

Genome Sequencing



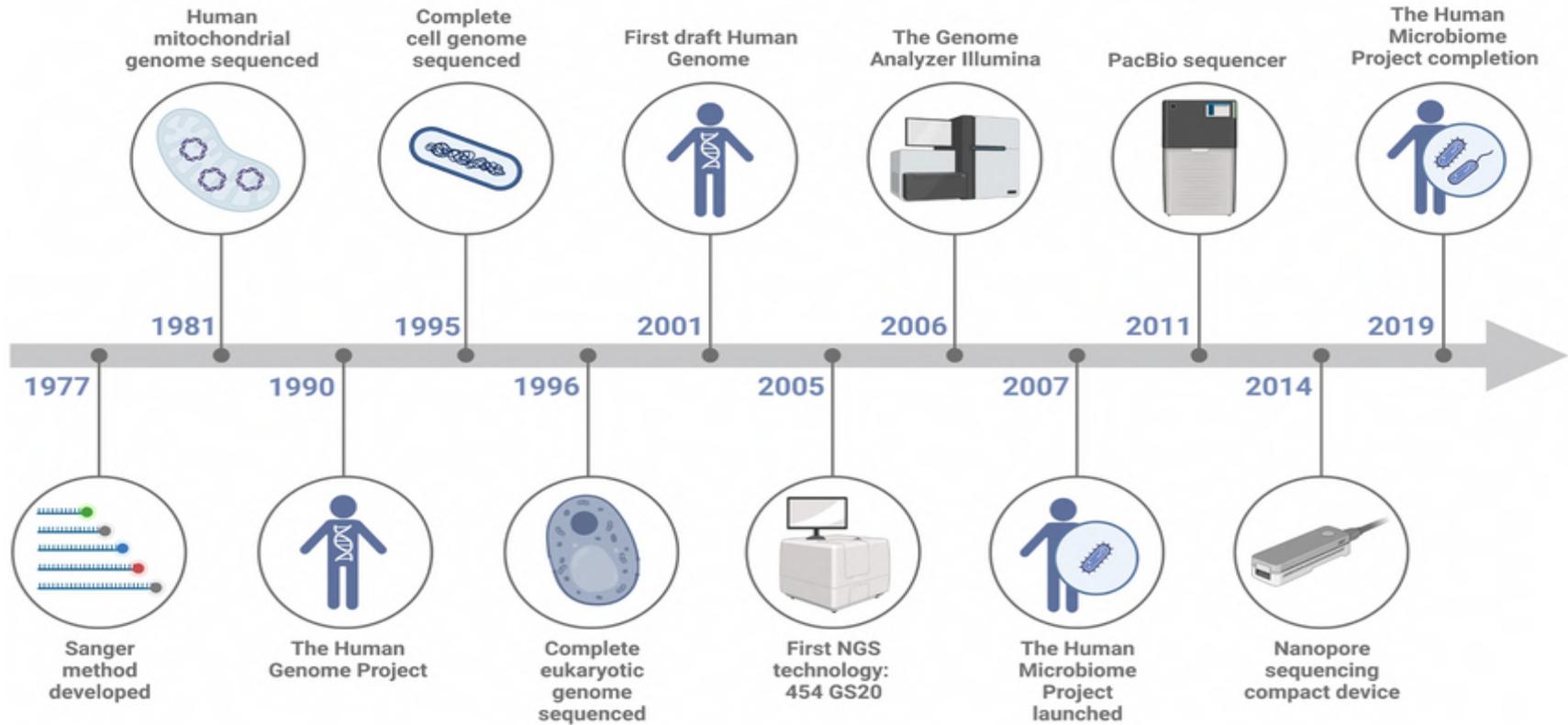
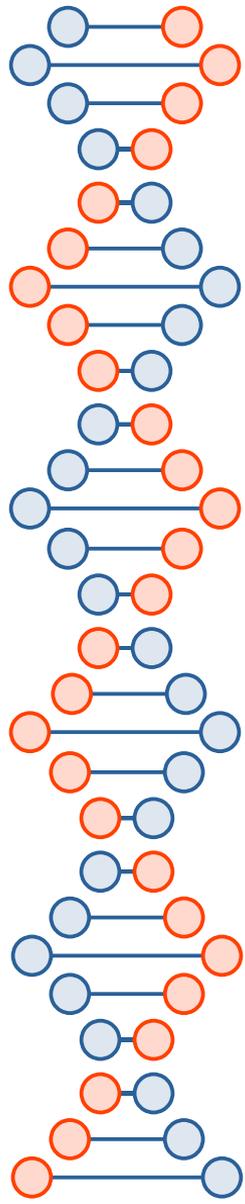
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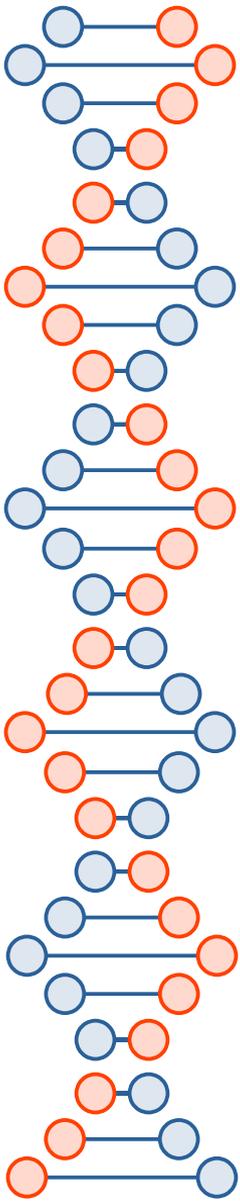
What are we discussing about ?

