# DNA Sequencing

## Lecture – 3.1 & 3.2

Presented By-
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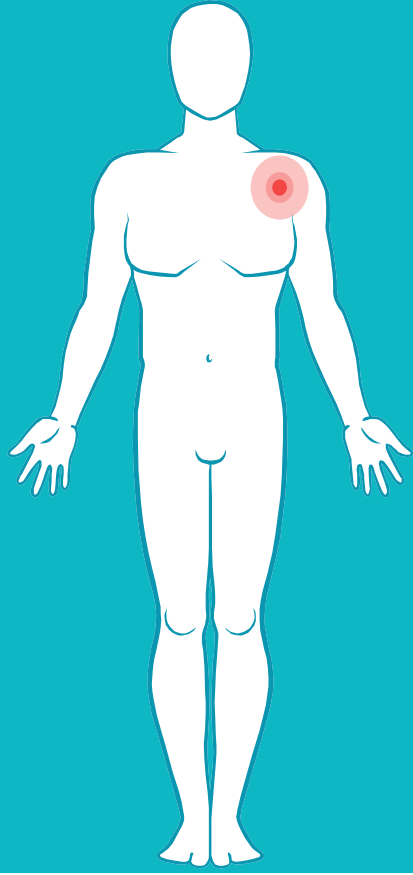

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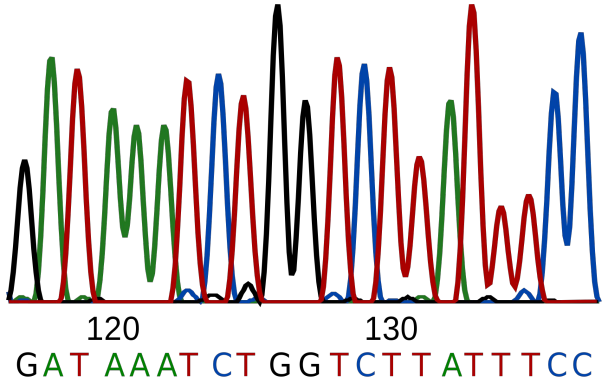
# 1. DNA Sequencing

Determining nucleotide sequences

# DNA Sequencing

- DNA sequencing is the process of determining the precise order of nucleotides (A, T, G, C) within a DNA molecule.

