SevenBridges

Next Generation Sequencing Technologies

Why Sequence the Genome?

What are the benefits of genome sequencing?

Refresher

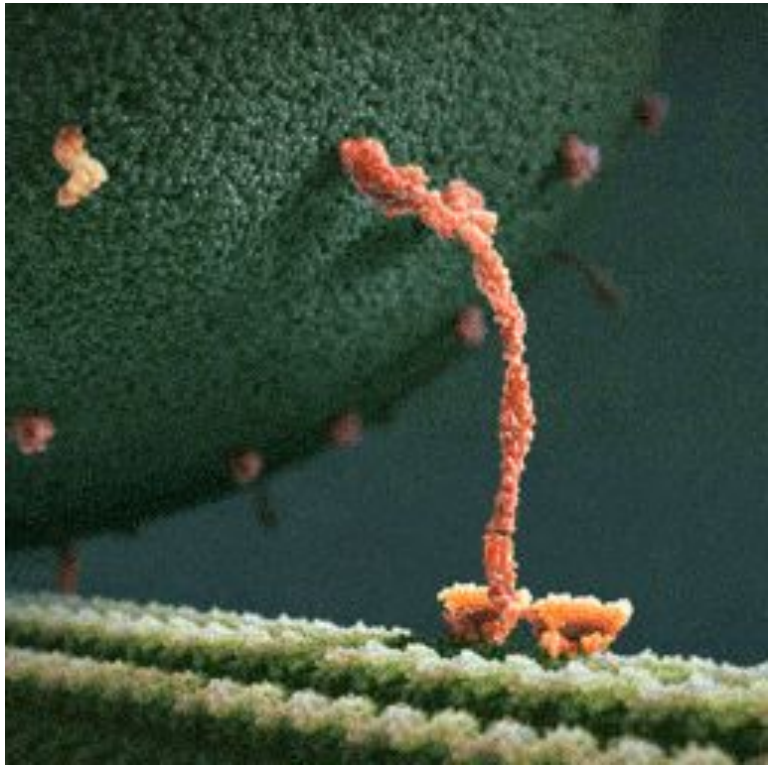
Central Dogma of Molecular Biology

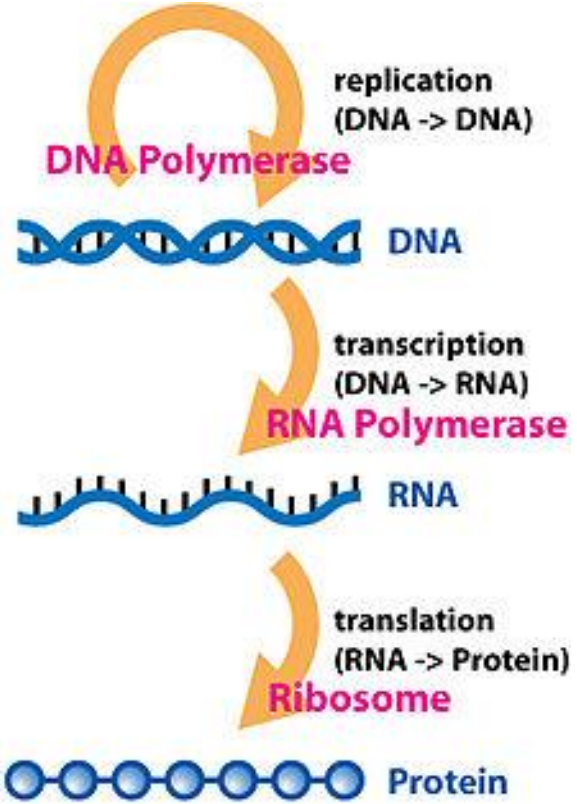
(proposed by Francis Crick):

DNA Replication: The process by which DNA makes an identical copy of itself.
Transcription: The synthesis of RNA using DNA as a template.
Translation: The synthesis of proteins using the information encoded in mRNA.

DNA➡ RNA➡ Protein

Refresher



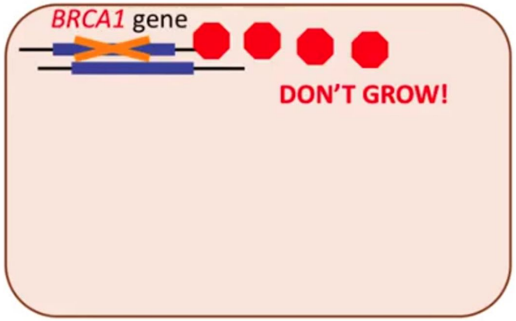

replication
(DNA -> DNA)
DNA Polymerase
DNA
transcription
(DNA -> RNA)
RNA Polymerase
RNA
translation
(RNA -> Protein)
Ribosome
Protein

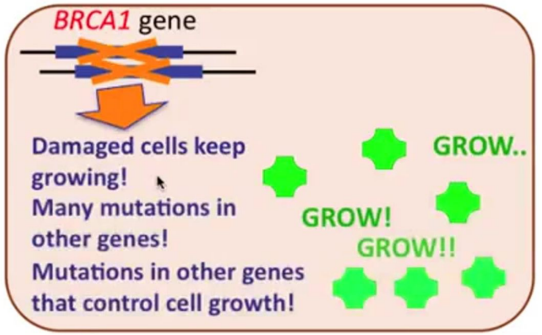
Sequencing

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

Why sequence DNA?

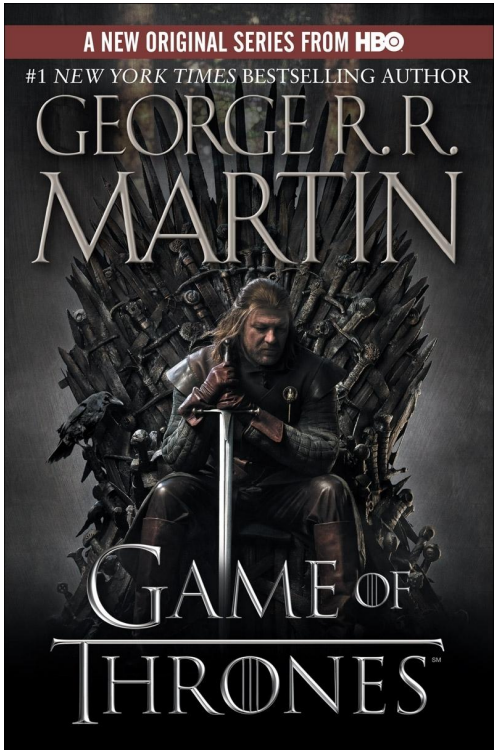
BRCA1 and BRCA2 genes (BReast CAncer susceptibility genes) code for proteins that block cell growth when cell is damaged