- Genome Sequencing with examples
 - First Generation
 - Second Generation
 - Third Generation
- Our Experience in sequencing Rabies Virus



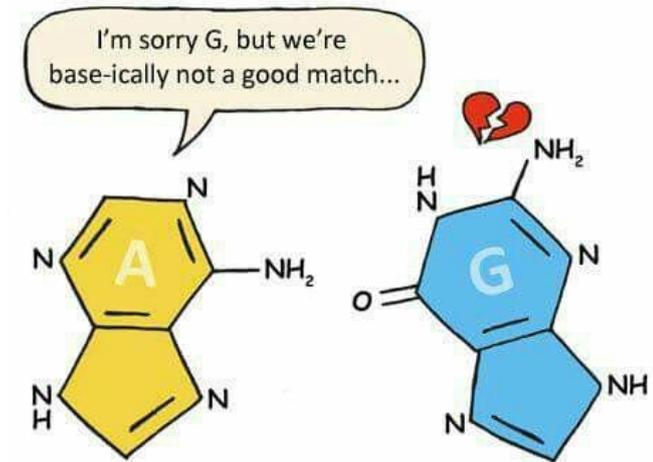
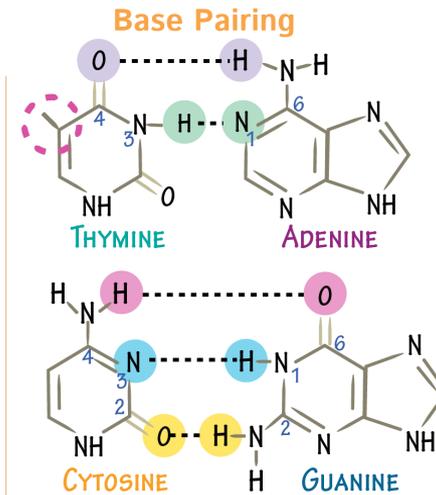
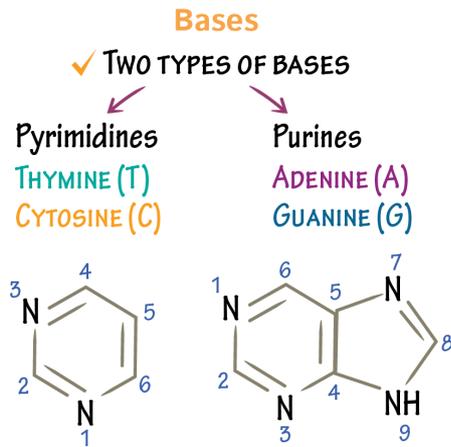
Genome sequencing (Landmark events)



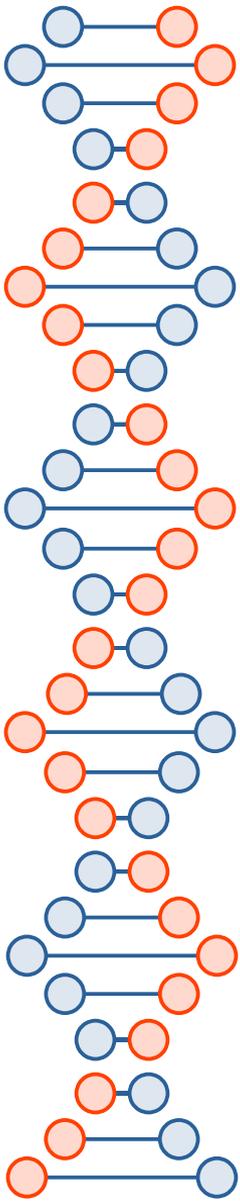


The core concept

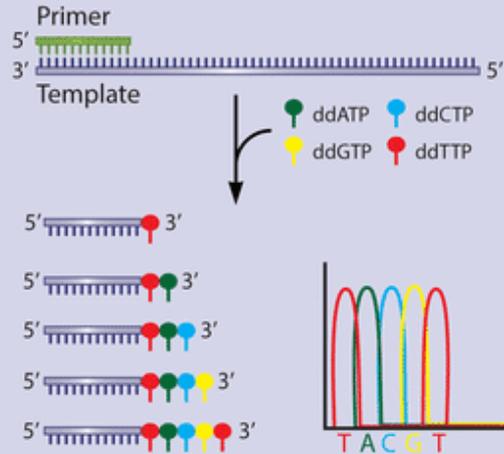
DNA Base Pairing



Sequencing Technologies



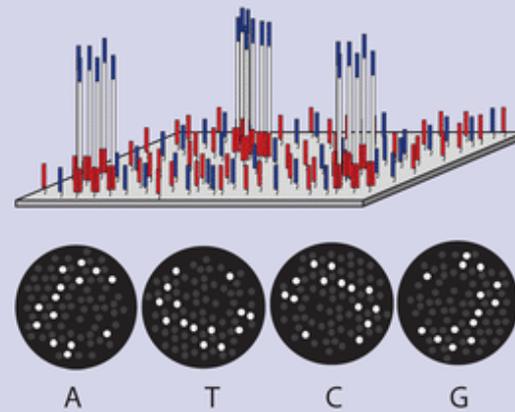
First Generation Shotgun Sequencing



- Sequencing by synthesis
- High accuracy
- Long read lengths
- Relatively small amount of data generated

e.g., ABI capillary sequencer (ABI)

