NGS Data Analysis

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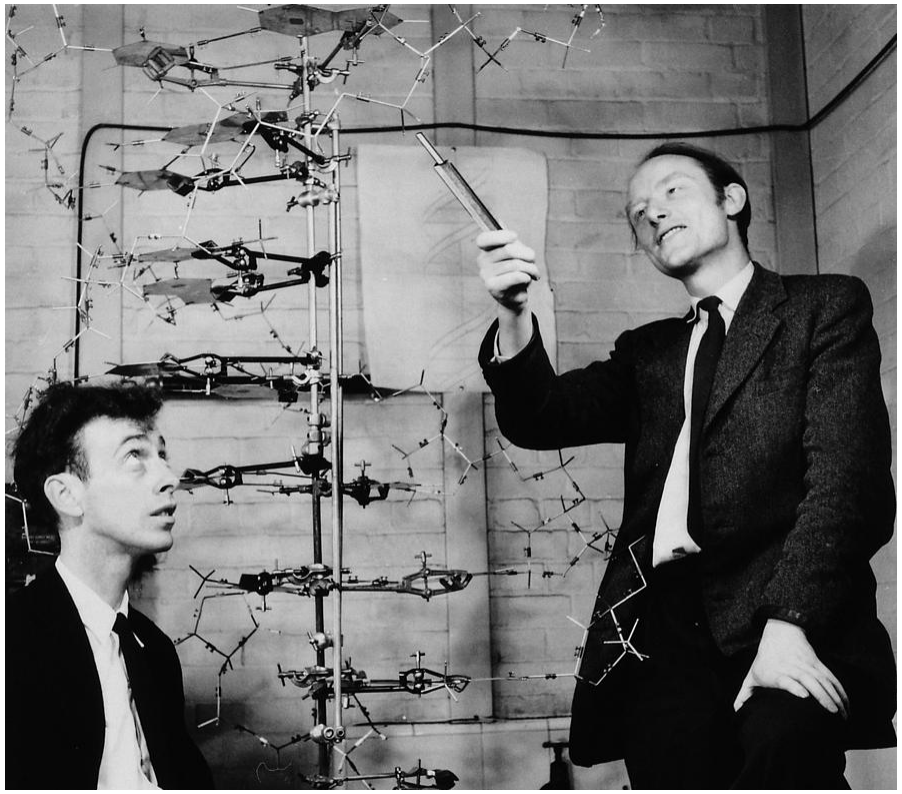
The Royal Melbourne Hospital

A joint venture between The University of Melbourne and The Royal Melbourne Hospital

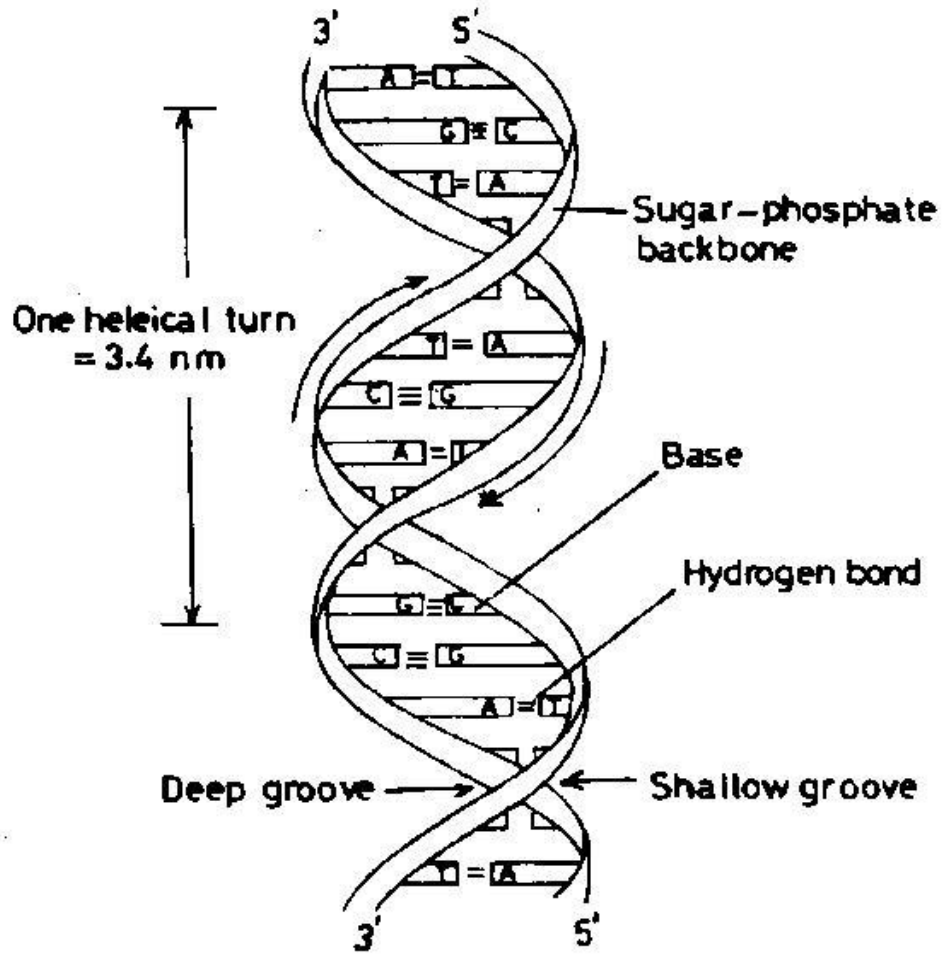
Objectives

- To understand the basics of sequence data analysis
- To provide an overview on the handling and analysis of high throughput NGS data

