SevenBridges

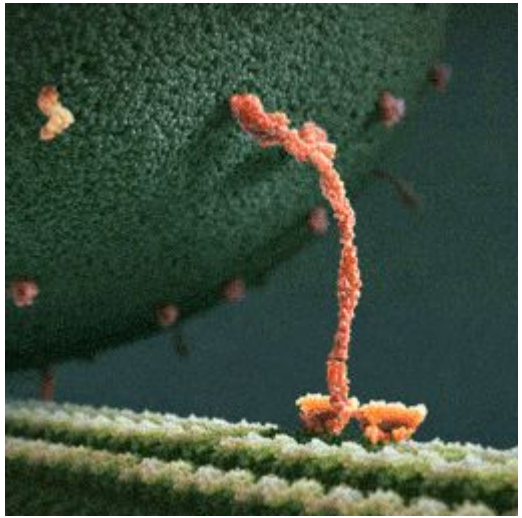
# Next Generation Sequencing Technologies

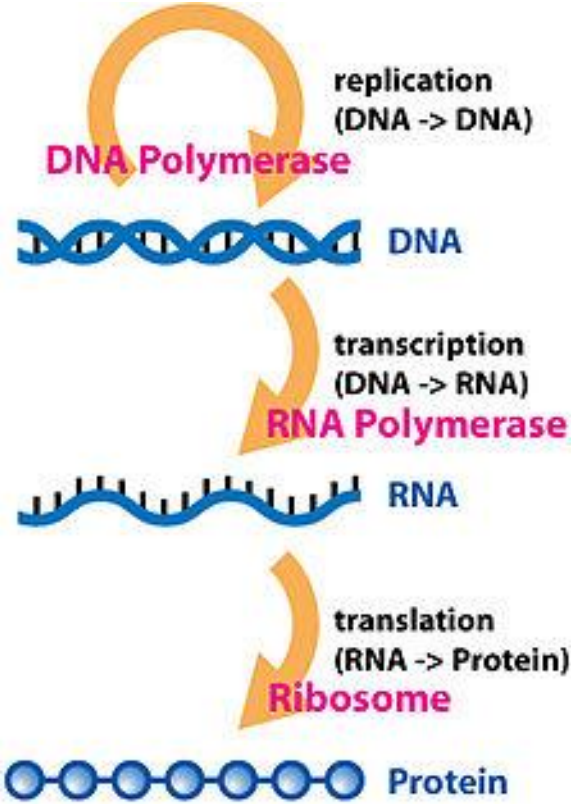
# Why Sequence the Genome?

What are the benefits of genome sequencing?

# Refresher

## Central dogma



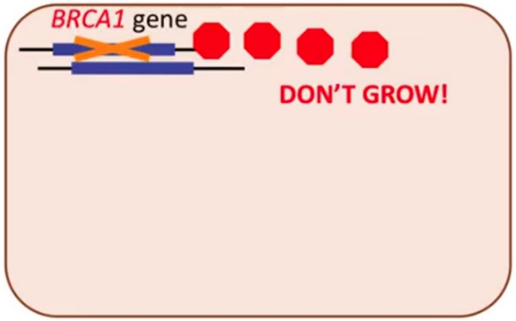


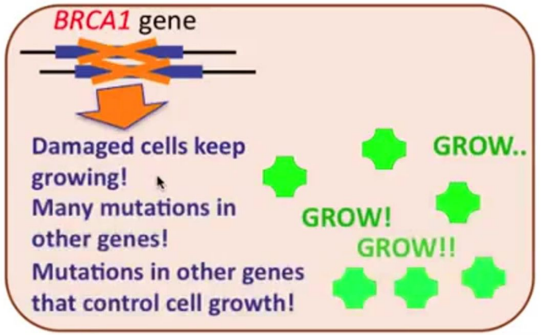
# Sequencing

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

# Why sequence DNA?

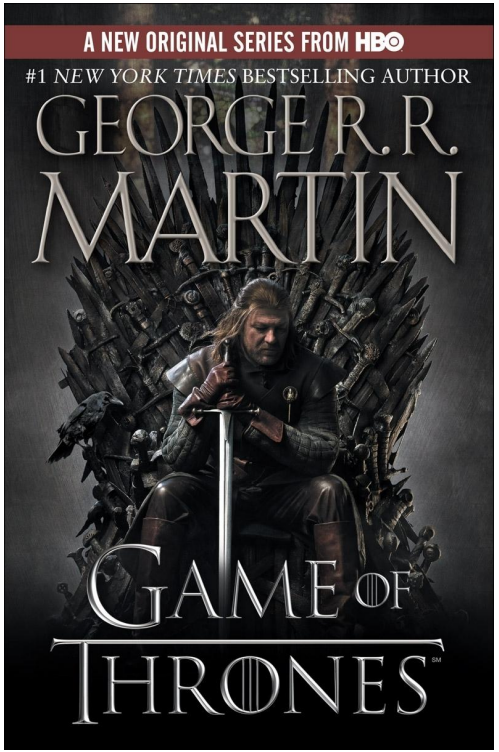
BRCA1 and BRCA2 genes (BReast CAncer susceptibility genes) code for proteins that block cell growth when cell is damaged



# Do we need computers?

- Human genome is 3 billion bps long
- How many GoT books is that?
  - 3.000 letters per page
  - 1.000 pages per book
- It would take 1.000 GoT books (stack 50m high) to write down a single human genome sequence
- Impossible to analyze manually

