

SEQUENCING TECHNIQUES

Professor Swakkhar Shatabda

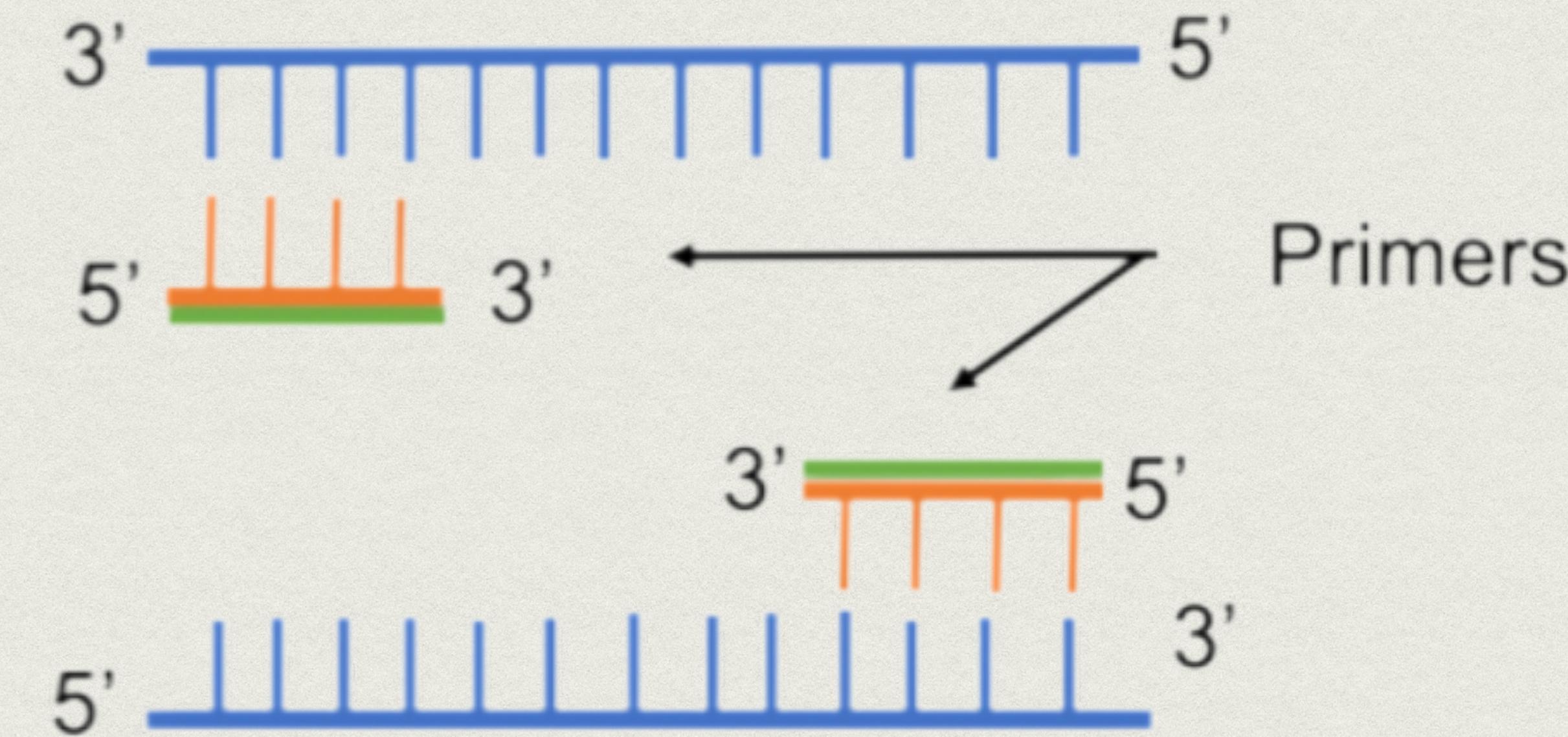
HOW TO COPY DNA?

