



# **Introduction to Bioinformatics**

## **Sequencing and PCR**

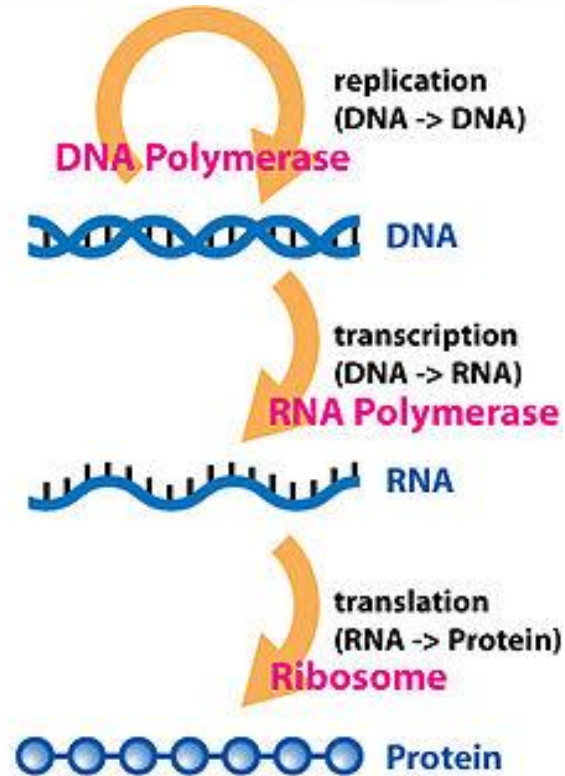
Stevan Radanović

# Resources

- Useful Genetics, <https://www.youtube.com/channel/UCtXCrx28msMBQ-vFUIOIReA>
- Codecademy

# Refresher

- Central dogma: DNA makes RNA makes Proteins

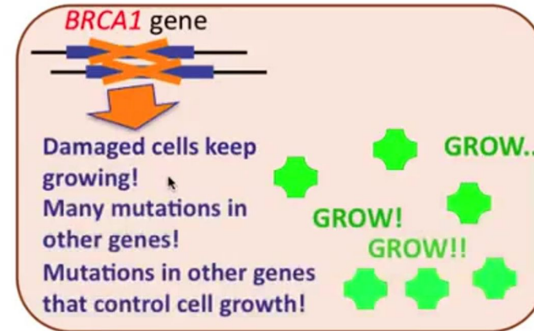
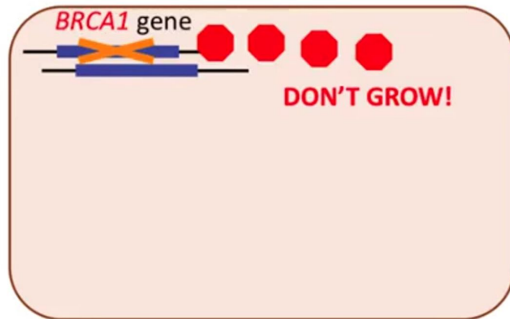


# Sequencing

- What does “sequencing” mean?
- Why sequence?
- Why sequence DNA and RNA?