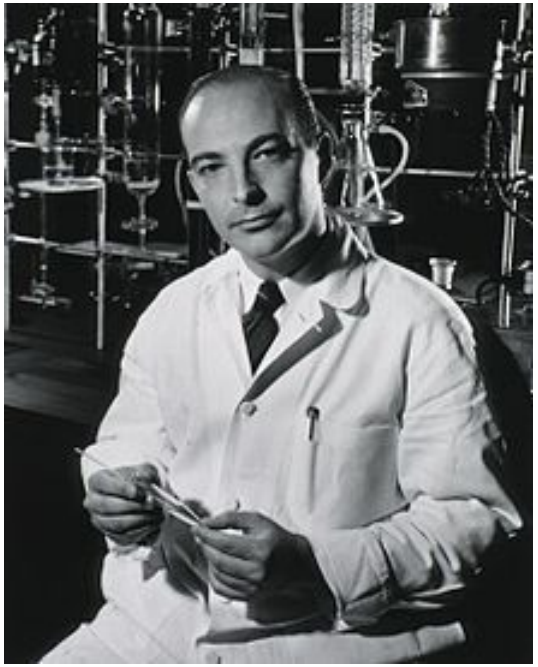


# The Birth of Sequencing Technologies

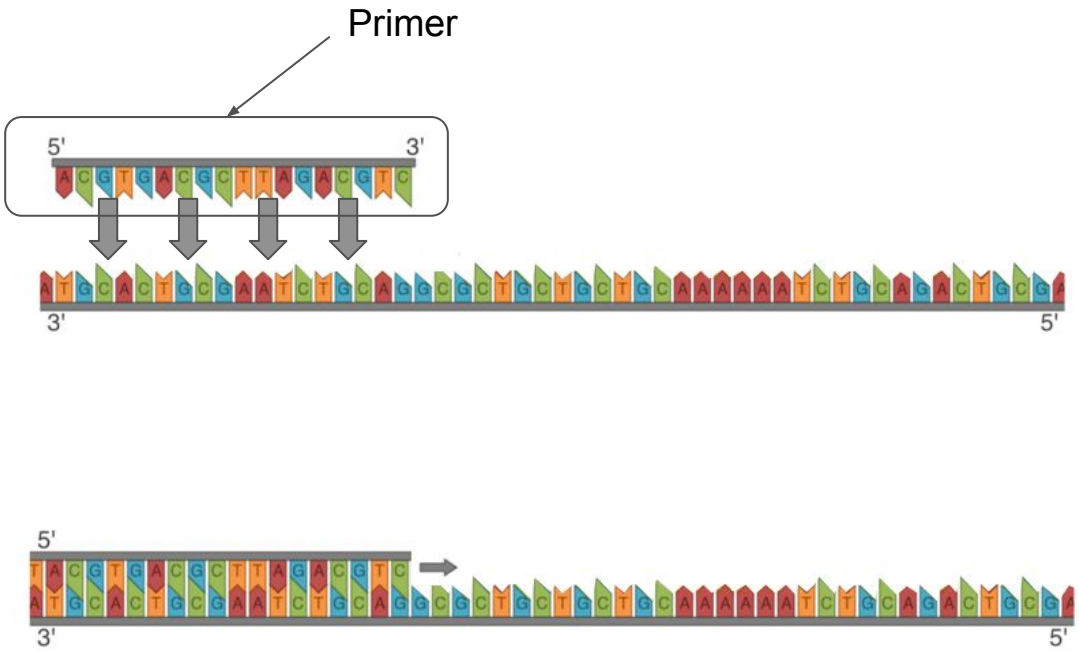
The discovery of DNA polymerase, modified nucleotides and the Chain termination method

# Crucial Concept: DNA Replication

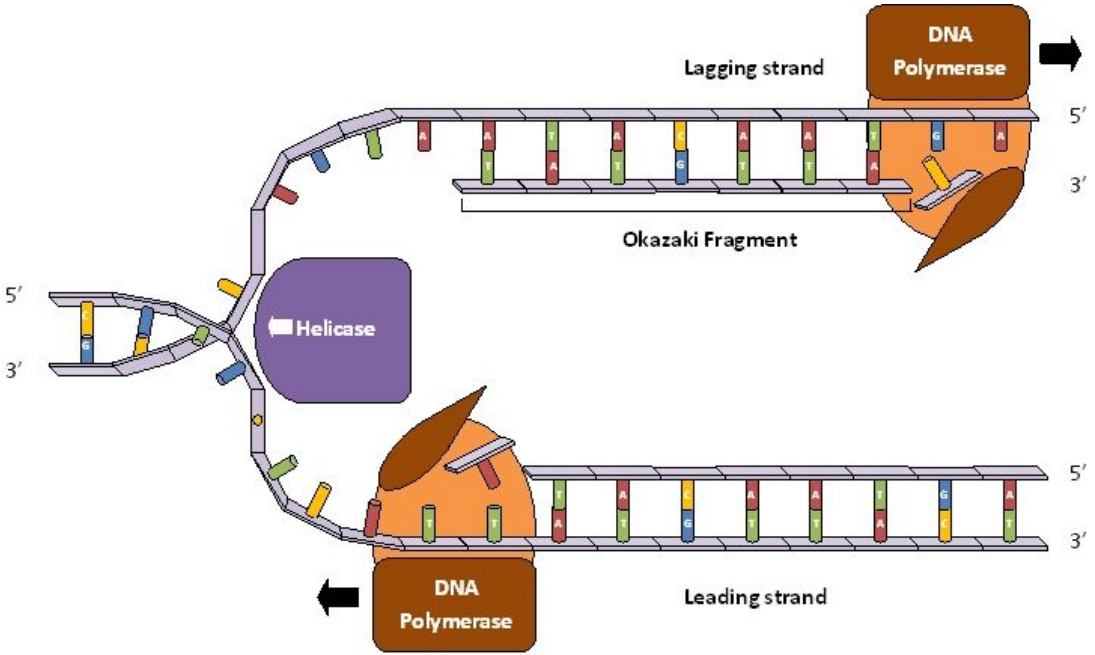
- **1956** - Arthur Kornberg et. al. (New York University)
  - discovered the **DNA Polymerase**
  - And that polymerase facilitates **DNA synthesis**
    - zipper molecule



# PCR Primers



# DNA Replication



# PCR: Polymerase Chain Reaction

- DNA Polymerase Chain Reaction (PCR) chemistry based on **thermo cycling**; acute and repeated i.e. cyclic temperature fluctuations for denaturation and hybridization of complementary strands of DNA
- **Taq Polymerase** - *Thermus Acquaticus*
  - Not human polymerase
  - Taq is more temperature resistant to high denaturation temperatures of thermocycler

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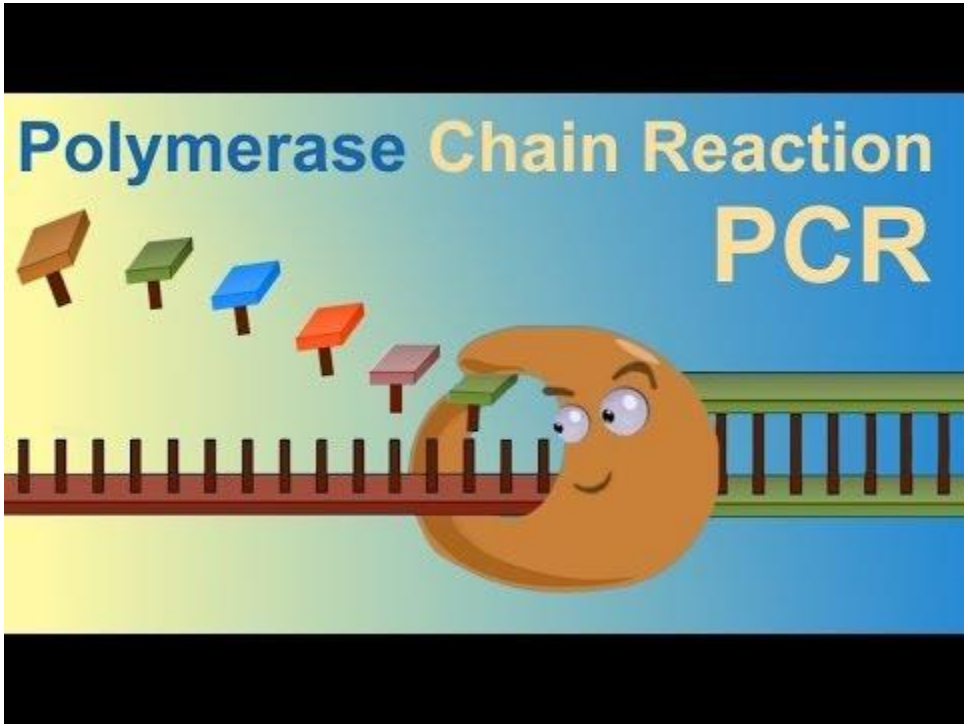
# PCR: Polymerase Chain Reaction

