

# Genome Sequencing



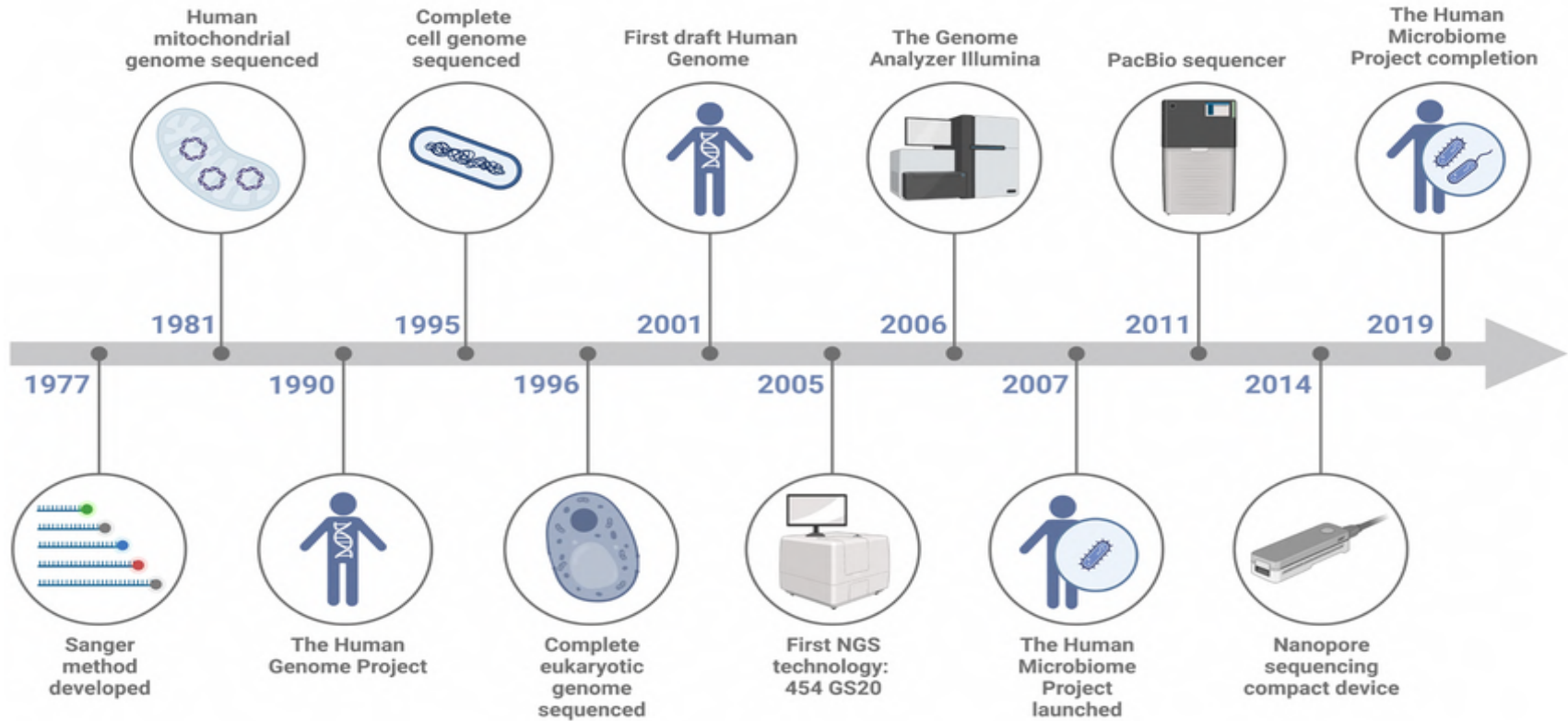
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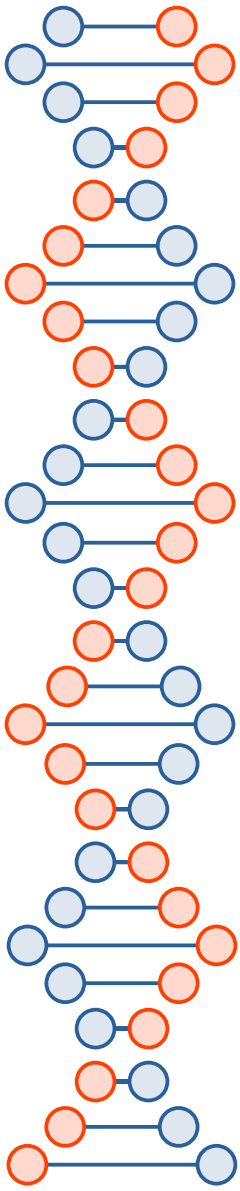
# What are we discussing about ?