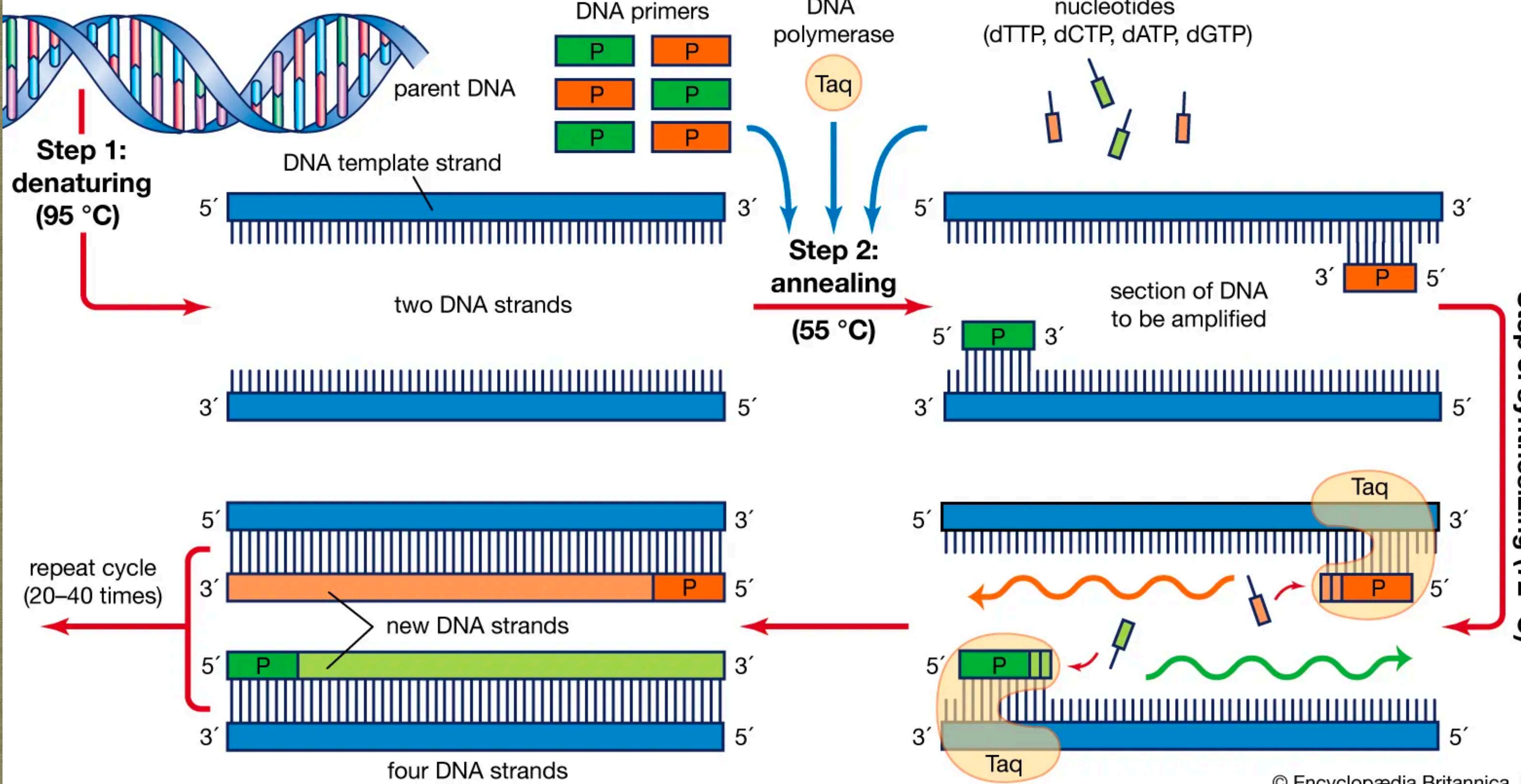


Polymerase Chain Reaction (PCR)

PRIMER

- A primer is a short single-stranded nucleic acid used by all living organisms in the initiation of DNA synthesis.