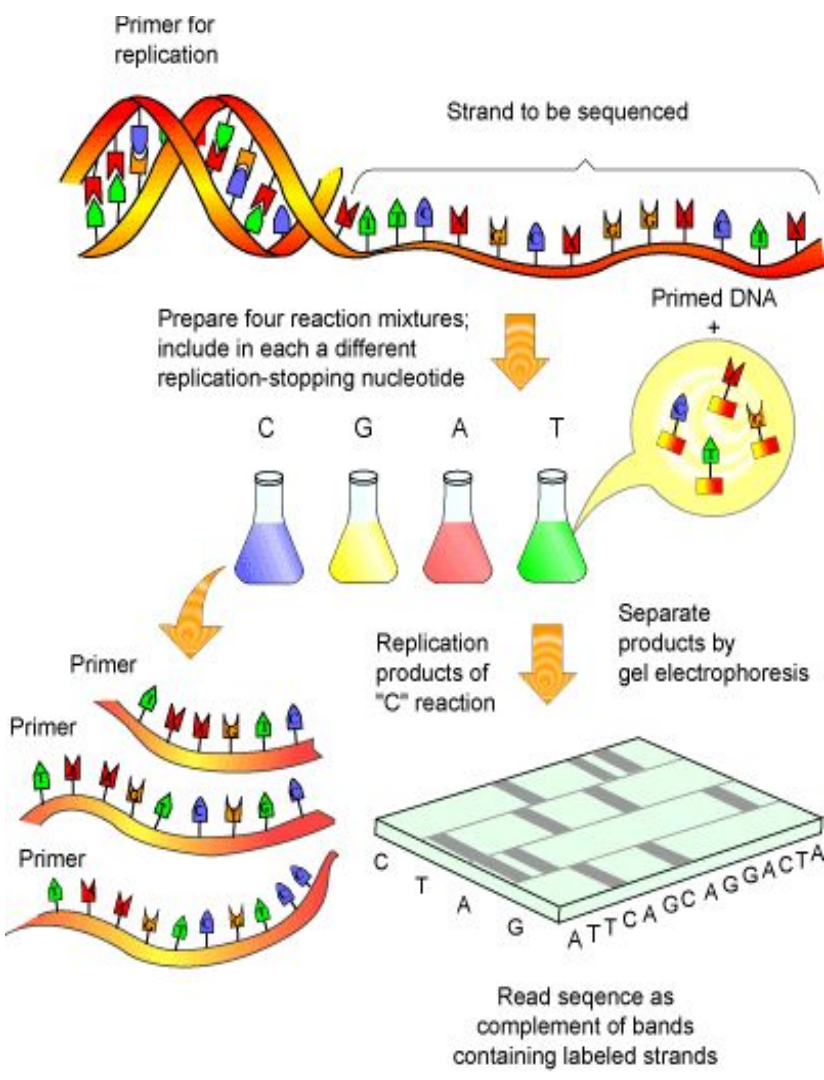


# 2. First Generation Sequencing

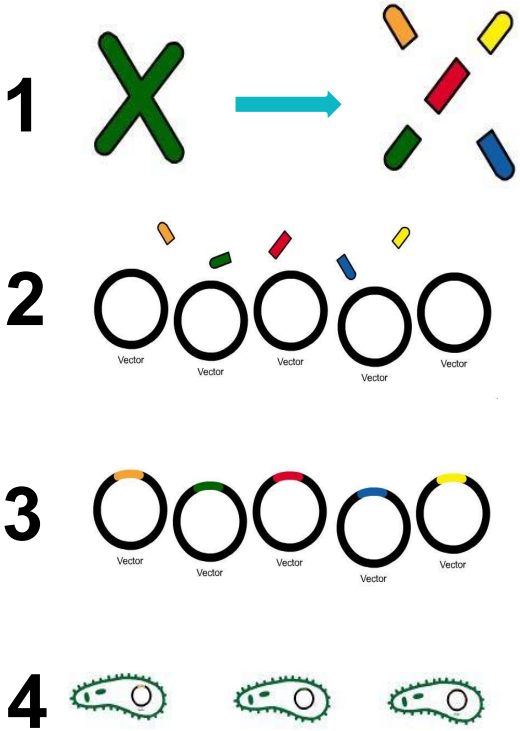
Predominant method for sequencing for decades

# Sanger Method

- ▹Developed by Frederick Sanger in 1977
- ▹Most popular and predominant method for DNA Sequencing for decades
- ▹Can read up to 2000 bps
- ▹Slow and expensive
- ▹Labor intensive
- ▹Human Genome Project was completed using Sanger Sequencing



# Step 1 - DNA Preparation




- Cut DNA into a smaller piece for sequencing
- Insert into Plasmid
- Insert Plasmid inside Bacteria Cell and let it multiply
- Extract all the necessary Plasmids and from Plasmid, isolate the DNA for sequencing

# Step 2 –Sequencing Reaction

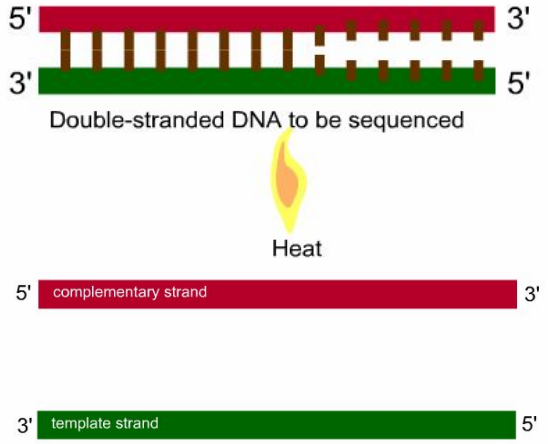
▹**Strand Separation**
- **Heat DNA in 96° C (denaturation)**