DNA



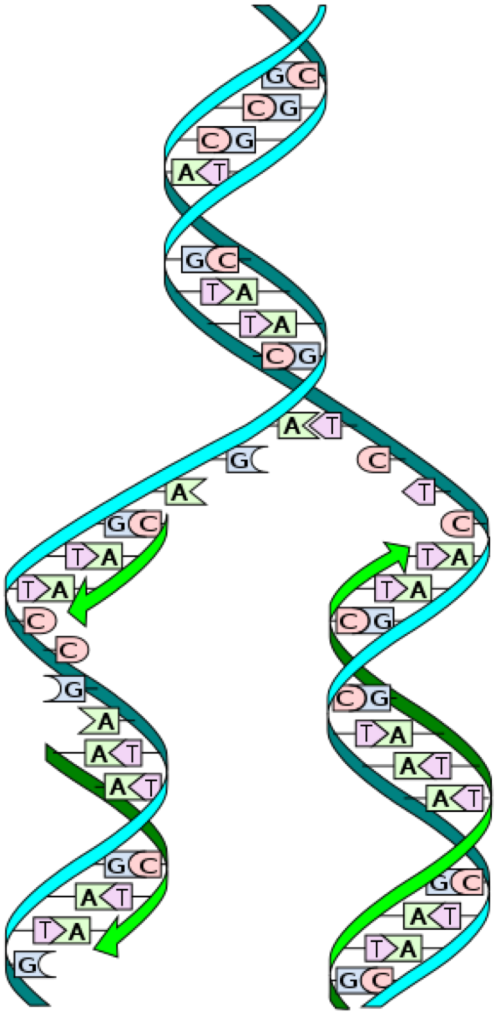
James Watson and Francis Crick



Nature (1953) 171:737-738

DNA sequencing

- Process of determining the order of sequence of nucleotides along the DNA strand

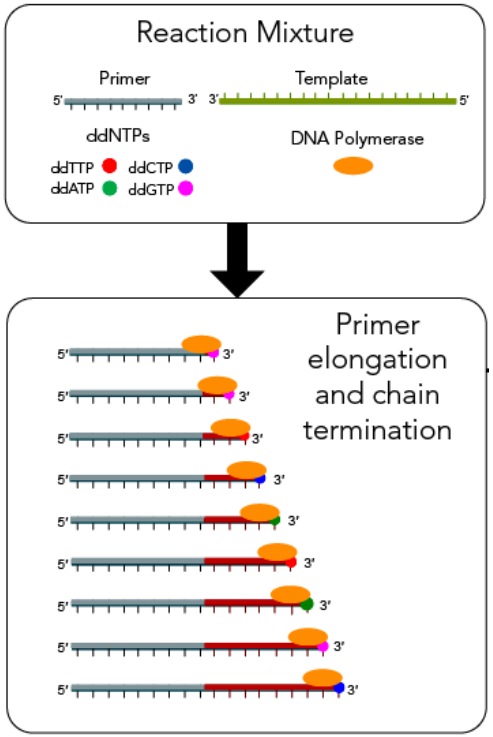


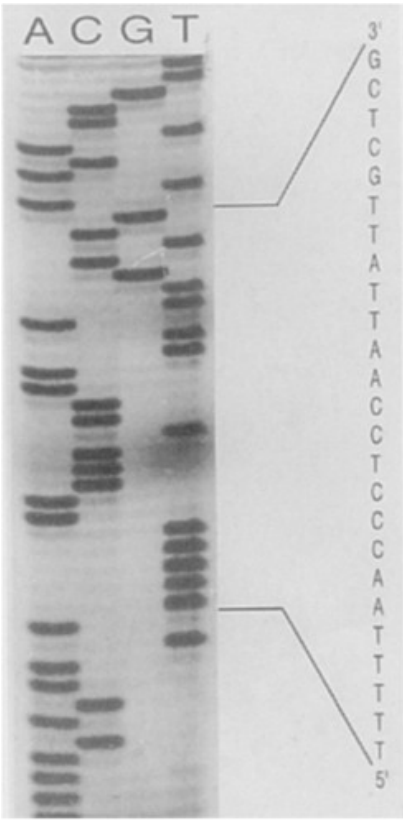
First generation sequencing

- Maxam-Gilbert, 1977
  - Chemical degradation method
  - Chemically breaks down radioactively labeled DNA at specific base positions
- Sanger, 1977
  - Enzyme-based termination of DNA strand elongation
  - Popular method of DNA sequencing