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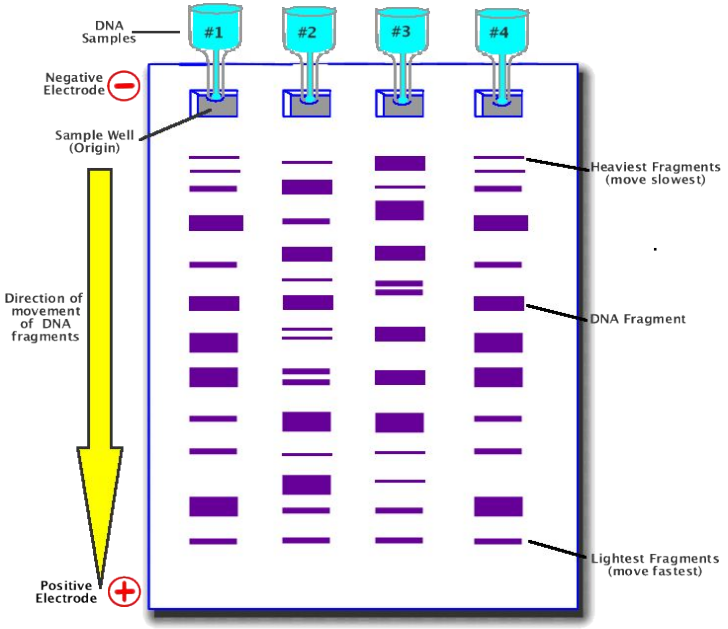
# Gel Electrophoresis

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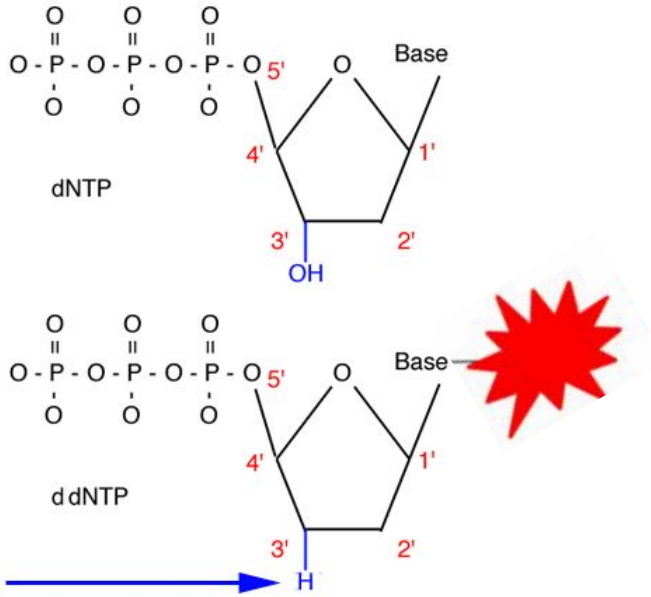
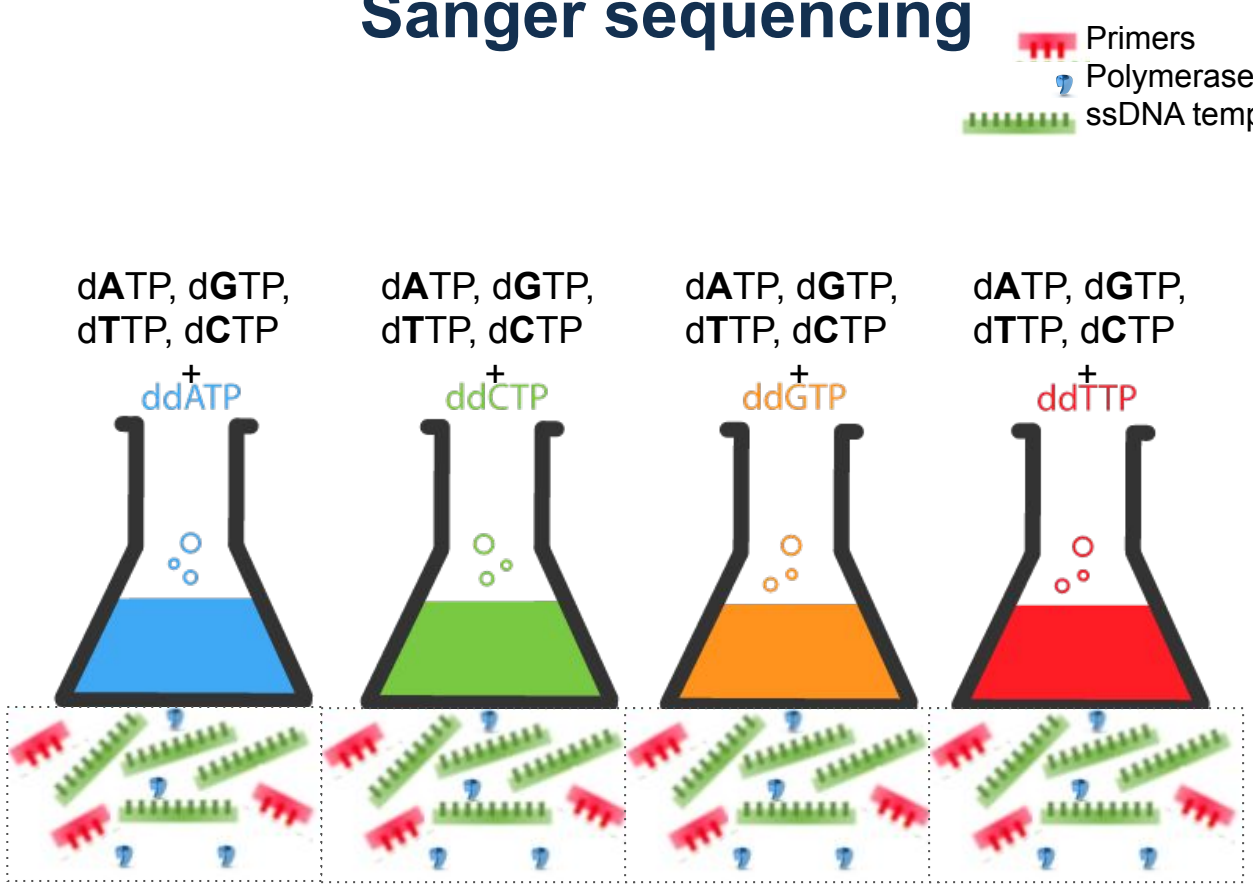
# Terminating and Labeling Nucleotides

- **Sanger**
  - Chain **Termination** Method
  - **dNTPs** + labeled **ddNTPs**
  - Southern Blotting on Electrophoresis gel
- **NGS**
  - Reversible termination
  - No termination
  - Slides