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



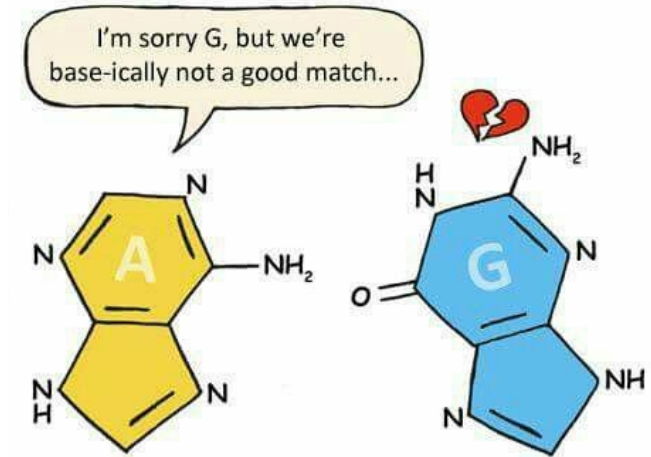
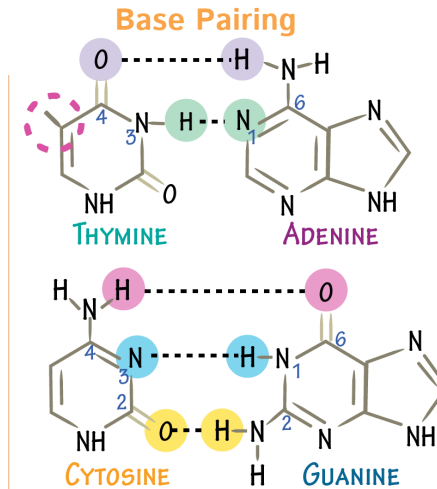
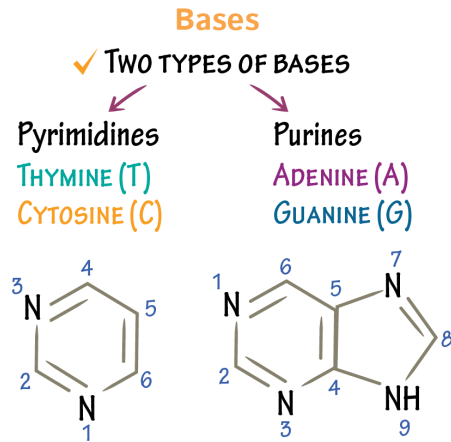
# Genome sequencing (Landmark events)





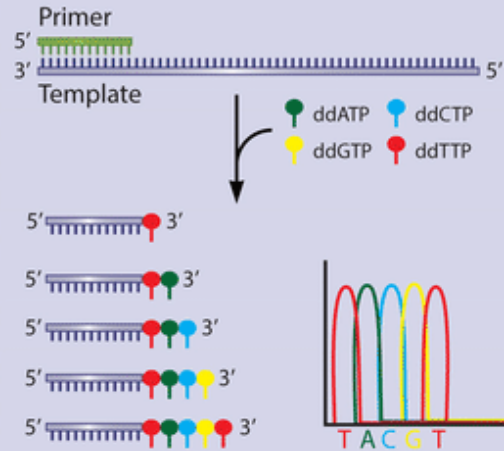
# The core concept

## DNA Base Pairing



# Sequencing Technologies

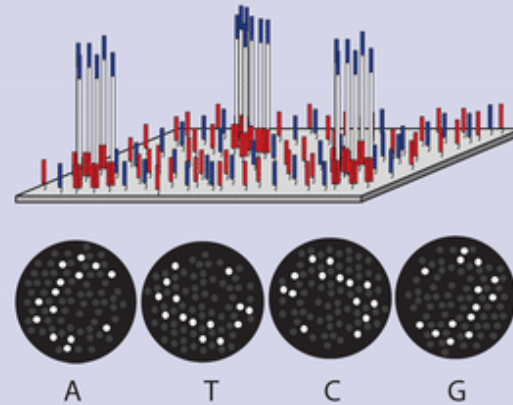
## First Generation Shotgun Sequencing



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

e.g., ABI capillary sequencer (ABI)

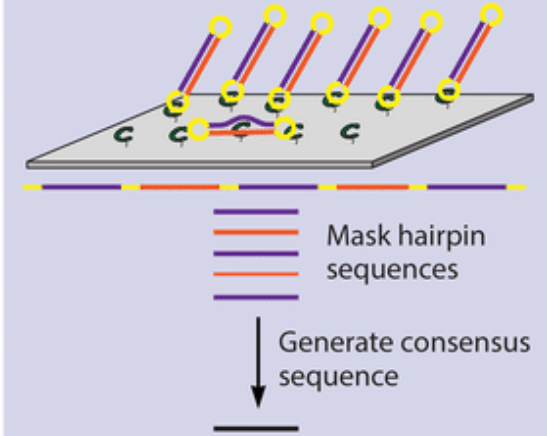
## Second Generation Massively Parallel Sequencing