▹**Primer Annealing**
- **Lower temperature to 50° C (annealing)**
- **Primer binds to DNA**

▹**Primer Extension**
- **Increase temperature to 60° C**
- **DNA Polymerase binds to Primer**
- **Add complementary bases (dNTP) after Primer until terminator base is added (ddNTP)**

▹**Termination**
- **Terminate chain after ddNTP is added**
- **ddNTP is fluorescently labelled (different colors for A, T, G, C)**

1.

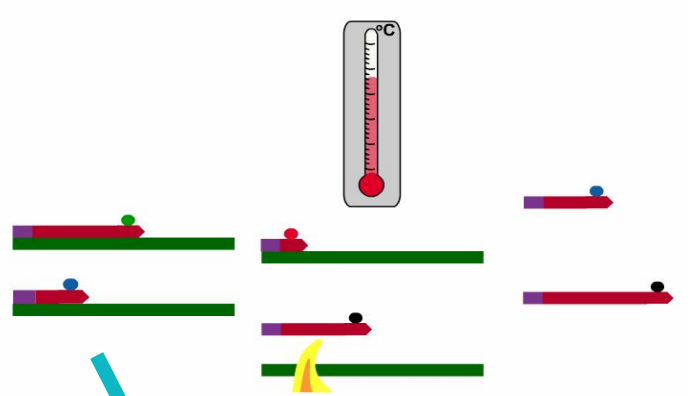


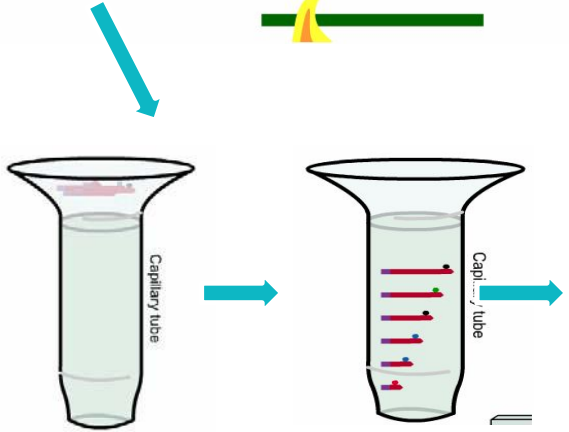

2.


3.

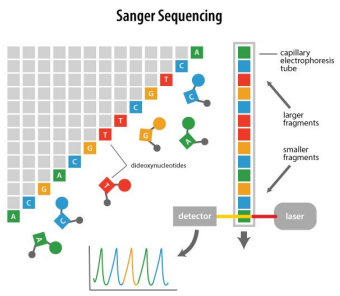
4.

# Step 3 – Electrophoresis in Capillary






- Sort the newly synthesized DNA strands by length (নতুন সংশ্লেষিত ডিএনএ স্ট্র্যান্ডগুলি দৈর্ঘ্য অনুসারে সাজান) Strands are loaded inside a capillary tube
- An electrical negative charge pulls positively charged DNA strands through the capillary
- Emerged strands pass through a laser beam that excites the ddNTP fluorescent dye at the end of each strand
- Beam causes dye to glow in a specific wavelength/color which is captured by photocell and stored in a computer
- Computer than maps each color to each nucleotide sequentially and generates final sequence output

# 3. Second / Next Gen Sequencing

Less Costly methods, mostly Short Read Sequences, High number of reads

# 454/Roche (2005)

- Pyrosequencing technique
- Long Read Sequencing (length up to 700 bps)
- Accuracy 99.9%
- Can sequence up to 1 Million reads/run
- Fast (around 24 hours/run)
- Expensive (costs around $10 per 1 million base)

# ABI SOLiD (2006)

- SOLiD (Sequence by Ligation)
- Short Read Sequencing (length up to 100 bps)
- Accuracy 99.9%
- Can sequence up to 1.4 Billion reads/run
- Time around 1-2 weeks, Slower than other sequencers
- Cheap (costs around $0.13 per 1 million base)