Sanger sequencing

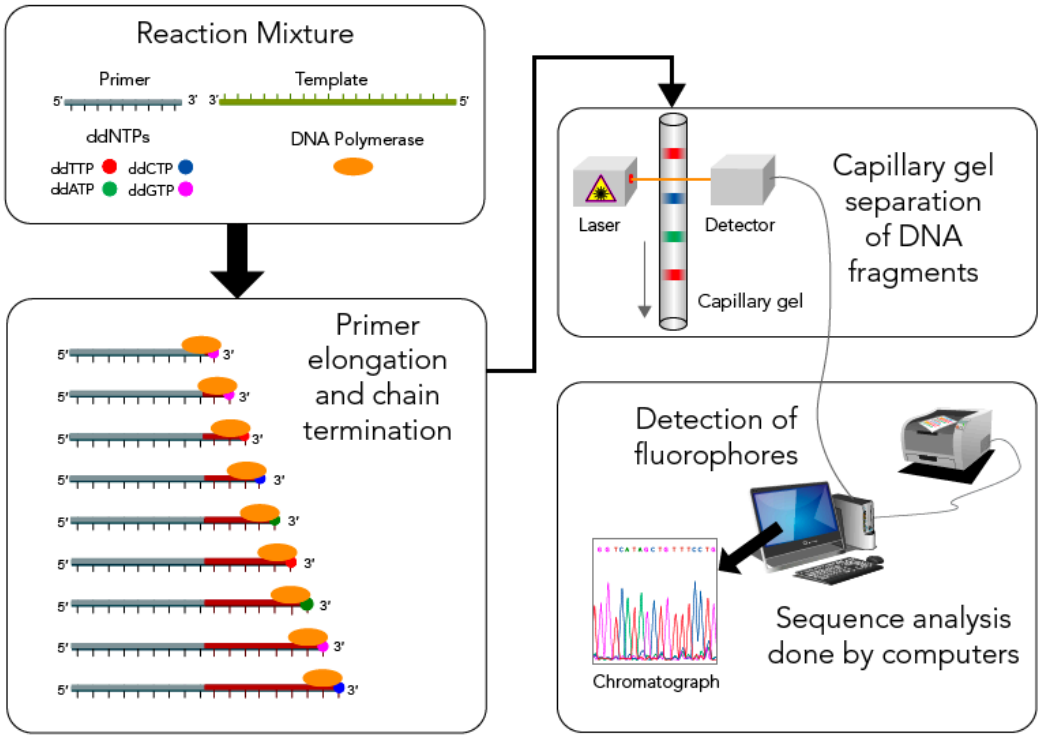
Frederick Sanger



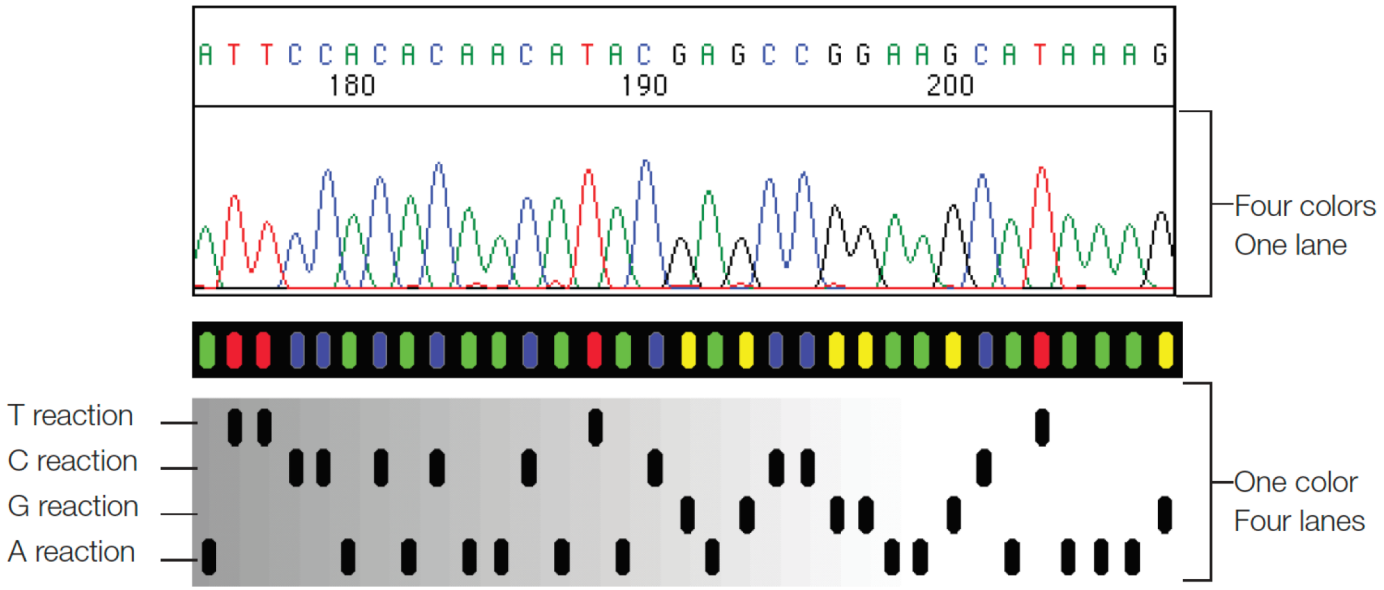


Capillary sequencing

- ABI 310, 1996
- Same underlying concept as Sanger method
- DNA fragments are separated on long thin capillaries
- Small diameter allows for high electric field, faster and efficient separation