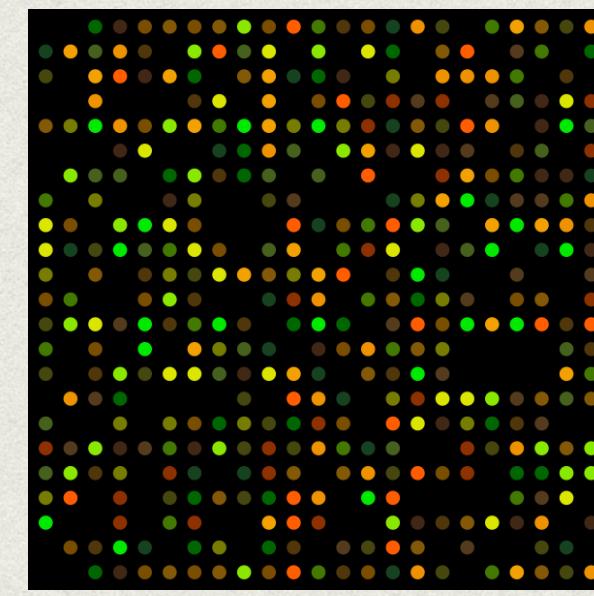




SEQUENCING



Sanger Sequencing
1970s



DNA microarrays
1990s



Second Generation
2000s



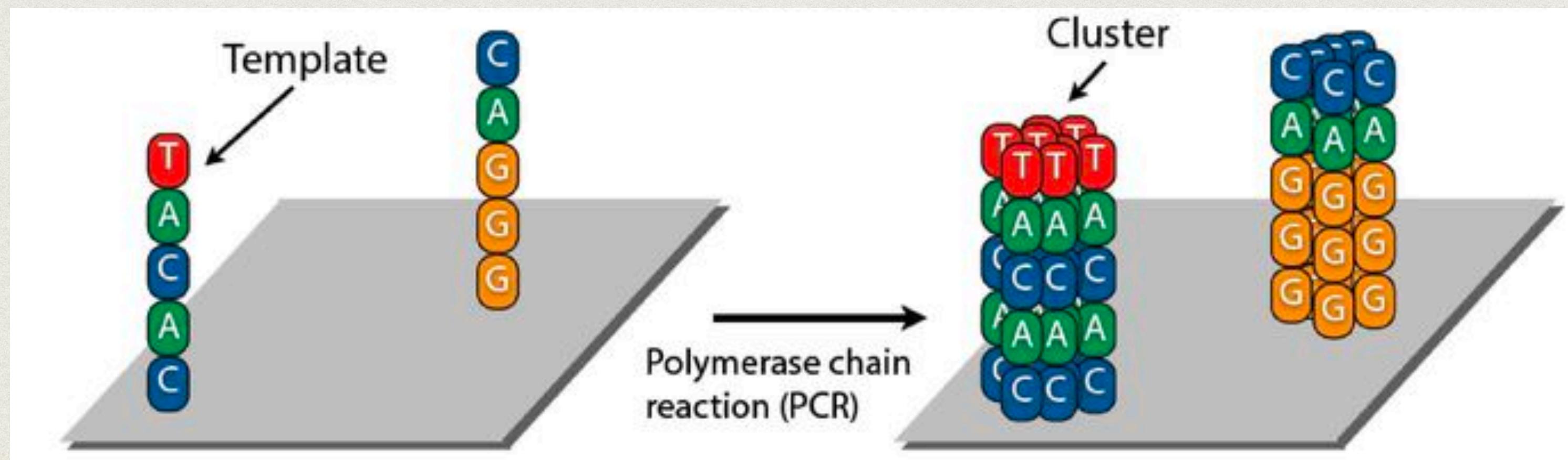
Pacific Biosciences
2010s



Oxford Nanopore
2010s

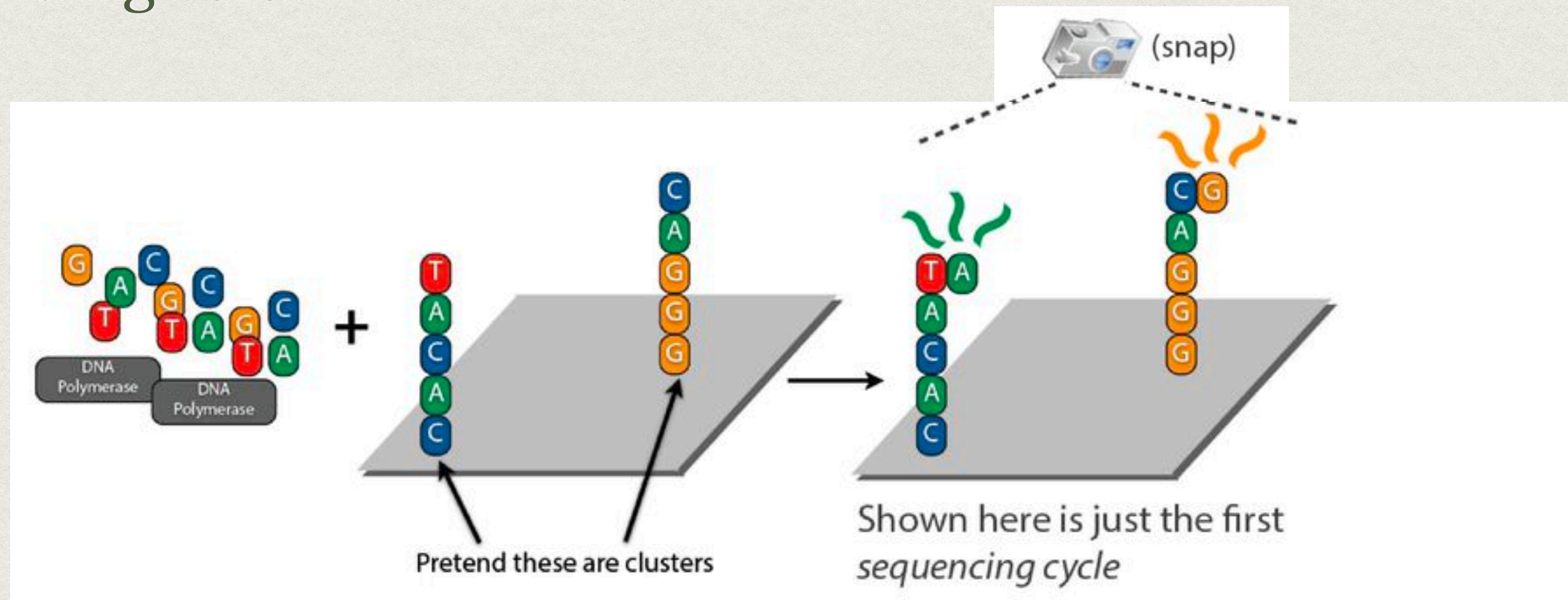
NGS

1. Take DNA sample, which includes many copies of the genome, and chop it into single stranded fragments or templates