# Illumina / Solexa (2007)

- Sequencing by Synthesis
- Short Read Sequencing (length up to 300 bps)
- Accuracy 99.9%
- Can sequence up to 3 Billion reads/run
- Moderately Slow (around 1-11 days/run)
- Expensive Equipment, run cost is low (costs around $0.05-$0.15)

# 4. Third / Next-Next Gen Sequencing

Long reads, Higher error rate

# Pacific Biosciences (PacBio)

- Single Molecule Real Time Sequencing
- Long Read Sequencing (length up to 40,000 bps)
- Accuracy 87%
- Can sequence up to 500-1000 Mega reads/run
- Time around 30 minutes – 4 hours, Faster
- Expensive Equipment, run cost is low (costs around $0.13-$0.60)

# Oxford Nanopore

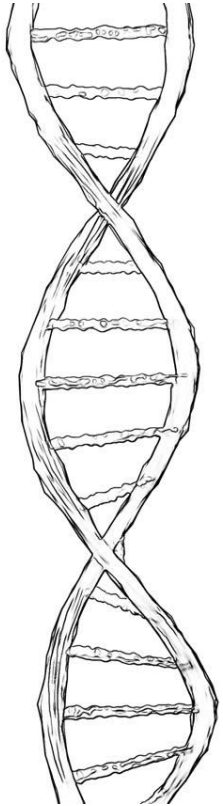
- Nanopore sequencing
- Very Long Read Sequencing (length up 500 kb), Portable
- Accuracy 92-97%
- Depends on read length selected by user
- Time around 1 minutes – 48 hours, Faster
- Expensive Equipment, run cost is low (costs around $500-$999 per flow cell)

# 5. Miscellaneous Terms

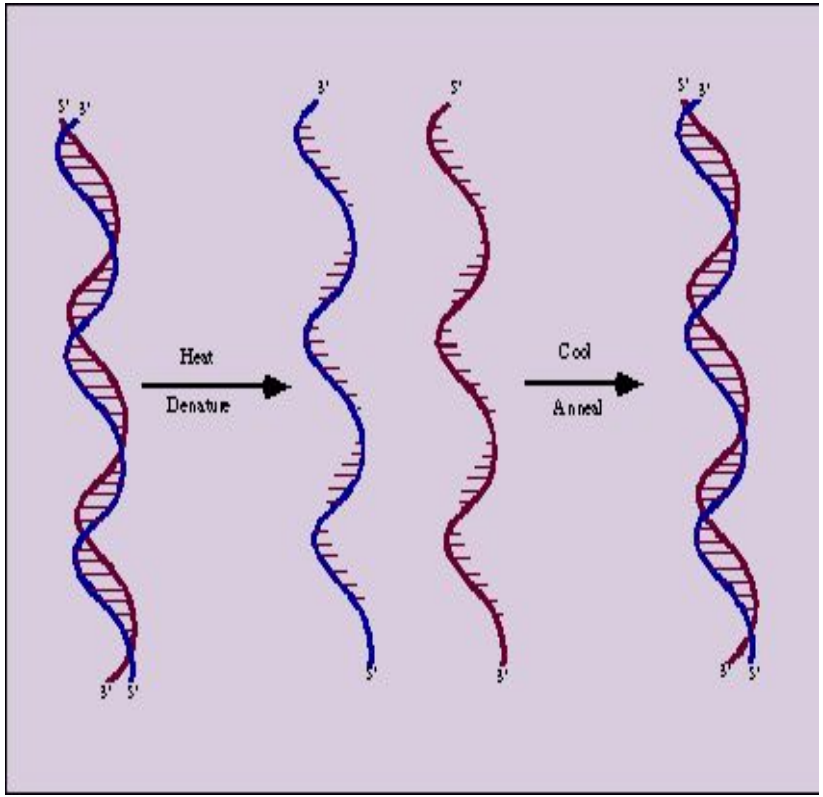
Some comparisons, terms etc.

