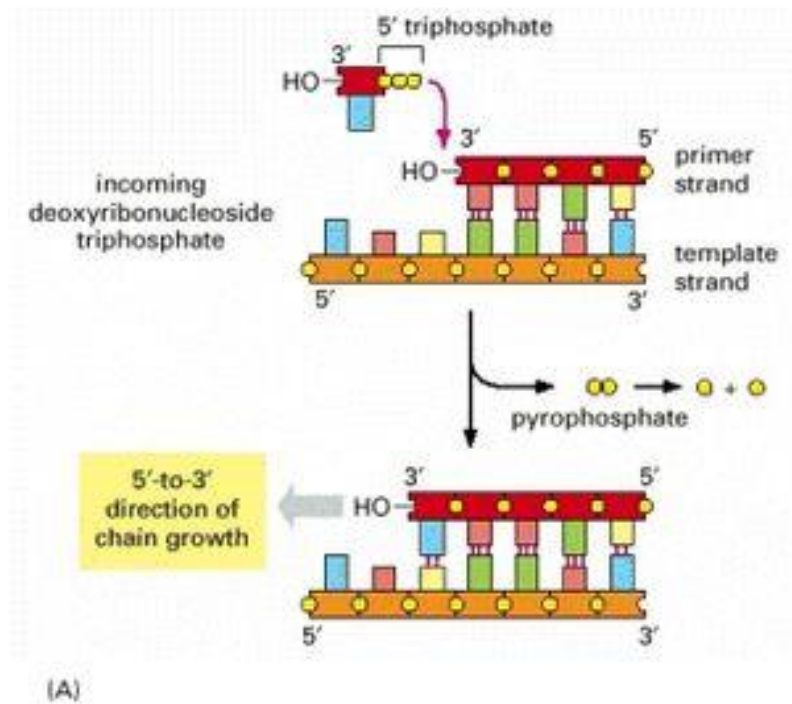


Figure 13-8 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

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



# 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

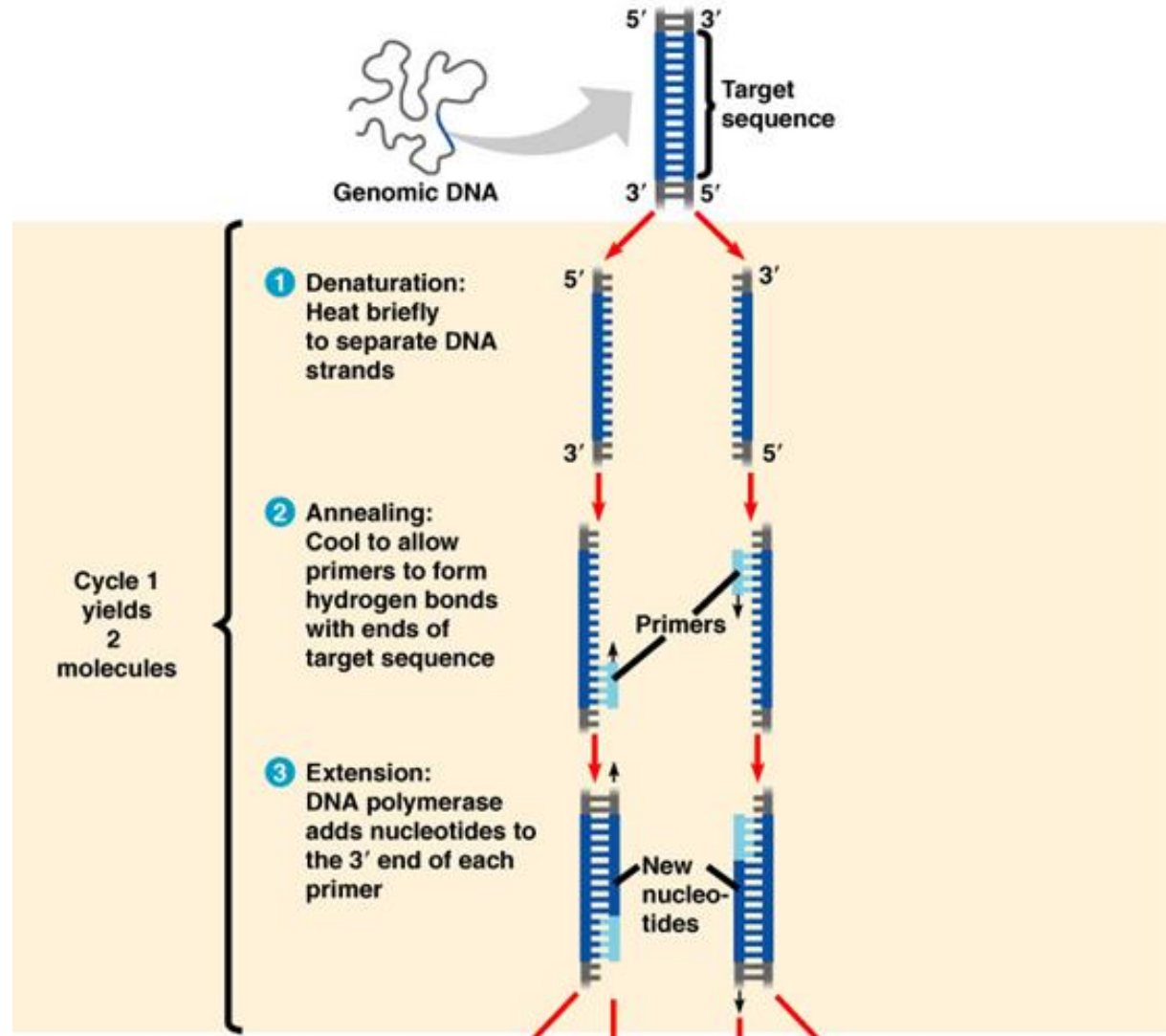
- *Taq* DNA polymerase was isolated from the bacterium *Thermus aquaticus*.
- *Taq* polymerase is stable at the high temperatures ( $\sim 95^{\circ}\text{C}$ ) used for denaturing DNA.