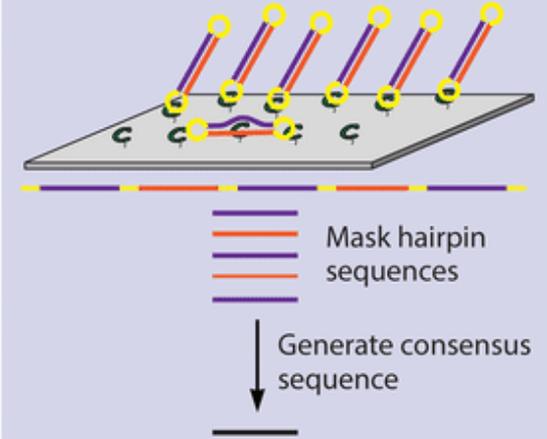
Second Generation Massively Parallel Sequencing



- Sequencing by synthesis
- Amplified templates are generated during sequencing, reducing the requirements for starting material
- High accuracy
- Short read lengths

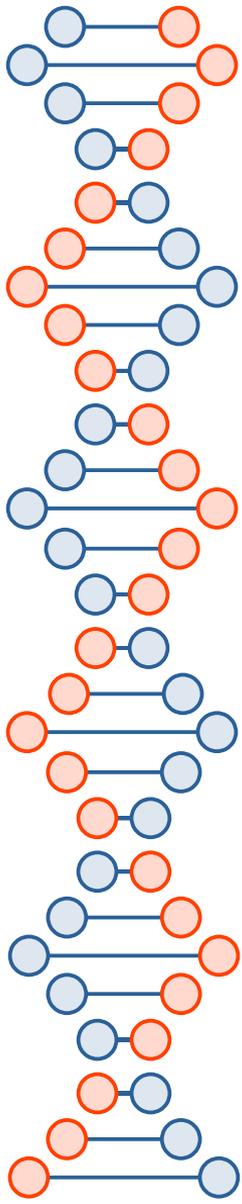
e.g., MiSeq (Illumina), Ion Torrent (Thermo Fisher Scientific)

Third Generation Single-molecule Sequencing



- Single-molecule templates
- Low accuracy
- Long read lengths

e.g., Single-Molecule Real-Time (SMRT) — Sequencing (Pacific Biosciences), MinION (Oxford Nanopore Technologies)

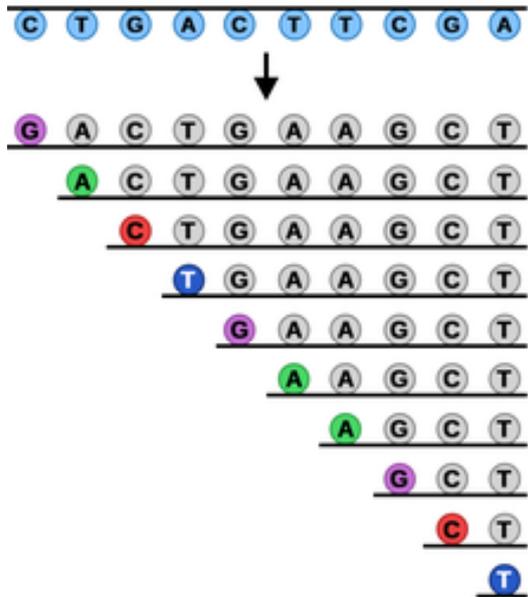


First Generation

Fluorescently labeled ddNTPs

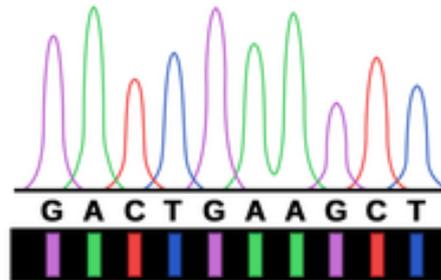
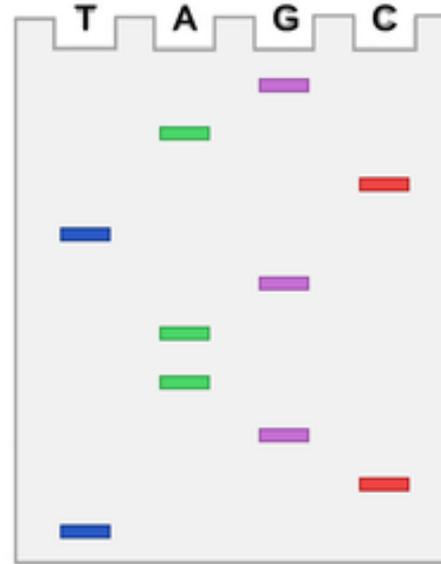