# Sequencing

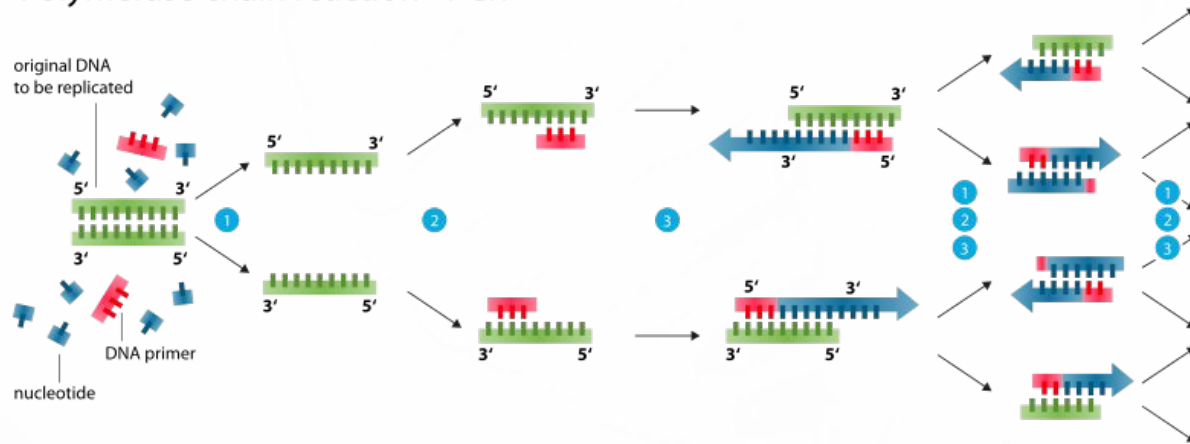
- BRCA1 and BRCA2 genes (BReast CAncer susceptibility genes) code for proteins that block cell growth when cell is damaged



# PCR

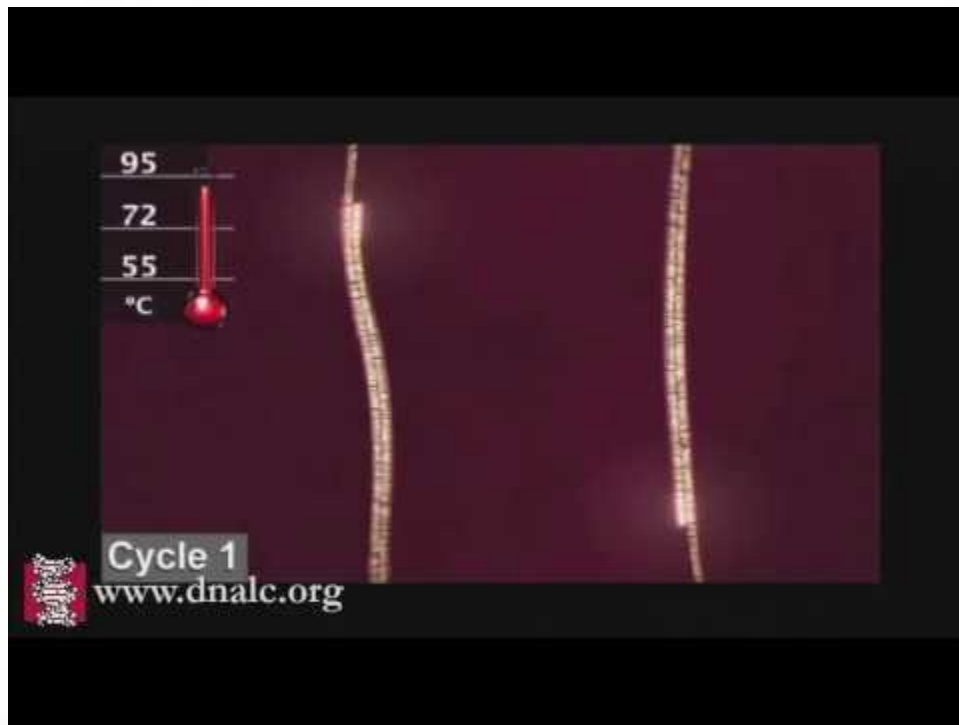
- PCR (Polymerase Chain Reaction) - DNA amplification