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

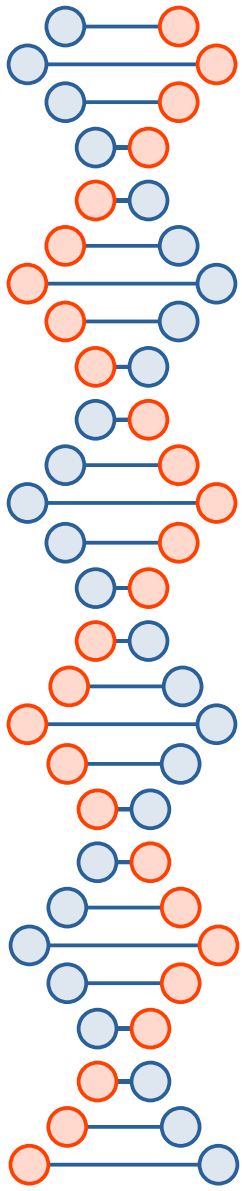
e.g., MiSeq (Illumina), Ion Torrent (Thermo Fisher Scientific)

## 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)

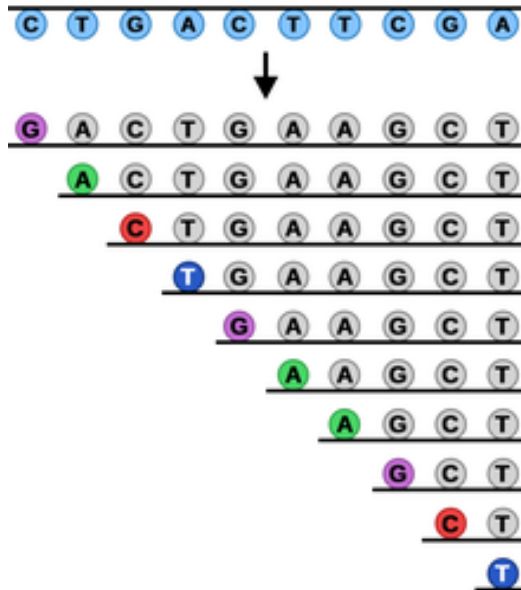


# First Generation

Fluorescently labeled ddNTPs

T A G C  
 ddTTP ddATP ddGTP ddCTP

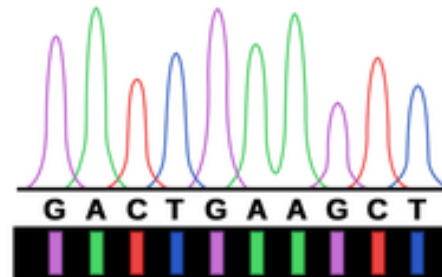
↓ DNA template



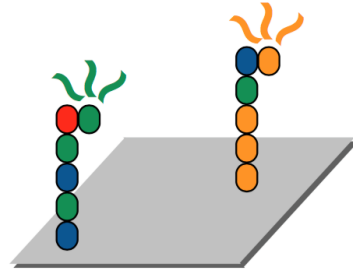
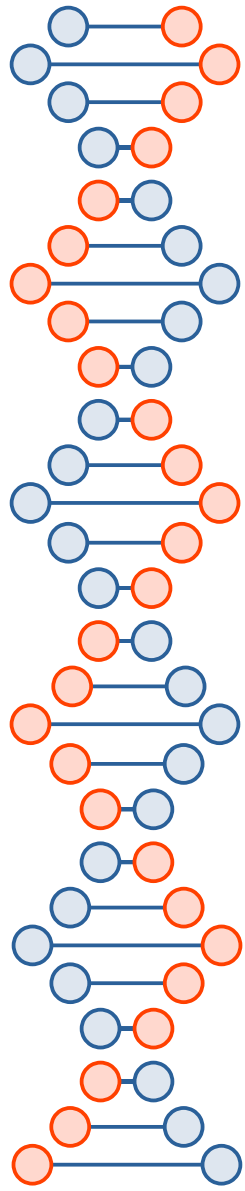
Separate with a gel



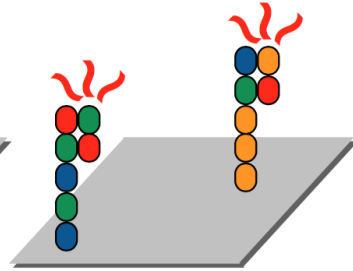
Use a sequencing machine



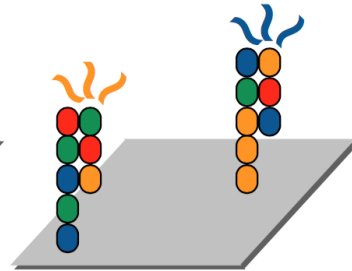
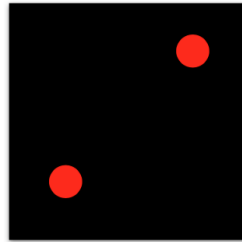
# Second Generation