# 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

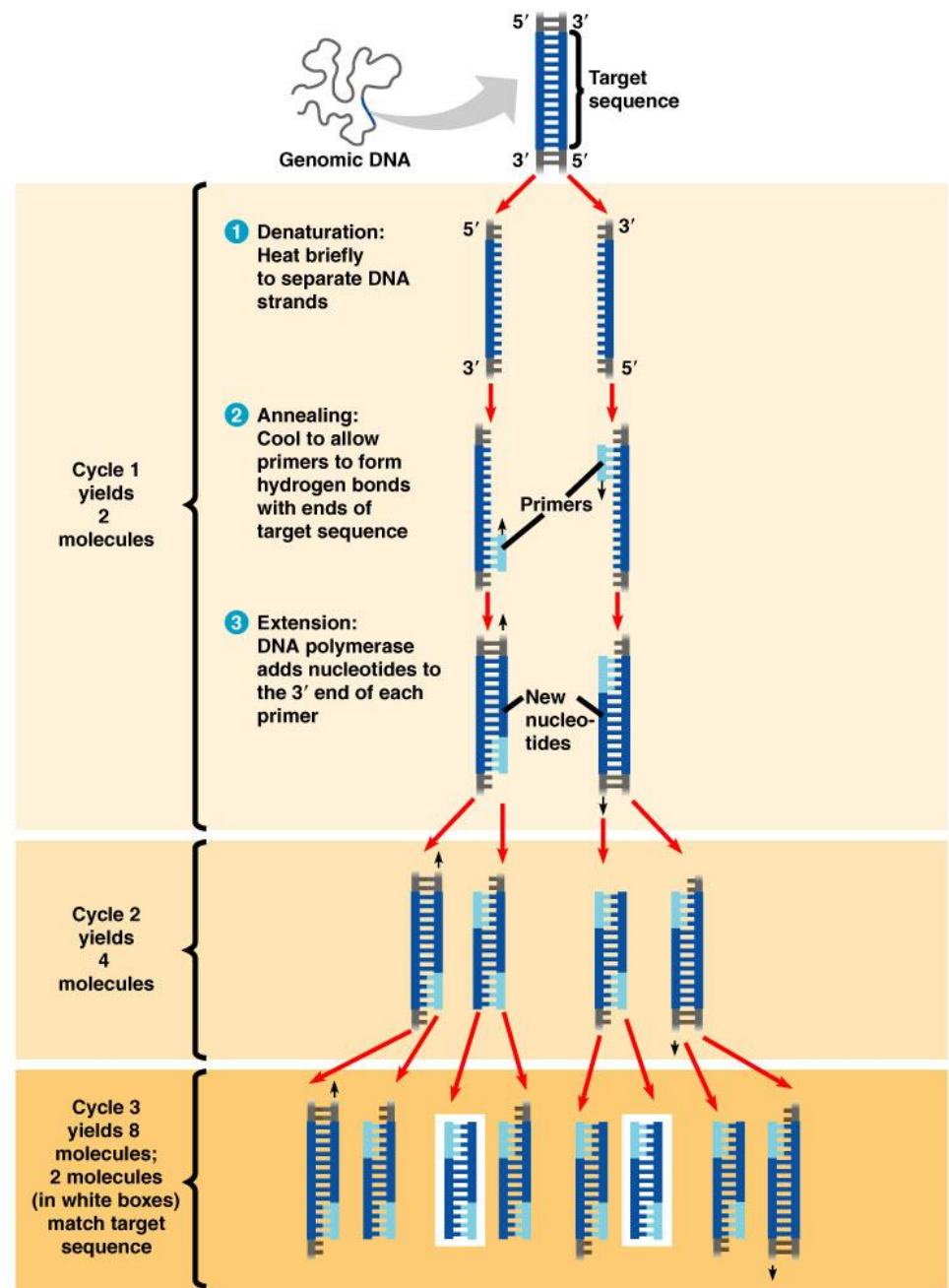


# More Cycles = More DNA

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 ( $2^n$ )