↓ DNA template

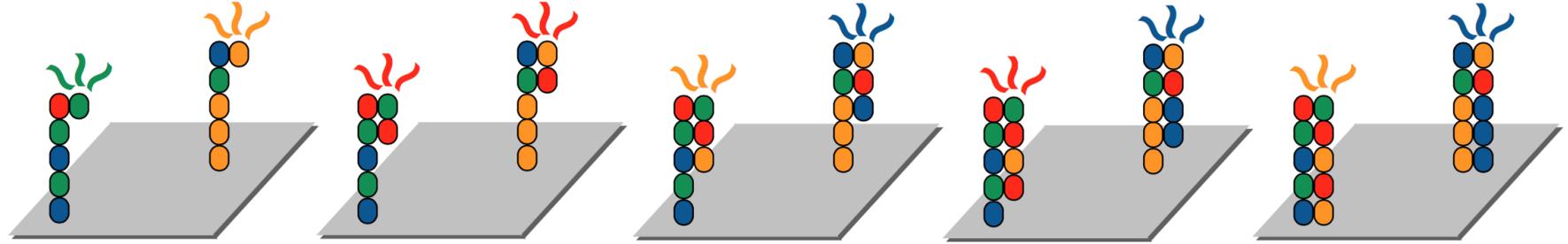
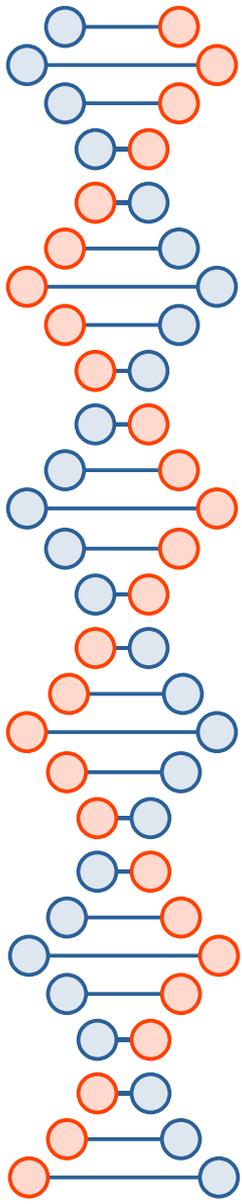