# Sanger sequencing

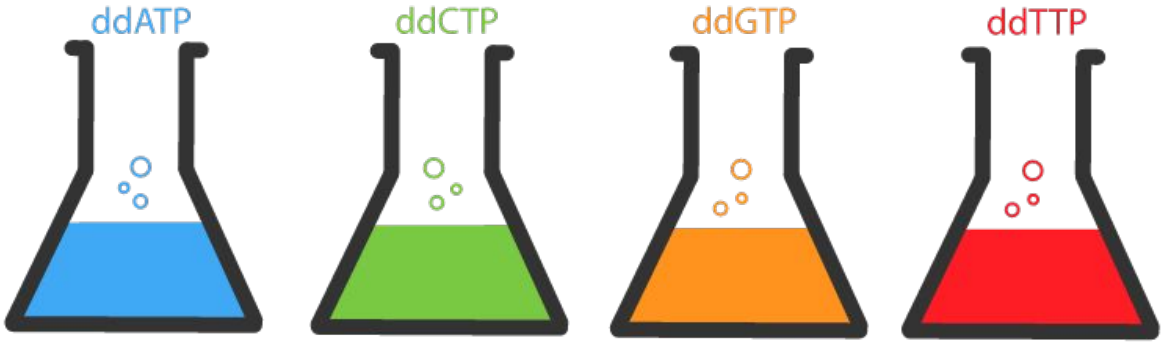


# Sanger sequencing example

**ACTAGCTA**

TGATCGAT

# Sanger sequencing




ACTAGCTA
TGATCGAT

ACTA
TGATCGAT

A
TGATCGAT

ACTAGC
TGATCG

AC
TGATCG

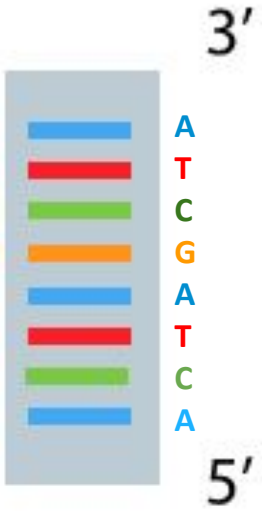
ACTAG
TGATC

ACTAGCT
TGATCGA

ACT
TGATCGA

# Sanger sequencing

# Sanger sequencing



# The Birth of Next Generation Sequencing Technologies

DNA polymerase in the sequencing by synthesis approach

# Sequencing Trends

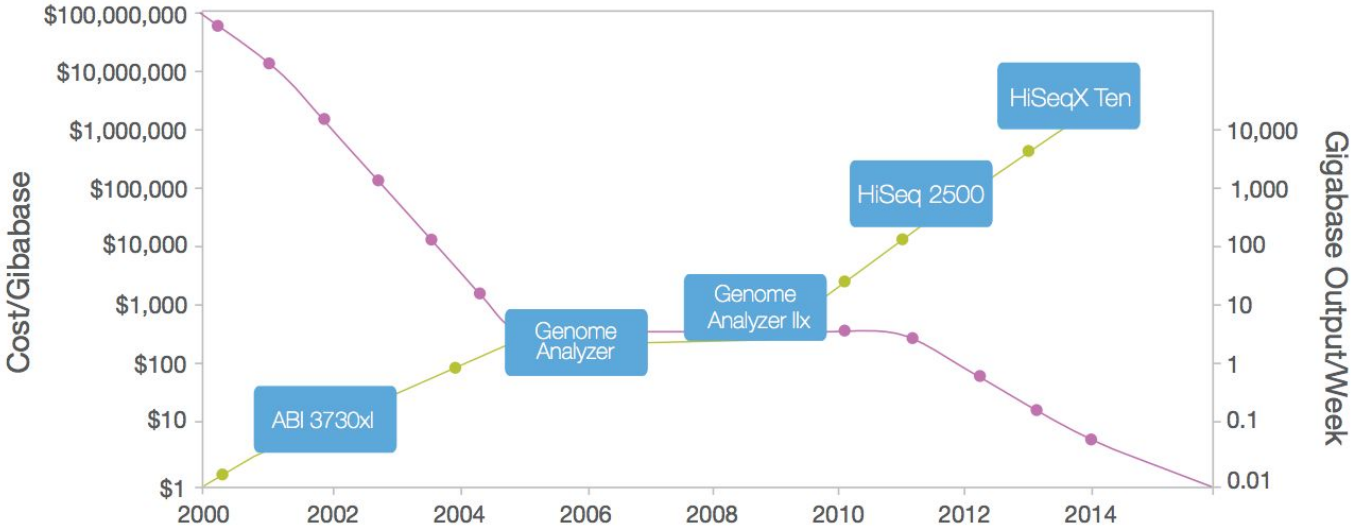
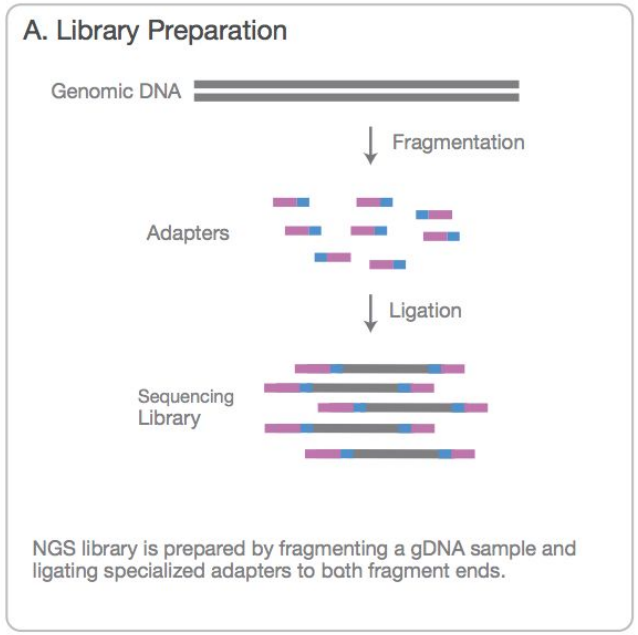
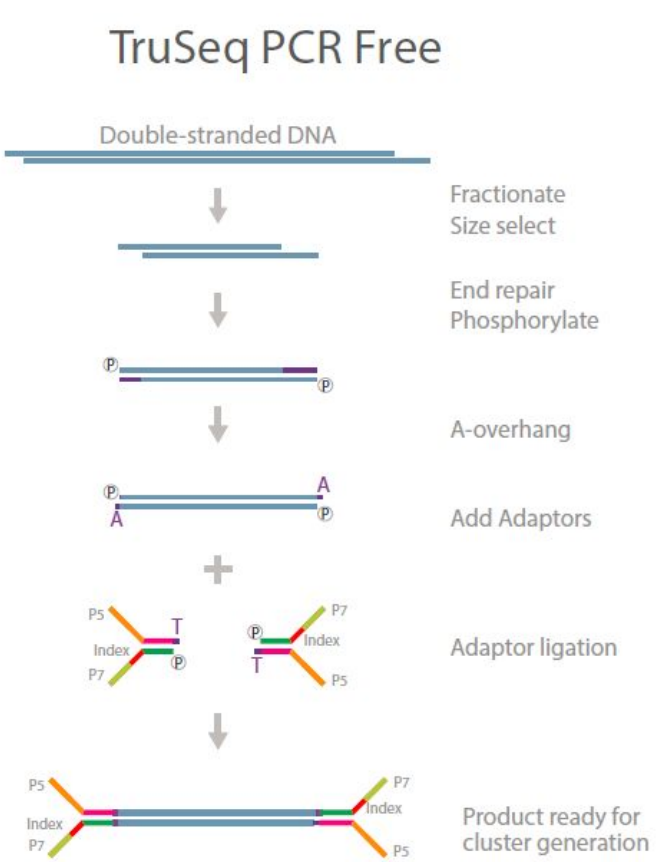



**Figure 1: Sequencing Cost and Data Output Since 2000**—The dramatic rise of data output and concurrent falling cost of sequencing since 2000. The Y-axes on both sides of the graph are logarithmic.