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

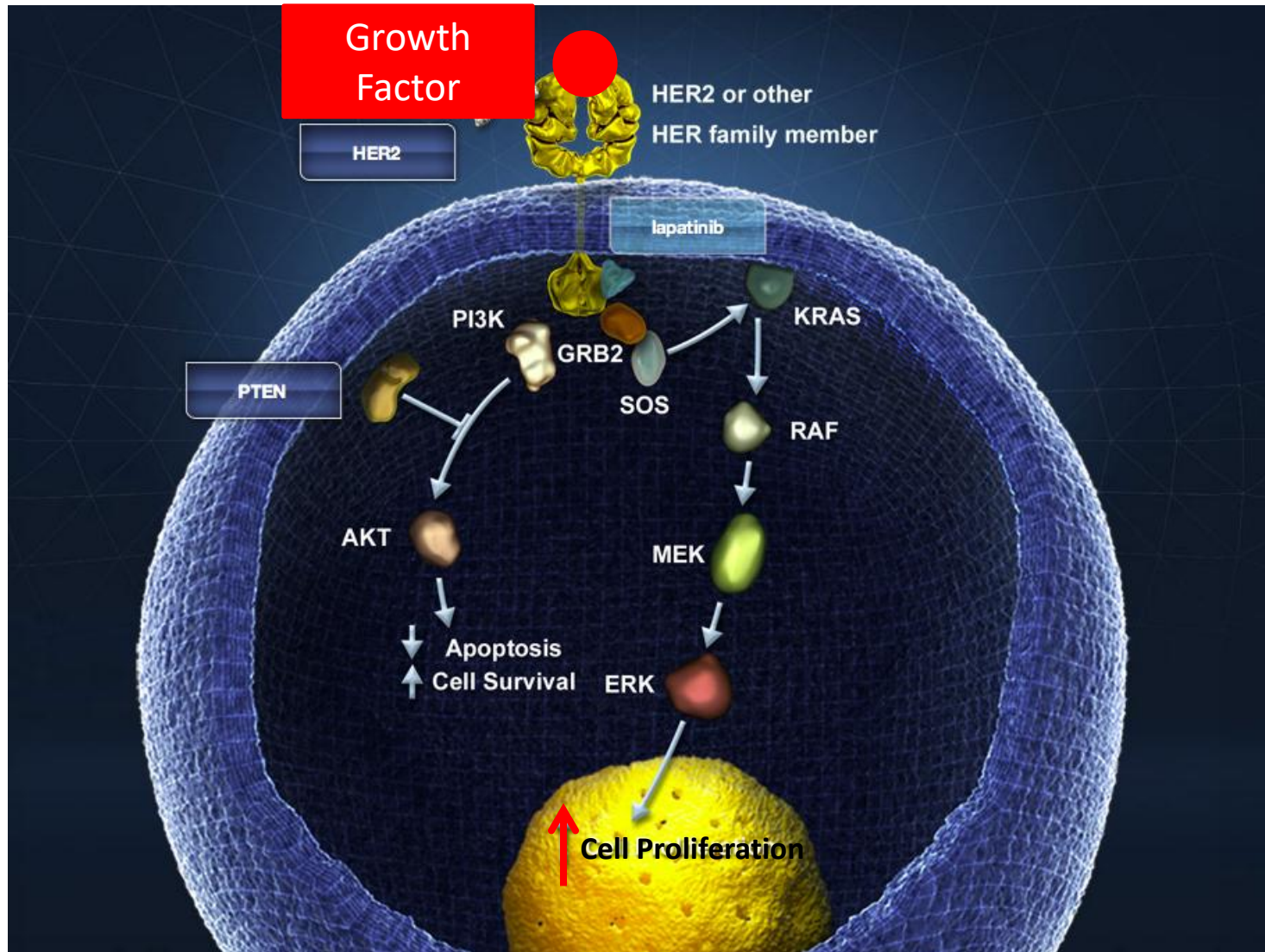
<https://www.youtube.com/watch?v=iQsu3Kz9NYo>