Separate with a gel

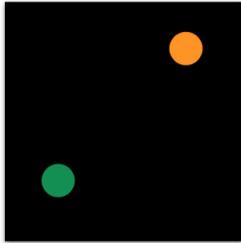
Use a sequencing machine



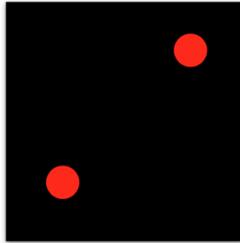
Second Generation



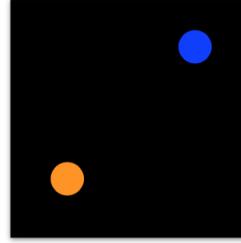
Cycle 1



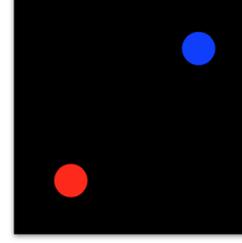
Cycle 2



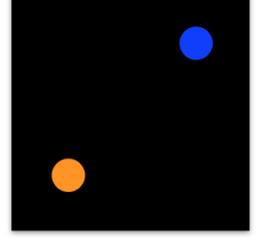
Cycle 3

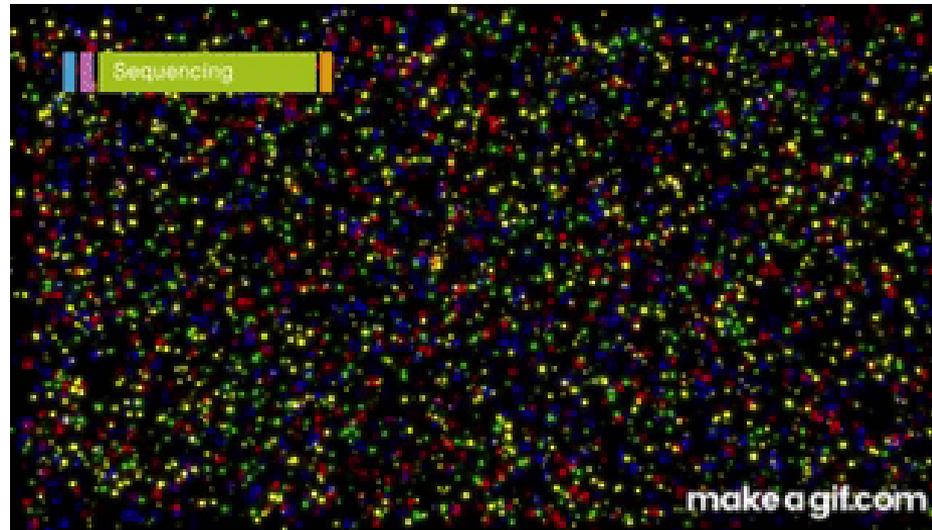
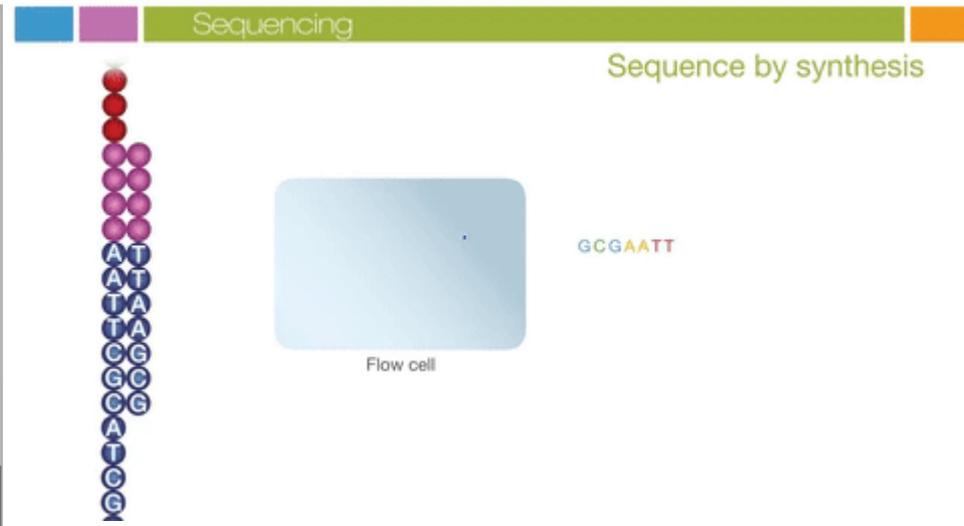
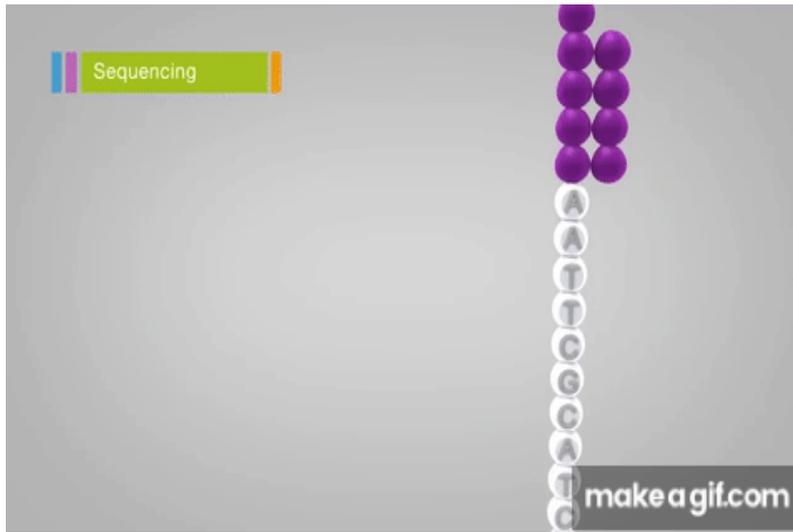
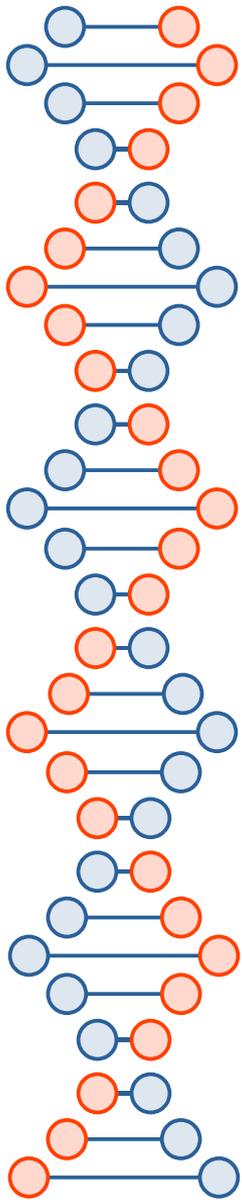