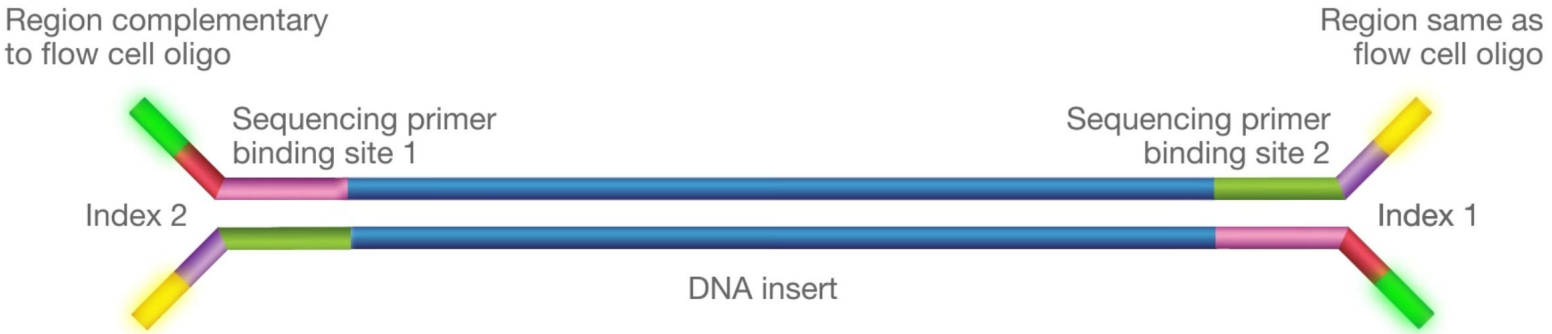
illumina®

# Illumina Library Prep

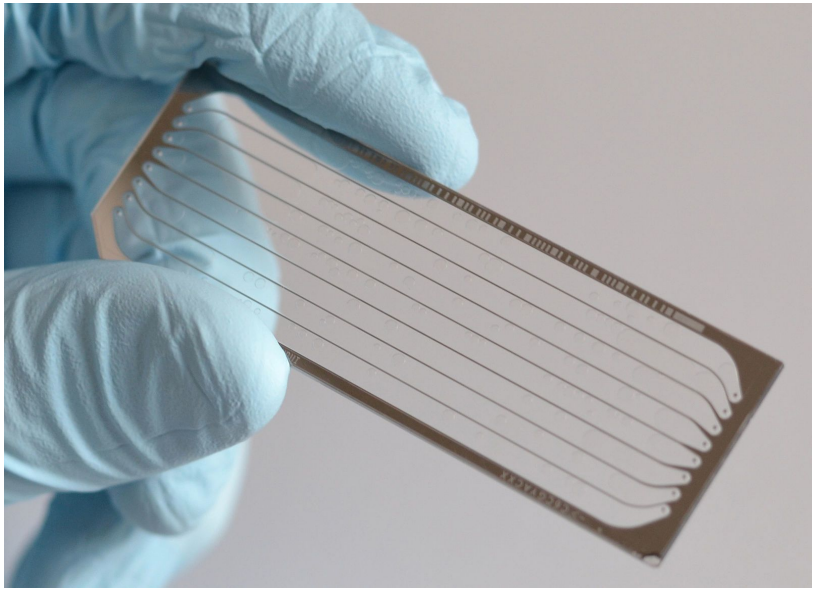


# Illumina library prep



# Illumina flow cell



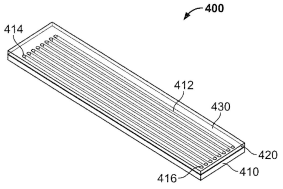



FIG. 3A

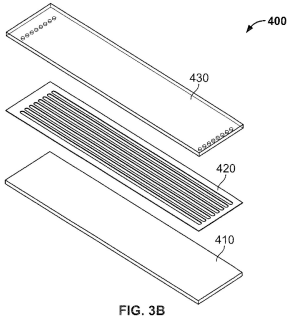
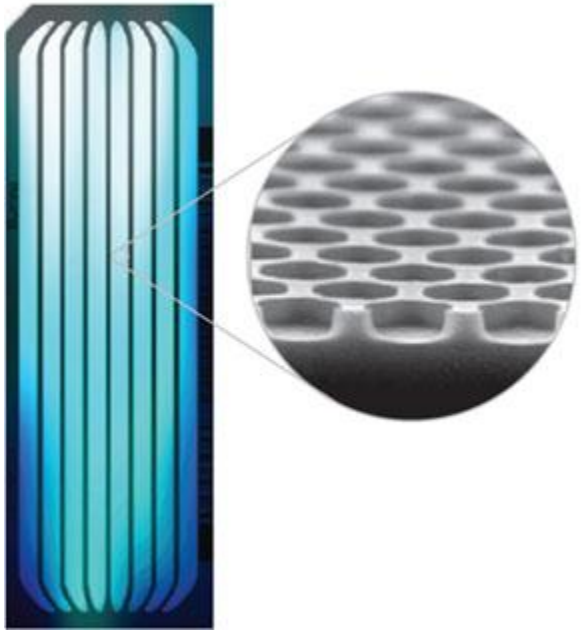
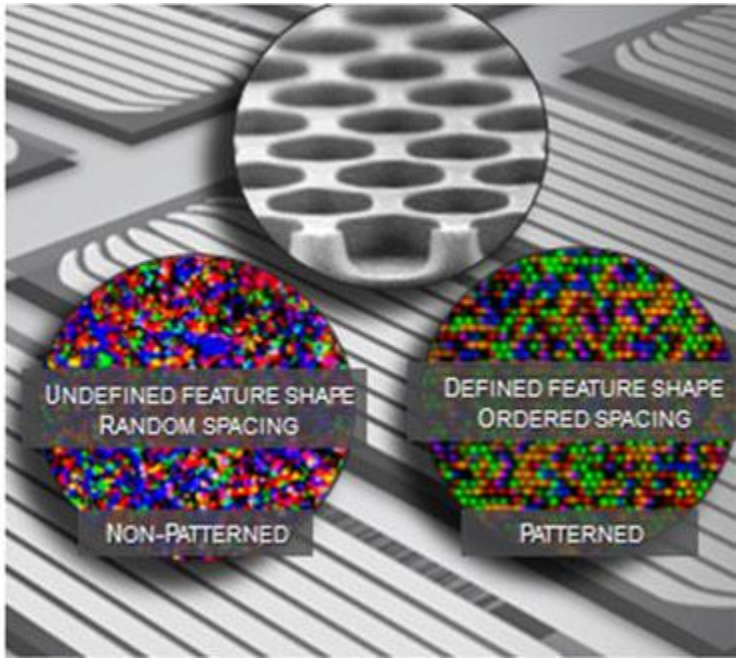