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

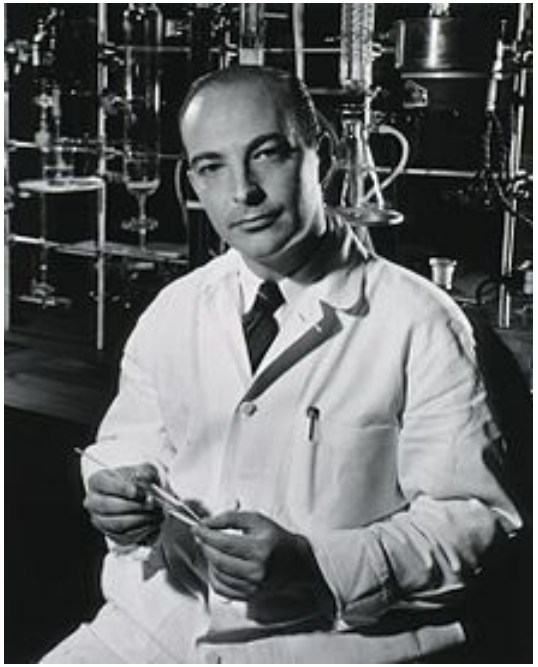


The Birth of Sequencing Technologies

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

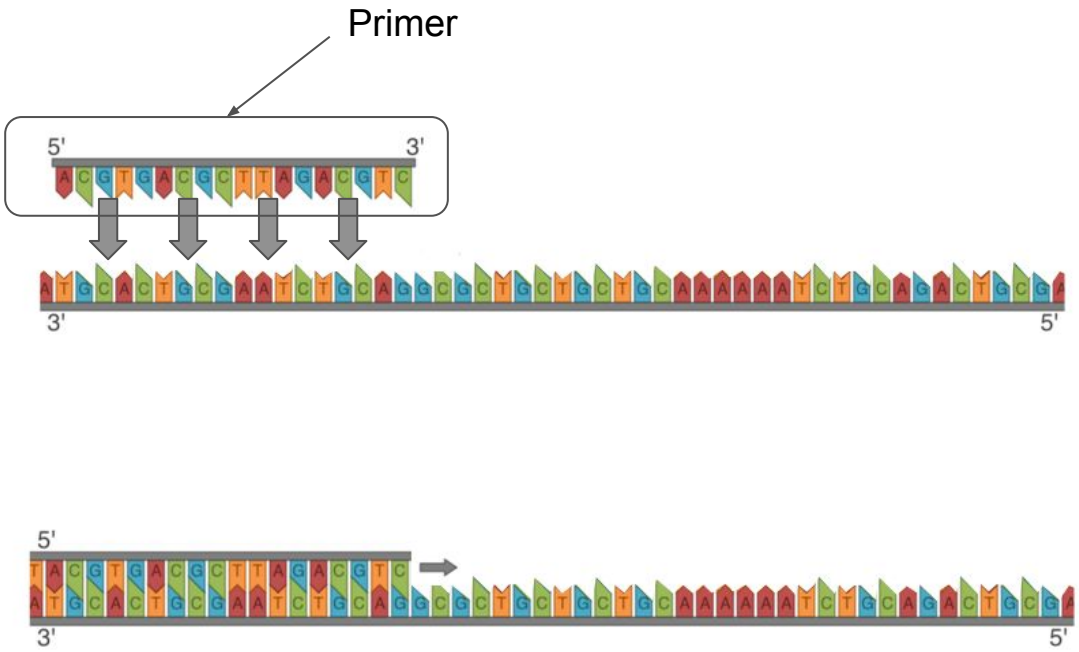
Crucial Concept: DNA Replication

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

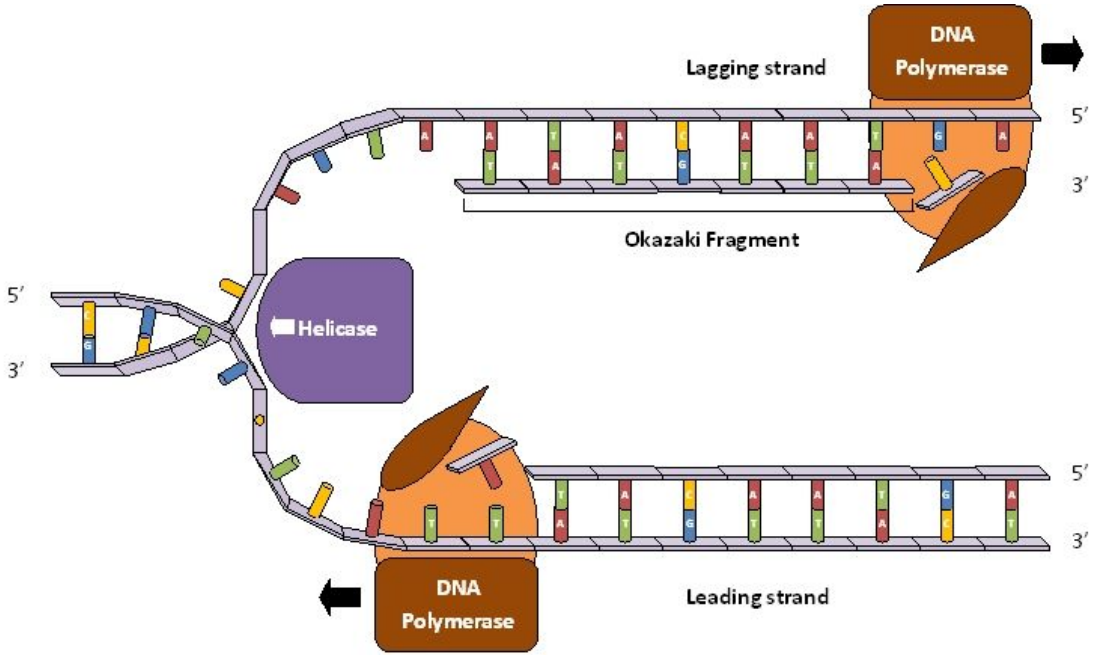


DNA Replication

PCR Primers

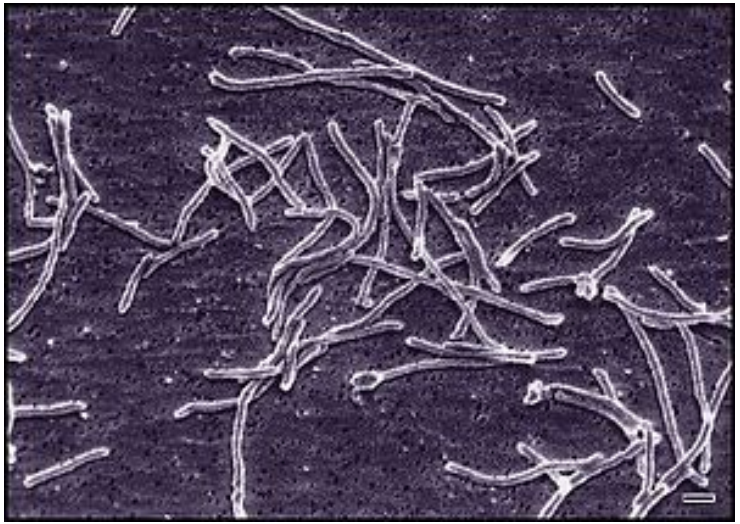


DNA Replication



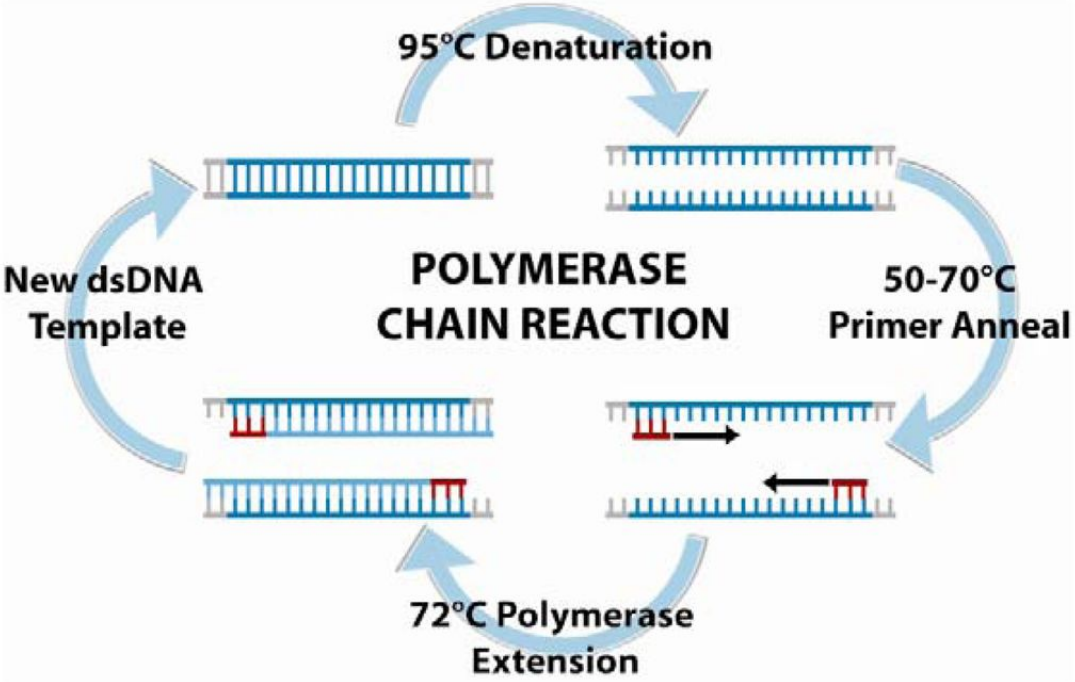
PCR: Polymerase Chain Reaction