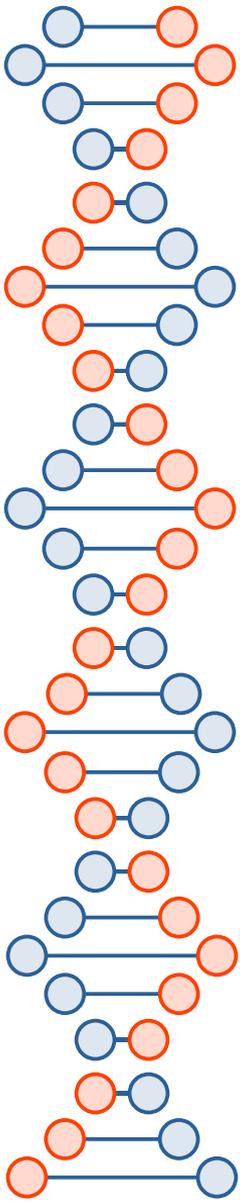
Cycle 4



Cycle 5



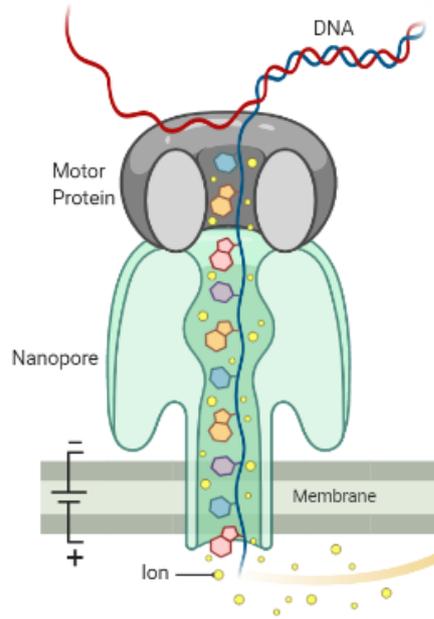




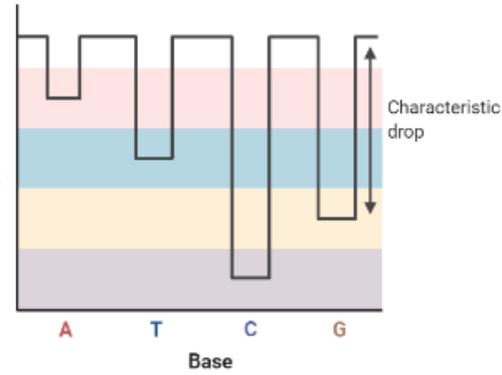
Third Generation

Nanopore Sequencing

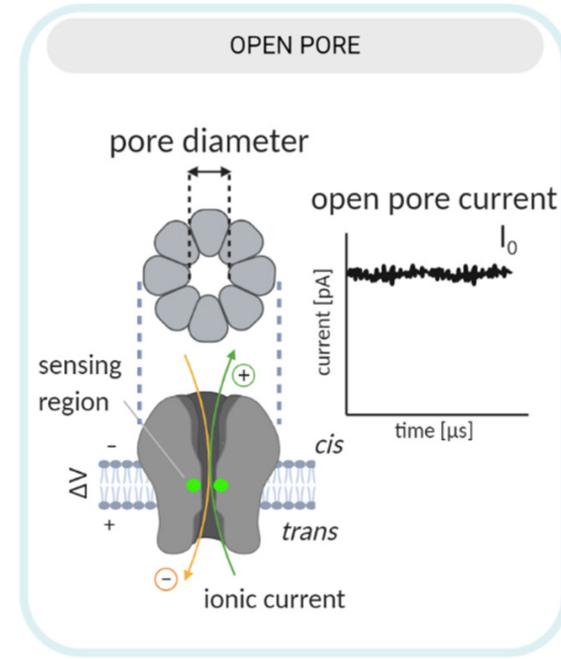