Capillary sequencing




15 February 2001
nature
$10.00
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the human genome
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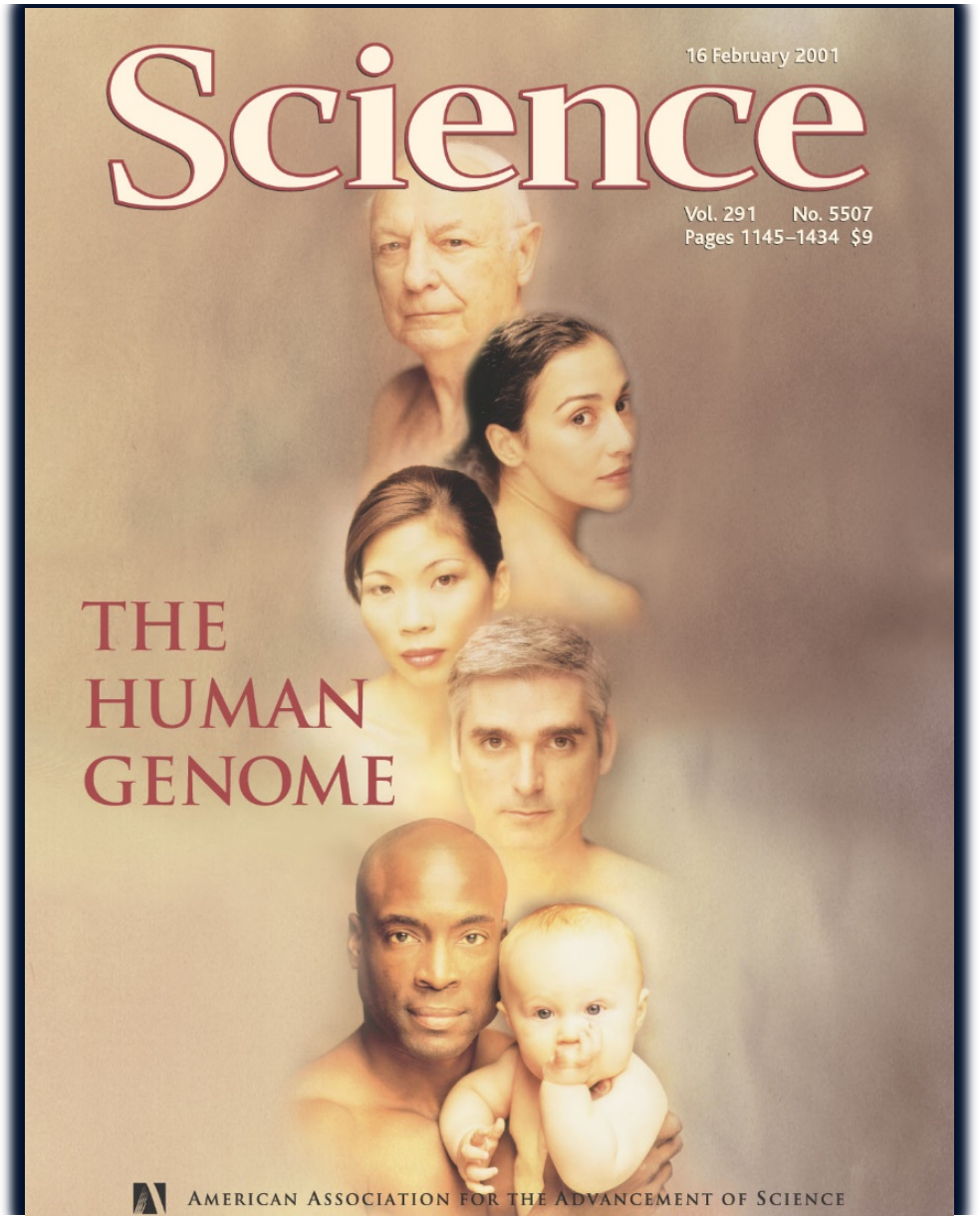
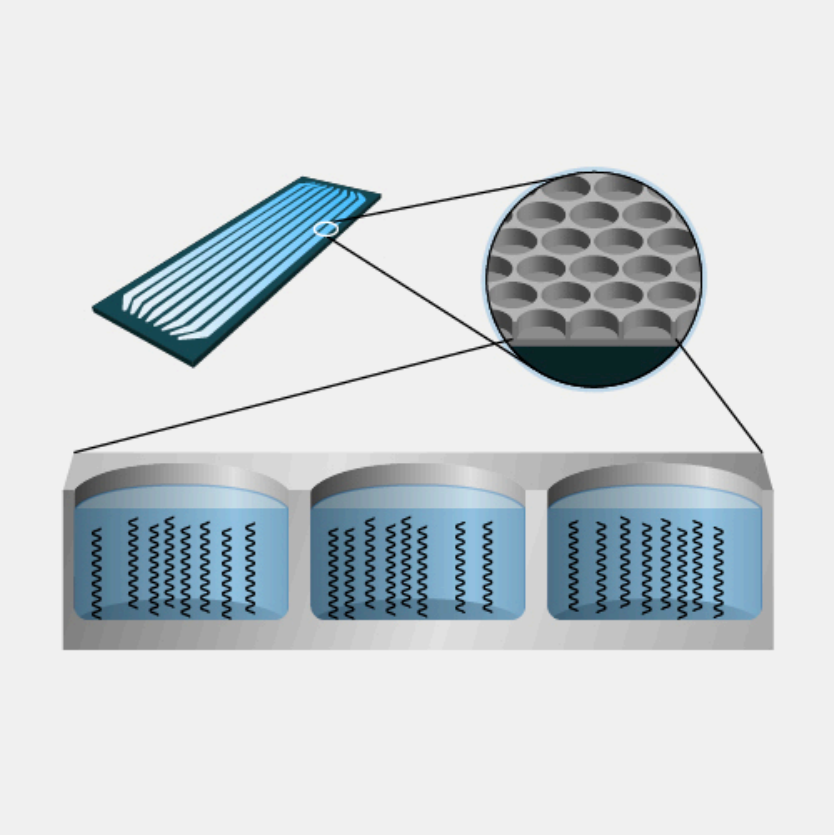

16 February 2001
Science
Vol. 291 No. 5507
Pages 1145–1434 $9
THE HUMAN GENOME
American Association for the Advancement of Science