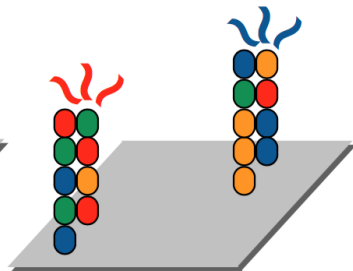
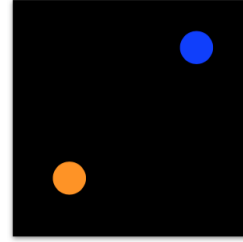
Cycle 1



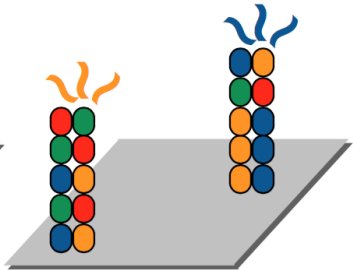
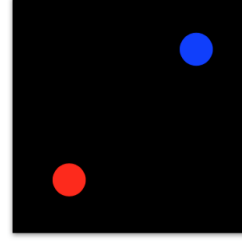
Cycle 2



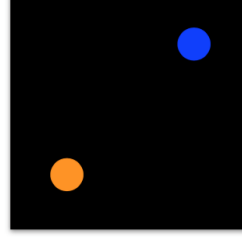
Cycle 3

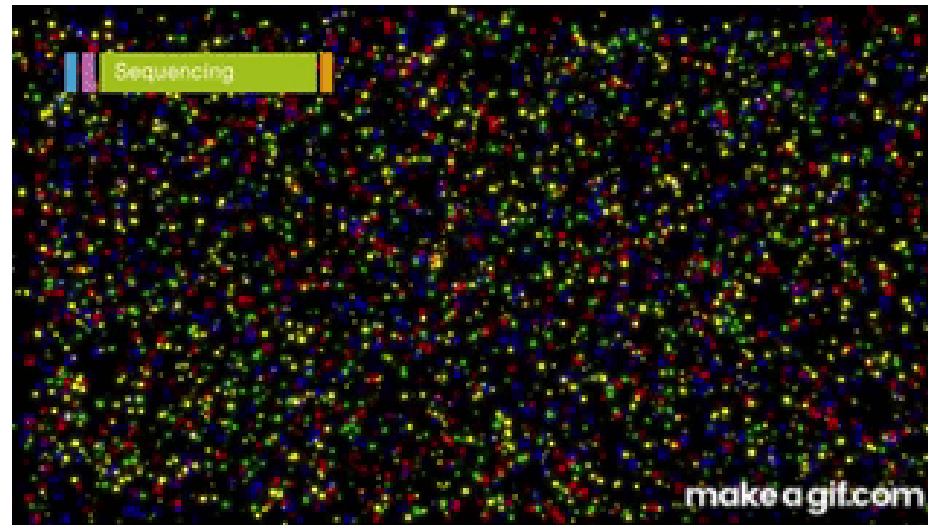
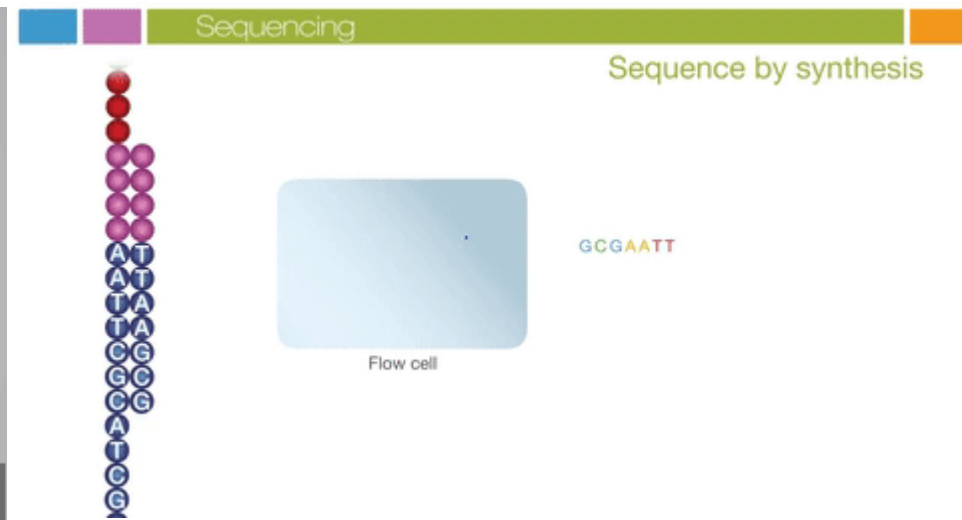
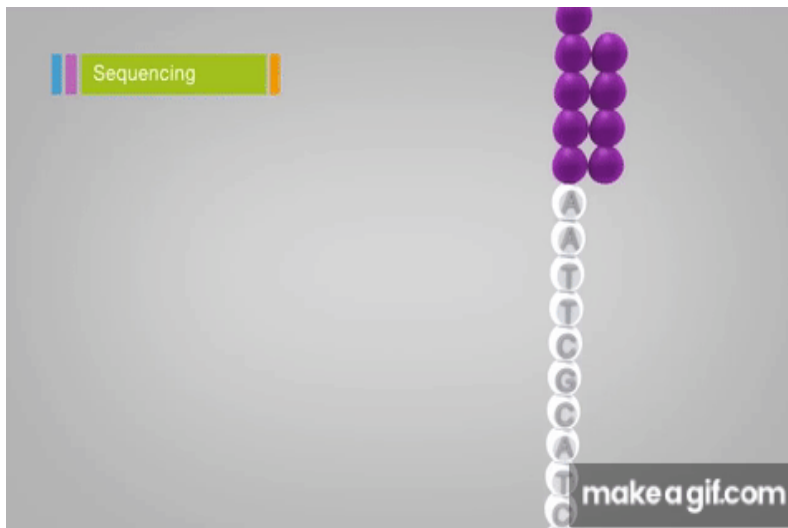
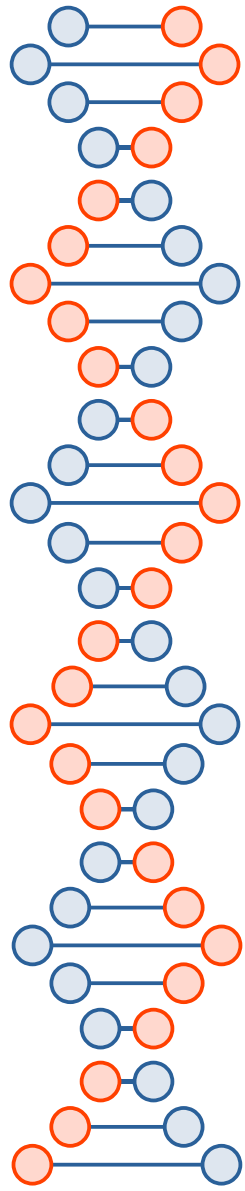