- 1 DNA is unwound by the motor protein and one strand is translocated through the pore to the +ve side of membrane

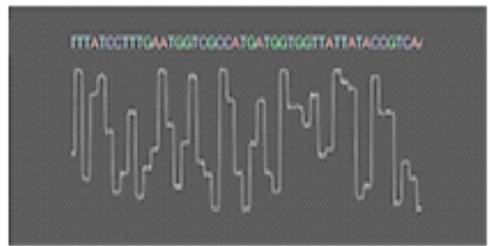
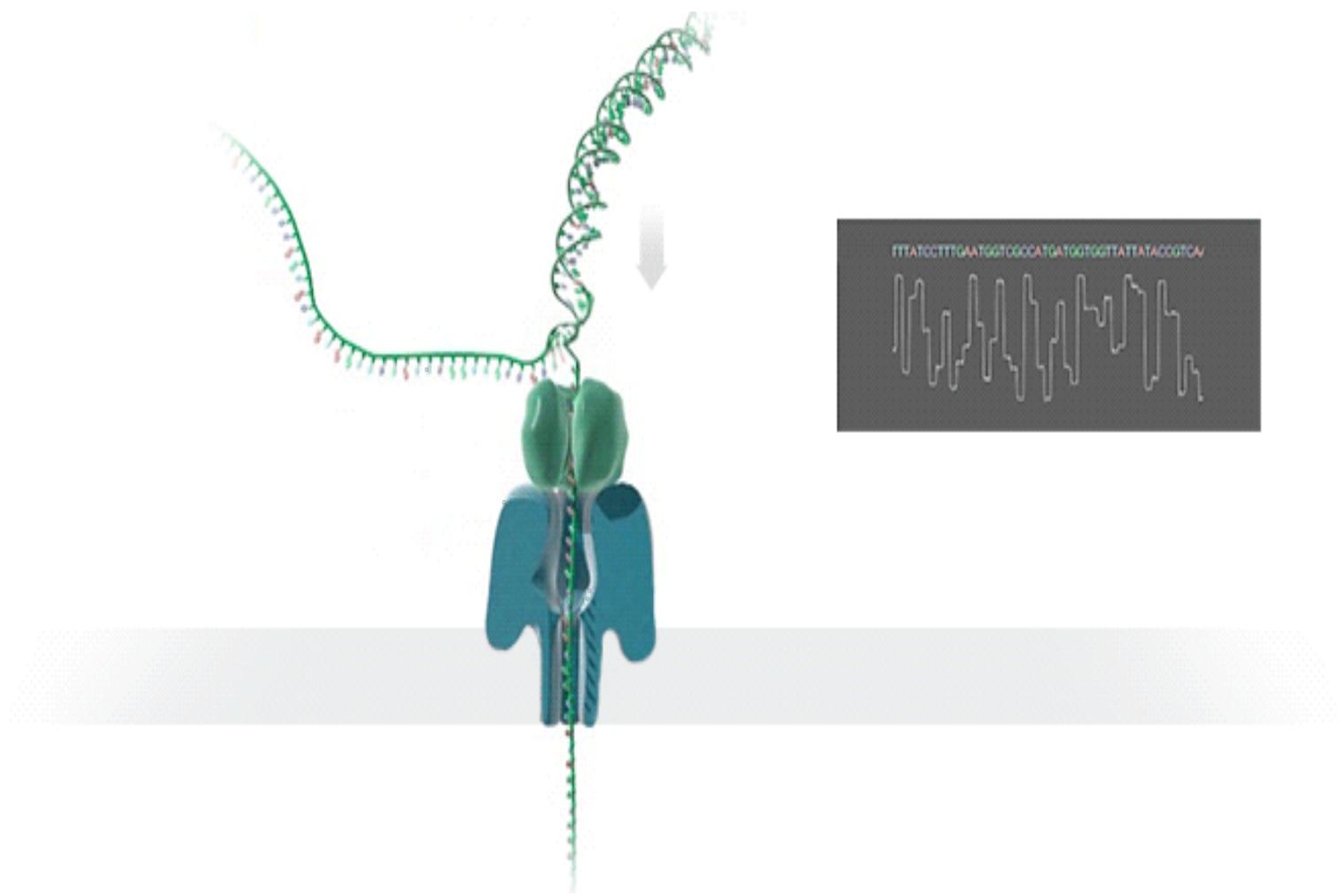
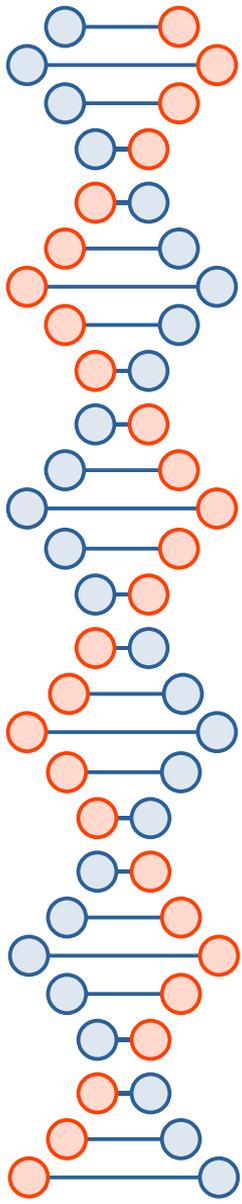