## Polymerase chain reaction - PCR



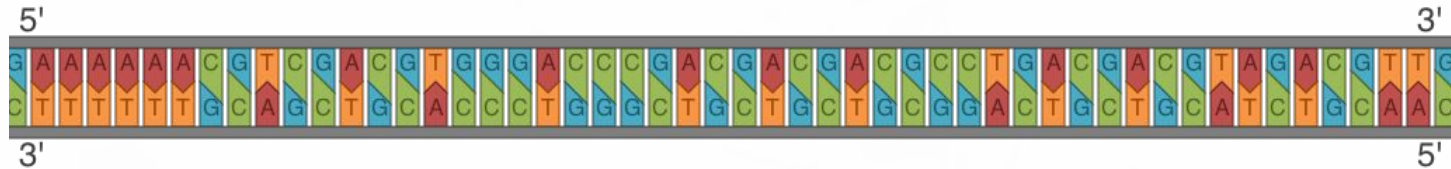
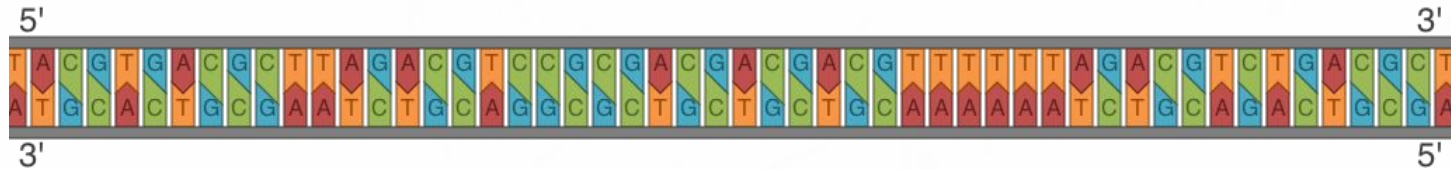
- 1 **Denaturation** at 94-96°C
- 2 **Annealing** at ~68°C
- 3 **Elongation** at ca. 72 °C

# PCR



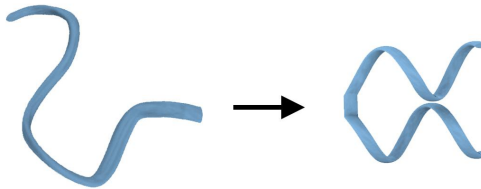
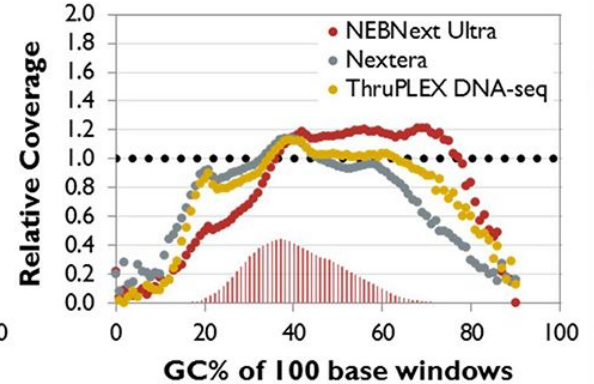
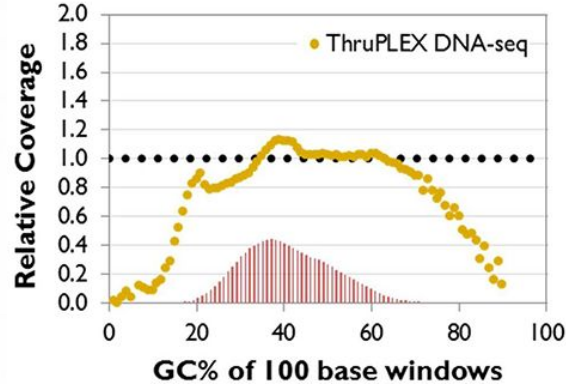
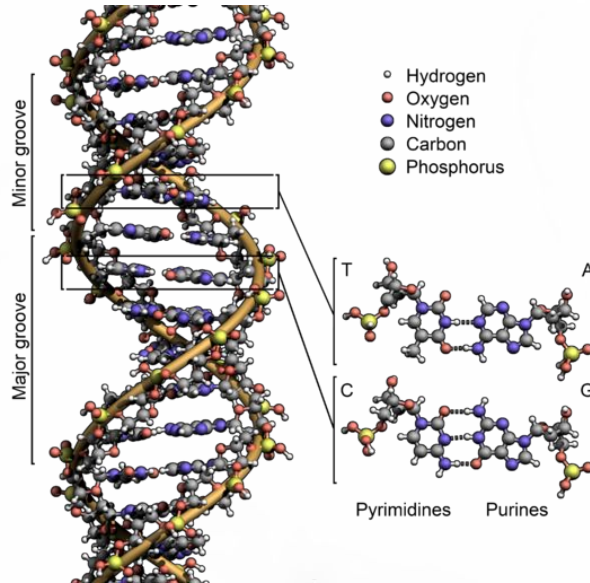
# PCR - Primers

