
DTU

DTU Health Technology
Bioinformatics

Introduction to NGS

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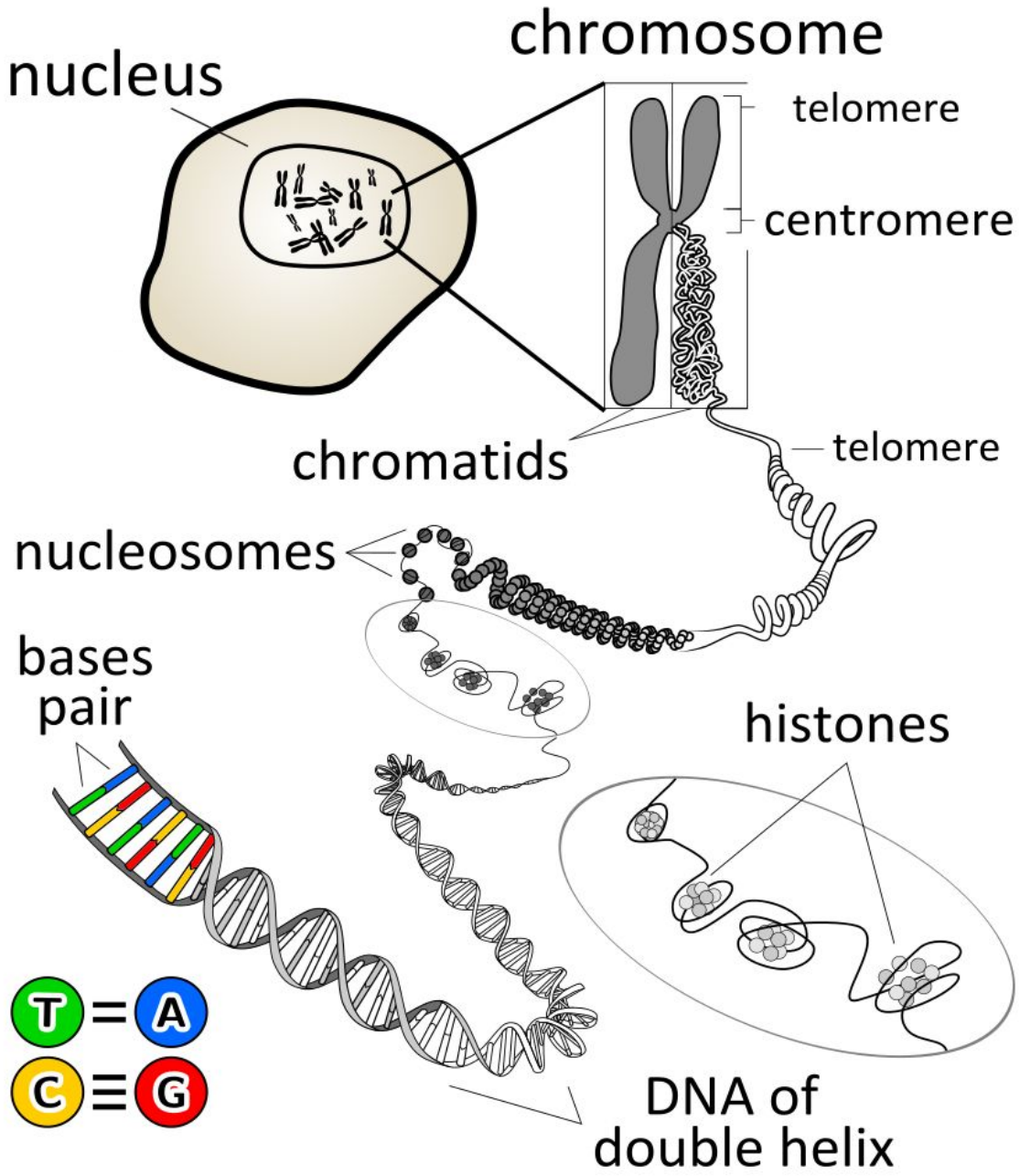
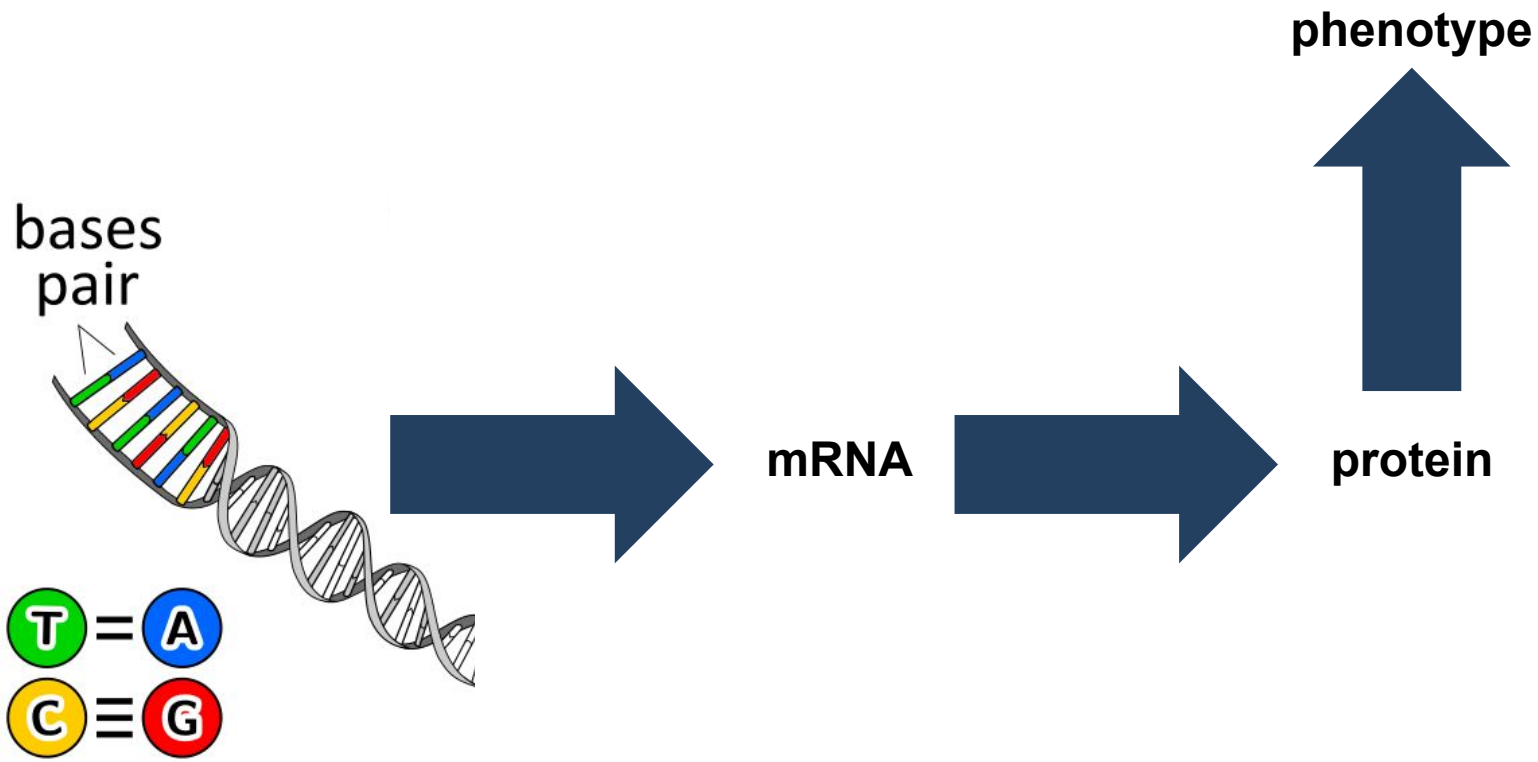
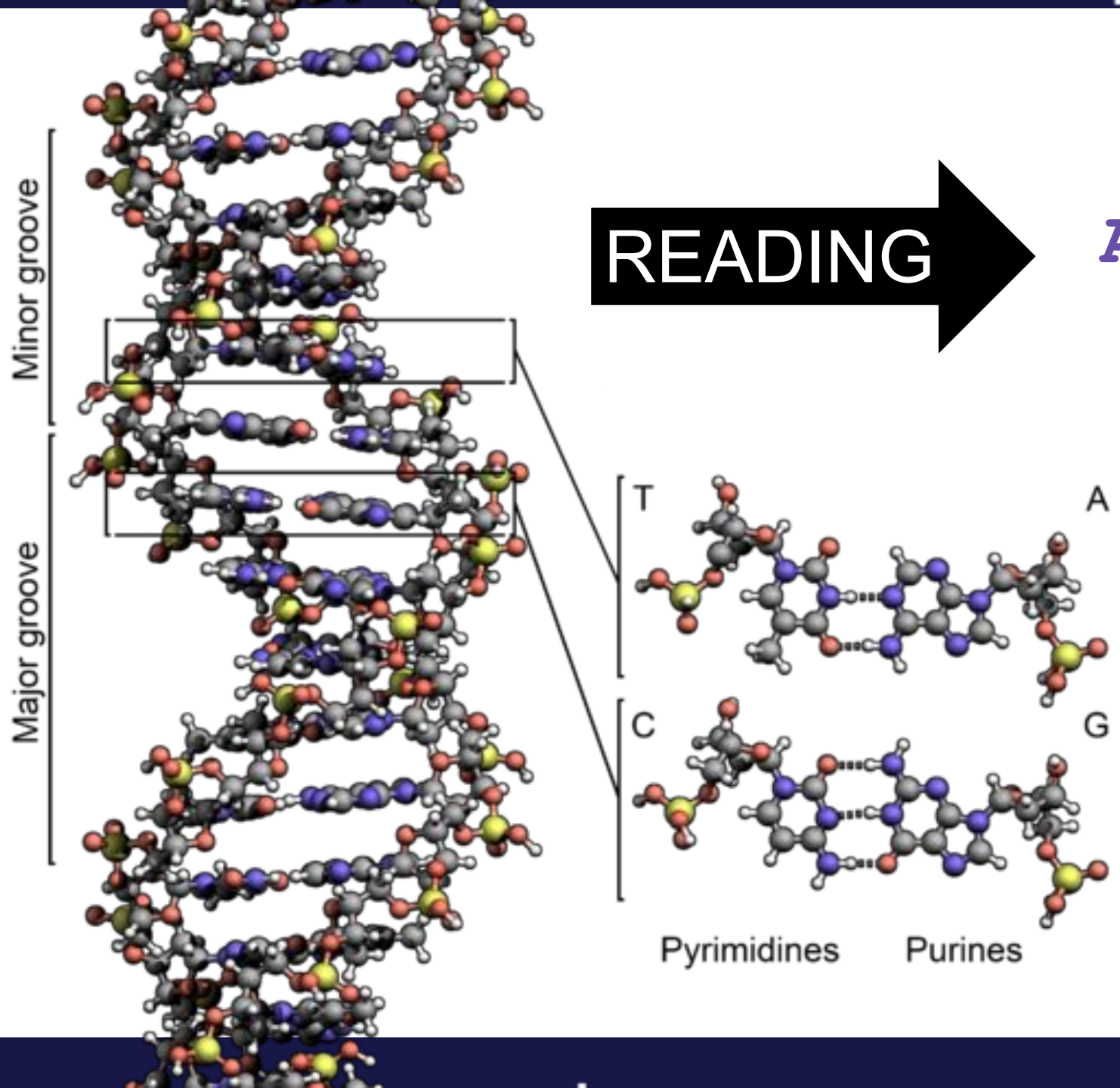
Menu