Ionic Current



- 2 Each base gives a characteristic reduction in the ionic current, allowing the DNA to be sequenced





Rabies Virus Genome Sequencing

Our Experience, thus far

Jan 28, 2024

 Rabies Virus Sequencing using Illumina- MiSeq

DOI

dx.doi.org/10.17504/protocols.io.ewov1qbrogr2/v1

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Jan 11, 2023

 MinION Sequencing protocol for Rabies virus of Arctic lineage

 Forked from [MinION Sequencing protocol for Rabies virus of Indian subcontinental lineage](#)

DOI

dx.doi.org/10.17504/protocols.io.3byl4jzb8lo5/v1

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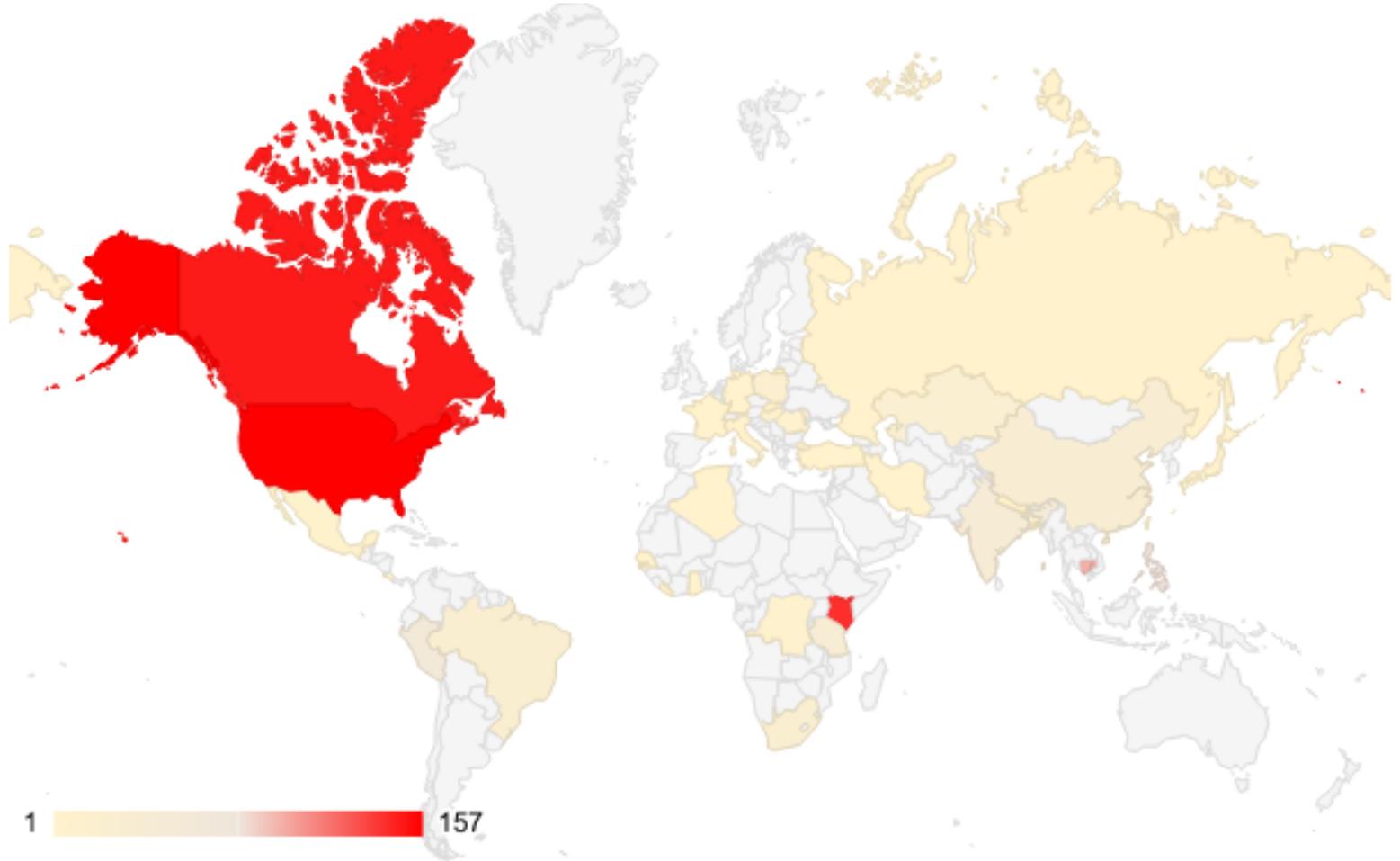
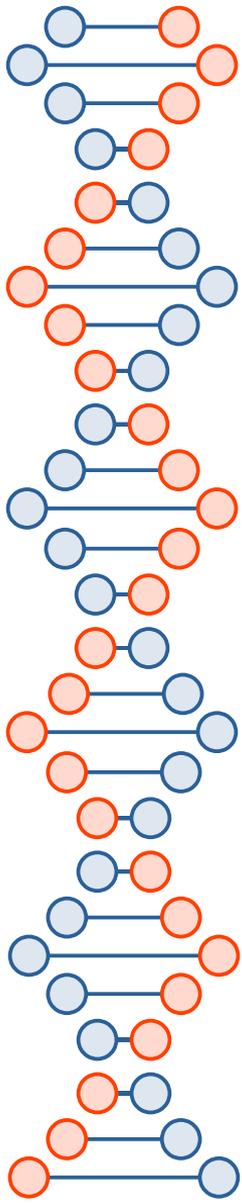
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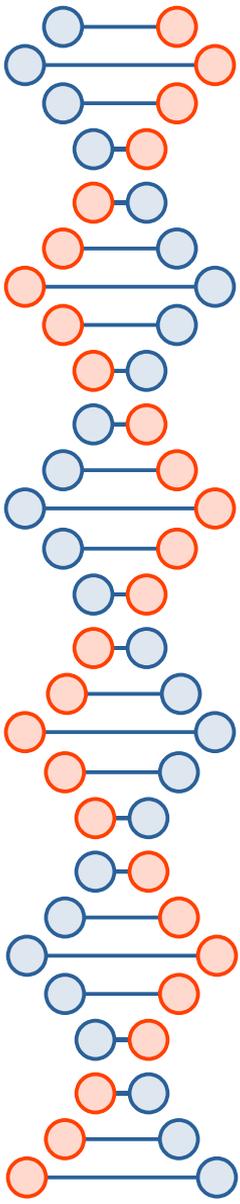


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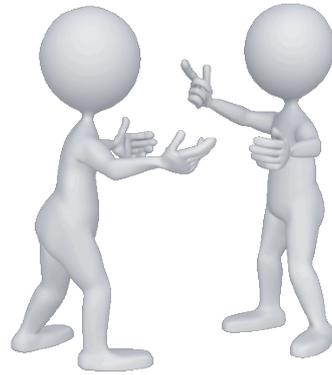
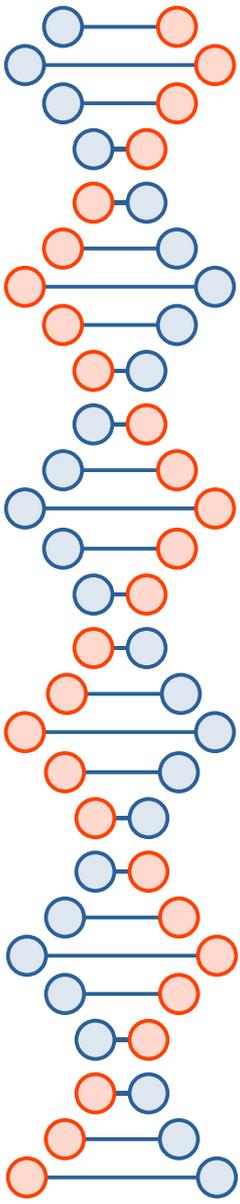
RABV Sequence Availability



Whole genome sequencing: Why



- **Epidemiological Studies**
 - Circulating Genotypes
 - Cross Contamination Event Predictions
- **Mutation**
 - Importance for successful vaccine designs
- **Phylodynamics**



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