- 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 Aquaticus*
  - Not human polymerase
  - Taq is more temperature resistant to high denaturation temperatures of thermocycler



Thermus Aquaticus

PCR: Polymerase Chain Reaction

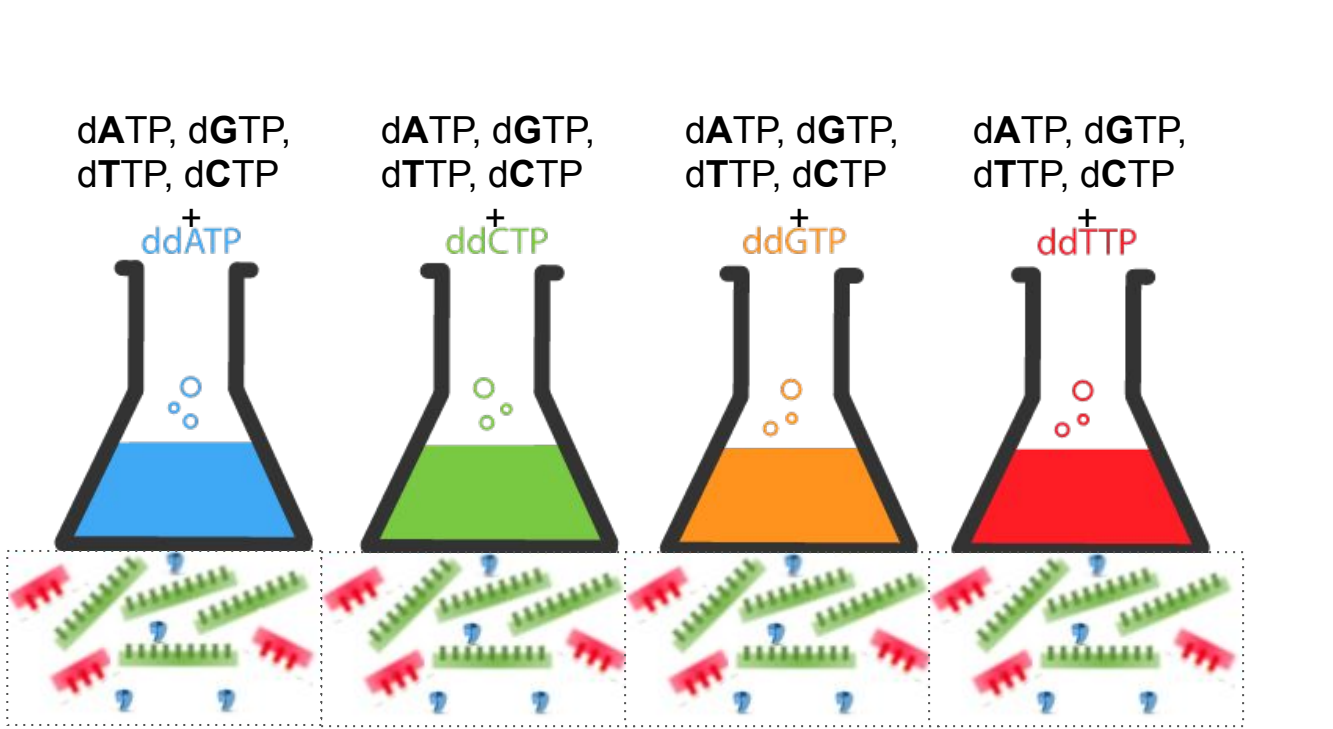


PCR: Polymerase Chain Reaction

Sanger sequencing

- Also known as chain termination sequencing and sequencing by capillary electrophoresis
- Developed by Frederick Sanger in the late 1970s
- Played a crucial role in DNA sequencing projects, including the Human Genome Project