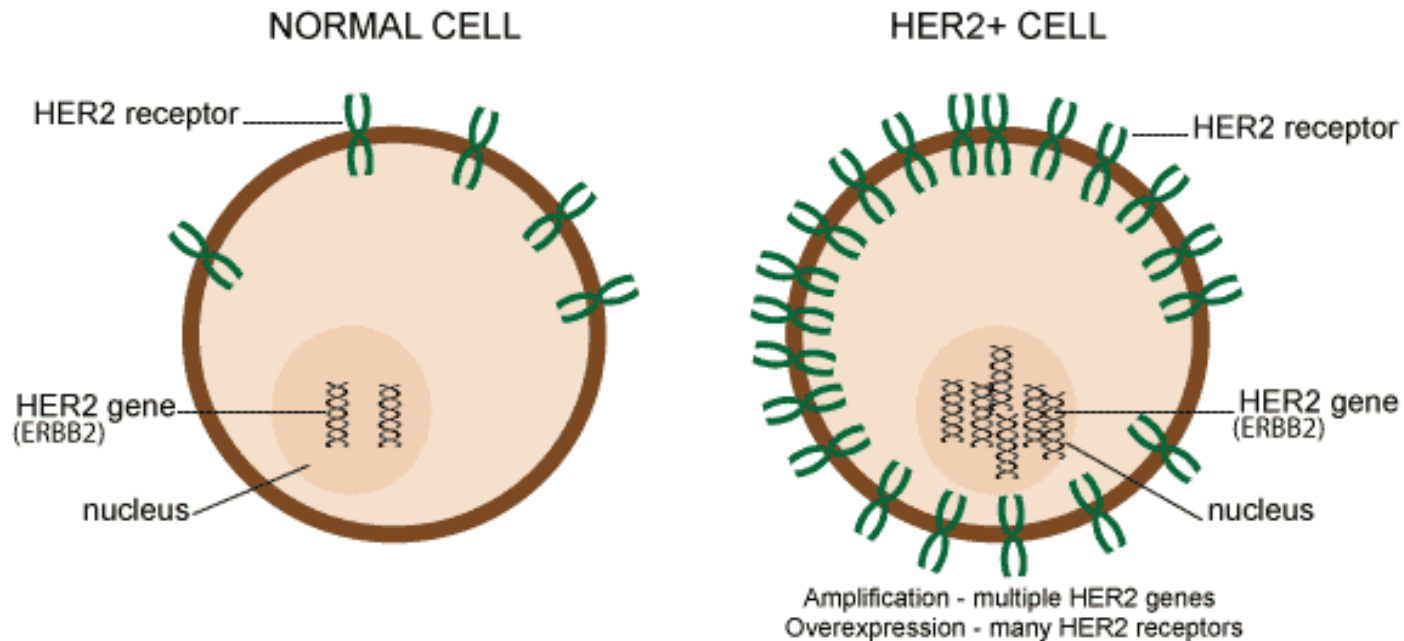
# HER2

- Receptors are proteins on the cell surface that receive signals, often causing the cell to divide
- HER2 is a type of **h**uman **e**pidermal growth factor **r**eceptor (binds to growth factors).
- **Gene Amplification** = new copies of the *HER2* gene in the