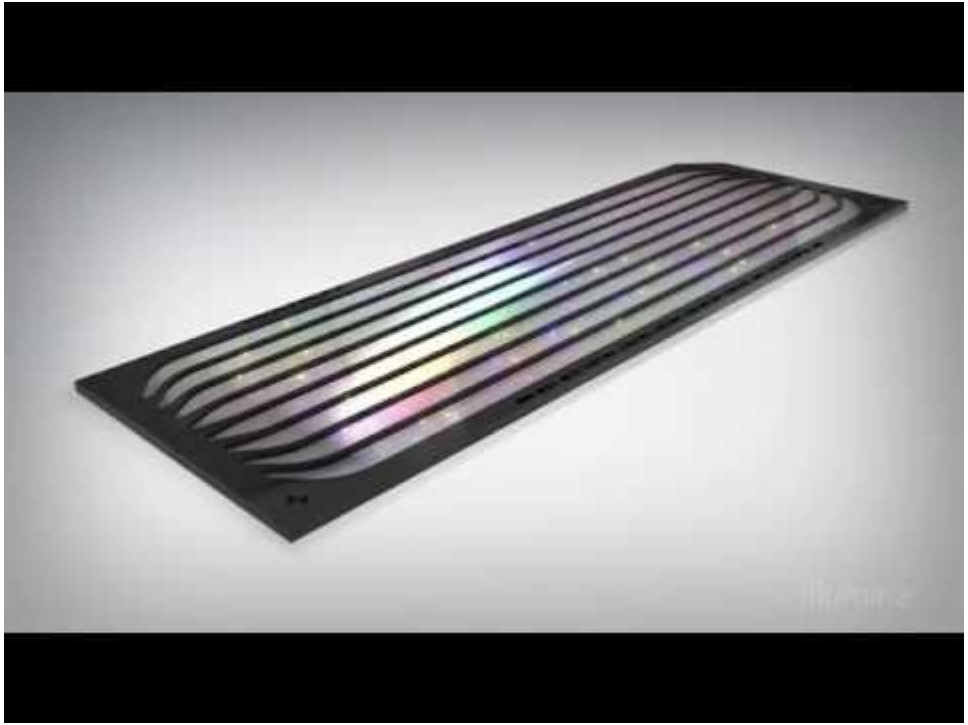

FIG. 3B

# Illumina flow cell





# Cluster Amplification



# Illumina sequencing

# Illumina sequencing: Paired ends

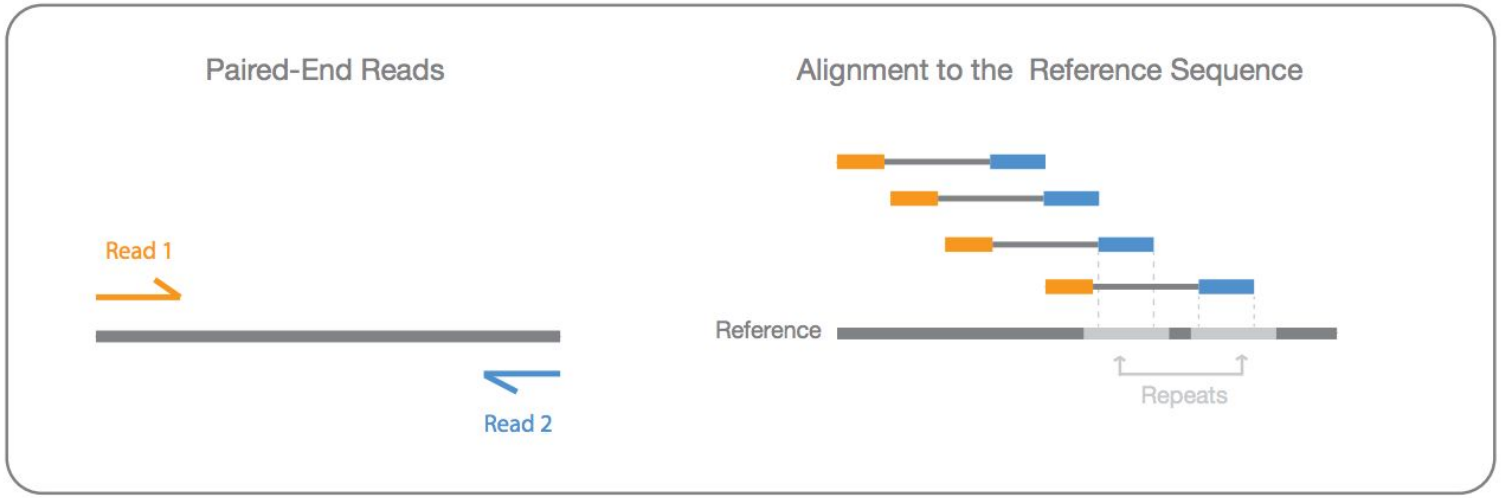
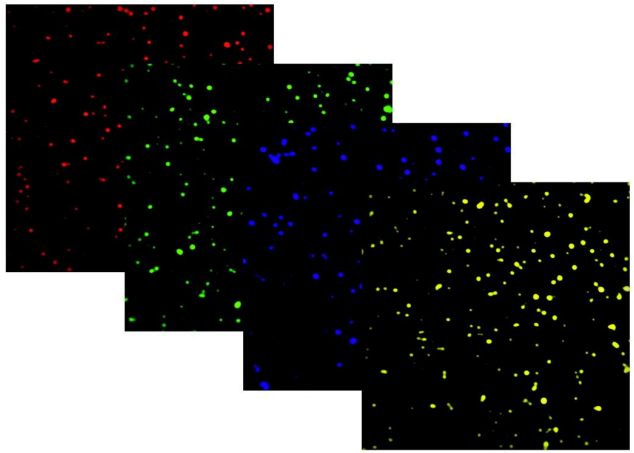


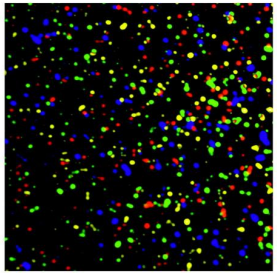

**Figure 4: Paired-End Sequencing and Alignment**—Paired-end sequencing enables both ends of the DNA fragment to be sequenced. Because the distance between each paired read is known, alignment algorithms can use this information to map the reads over repetitive regions more precisely. This results in much better alignment of the reads, especially across difficult-to-sequence, repetitive regions of the genome.

# Recording called bases or reads

The FASTQ file format

# Image processing

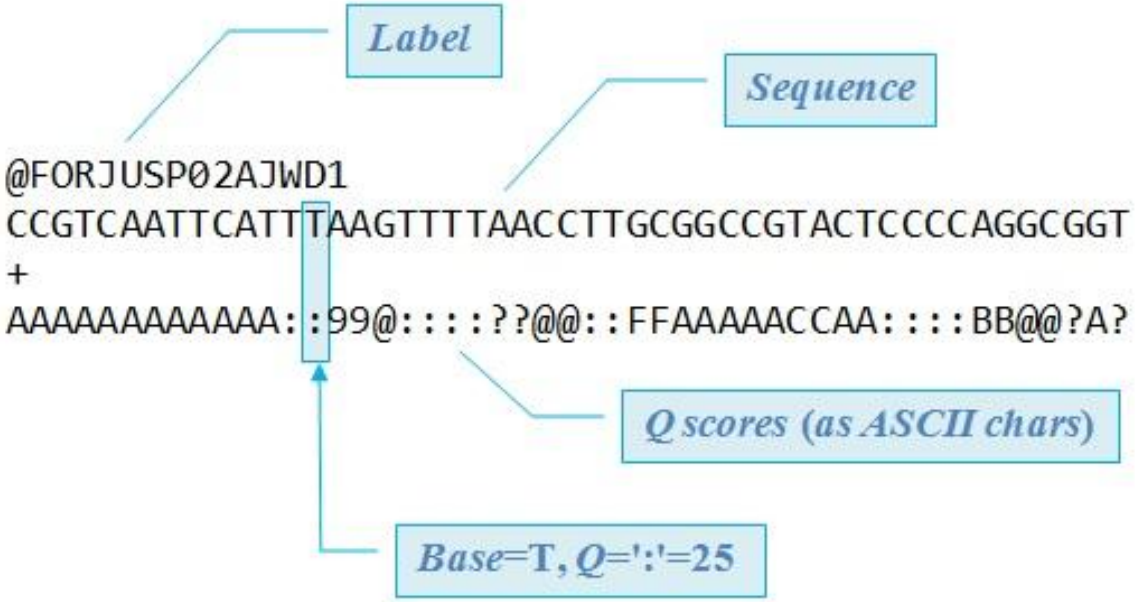




- Sequencing produces high-resolution TIFF images
- 100 tiles per lane, 8 lanes per flow cell, 100 cycles
- 4 images (A,G,C,T) per tile per cycle = 320,000 images
- Each TIFF image ~ 7Mb = 2,240,000 Mb of data (2.24TB)

Base calling - uses cluster intensities and noise estimate to output the sequence of bases read from each cluster, along with a confidence level for each base.