2. Attach templates to surface
3. Make copies of templates so that each template becomes a cluster of clones.



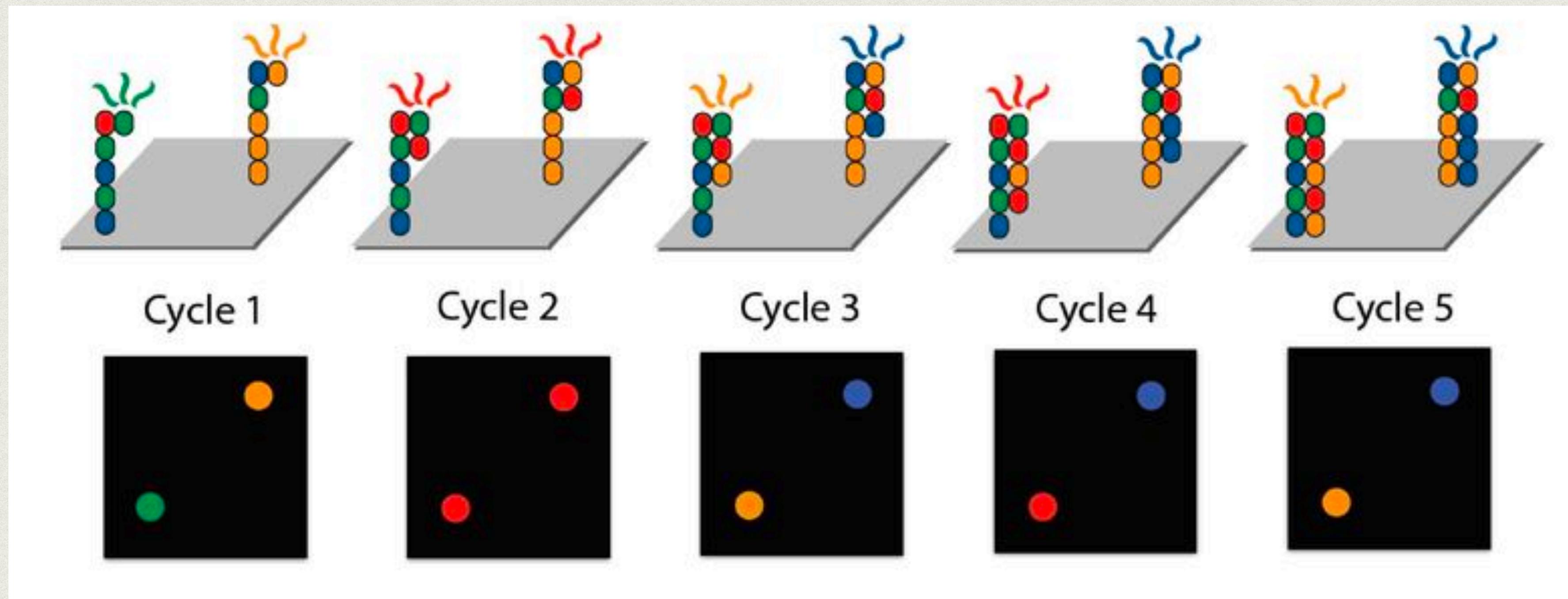
SEQUENCING

- Repeatedly inject mixture of color labeled nucleotides and DNA polymerase. When a complimentary nucleotide is added, the corresponding colour is emitted.