genome → more HER2 protein on the cell surface → more signaling → development of breast cancer.
- Found in approximately 30% of breast cancer patients.

# HER2 Function



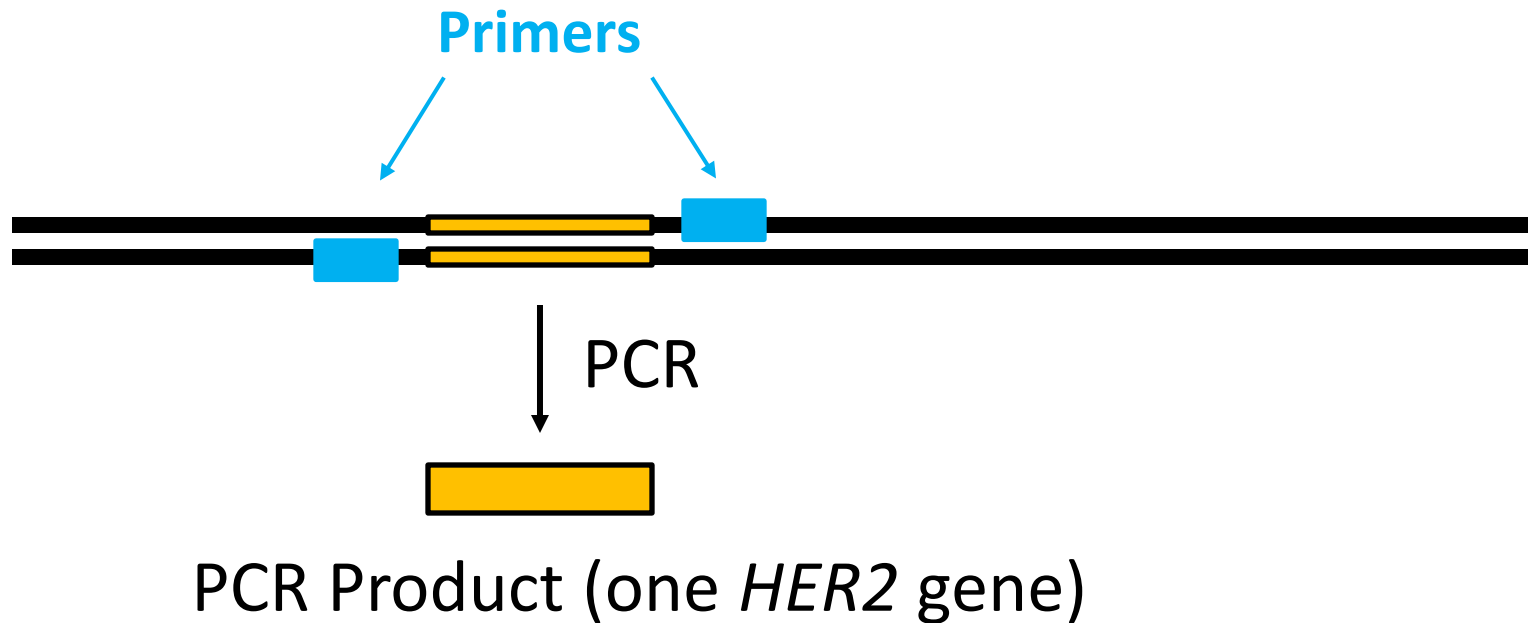
# *HER2 Gene Amplification & HER2 Over-Expression Leads to an Abnormal Increase in Cell Division*