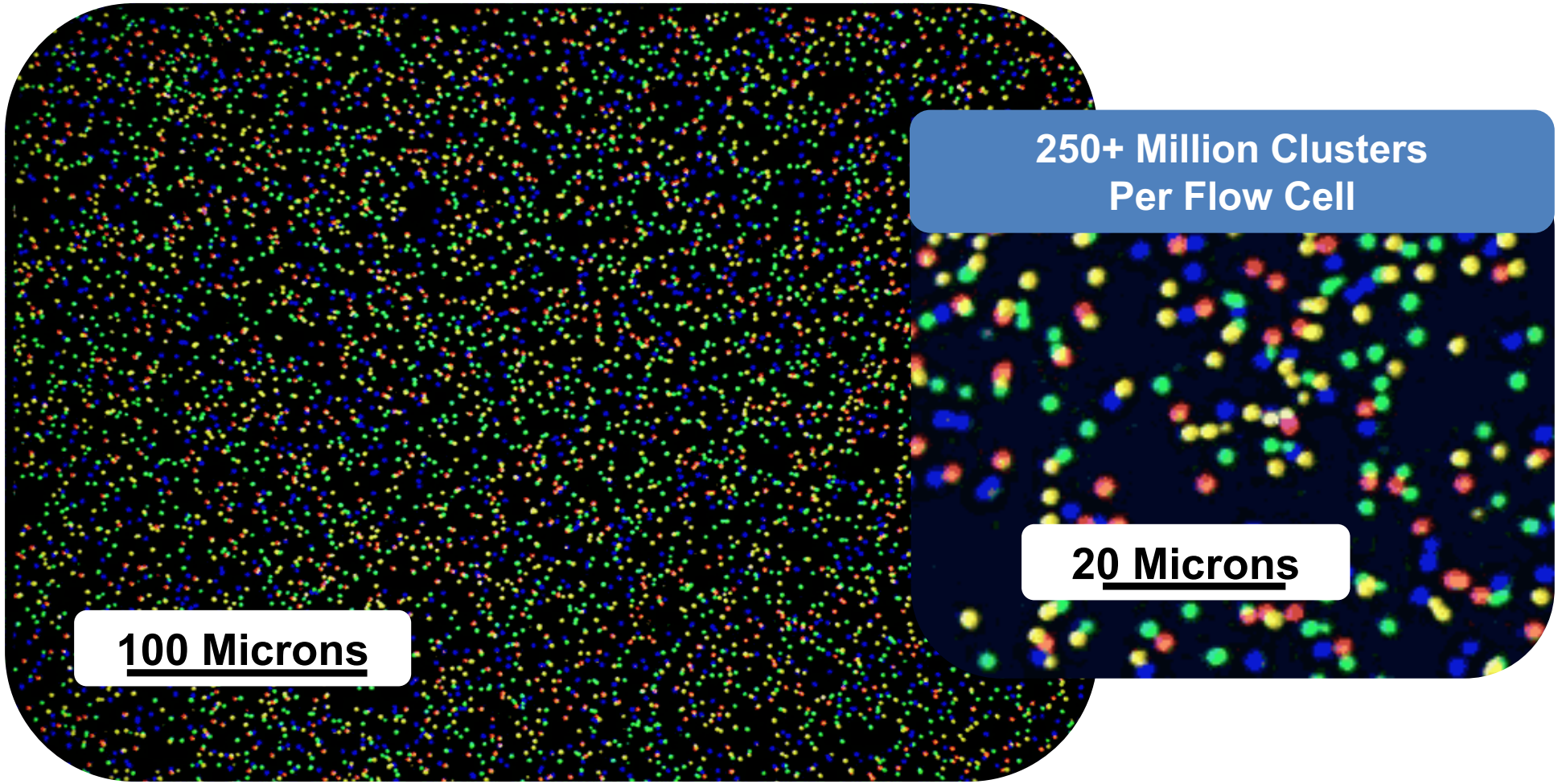
Next generation sequencing

Massively parallel

- Solexa (Illumina), 2006
- Uses micro- and nanotechnology for simultaneous sequencing of millions of short DNA fragments at a time