Cycle 4

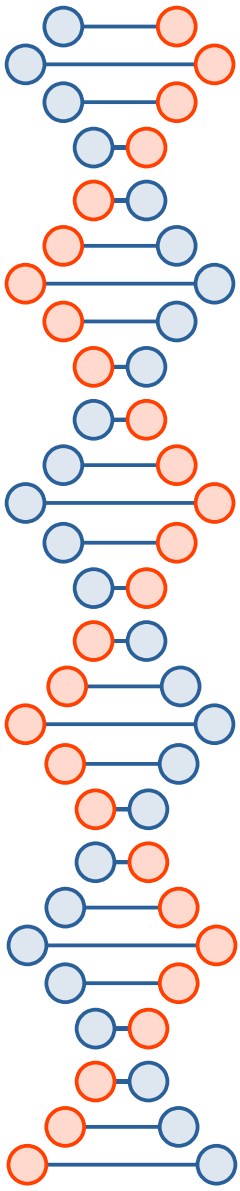


Cycle 5



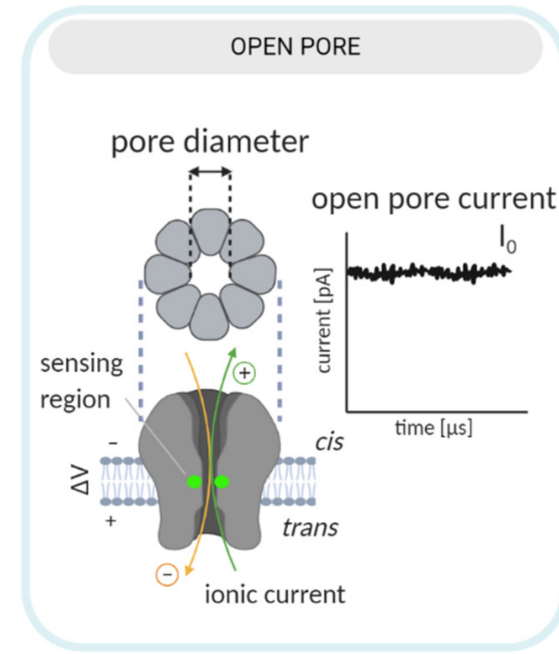
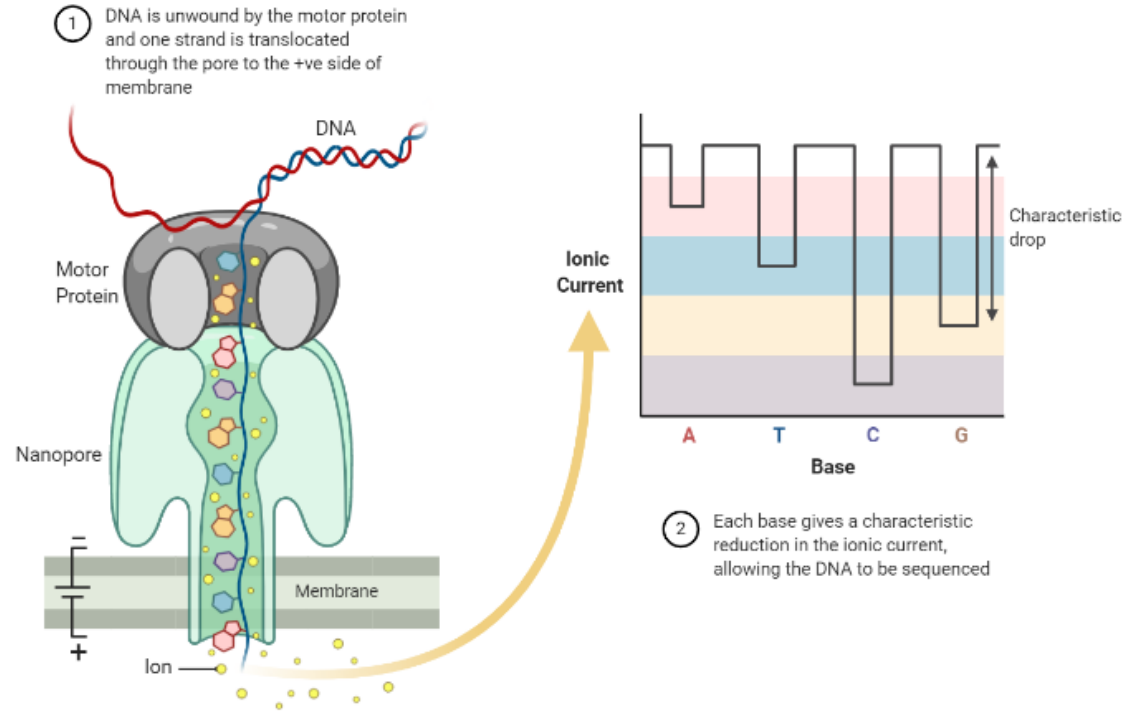