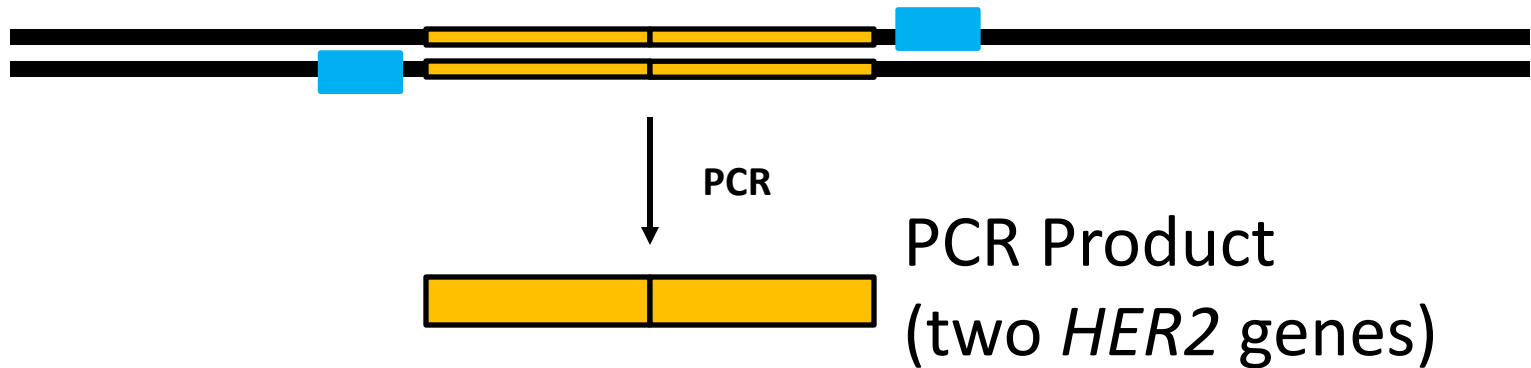
Normal number of signals  
telling cells to grow and divide

Too many signals telling cells to  
grow and divide causes  
increased cell division

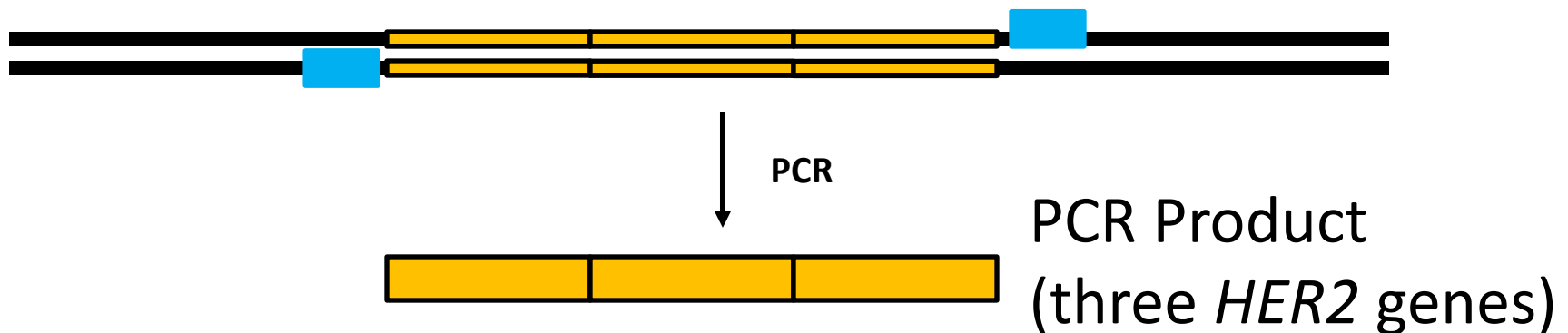
# PCR Can Be Used to Detect a Gene Amplification