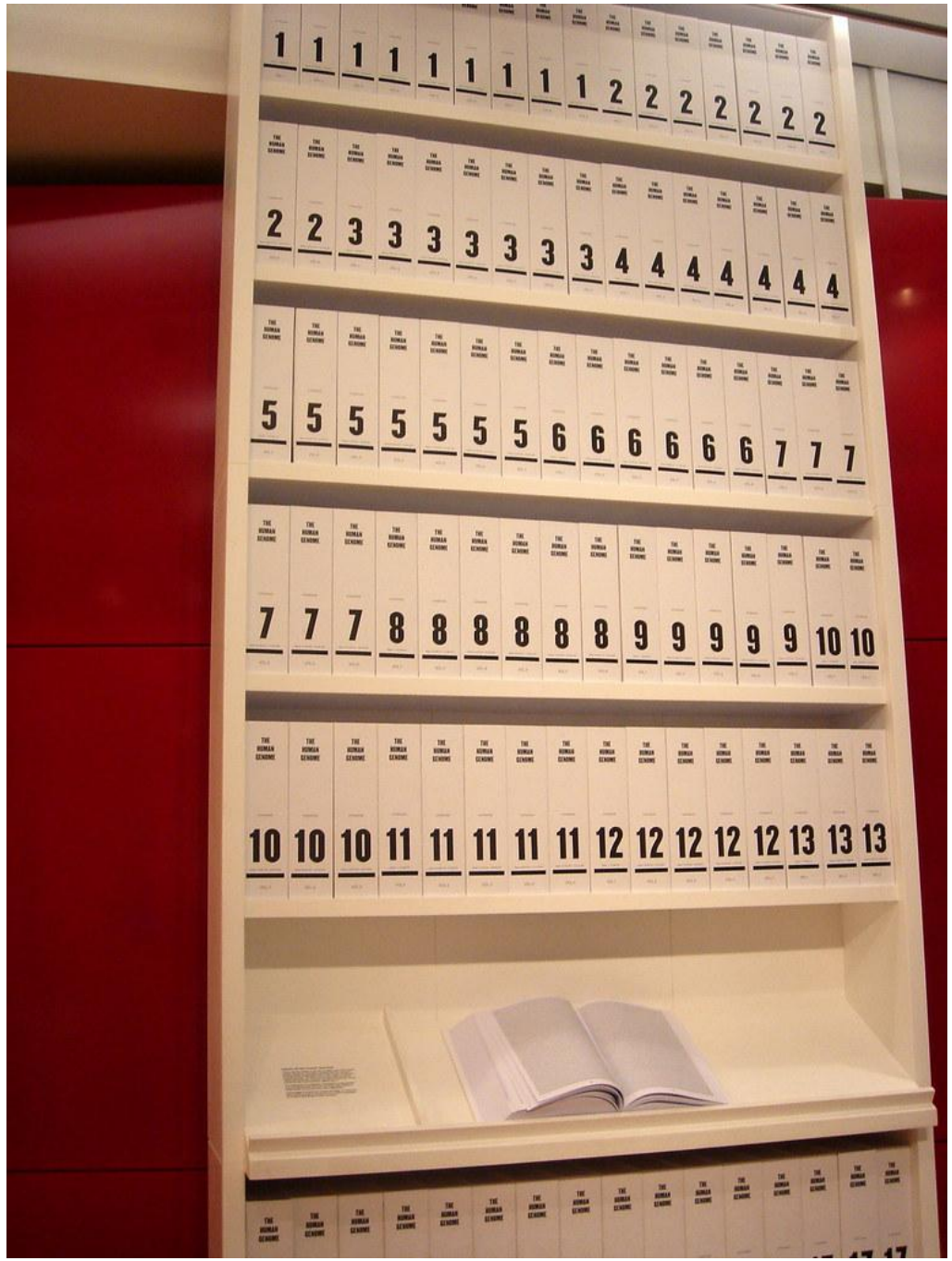
- What is sequencing? why?
- Basic nomenclature

What is sequencing?


chromosome
nucleus
telomere
centromere
chromatids
telomere
nucleosomes
bases
pair
histones
T = A
C ≡ G
DNA of
double helix

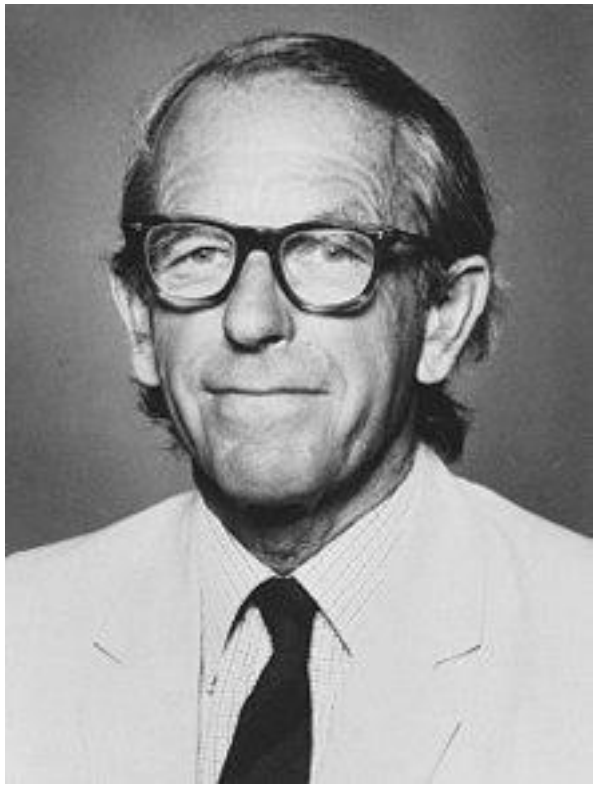

phenotype
bases
pair
T = A
C ≡ G
mRNA
protein


Minor groove
Major groove
READING
AGCAATCTCAATTACA
T
A
C
G
Pyrimidines
Purines



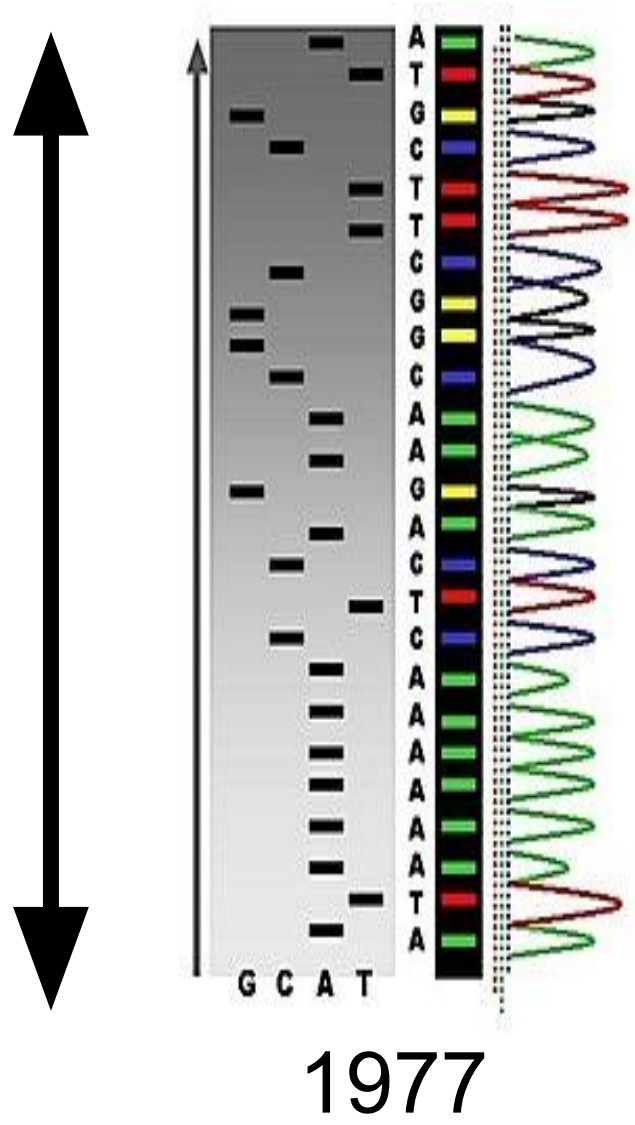
Human genome
3 billion letters

If we study Next-Generation Sequencing, why “next”? What was before?