Sanger sequencing

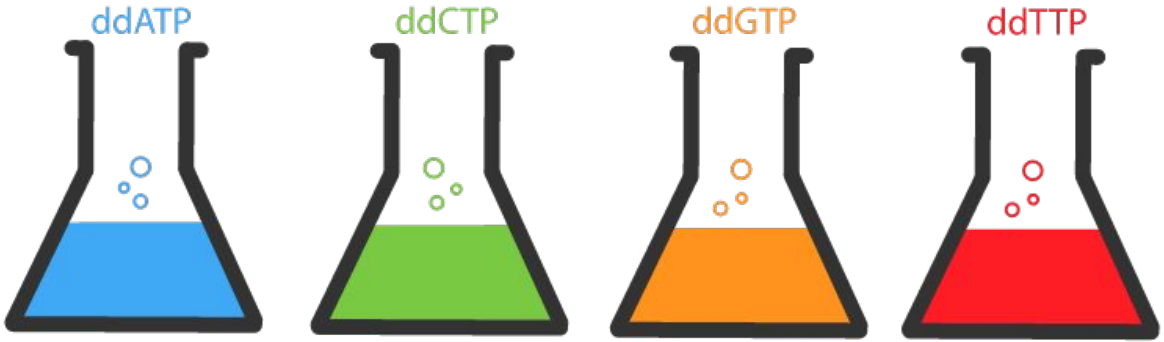


Sanger sequencing example

ACTAGCTA

TGATCGAT

Sanger sequencing



ACTAGCTA
TGATCGAT

ACTA
TGATCGAT

A
TGATCGAT

ACTAGC
TGATCG

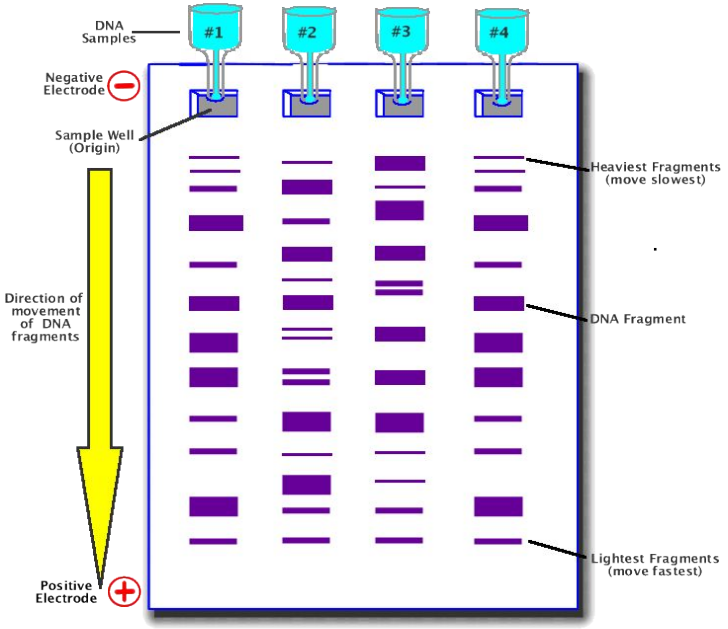
AC
TGATCG

ACTAG
TGATC

ACTAGCT
TGATCGA

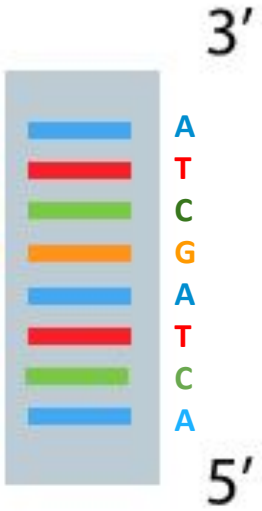
ACT
TGATCGA

Gel Electrophoresis



Sanger sequencing

Sanger sequencing



Sequencing Trends

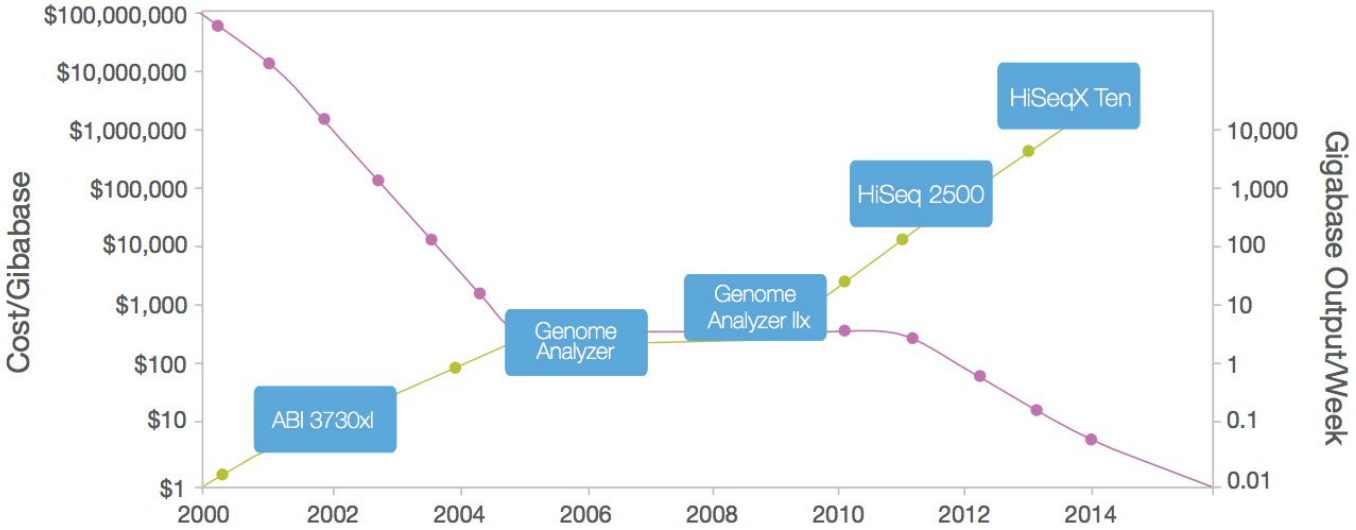


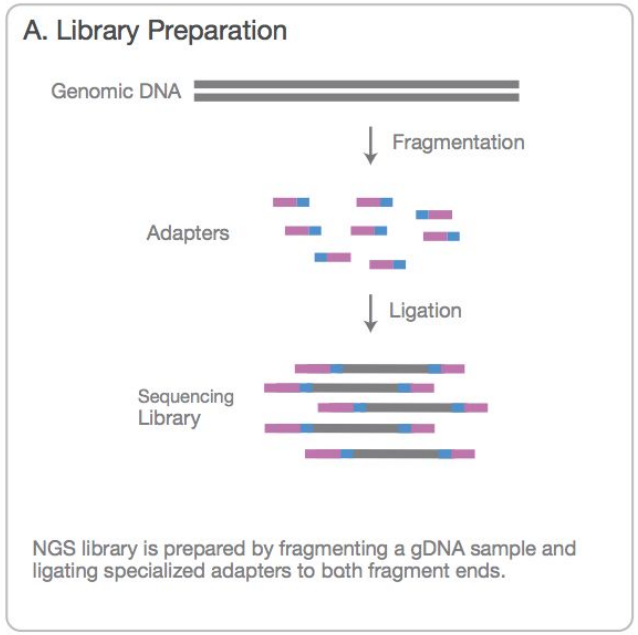
Figure 1: Sequencing Cost and Data Output Since 2000—The dramatic rise of data output and concurrent falling cost of sequencing since 2000. The Y-axes on both sides of the graph are logarithmic.