Flow cells and imaging

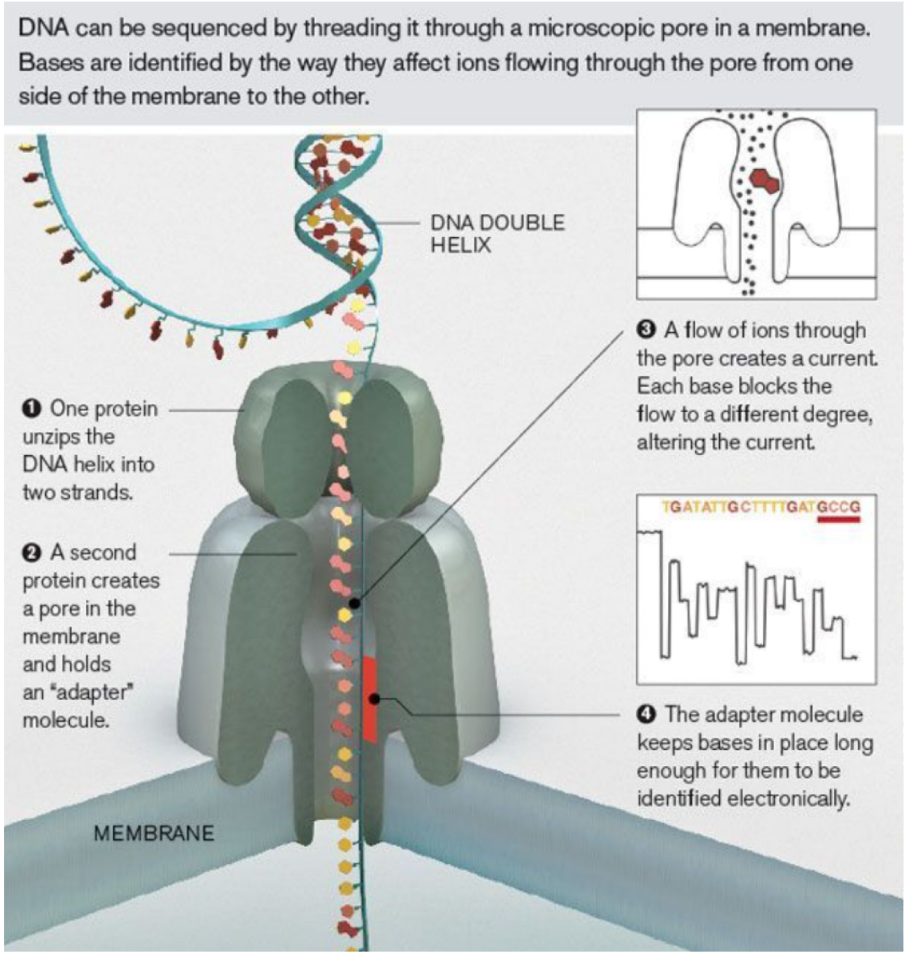


Third generation sequencing

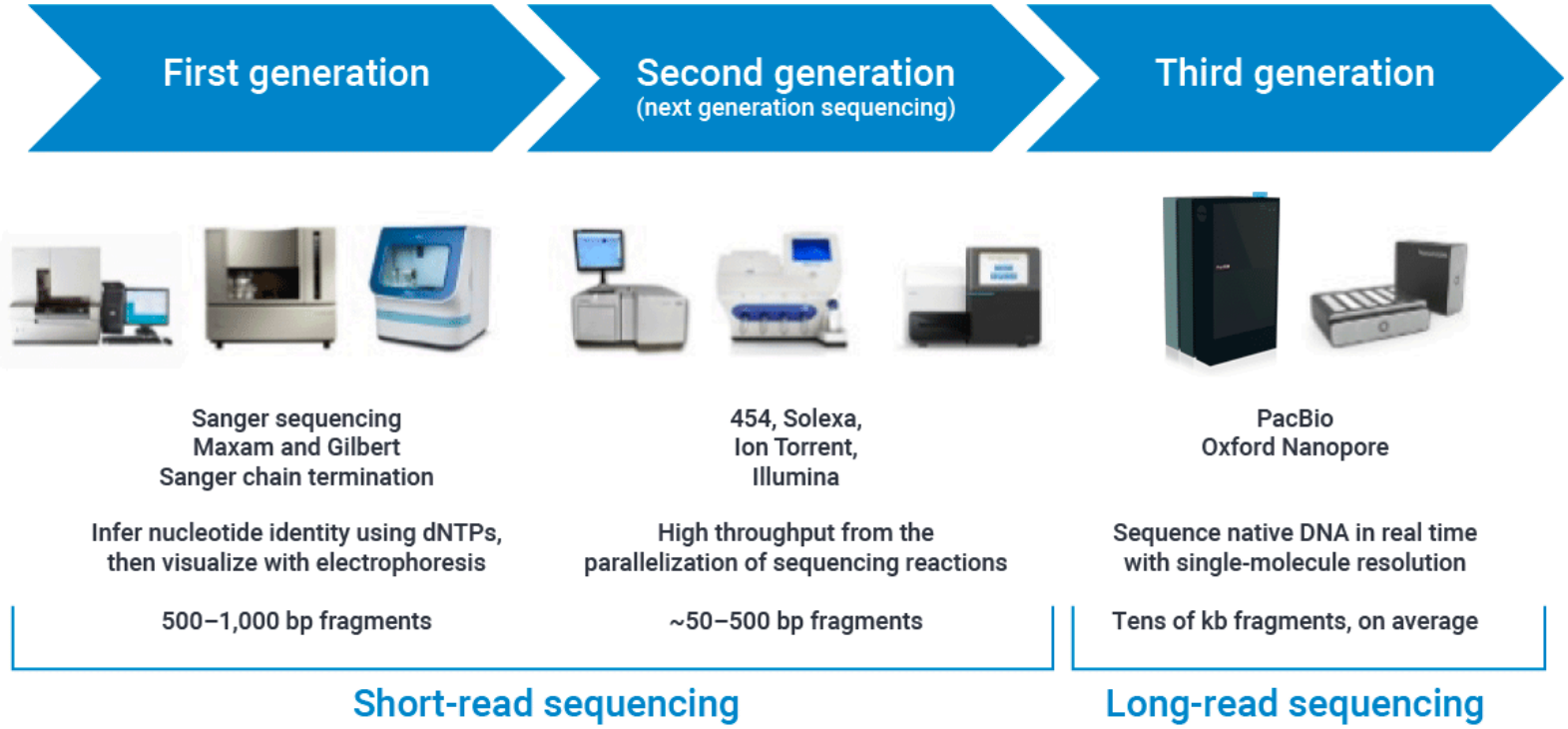
- Single molecule sequencing
- Obviates the need for DNA amplification
- Longer read lengths
- Minimal sample preparation
- Higher error rates in base calling

Nanopore sequencing

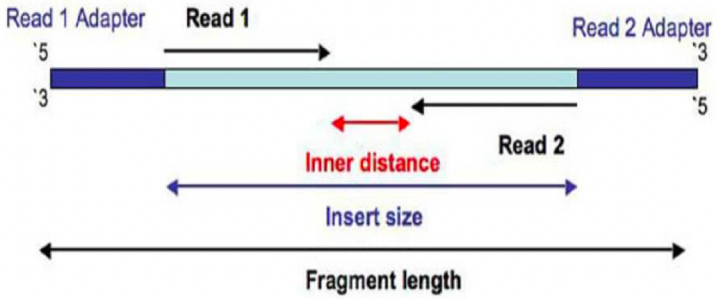
- Nanopore sequencing is based on threading a DNA strand through a pore (e.g. bacterial alpha-hemolysin) and
- The base sequence is inferred based on the shape and size of the nucleotides passing through the center of the pore
- Scalable and portable
- Real-time long read sequencing