# FASTQ file format



# Phred Scoring

## Quality score interpretation

$$Q = -10 \log_{10} P \quad \Longrightarrow \quad P = 10^{\frac{-Q}{10}}$$

| Phred Quality Score | Probability of incorrect base call | Base call accuracy |
|---|---|---|
| 10 | 1 in 10 | 90% |
| 20 | 1 in 100 | 99% |
| 30 | 1 in 1000 | 99.9% |
| 40 | 1 in 10000 | 99.99% |
| 50 | 1 in 100000 | 99.999% |

Materials from Wikepedia

# ASCII Base + 33 encoding

ASCII_BASE=33 Illumina, Ion Torrent, PacBio and Sanger

| Q | P_error | ASCII | Q | P_error | ASCII | Q | P_error | ASCII | Q | P_error | ASCII |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 1.00000 | 33 ! | 11 | 0.07943 | 44 , | 22 | 0.00631 | 55 7 | 33 | 0.00050 | 66 B |
| 1 | 0.79433 | 34 " | 12 | 0.06310 | 45 - | 23 | 0.00501 | 56 8 | 34 | 0.00040 | 67 C |
| 2 | 0.63096 | 35 # | 13 | 0.05012 | 46 . | 24 | 0.00398 | 57 9 | 35 | 0.00032 | 68 D |
| 3 | 0.50119 | 36 $ | 14 | 0.03981 | 47 / | 25 | 0.00316 | 58 : | 36 | 0.00025 | 69 E |
| 4 | 0.39811 | 37 % | 15 | 0.03162 | 48 0 | 26 | 0.00251 | 59 ; | 37 | 0.00020 | 70 F |
| 5 | 0.31623 | 38 & | 16 | 0.02512 | 49 1 | 27 | 0.00200 | 60 < | 38 | 0.00016 | 71 G |
| 6 | 0.25119 | 39 ' | 17 | 0.01995 | 50 2 | 28 | 0.00158 | 61 = | 39 | 0.00013 | 72 H |
| 7 | 0.19953 | 40 ( | 18 | 0.01585 | 51 3 | 29 | 0.00126 | 62 > | 40 | 0.00010 | 73 I |
| 8 | 0.15849 | 41 ) | 19 | 0.01259 | 52 4 | 30 | 0.00100 | 63 ? | 41 | 0.00008 | 74 J |
| 9 | 0.12589 | 42 * | 20 | 0.01000 | 53 5 | 31 | 0.00079 | 64 @ | 42 | 0.00006 | 75 K |
| 10 | 0.10000 | 43 + | 21 | 0.00794 | 54 6 | 32 | 0.00063 | 65 A | | | |

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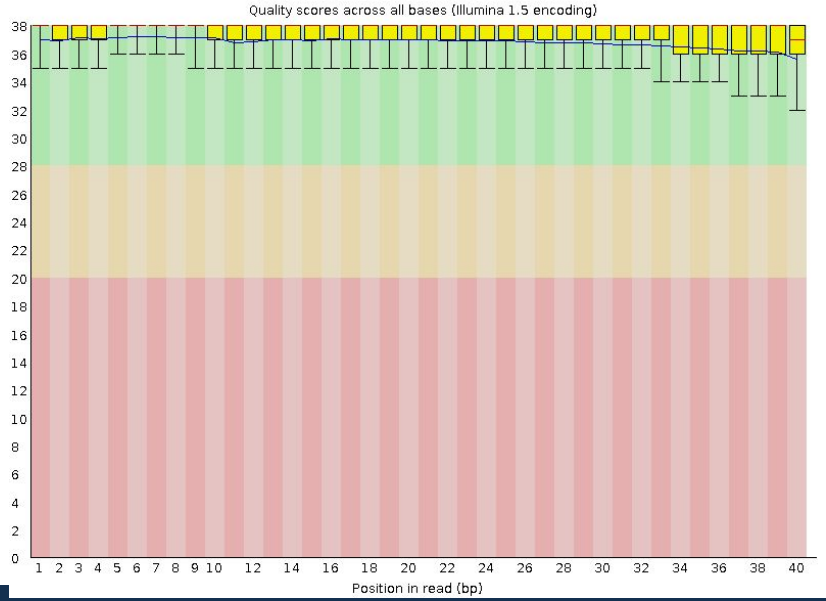
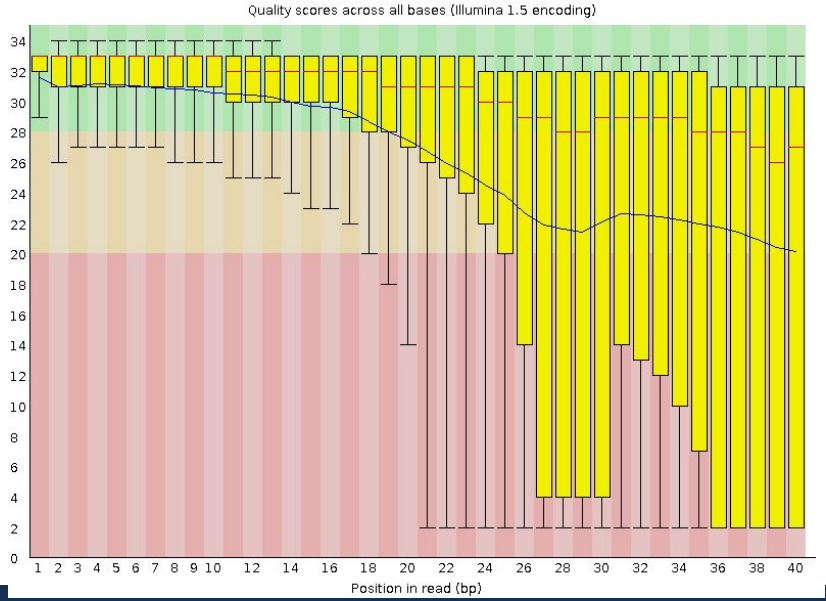
# FASTQ - Analysis