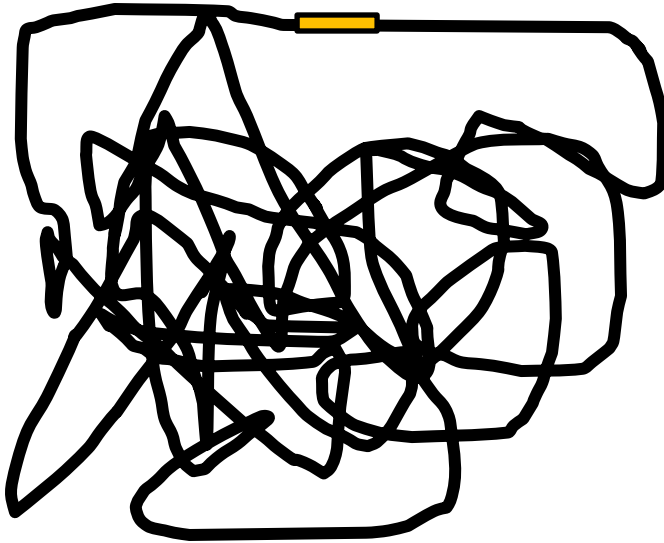
# Detecting a Gene Duplication



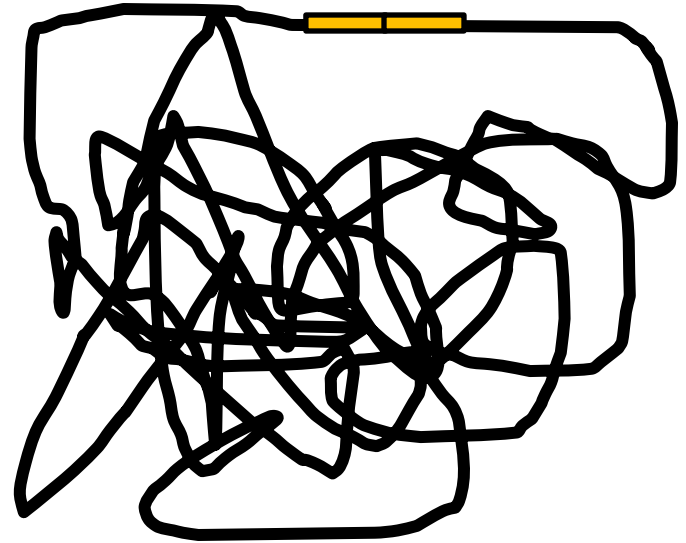
## Or Gene Triplication...