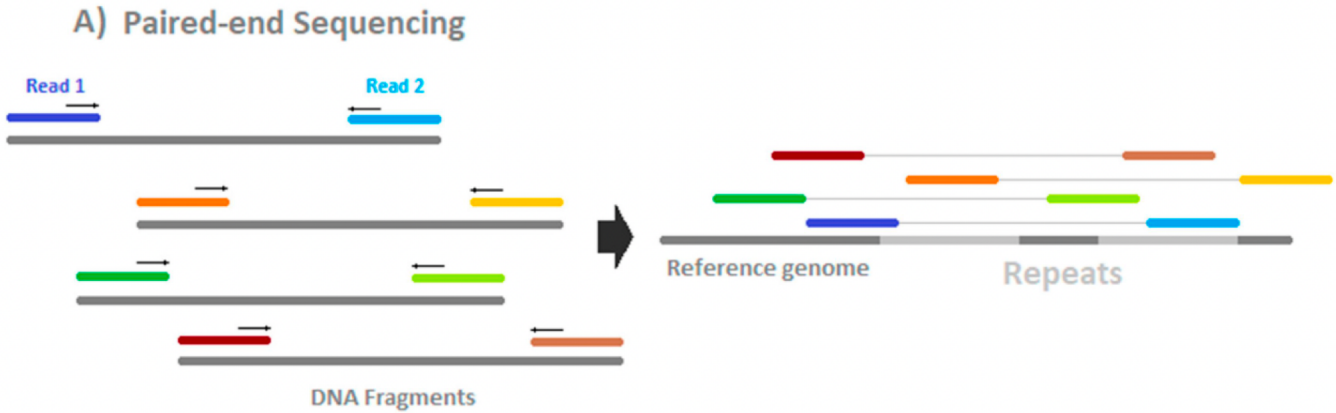
NGS technologies

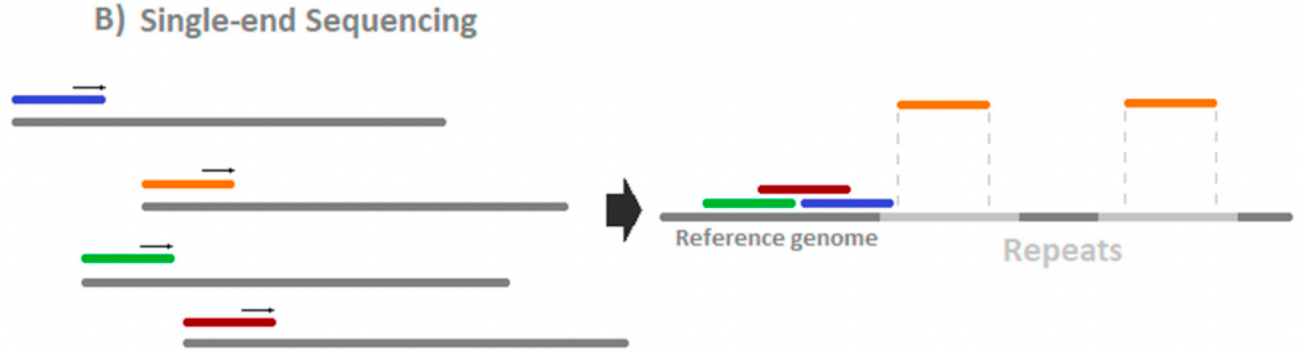


Single-end vs paired-end reads



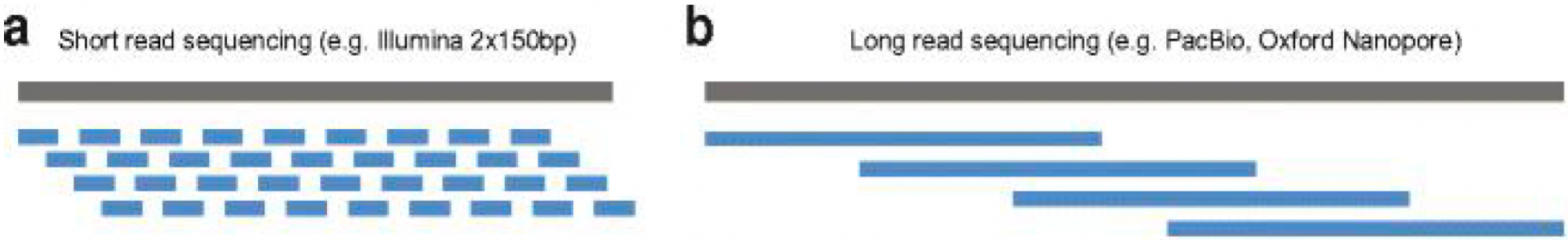
https://thesequencingcenter.com/knowledge-base/what-are-paired-end-reads/





https://www.mdpi.com/epigenomes/epigenomes-02-00021/article_deploy/html/images/epigenomes-02-00021-g004.png

Short vs long reads



Sequence data analysis

- Problem

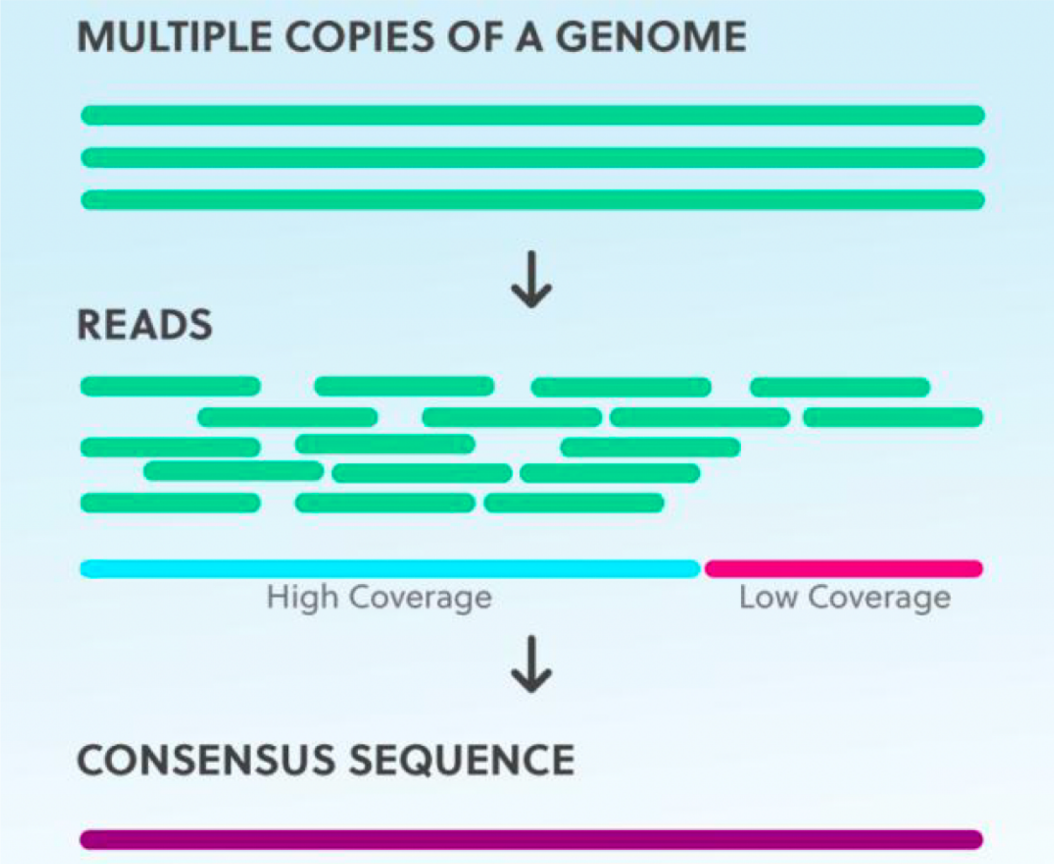
copies of target genome

sequence reads

reconstruct genomic sequence

- Assembly – process of putting individual short reads in the right order to create a consensus sequence