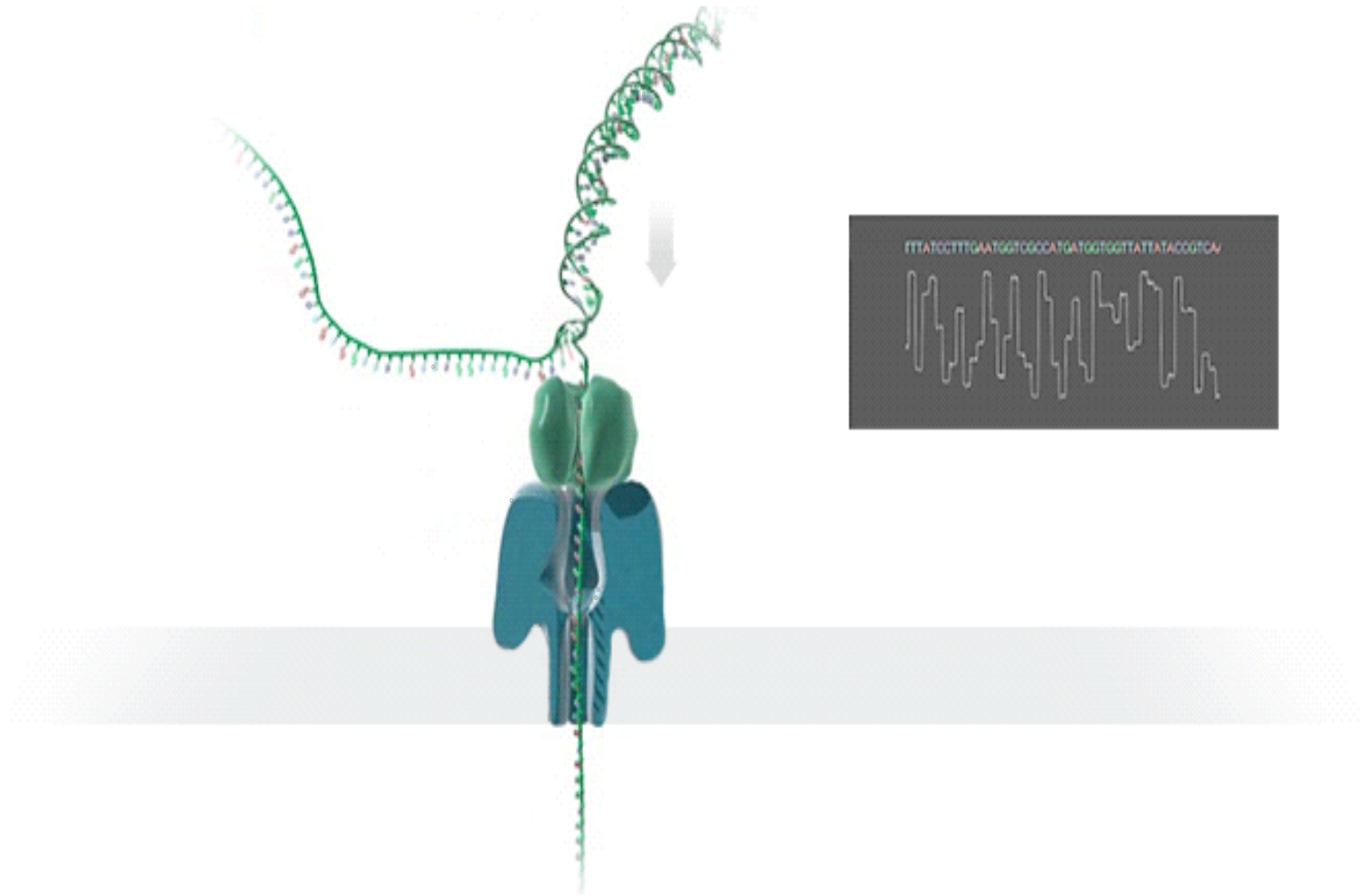
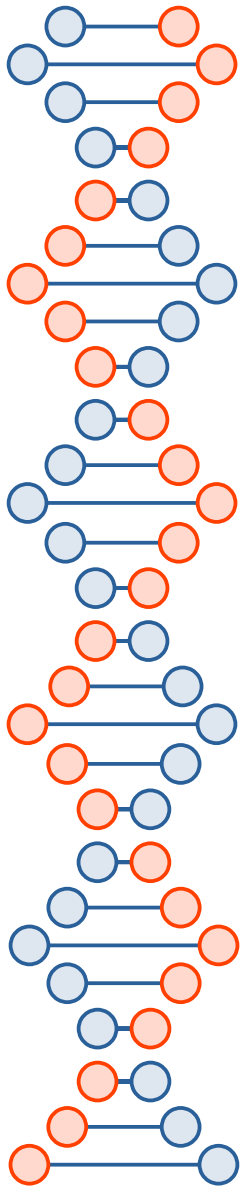




# Third Generation

## Nanopore Sequencing






# 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](https://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](https://dx.doi.org/10.17504/protocols.io.3byl4jzb8lo5/v1)