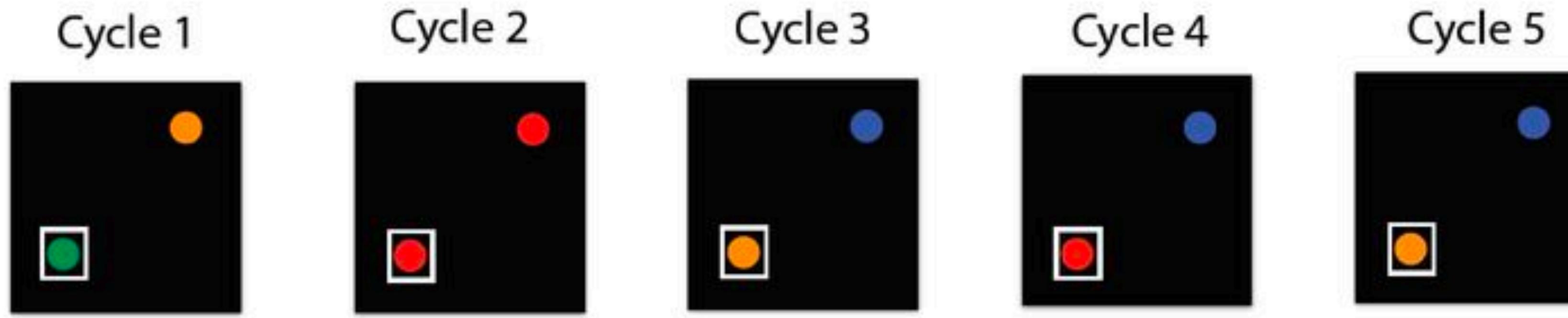


SEQUENCING

- Line up images and for each cluster turn the series of light signals into corresponding series of nucleotides



BASE CALLER



"Base caller" software looks at this cluster across all images and "calls" the complementary nucleotides: **TACAC**, corresponding to the template sequence



TACAC is a "sequence read," or "read." Actual reads are usually 100 or more nucleotides long.

BASE CALLER

Identifier	• @SRR566546.970 HWUSI-EAS1673_11067_FC7070M:4:1:2299:1109 length=50
Sequence	• TTGCCTGCCTATCATTAGTGCCTGTGAGGTGGAGATGTGAGGATCAGT
'+' sign	• +
Quality scores	• hhhhhhhhhghhhhhfhhhhfffffe'ee['X]b[d[ed' [Y[^Y
Identifier	• @SRR566546.971 HWUSI-EAS1673_11067_FC7070M:4:1:2374:1108 length=50
Sequence	• GATTGTATGAAAGTATAACAACACTAAAATGCAGGTGGATCAGAGTAAGTC
'+' sign	• +
Quality scores	• hhggfhhcghghggfcffdhfehhhhcehdchhdhahehffffde'bVd