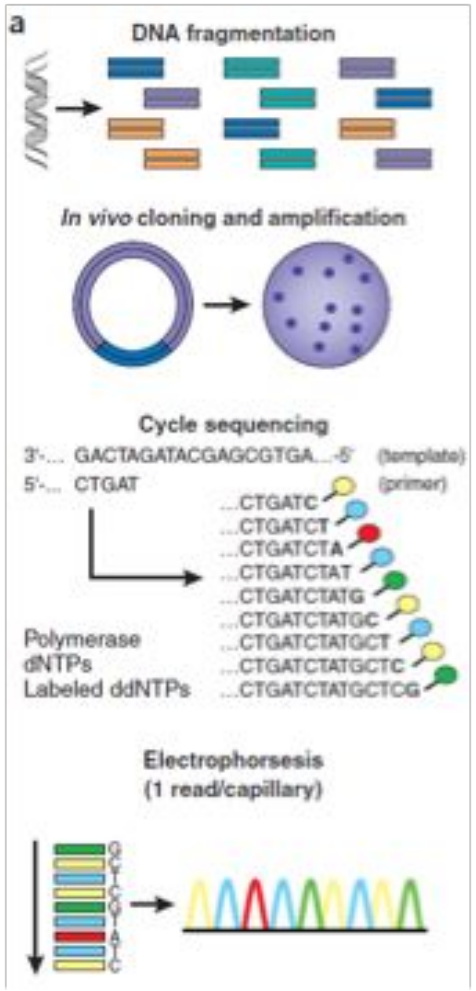
Frederick Sanger 1918 - 2013

1000 bases
x
96



1977

First generation: Sanger



- Fragment DNA
- Clone into plasmid and amplify
- DNA polymerase and only 1 primer
- Sequence using labeled dinucleotides which cap seqs.
- Run capillary electrophoresis/gel and “read” DNA code
- Low output, long reads (~800-1200 nt), high quality
- Produces 96 reads / run

Why sequence?

AGGATTATTGGTACT

AGGATTATTGGTACT

AGGATTATCGGTACT

AGGATTATTGGTACT

AGGATTATCGGTACT

AGGATTATTGGTACT

AGGATTATCGGTACT

AGGATTATTGGTACT

AGGTTTATTGGTACT

AGGATTATCGGTACT

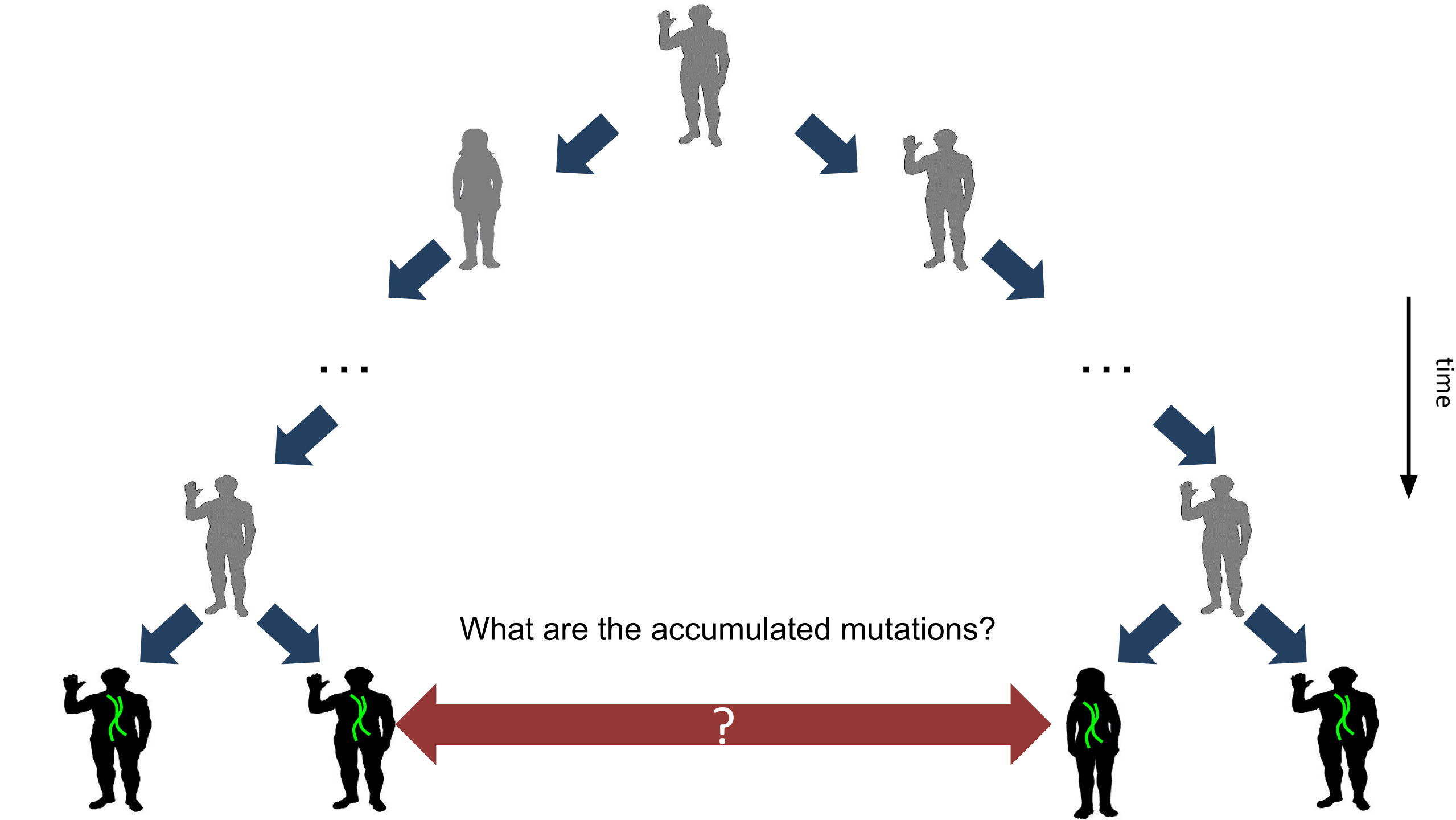
AGGATTATCGGTACT

AGGTTTATTGGTACT

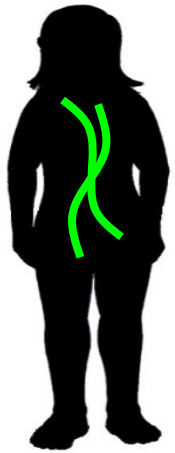
AGGTTTATTGGTAGT

AGGATTATCGGTACT

AAGATTATCGGTACT

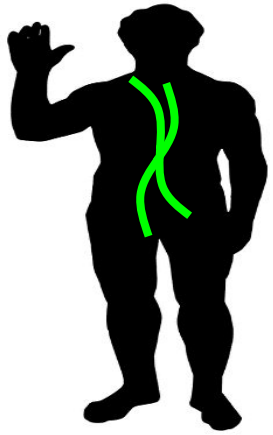

time
What are the accumulated mutations?
?

Investigate differences within a species

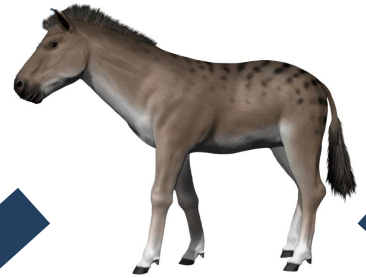


AGGTTTATTGGTAGT

AAGATTATCGGTACT

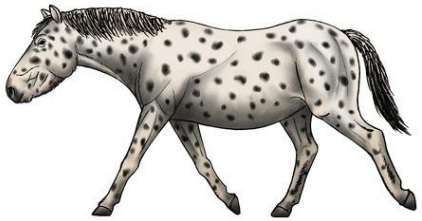


Investigate differences between species



?

...



...

?

time

"Nothing in Biology Makes Sense Except in the Light of Evolution"

Theodosius Dobzhansky, 1973

"Nothing in Biology Makes Sense Except in the Light of Evolution"

Theodosius Dobzhansky, 1973

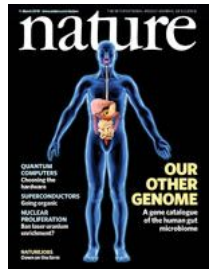
NGS

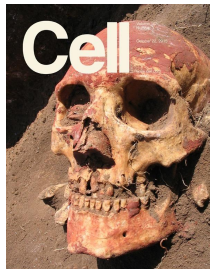
"Nothing in ~~Biology~~ Makes Sense Except in the Light of Evolution"

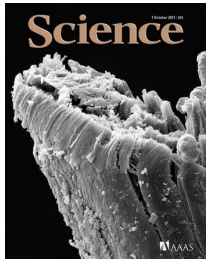
me, I made that up just now

What can we use it for?