# Oligonucleotide

- **Short sequences of DNA or RNA**
- **Typically less than 20bp**
- **Oligonucleotide of ‘k’ bases length is called k-mer.**



# Denaturation and Annealing




## Denaturation

- Energy of heat pull apart two DNA strands
- Happens at a critical temperature denoted $T_m$

## Annealing

- Decrease temperature, and strands are joined back together
- Only complementary bases will bond

# Plasmid

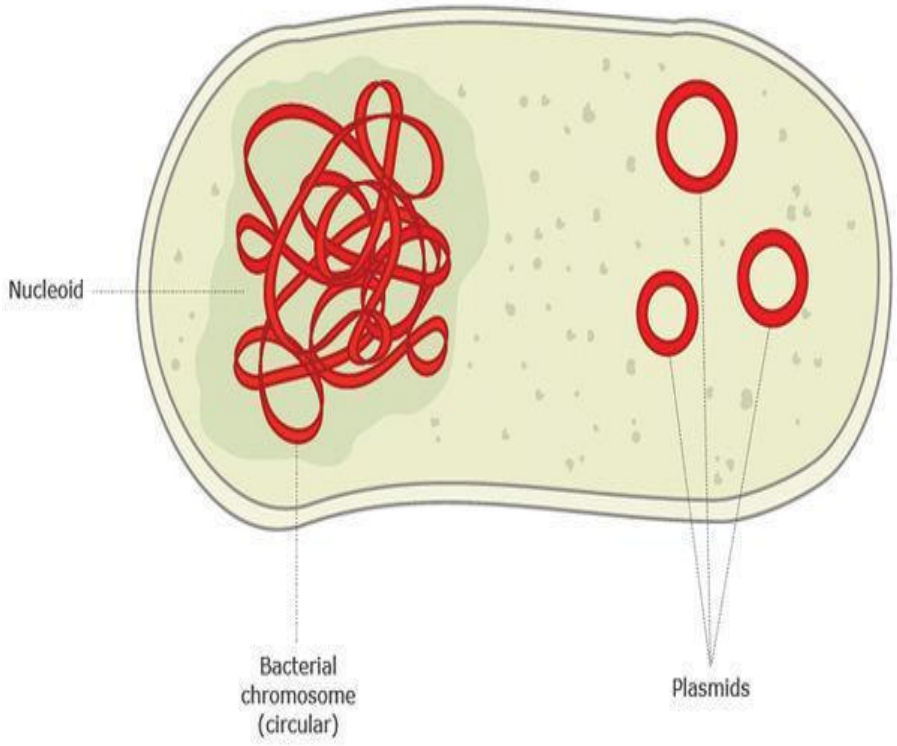
- Small, circular piece of DNA often found in bacteria.
- Sizes of 2.5-20 kb
- Plasmid using method -

    * Isolate them in large quantities

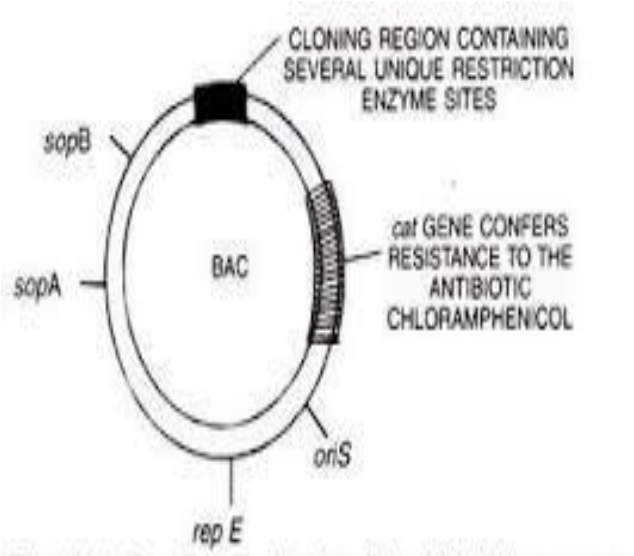
    * Cut and splice them, adding whatever DNA needed