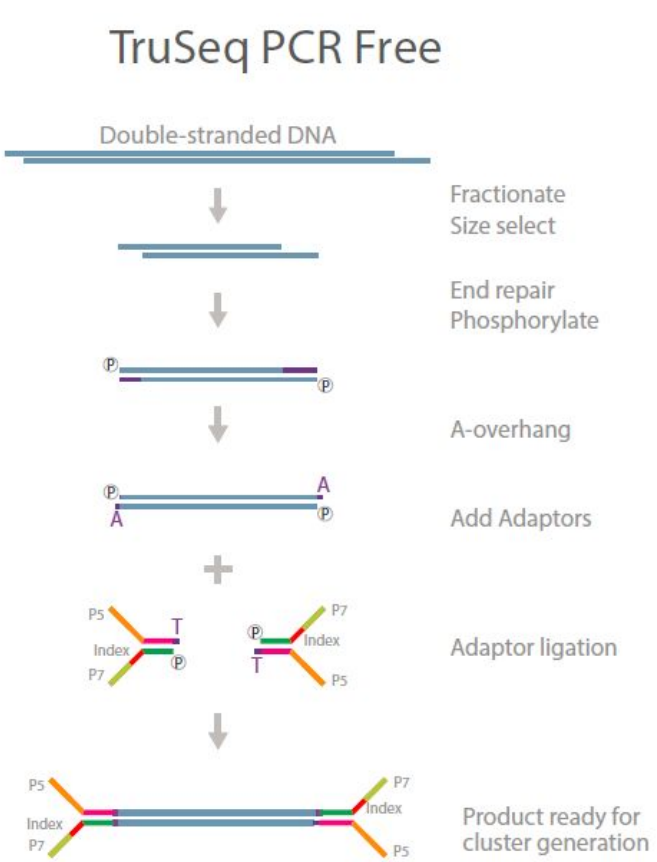
The Birth of Next Generation Sequencing Technologies