Forward primer





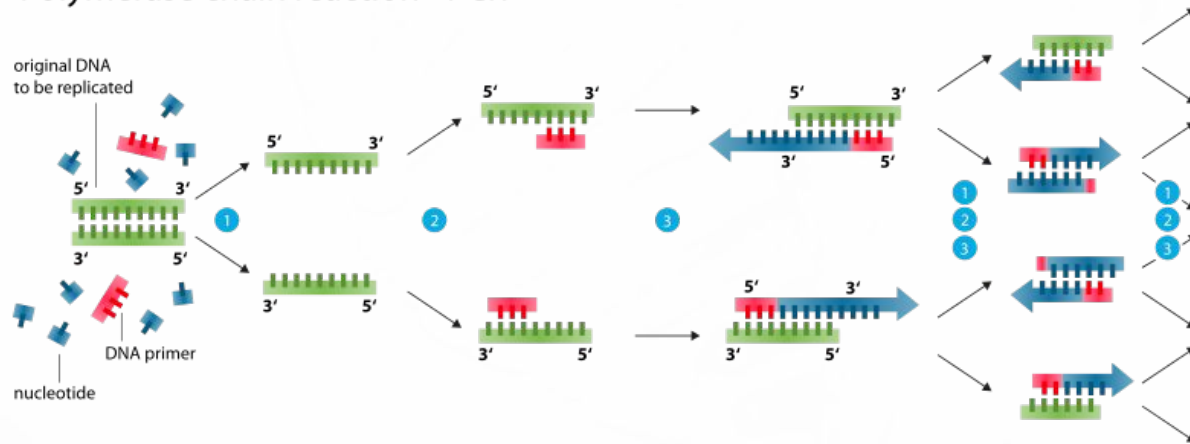
# PCR - GC bias



# PCR Errors

- 1 in 10k error rate

## Polymerase chain reaction - PCR

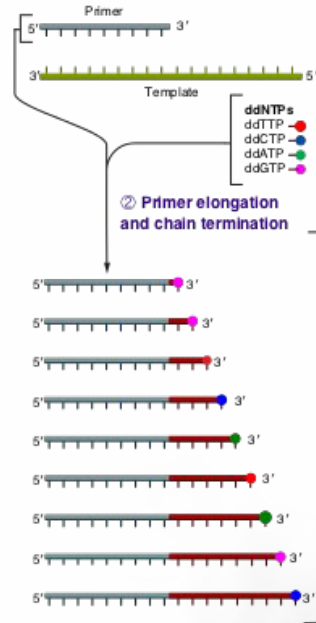


- 1 Denaturation** at 94-96°C
- 2 Annealing** at ~68°C
- 3 Elongation** at ca. 72 °C

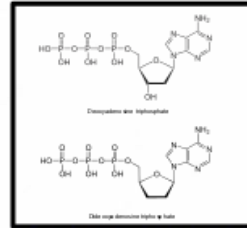
# Sequencing - Example

## ① Reaction mixture

- Primer and DNA template
- DNA polymerase
- ddNTPs with flouorochromes
- dNTPs (dATP, dCTP, dGTP, and dTTP)