- Whole genome re-sequencing
- Population genomics
- Diagnostics
- Cancer genomics
- Ancient genomes
- Metagenomics
- RNA sequencing
- Single cell sequencing
- Genomic Epidemiology
- anything with DNA





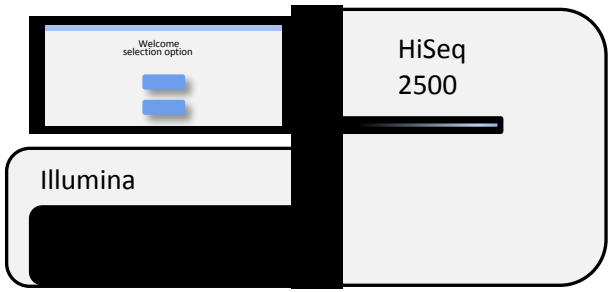


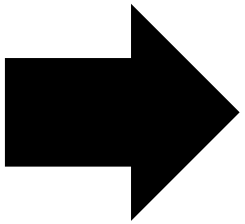
Basic concepts

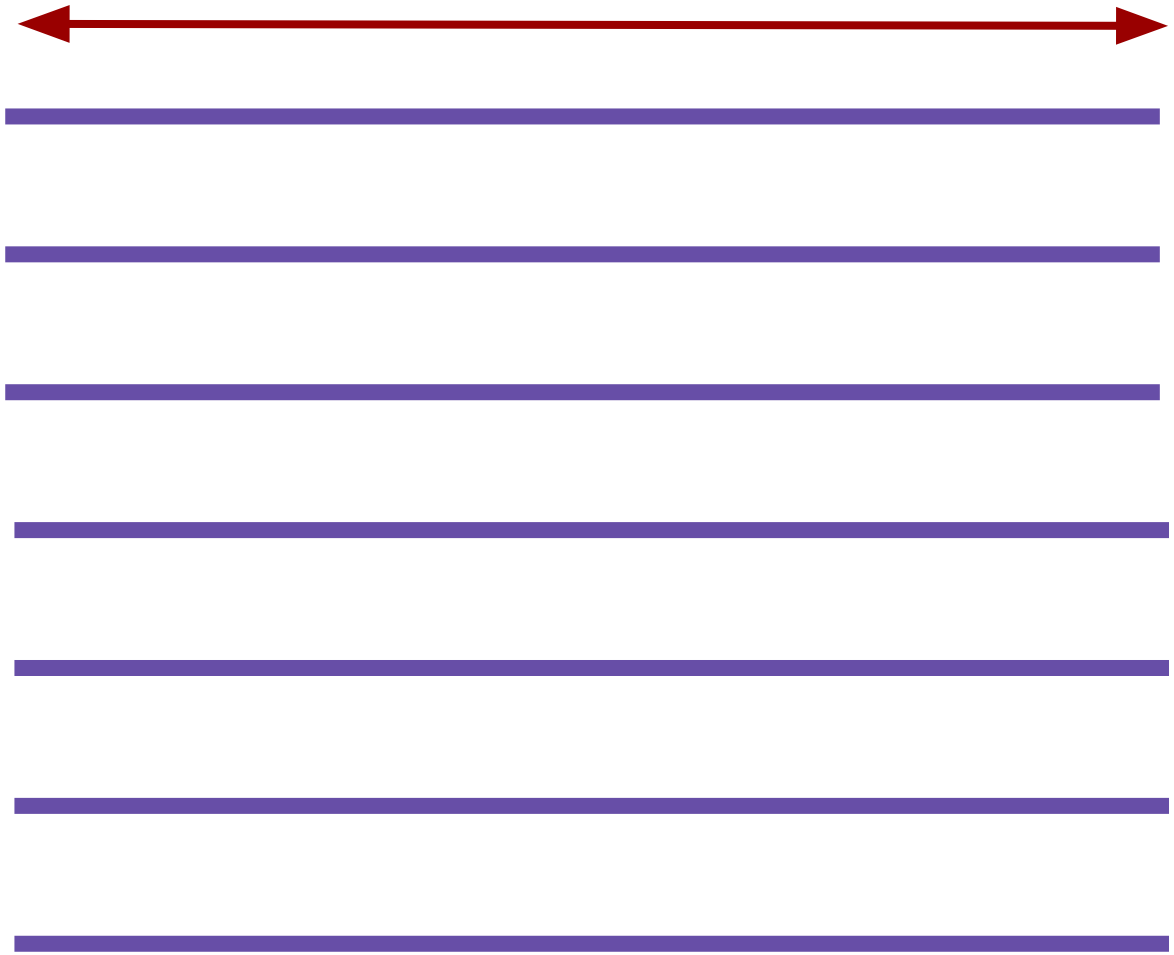
3 key concepts

- Read length
- Throughput
- Types of errors

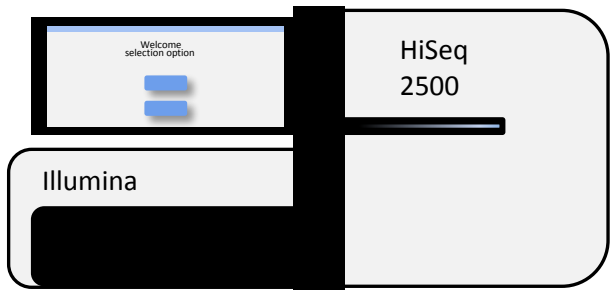
read length

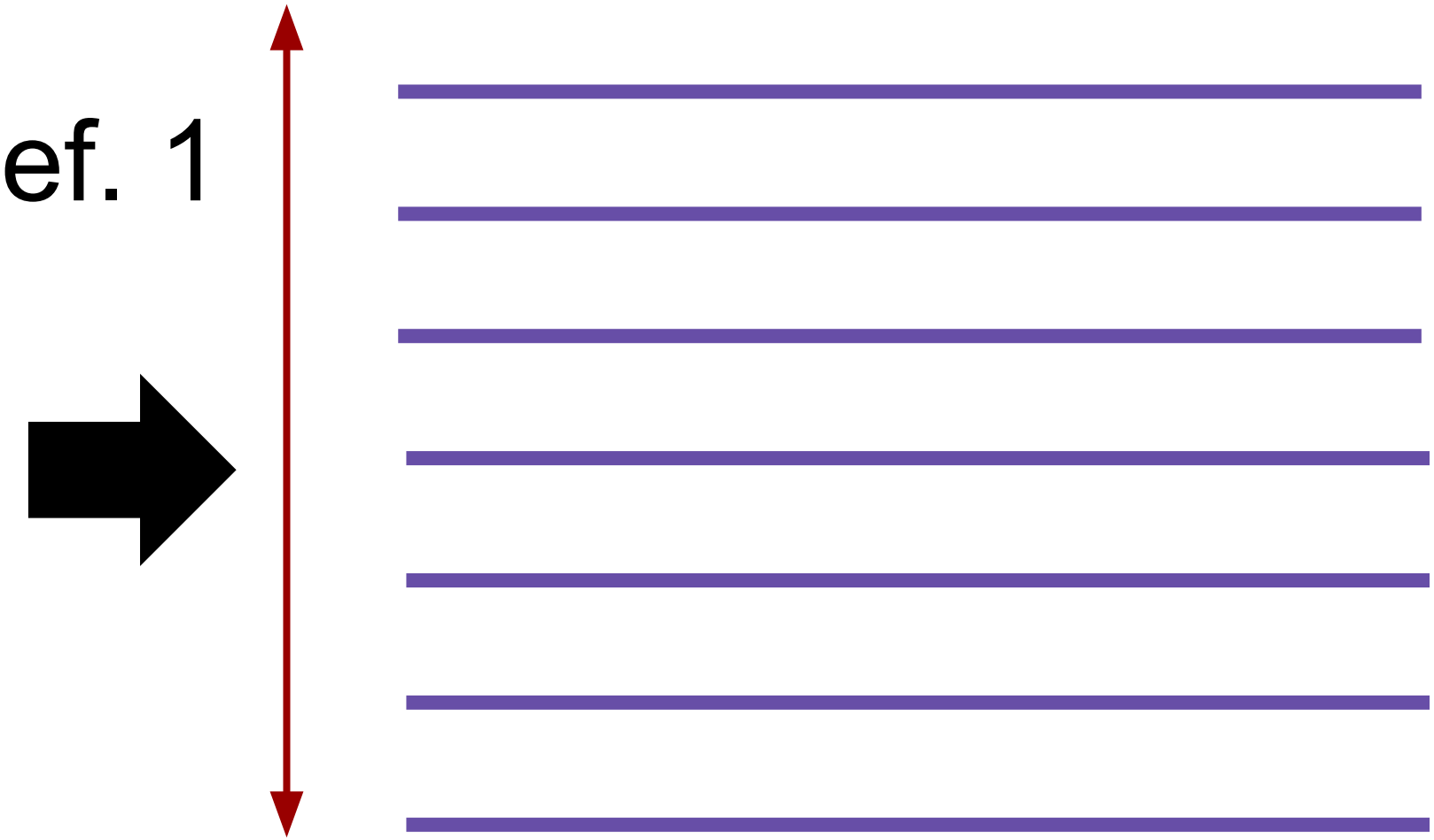




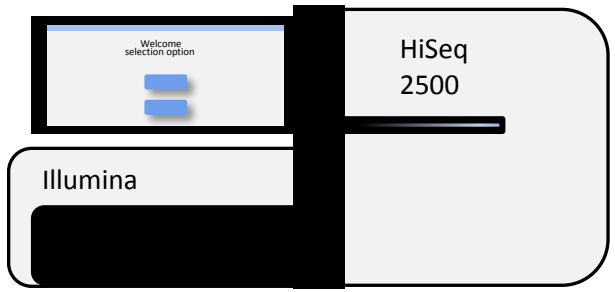