DNA polymerase in the sequencing by synthesis approach

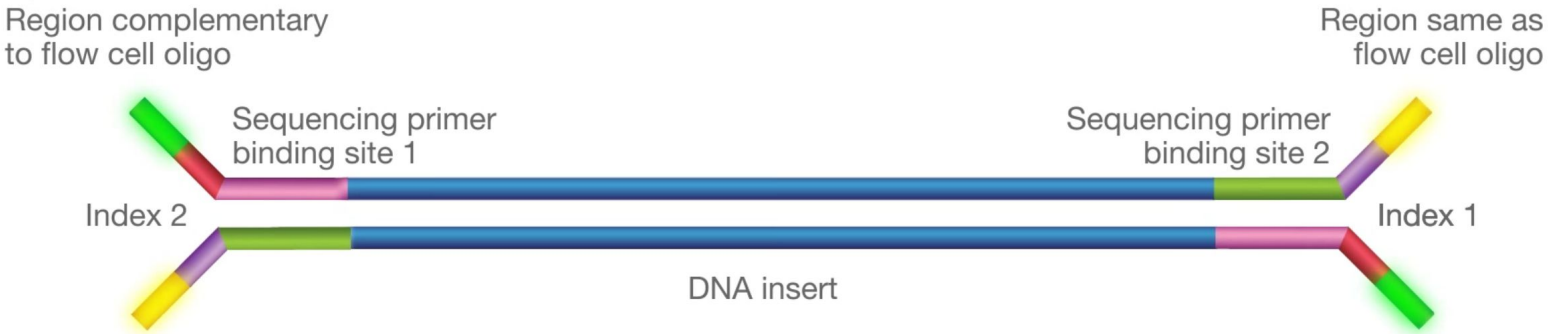

illumina®

Illumina Library Prep

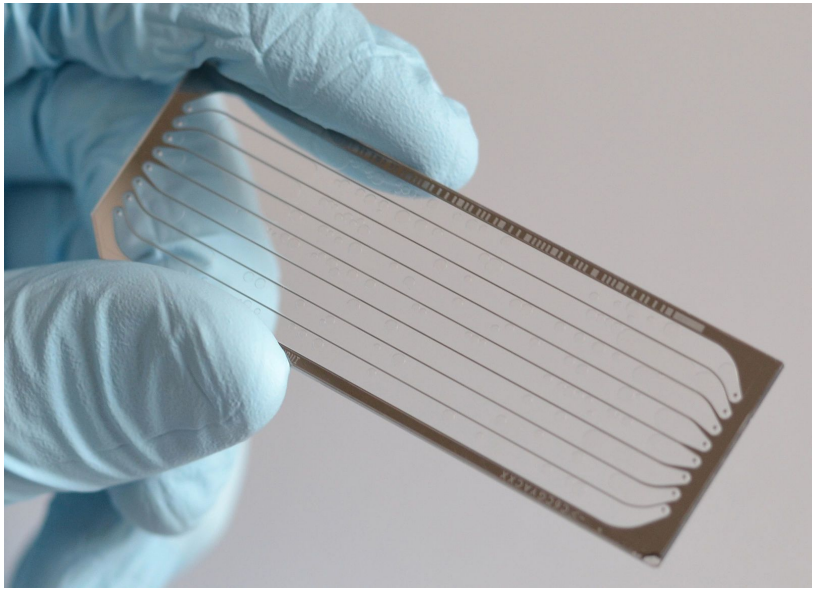




Illumina library prep



Illumina flow cell



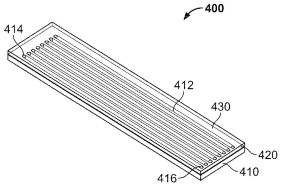


FIG. 3A

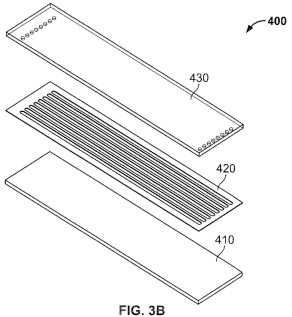
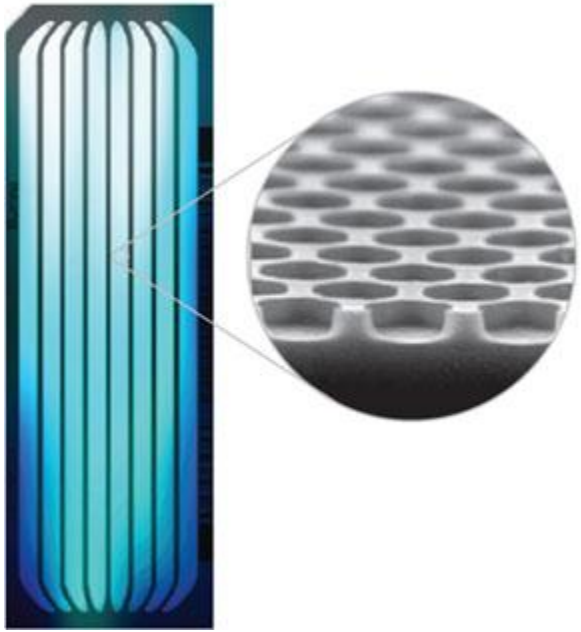
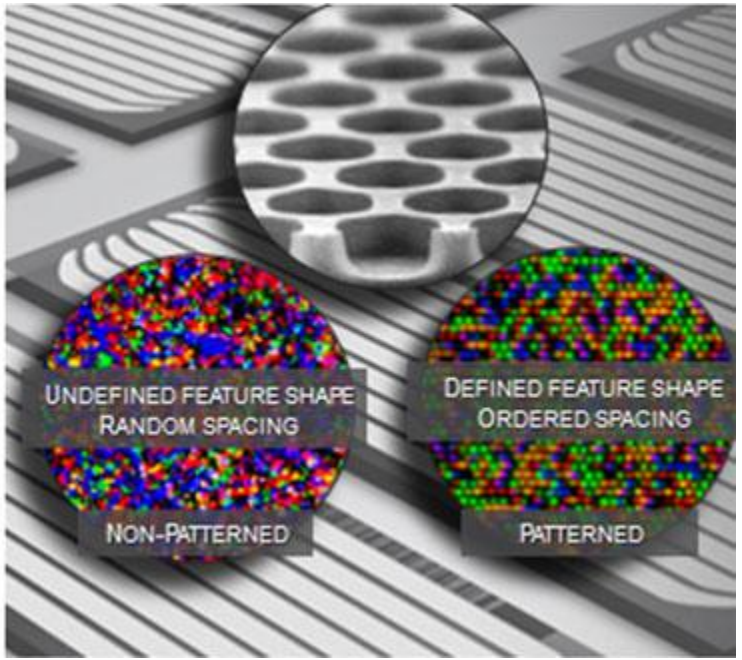


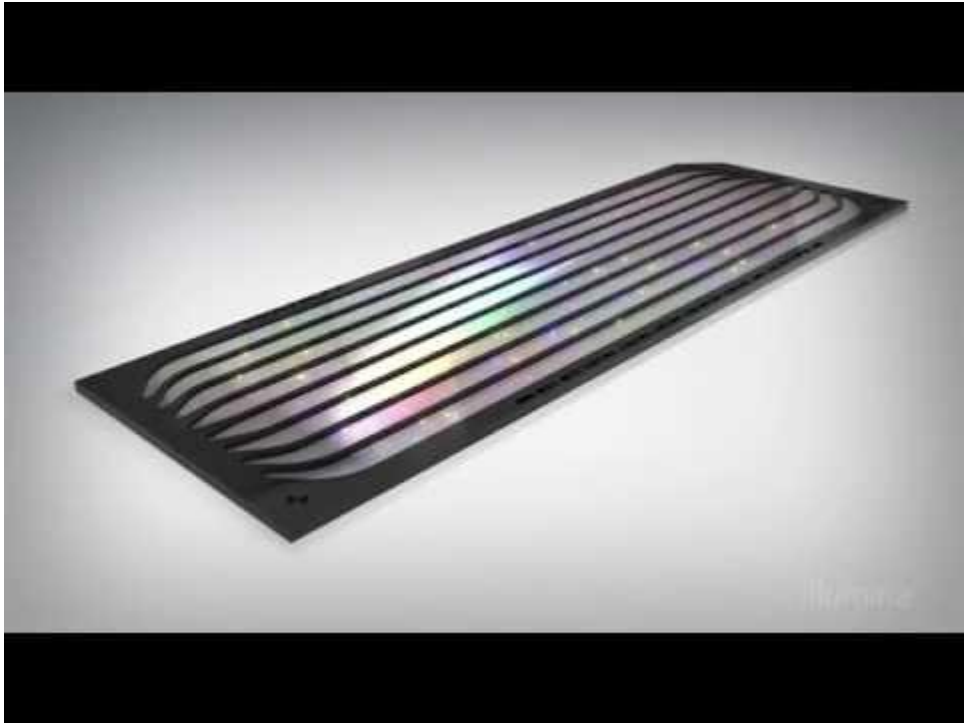
FIG. 3B

Illumina flow cell





Cluster Amplification



Illumina sequencing

Illumina sequencing: Paired ends

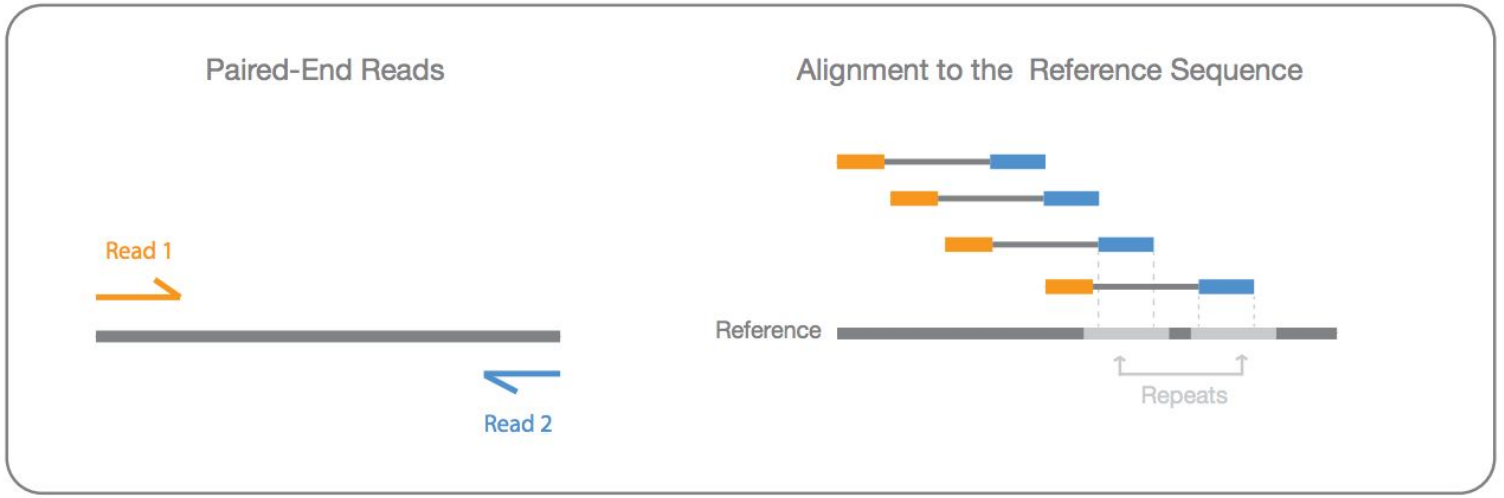
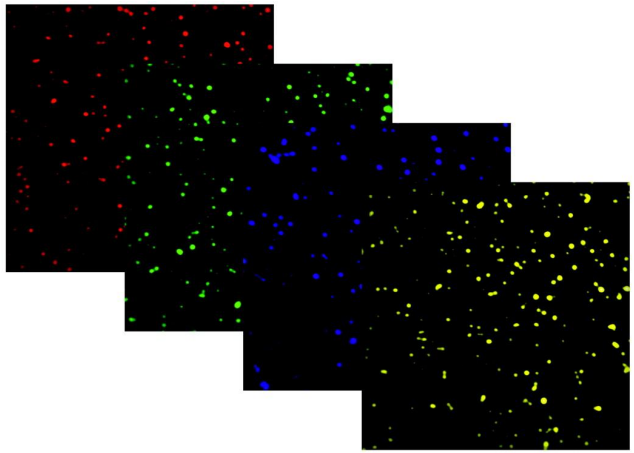