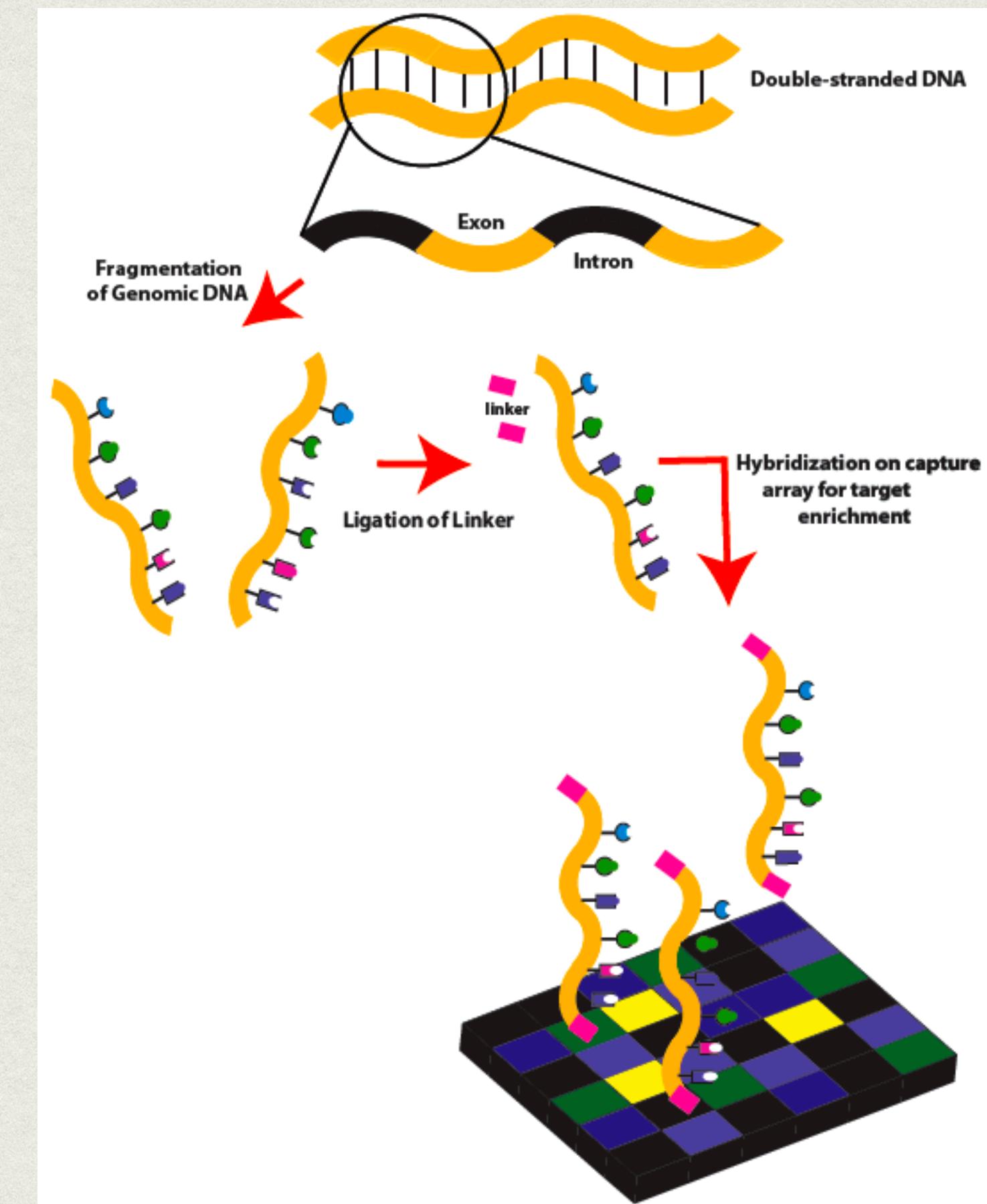
NGS APPLICATION

1.Convert Molecules to DNA

2.Apply second generation sequencing

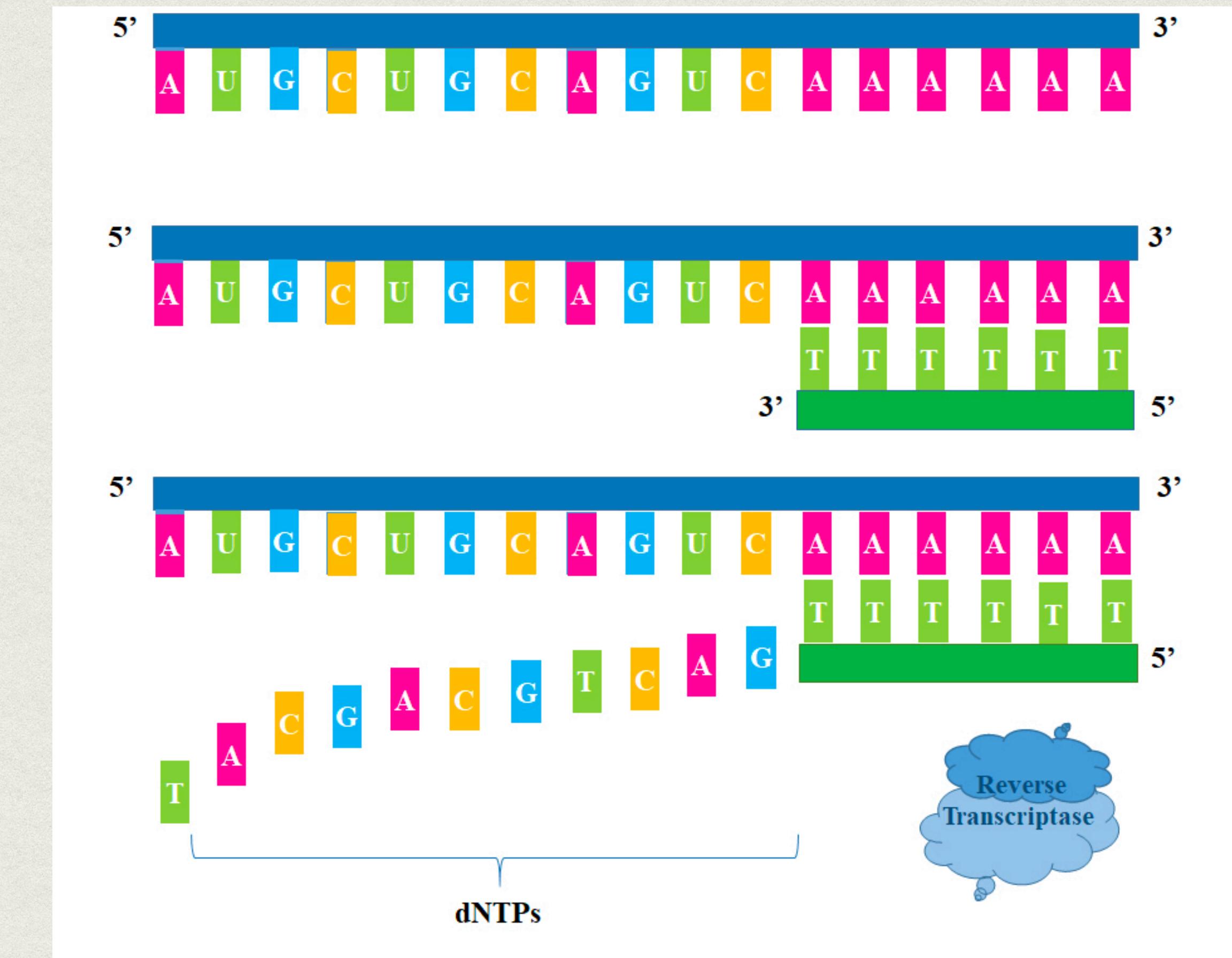
EXOME SEQUENCING

- Exome sequencing, also known as whole exome sequencing (WES), is a genomic technique for sequencing all of the protein-coding regions of genes in a genome (known as the exome).