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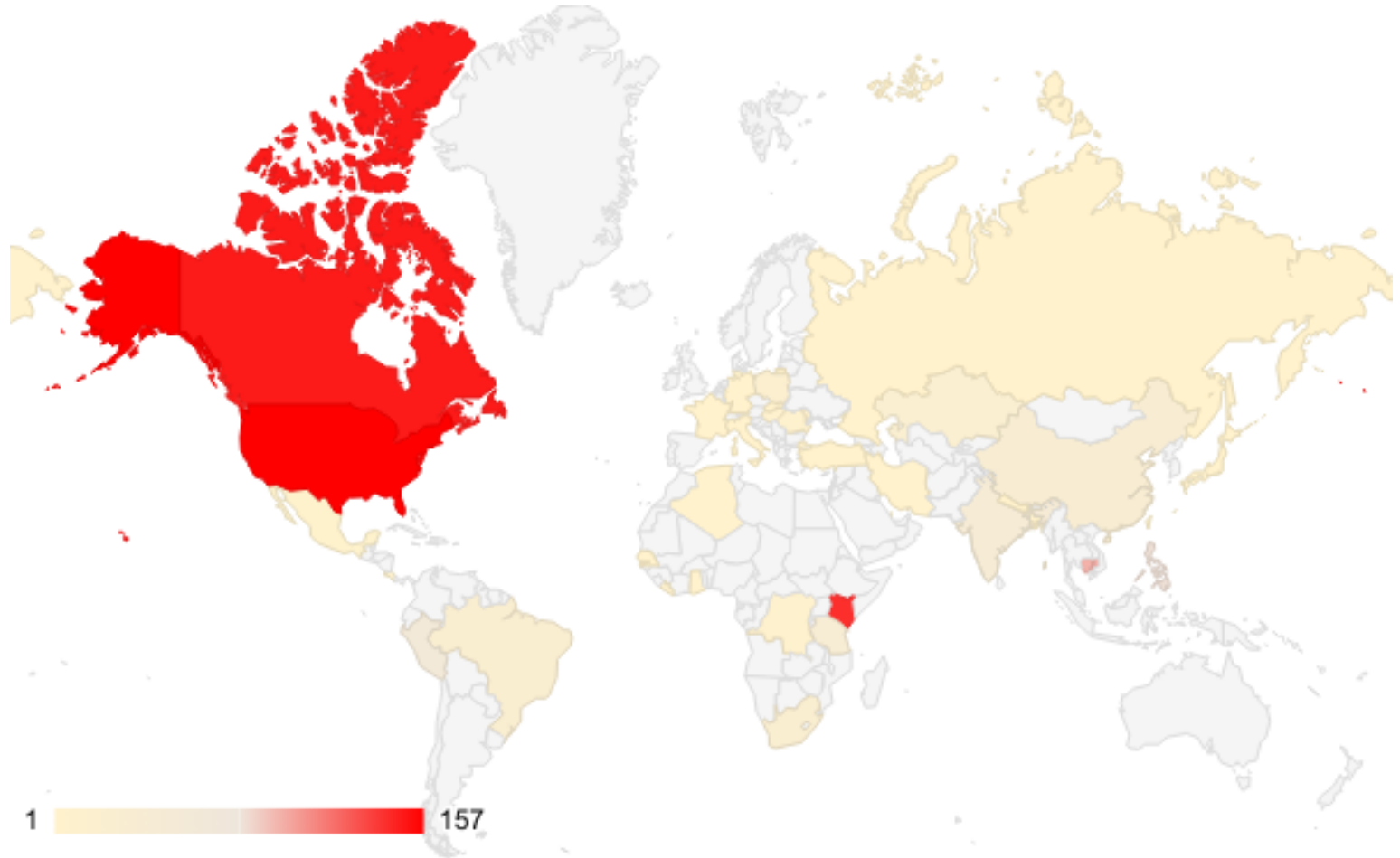
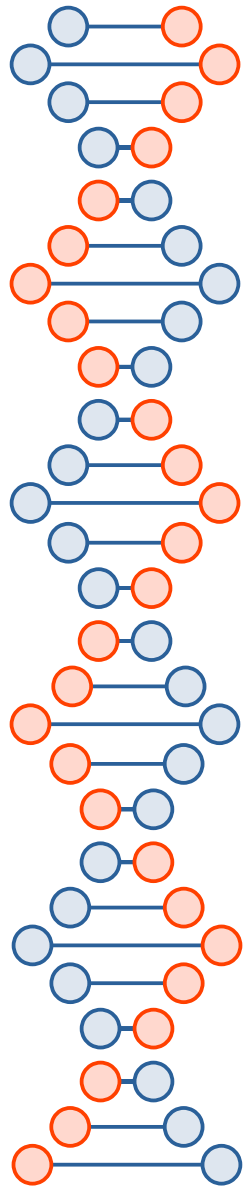
pramadaprasad

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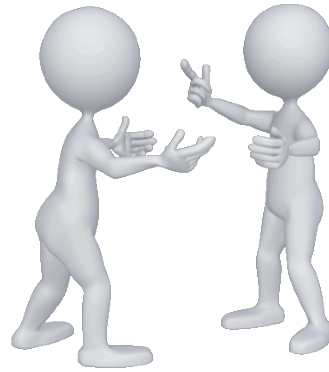
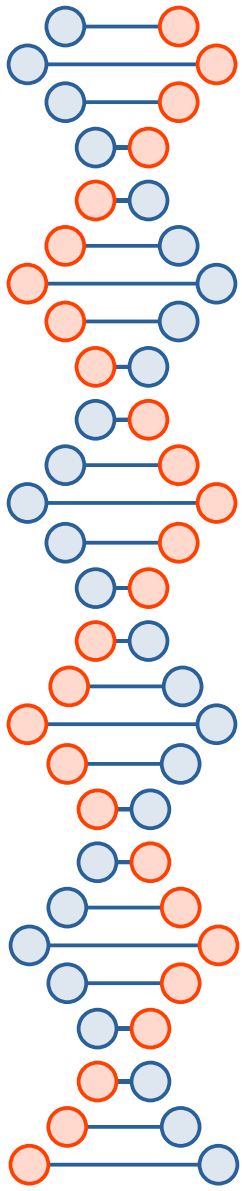
# RABV Sequence Availability





# Whole genome sequencing: Why

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



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