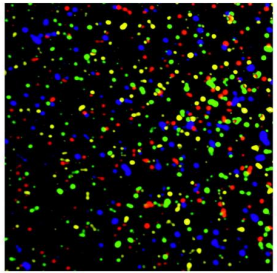
Figure 4: Paired-End Sequencing and Alignment—Paired-end sequencing enables both ends of the DNA fragment to be sequenced. Because the distance between each paired read is known, alignment algorithms can use this information to map the reads over repetitive regions more precisely. This results in much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

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%

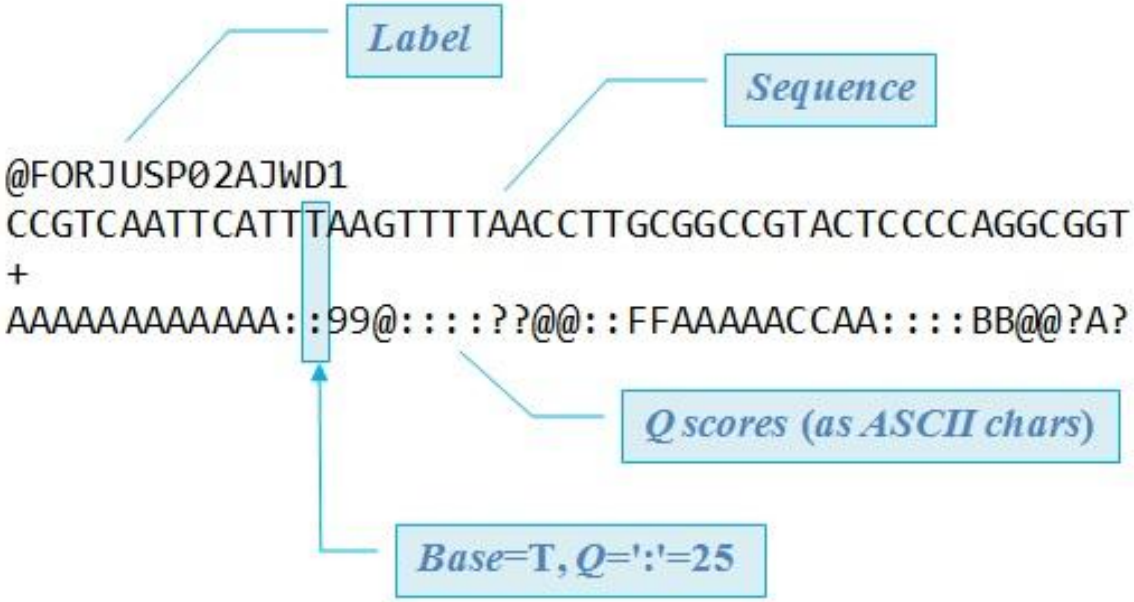
Materials from Wikepedia

ASCII Base + 33 encoding

ASCII_BASE=33 Illumina, Ion Torrent, PacBio and Sanger

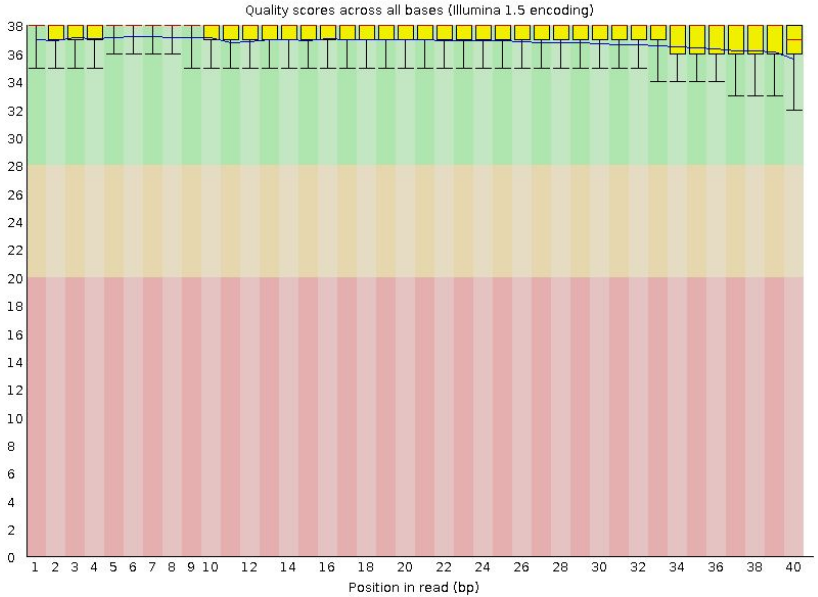
Q	P_error	ASCII	Q	P_error	ASCII	Q	P_error	ASCII	Q	P_error	ASCII
0	1.00000	33 !	11	0.07943	44 ,	22	0.00631	55 7	33	0.00050	66 B
1	0.79433	34 "	12	0.06310	45 -	23	0.00501	56 8	34	0.00040	67 C
2	0.63096	35 #	13	0.05012	46 .	24	0.00398	57 9	35	0.00032	68 D
3	0.50119	36 $	14	0.03981	47 /	25	0.00316	58 :	36	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			