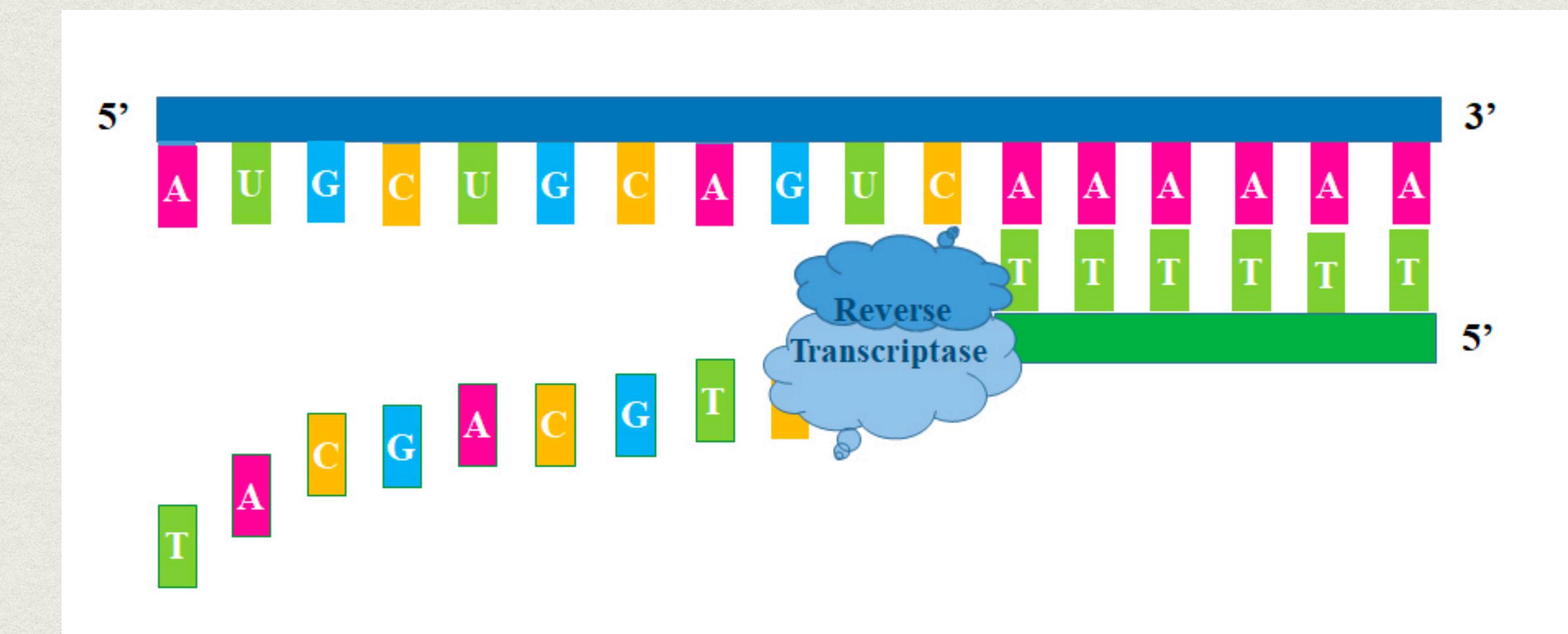
RNA-SEQ

- RNA-seq (RNA-sequencing) is a technique that can examine the quantity and sequences of RNA in a sample using next-generation sequencing (NGS)



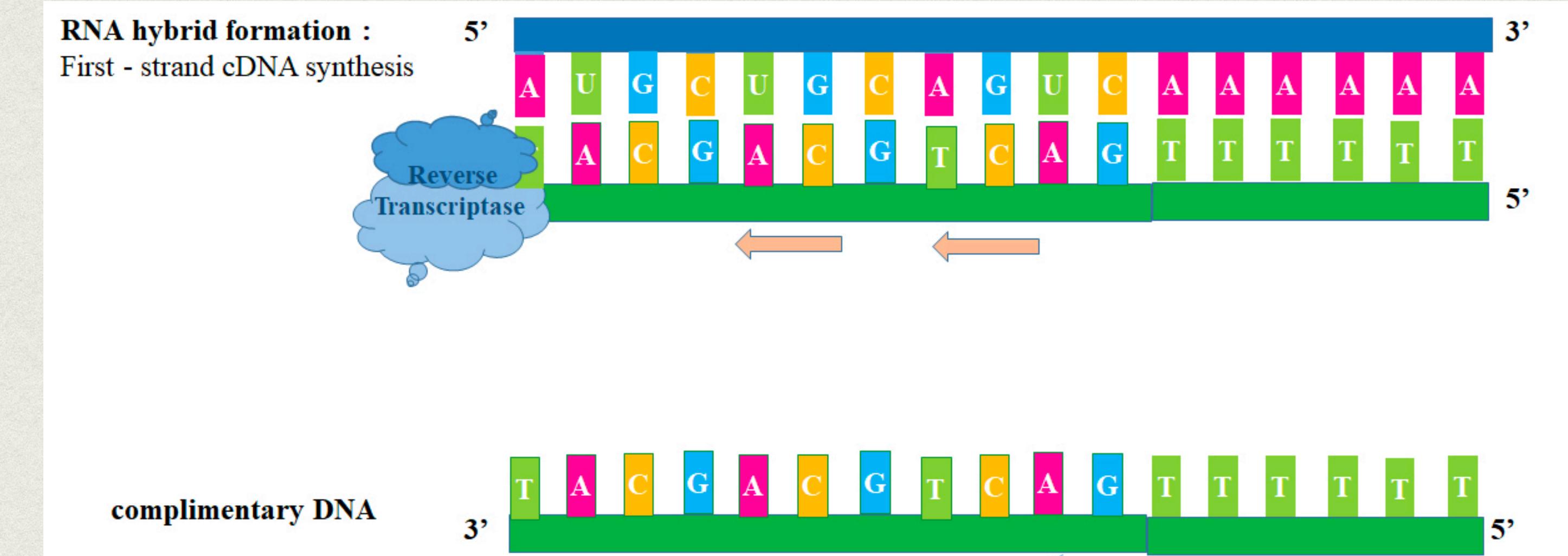
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CHIP-SEQ

- Chromatin immunoprecipitation sequencing
- ChIP-sequencing, also known as ChIP-seq, is a method used to analyze protein interactions with DNA.