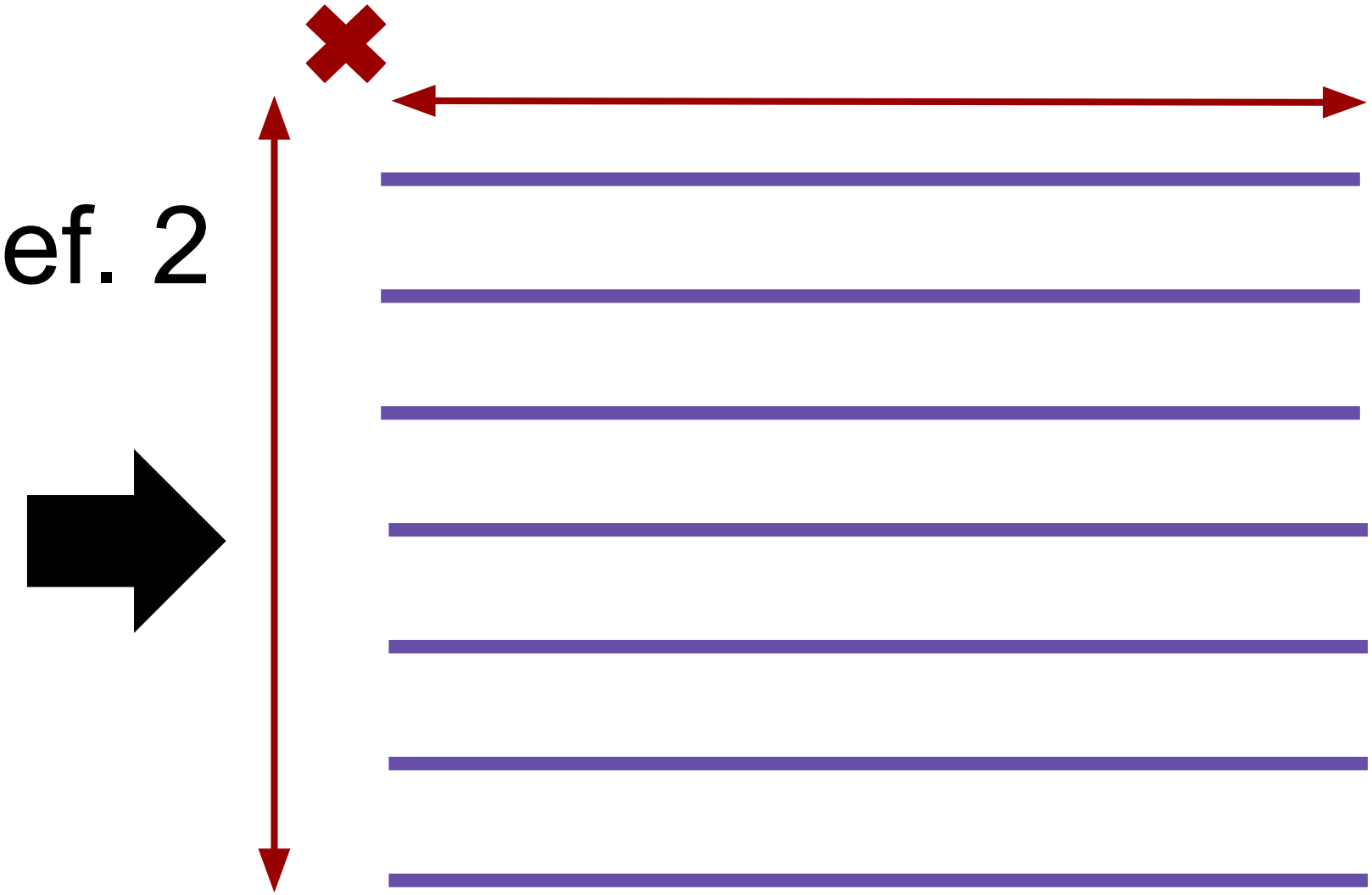
throughput def. 1



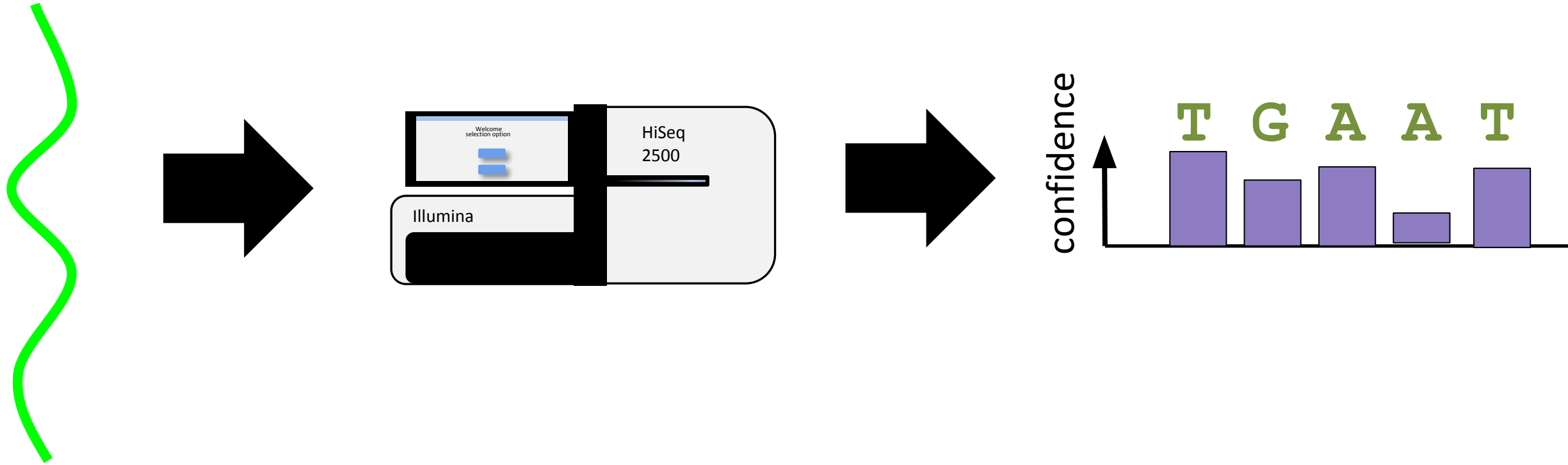


throughput def. 2

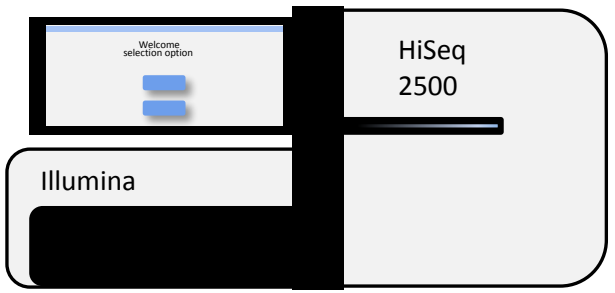


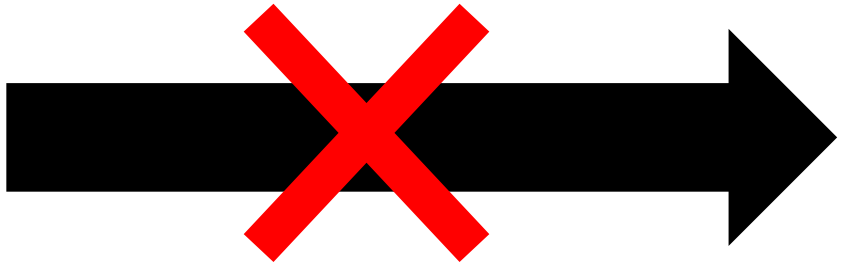


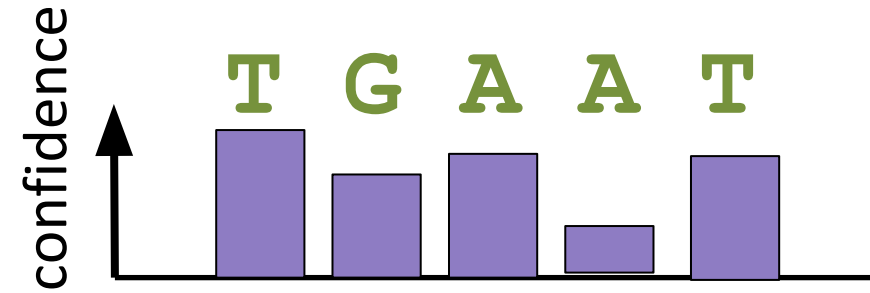
template

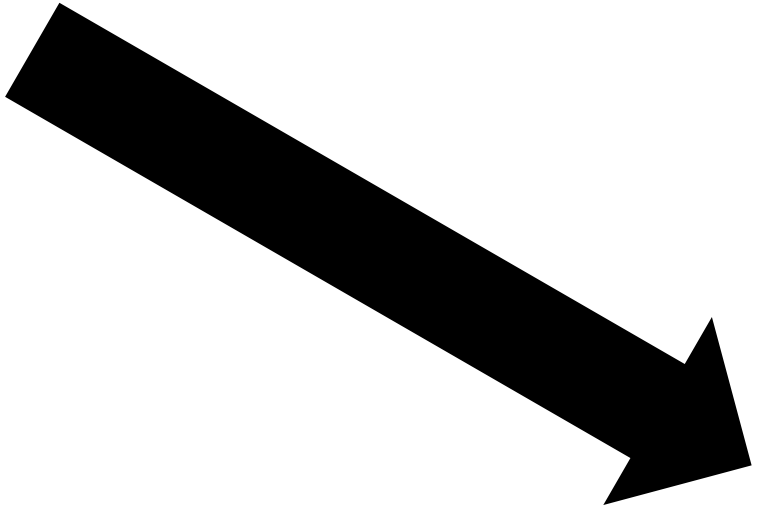

Welcome
selection option
HiSeq
2500
Illumina
confidence
T G A A T

Key concept: basecalling

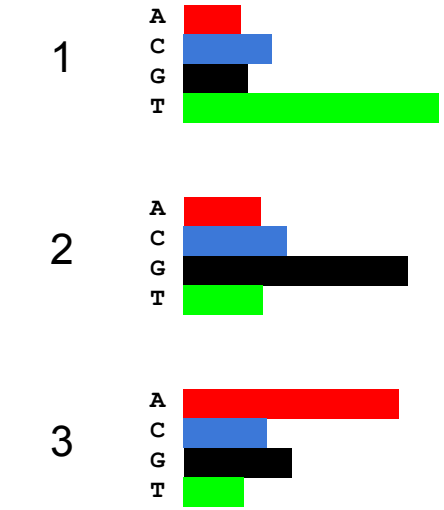




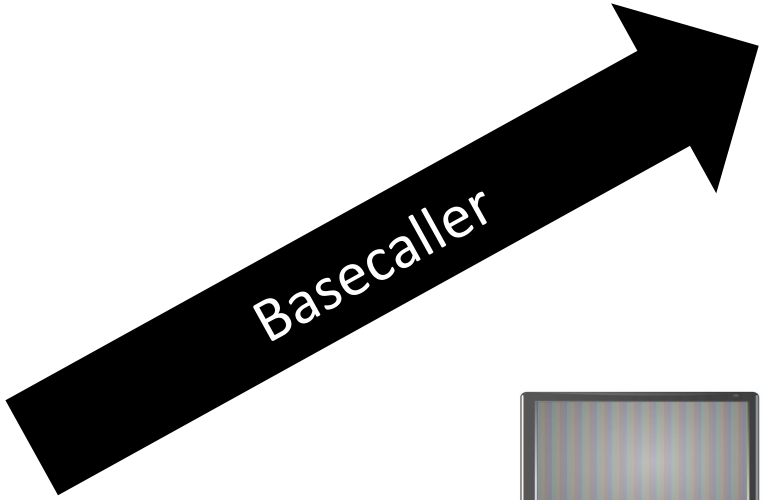




Base#

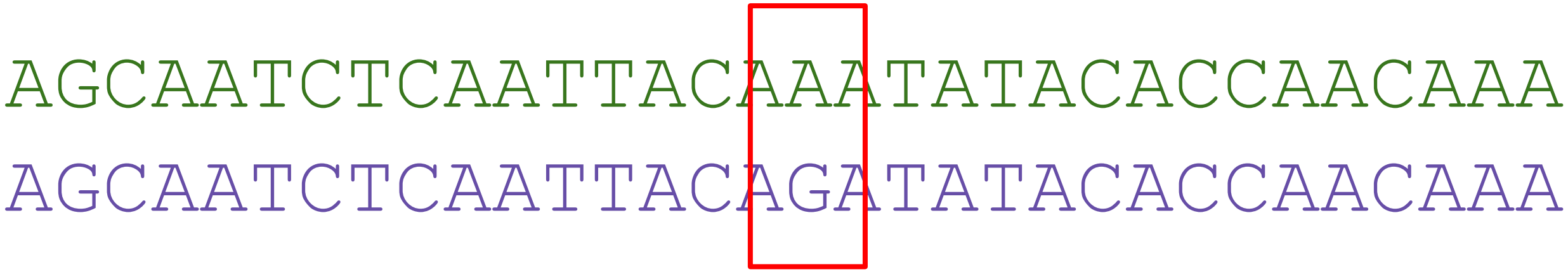


Raw Data



mismatch