    * Put them back into bacteria, where they'll replicate along with the bacteria's own DNA

    * Isolate them again - getting billions of copies of whatever DNA was inserted into the plasmid



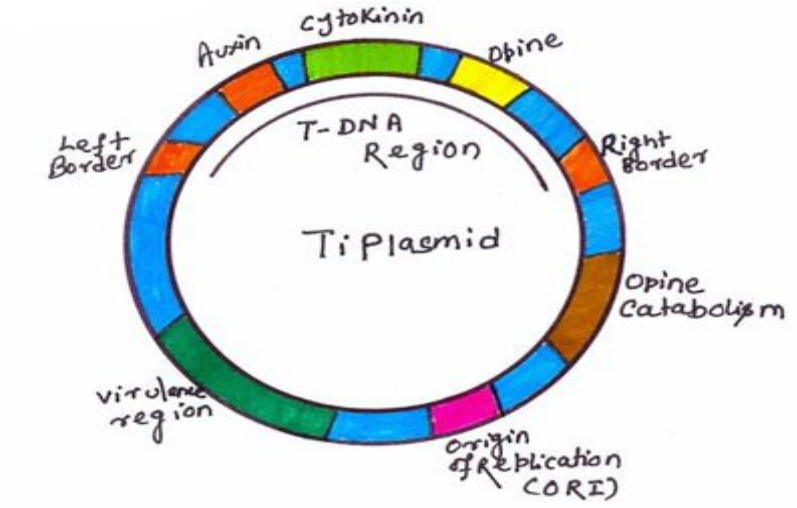
# Bacterial Artificial Chromosome (BAC)




- Used like a plasmid
- BACs carry DNA from humans or mice or any other living being, and is inserted into a host bacterium for replication
- BAC is artificially constructed, unlike Plasmid

# Cloning Vector

- A cloning vector is a small piece of DNA, taken from a virus, a plasmid, or the cell of a higher organism, that can be stably maintained in an organism, and into which a foreign DNA fragment can be inserted for cloning purposes.



8%

Of Human DNA is made of Ancient Viruses

700 Terabytes

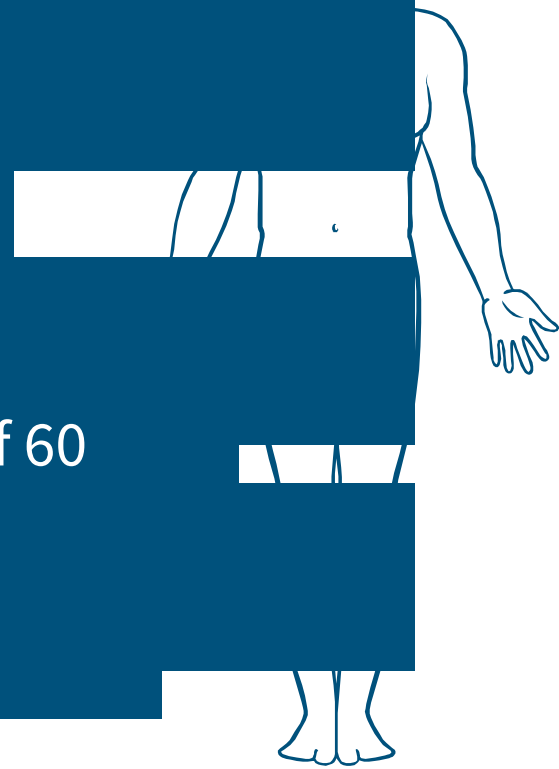
Data can be stored in 1gm DNA

50 Years Time

Type entire human genome at a speed of 60

99.9%

Human DNA is identical, 0.01% creates



TO BE CONTINUED

# Impressed?

# Youtube Links

- Sanger Sequencing - https://www.youtube.com/watch?v=0NGdehkB8jU