# Testing Patient Samples for Gene Amplification



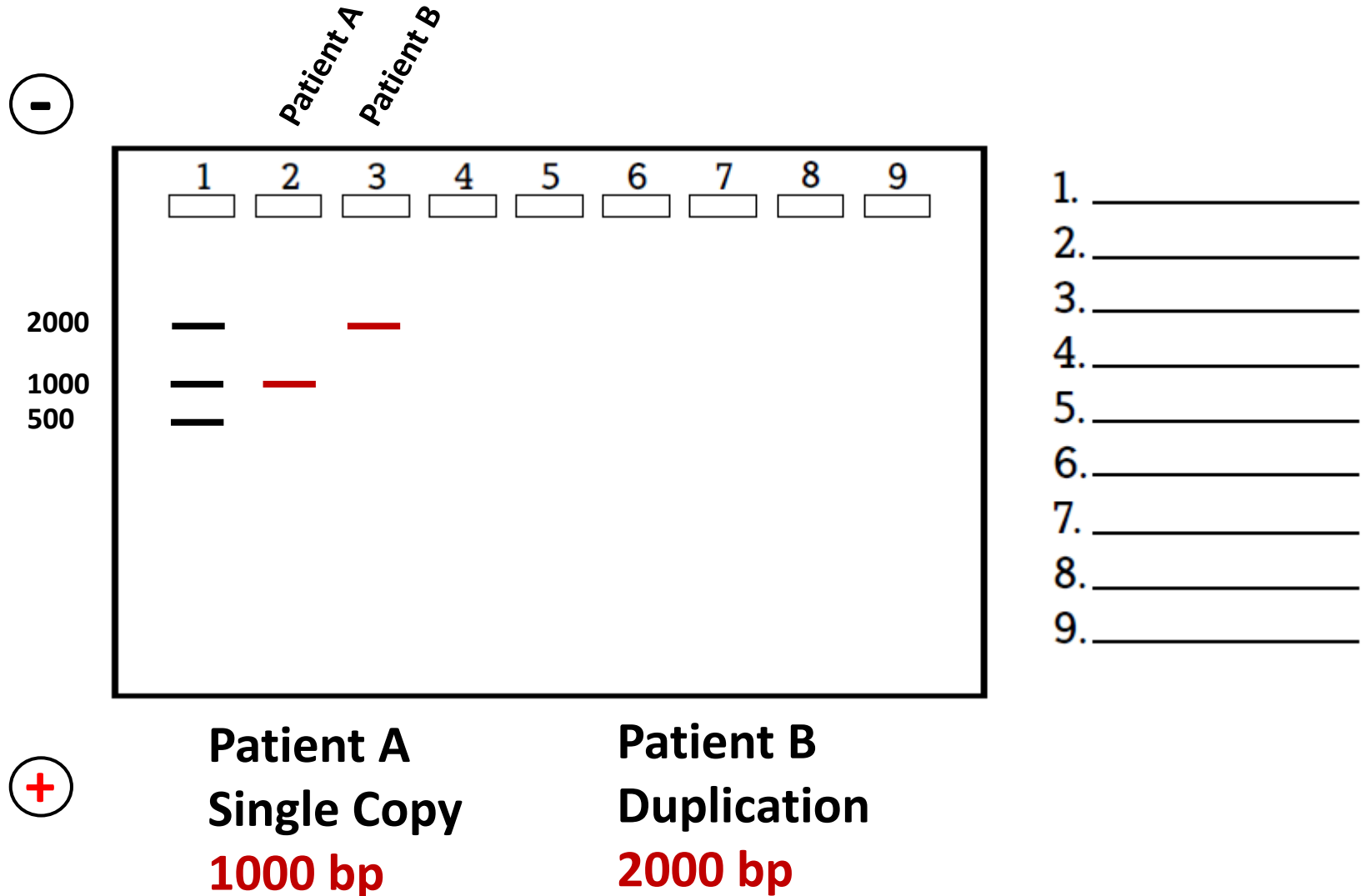
Patient A Genome



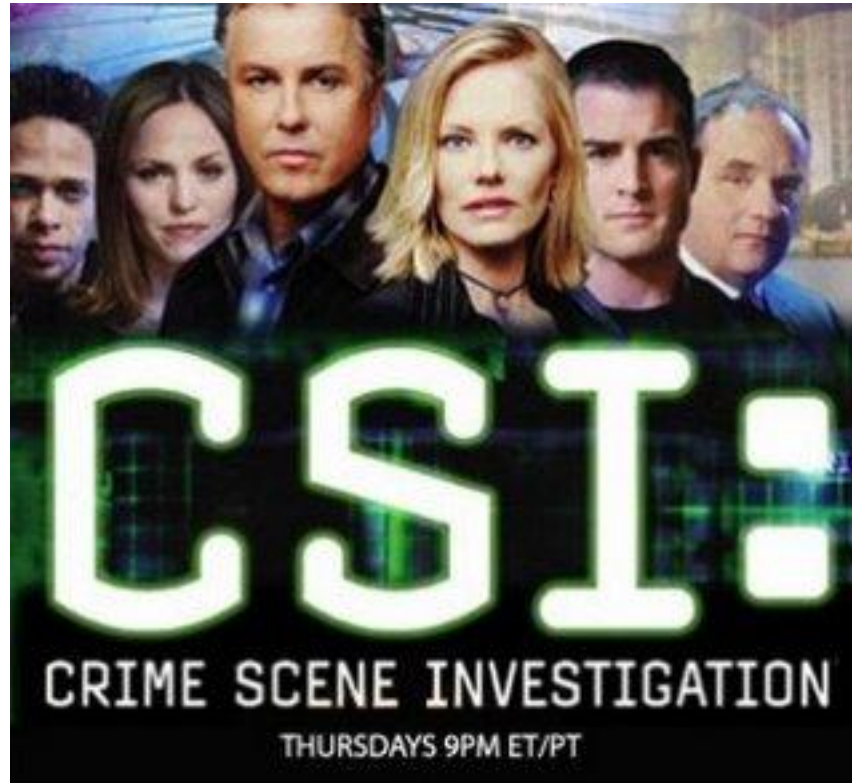
Patient B Genome



# View PCR Product Using Agarose Gel Electrophoresis



# 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>