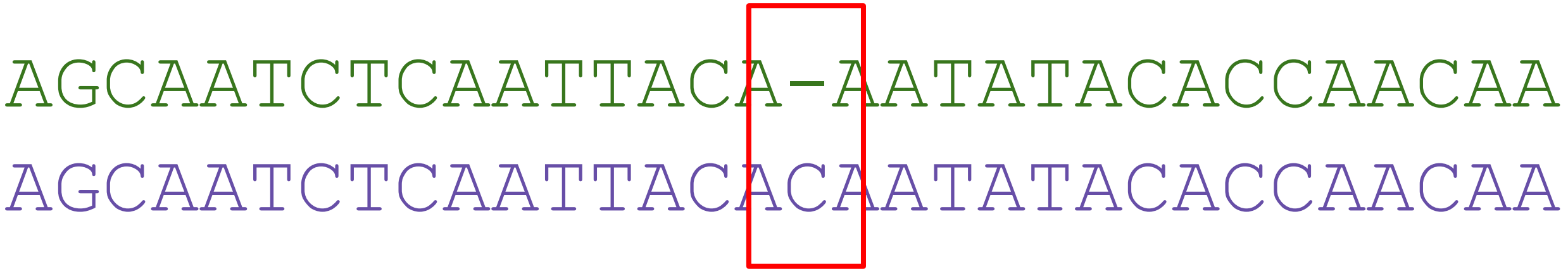
template



read

insert

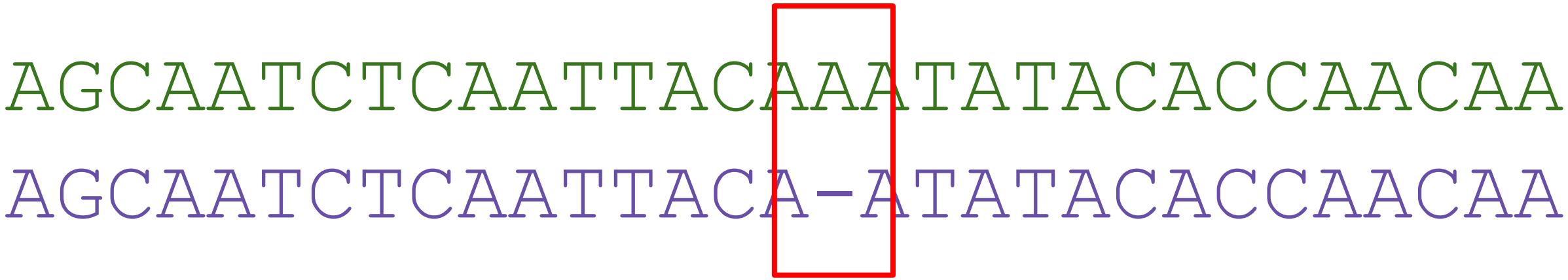
template



read

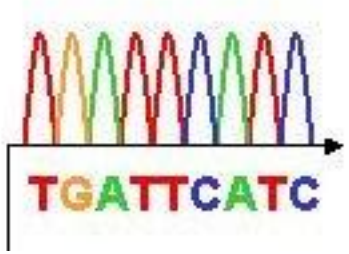
deletion

template



read

1977 1980 1983 1986 1989 1992 1995 1998 2001 2004 2007 2010 2013 2016 2019



Sanger

454

PacBio

Ion Torrent

Oxford Nanopore

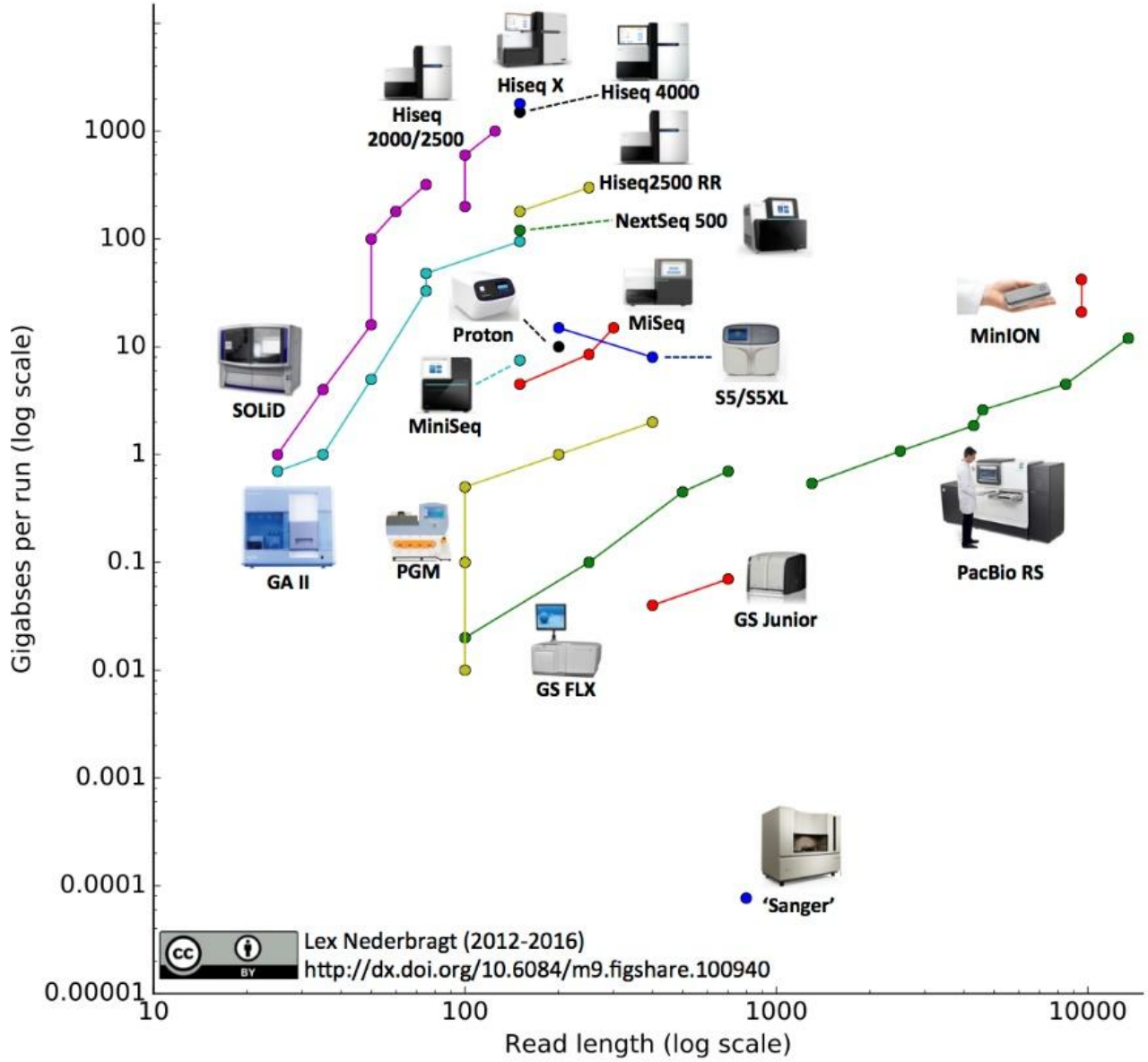
SOLiD

BGI

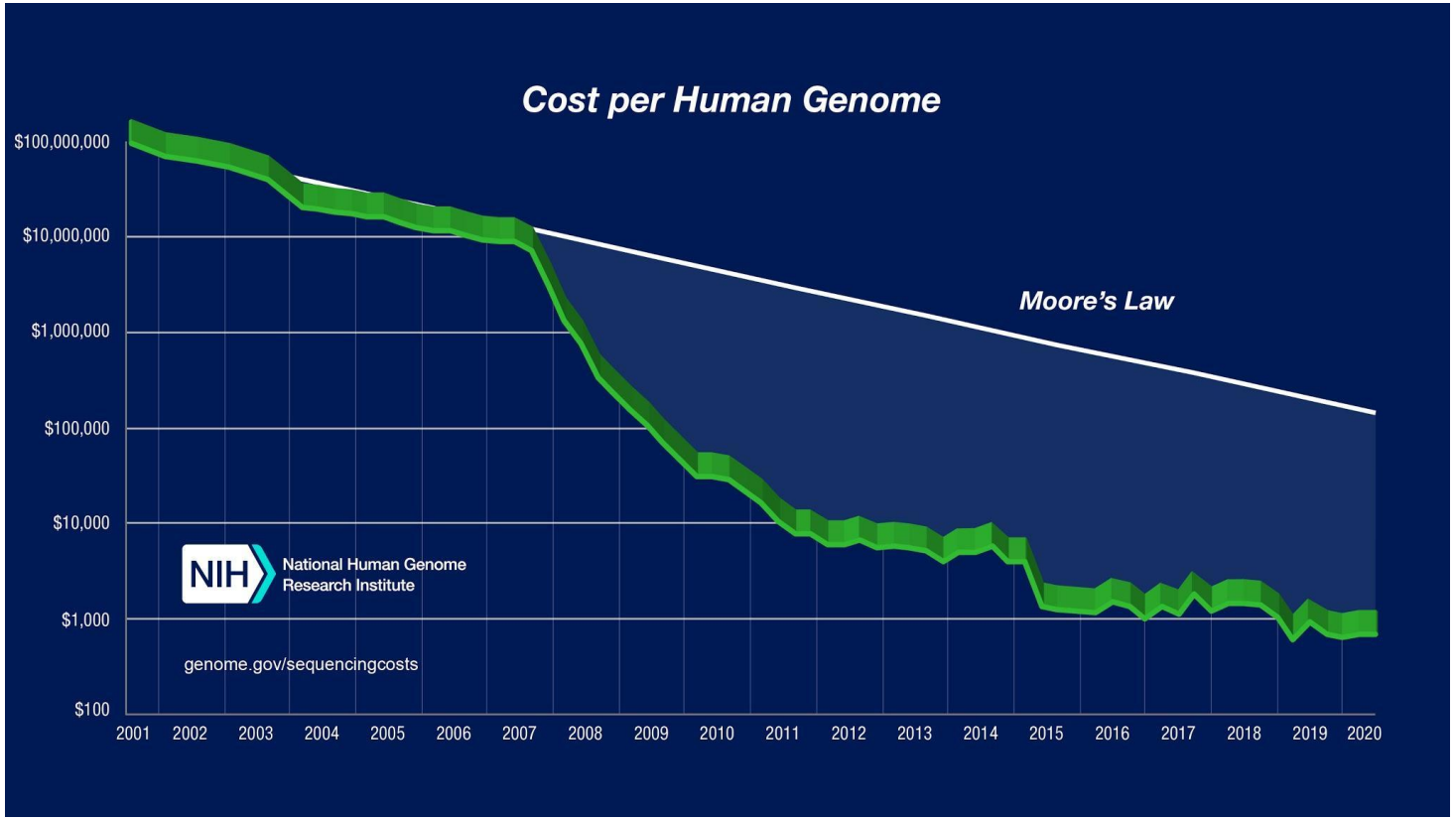
Illumina

1st generation of NGS

- 454 Life Sciences.
  – Bought by Roche 2007.
- Illumina/BGI is currently cheapest per GB