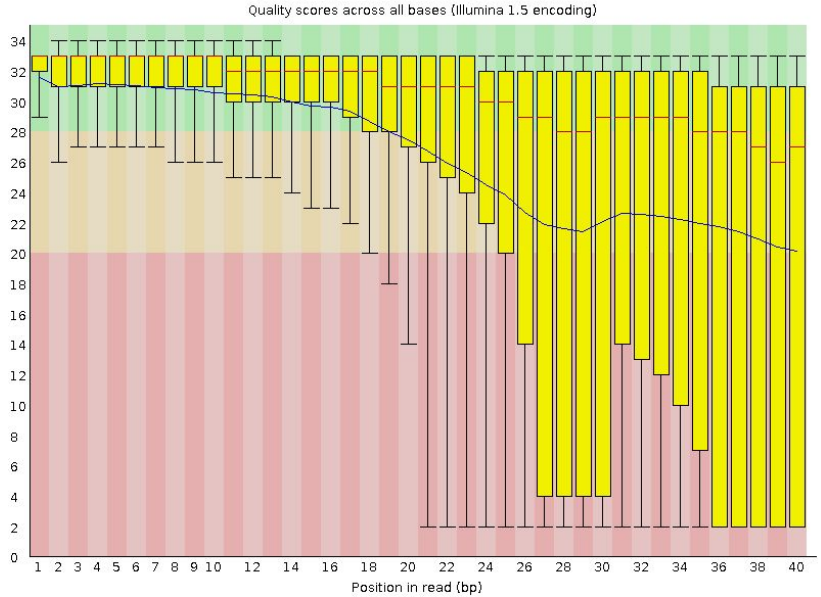
FASTQ file format



FASTQ - Analysis

Per Base Sequence Quality





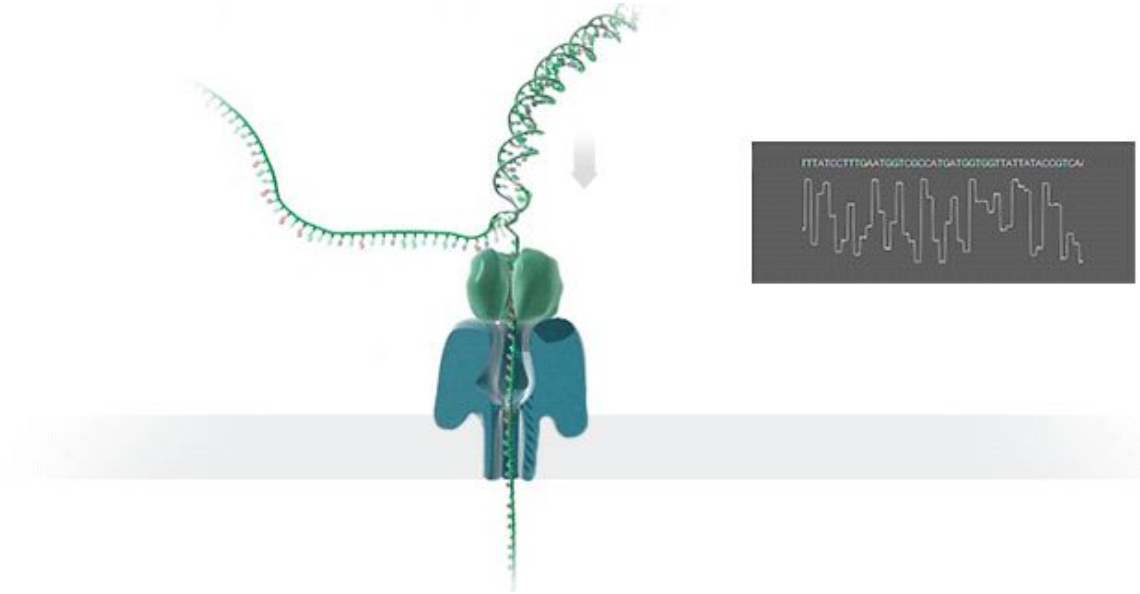
Other NGS Technologies

Nanopore sequencing

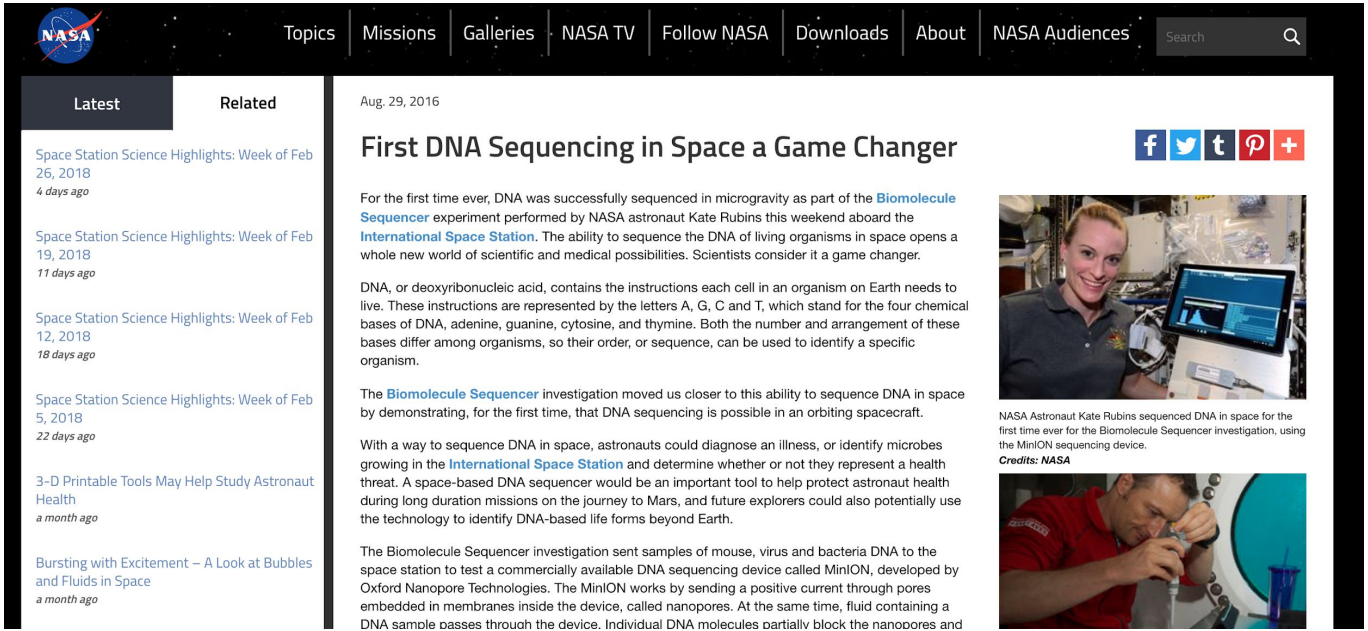
SMRT/ZMV Sequencing

PACIFIC
BIOSCIENCES®

Oxford NANOPORE Technologies



Oxford Nanopore MinION



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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]

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

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