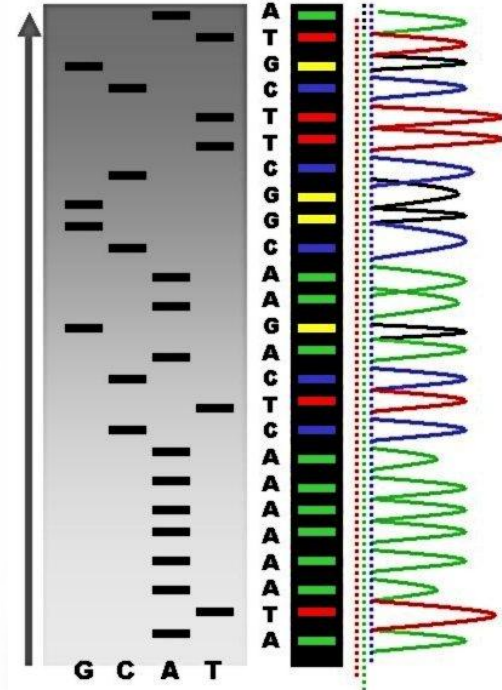
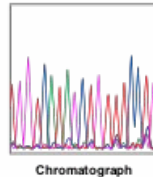
## ② Primer elongation and chain termination



## ③ Capillary gel electrophoresis separation of DNA fragments



## ④ Laser detection of flourochromes and computational sequence analysis



# Shotgun Sequencing

Strand	Sequence
Original	AGCATGCTGCAGTCATGCTTAGGCTA
First shotgun sequence	AGCATGCTGCAGTCATGCT-----
	-----TAGGCTA
Second shotgun sequence	AGCATG-----
	-----CTGCAGTCATGCTTAGGCTA
Reconstruction	AGCATGCTGCAGTCATGCTTAGGCTA



# Command Line

# Command Line

- The command line is a text interface for your computer. It's a program that takes in commands, which it passes on to the computer's operating system to run.

# Command Line

- ls

# Command Line

- ls
  - ls -l



# Command Line

- ls
  - ls -l
  - ls -a

# Command Line

- ls
  - ls -l
  - ls -a
  - ls -t

# Command Line

- ls
  - ls -l
  - ls -a
  - ls -t
  - ls -la

# Command Line

- ls
  - ls -l
  - ls -a
  - ls -t
  - ls -la
- pwd

# Command Line

- ls
  - ls -l
  - ls -a
  - ls -t
  - ls -la
- pwd
- cd

# Command Line

- mkdir

# Command Line

- mkdir
- touch

# Command Line

- mkdir
- touch
- cp



# Command Line

- mkdir
- touch
- cp
- mv

# Command Line

- mkdir
- touch
- cp
- mv
- rm

# Command Line

- `mkdir`
- `touch`
- `cp`
- `mv`
- `rm`
- `nano`

# Command Line

- echo

# Command Line

- echo
- cat

# Command Line

- echo
- cat
- less

# Command Line

- echo
- cat
- less
- >

# Command Line

- echo
- cat
- less
- >
- >>



# Command Line

- echo
- cat
- less
- >
- >>
- <

# Command Line

- WC

# Command Line

- WC
- |

# Command Line

- `wc`
- `|`
- `sort`

# Command Line

- `wc`
- `|`
- `sort`
- `uniq`

# Command Line

- grep

# Command Line

- grep
  - grep -i

# Command Line

- `grep`
  - `grep -i`
  - `grep -R`



# Command Line

- **grep**
  - grep -i
  - grep -R
- **sed**

# Command Line

- **grep**
  - `grep -i`
  - `grep -R`
- **sed**
- `nano ~/.bash_profile`

# Command Line

- **grep**
  - `grep -i`
  - `grep -R`
- **sed**
- **nano ~/.bash\_profile**
- **source**

# Command Line

- alias

# Command Line

- alias
- export

# Command Line

- alias
- export
- \$VAR

# Command Line

- alias
- export
- \$VAR
- env