- Long-read sequencing is revolutionizing assembly



Sequencing costs



NHGRI - August 2020

- Computer speed and storage capacity is **doubling every 18 months** and this rate is steady

- DNA sequence data **is doubling faster than computer speeds!**

1990 - 2003

~5.16G USD (adj. for FY 2020)

20 research centers, 6 countries

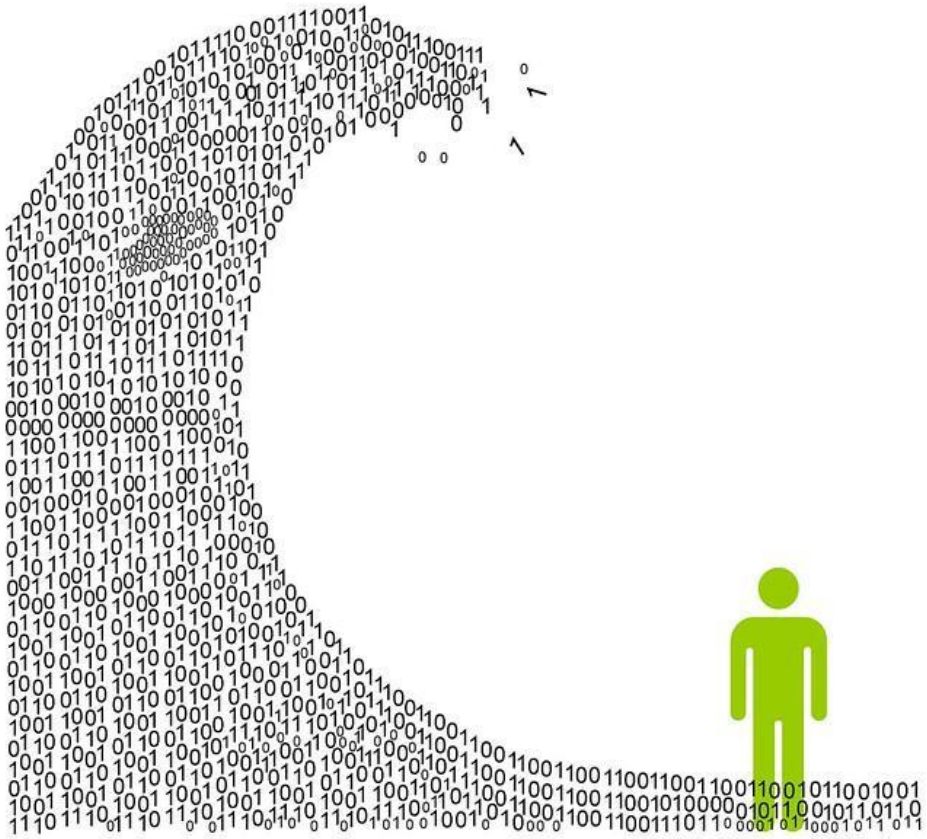
Picture: The Guardian

Human sequencing

- First draft genome of human in 2001, final 2004
- Estimated costs $5.16 billion USD, time 13 years

- Today:
  - 1000-2000$ for one genome
  - A couple of days!