## Per Base Sequence Quality



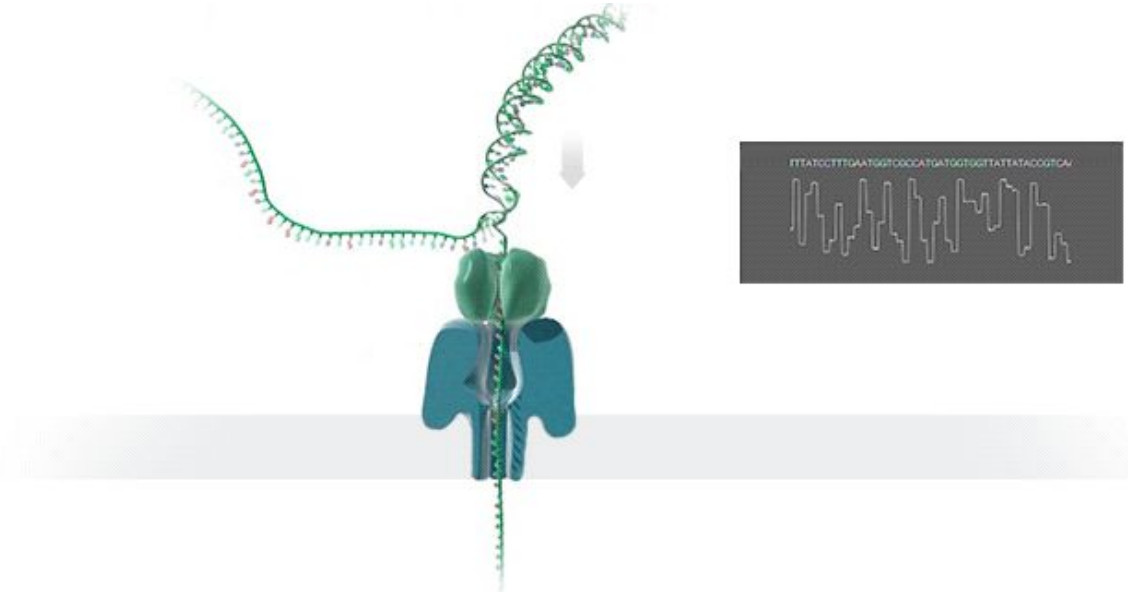
# Other NGS Technologies

Nanopore sequencing

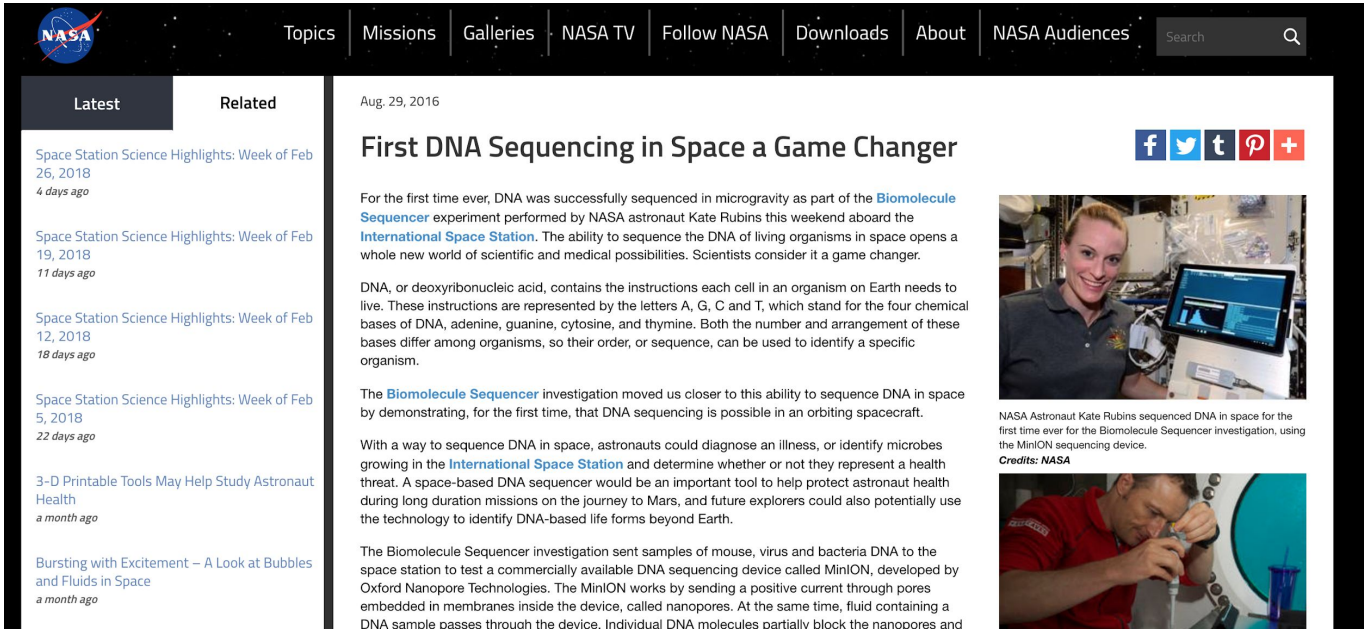
# SMRT/ZMV Sequencing

PACIFIC
BIOSCIENCES®

Oxford NANOPORE Technologies



# Oxford Nanopore MinION



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*a month ago*

Aug. 29, 2016

## First DNA Sequencing in Space a Game Changer

For the first time ever, DNA was successfully sequenced in microgravity as part of the **Biomolecule Sequencer** experiment performed by NASA astronaut Kate Rubins this weekend aboard the **International Space Station**. The ability to sequence the DNA of living organisms in space opens a whole new world of scientific and medical possibilities. Scientists consider it a game changer.

DNA, or deoxyribonucleic acid, contains the instructions each cell in an organism on Earth needs to live. These instructions are represented by the letters A, G, C and T, which stand for the four chemical bases of DNA, adenine, guanine, cytosine, and thymine. Both the number and arrangement of these bases differ among organisms, so their order, or sequence, can be used to identify a specific organism.

The **Biomolecule Sequencer** investigation moved us closer to this ability to sequence DNA in space by demonstrating, for the first time, that DNA sequencing is possible in an orbiting spacecraft.

With a way to sequence DNA in space, astronauts could diagnose an illness, or identify microbes growing in the **International Space Station** and determine whether or not they represent a health threat. A space-based DNA sequencer would be an important tool to help protect astronaut health during long duration missions on the journey to Mars, and future explorers could also potentially use the technology to identify DNA-based life forms beyond Earth.

The Biomolecule Sequencer investigation sent samples of mouse, virus and bacteria DNA to the space station to test a commercially available DNA sequencing device called MinION, developed by Oxford Nanopore Technologies. The MinION works by sending a positive current through pores embedded in membranes inside the device, called nanopores. At the same time, fluid containing a DNA sample passes through the device. Individual DNA molecules partially block the nanopores and

NASA Astronaut Kate Rubins sequenced DNA in space for the first time ever for the Biomolecule Sequencer investigation, using the MinION sequencing device.
***Credits: NASA***

[MinION in Space Video]

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# Rosalind

Counting DNA Nucleotides

Transcribing DNA into RNA

Complementing a Strand of DNA

Parsing FASTQ file assignment:

- Count the number of reads
- Calculate average read quality
- Calculate per base average quality
- Create histogram of average read quality
- Create histogram of read quality per base

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# Resources

- Useful Genetics, https://www.youtube.com/channel/UCtXCrx28msMBQ-vFUIOIReA
- Codecademy, http://www.codeacademy.com
- Illumina inc., https://www.youtube.com/channel/UCxWMU29FF4kIG8YmQf6Zv0g
- AWS https://aws.amazon.com/documentation/ec2/
- Ion Torrent sequencing, Thermo Fisher Sci., Life Tech., https://www.youtube.com/user/LifeTechnologiesCorp
- Oxford Nanopore, https://www.youtube.com/channel/UC5yMlYjHSgFfZ37LYq-dzig
- PacBio, https://www.youtube.com/user/PacificBiosciences
- IPython, virtualenv, virtualenvwrapper
- Jupyter Notebook
- nbviewer, http://nbviewer.ipython.org/
- Sequencing, http://mol-biol4masters.masters.grkraj.org/html/Genetic_Engineering5A-DNA_Synthesis_&_Sequencing.htm