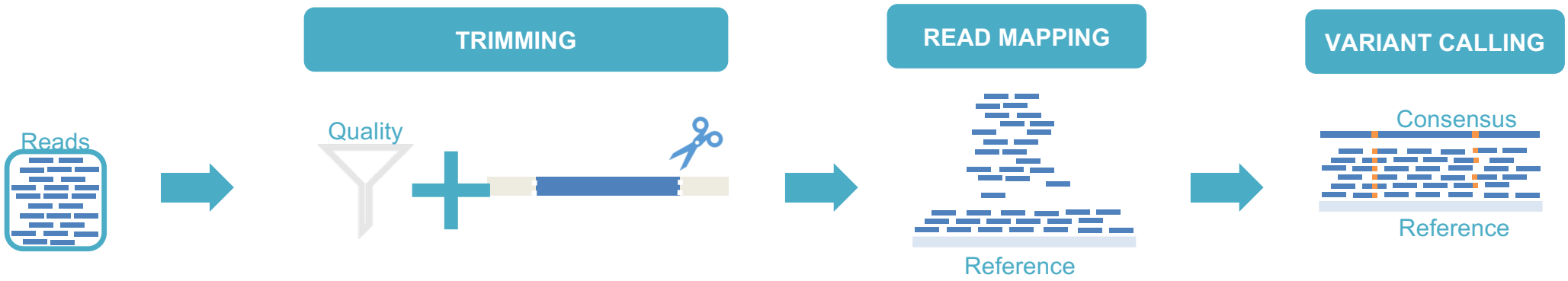
Sequence data analysis



Sequence Reads

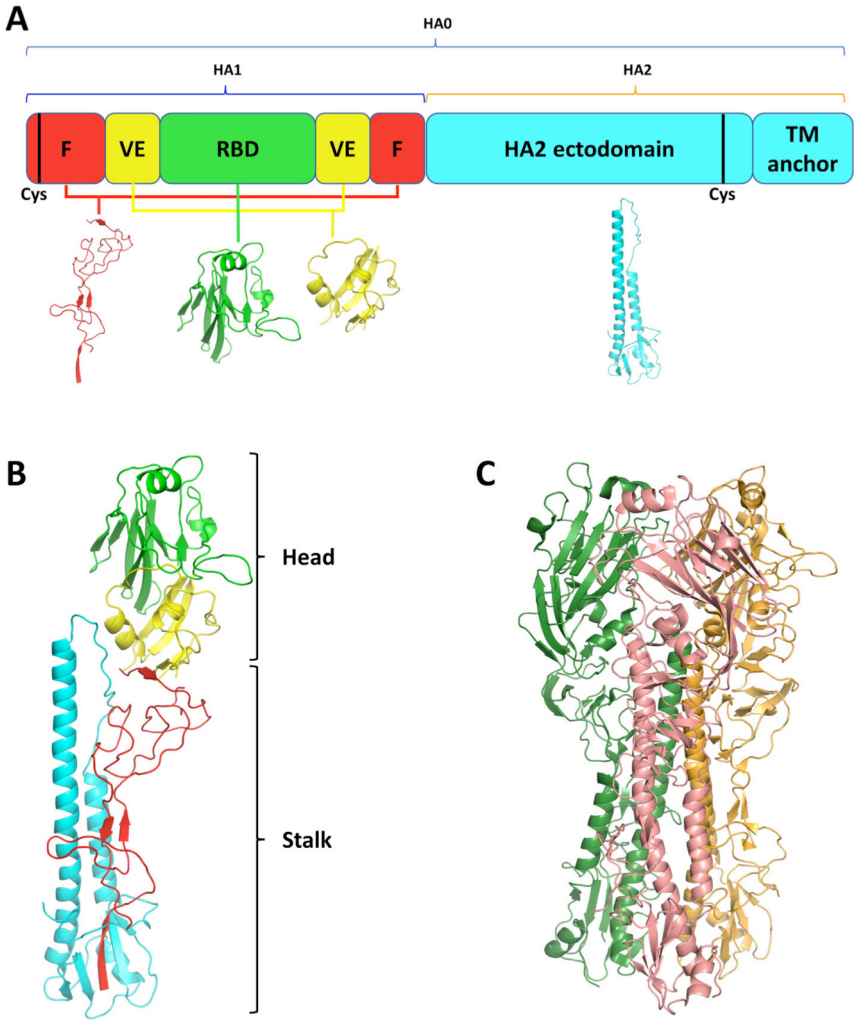
- Fragments of the original genome or any template sequence (FASTQ format)
- One of the initial goals of bioinformatics is to reconstruct the template sequence based on the read fragment information (sequence assembly)

Sequence data analysis



Annotation

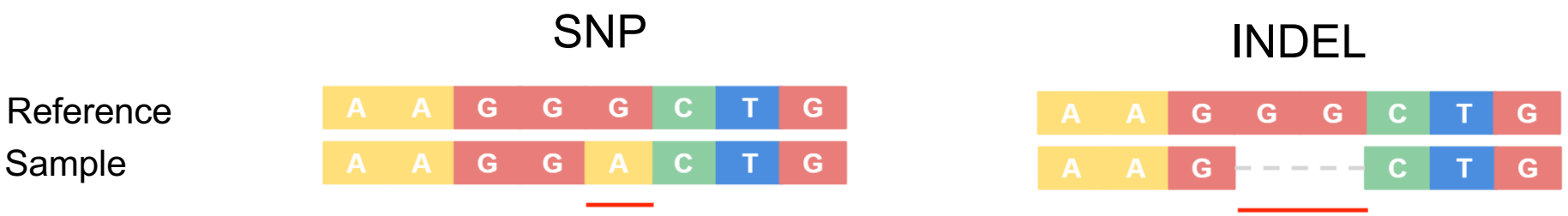
- Genome annotation is the process of identifying structure and function of the components of a genome



Sci Rep (2023) 13:6940

Variant calling

- Variant calling is the process of identifying single nucleotide polymorphisms (SNPs), insertions and deletions (indels) from NGS data



Thank you !

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A joint venture between The University of Melbourne and The Royal Melbourne Hospital