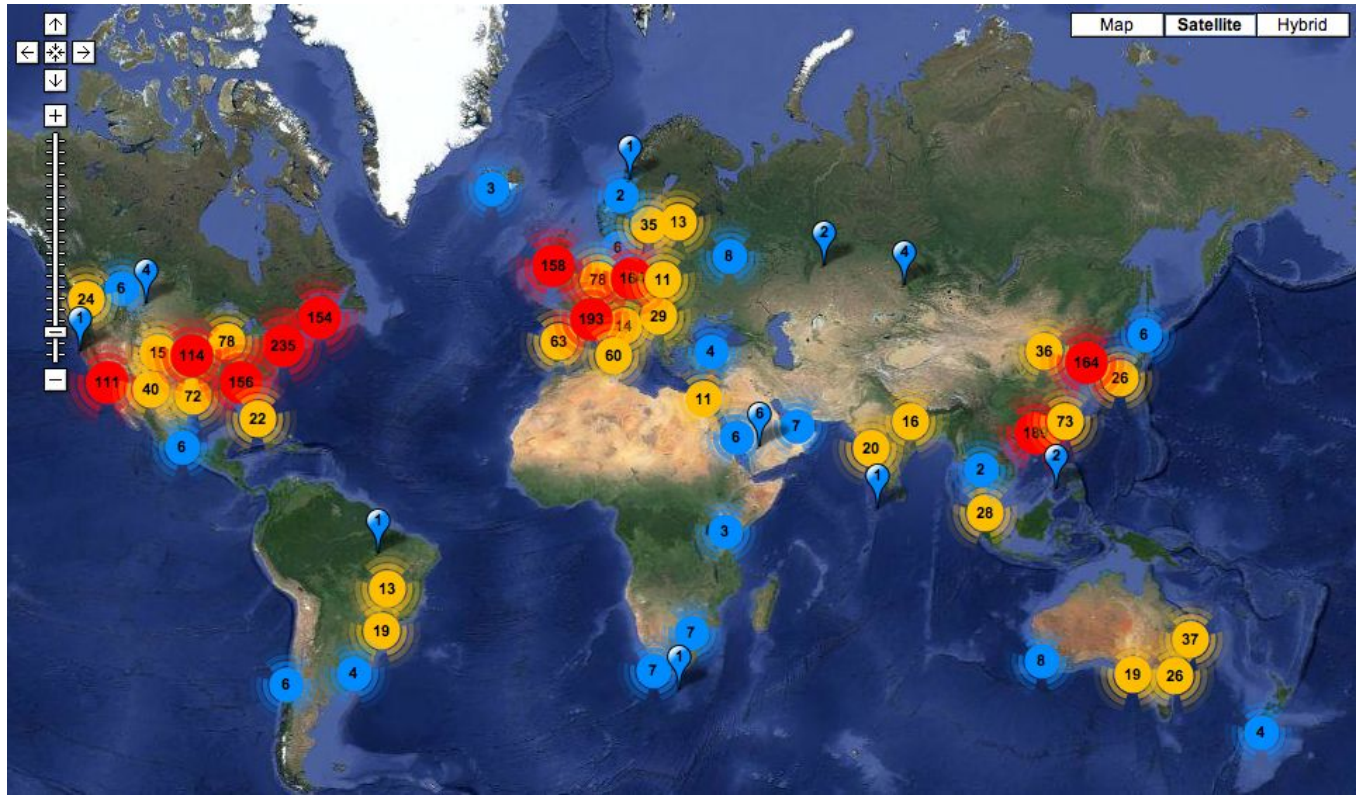
Storage and analysis



- Cost of sequencing is almost less than the cost of storage and analysis
- One Illumina NovaSeq system: almost 10,000 human genomes per year!
- A standard human (30-40x) whole-genome sequencing exp. would create 30-150 Gb of data

Distributed data production

- Worldwide >900 centers
- >60 Pb pr year (2014)
- 20,000 Pb pr year (2025)
- Data transfer and storage becomes an issue

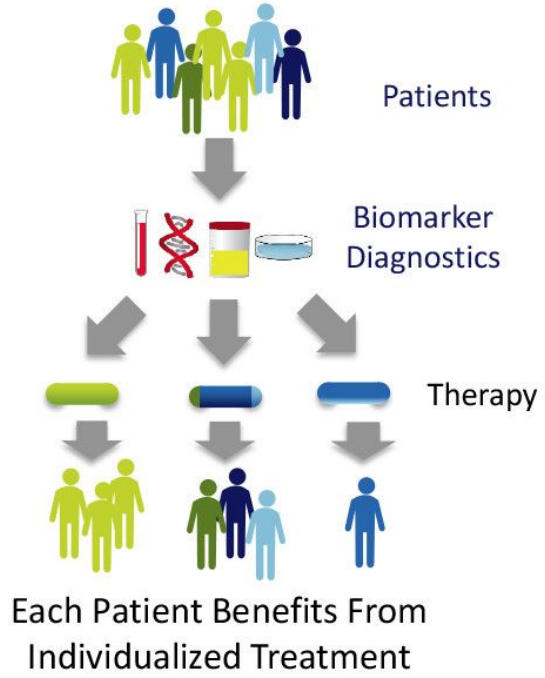


The X Genomes projects

- Human population projects
  - 1000 genomes project (2500 individuals)
  - Genomics England (100k individuals)
  - US Precision Medicine (1 million individuals)
- 100K pathogens project, Earth Microbiome project, Cancer genome project, Plants and animals, Insects,…

NGS in the clinic

- Diagnostics of patients (+ fetus)
- Focused treatment of cancer patients
- Sequencing of bacterial isolates
- Country-wide projects:
  - UK, US, UAE, Qatar, Finland, China, …
  - DK: Danish regions want to sequence 100k individuals



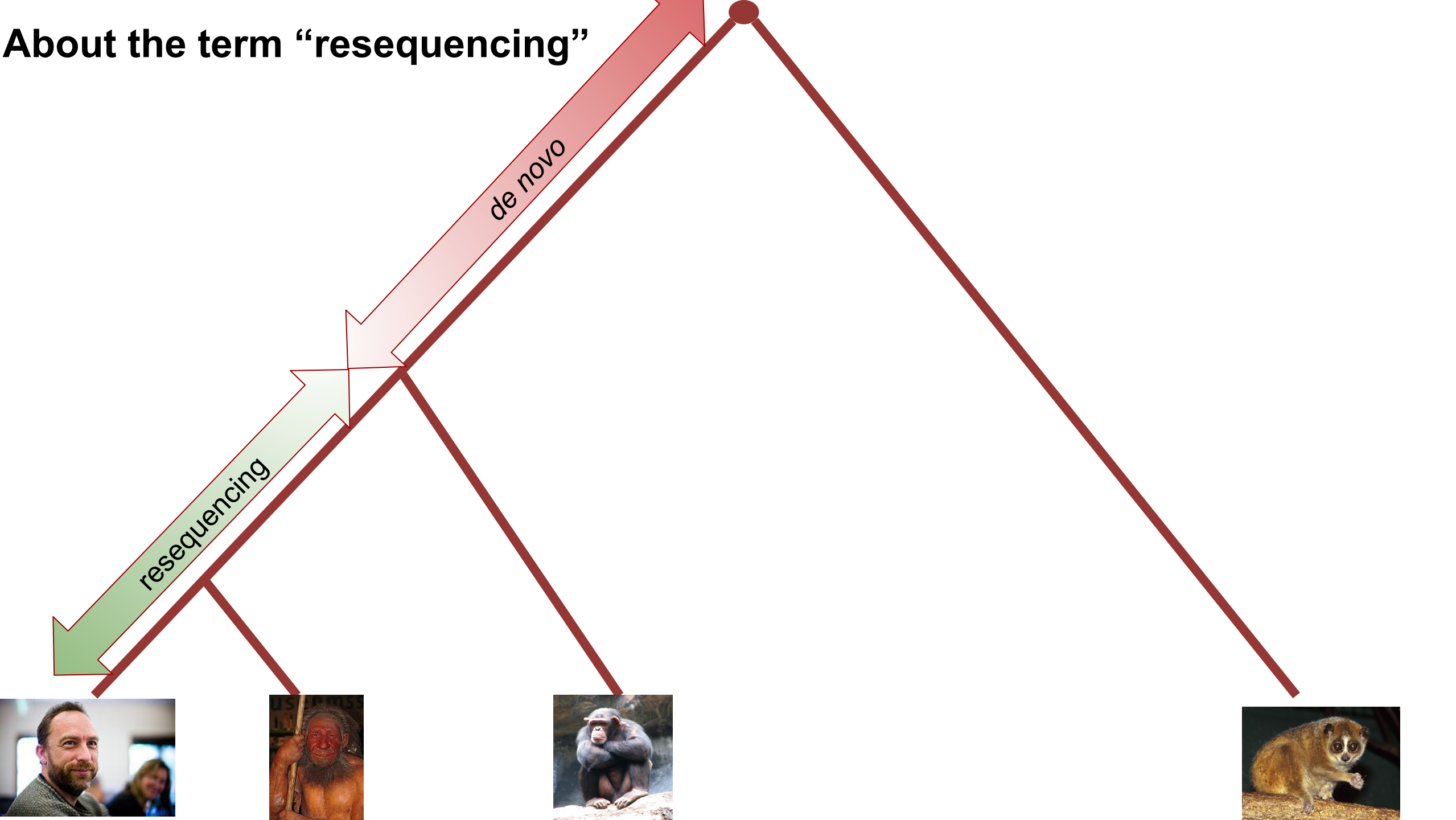
Personalized medicine

- Giving the same medication to all will not work
- Personalized medicine initiative in DK
- Sequence 100,000 patients on hospitals
- Use extensive registry data
- Current: 100M DKK (estimated 2G DKK)

NGS & bioinformatics

- Extreme data size causes problems
- Just transferring and storing the data
- Standard comparisons fail (N*N)
- Standard/old tools cannot be used
- Think in fast and parallel programs

About the term “resequencing”



Whole genome sequencing