

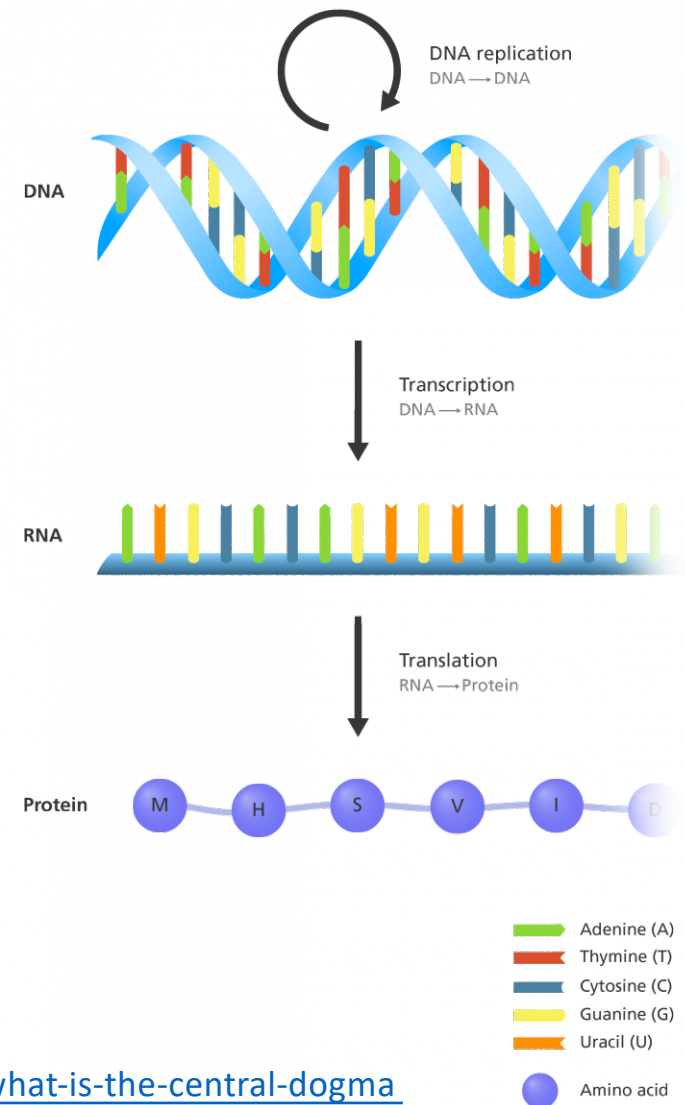
CMSC 423

Introduction to Biology

Part 2

- DNA encodes genetic information necessary for life

- DNA replication
- Transcription of DNA into RNA
- Translation of RNA into proteins

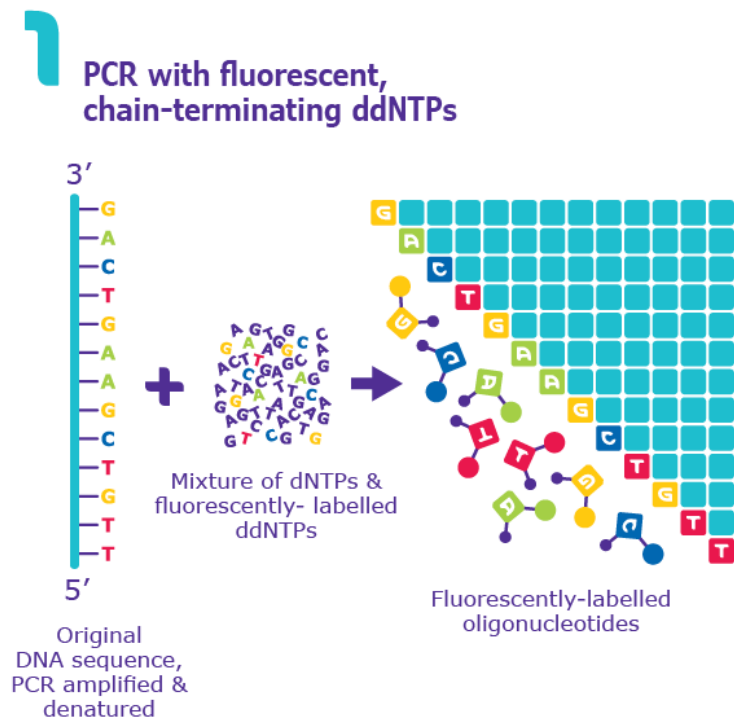


How do we study the DNA
sequence of an organism?

DNA Sequencing

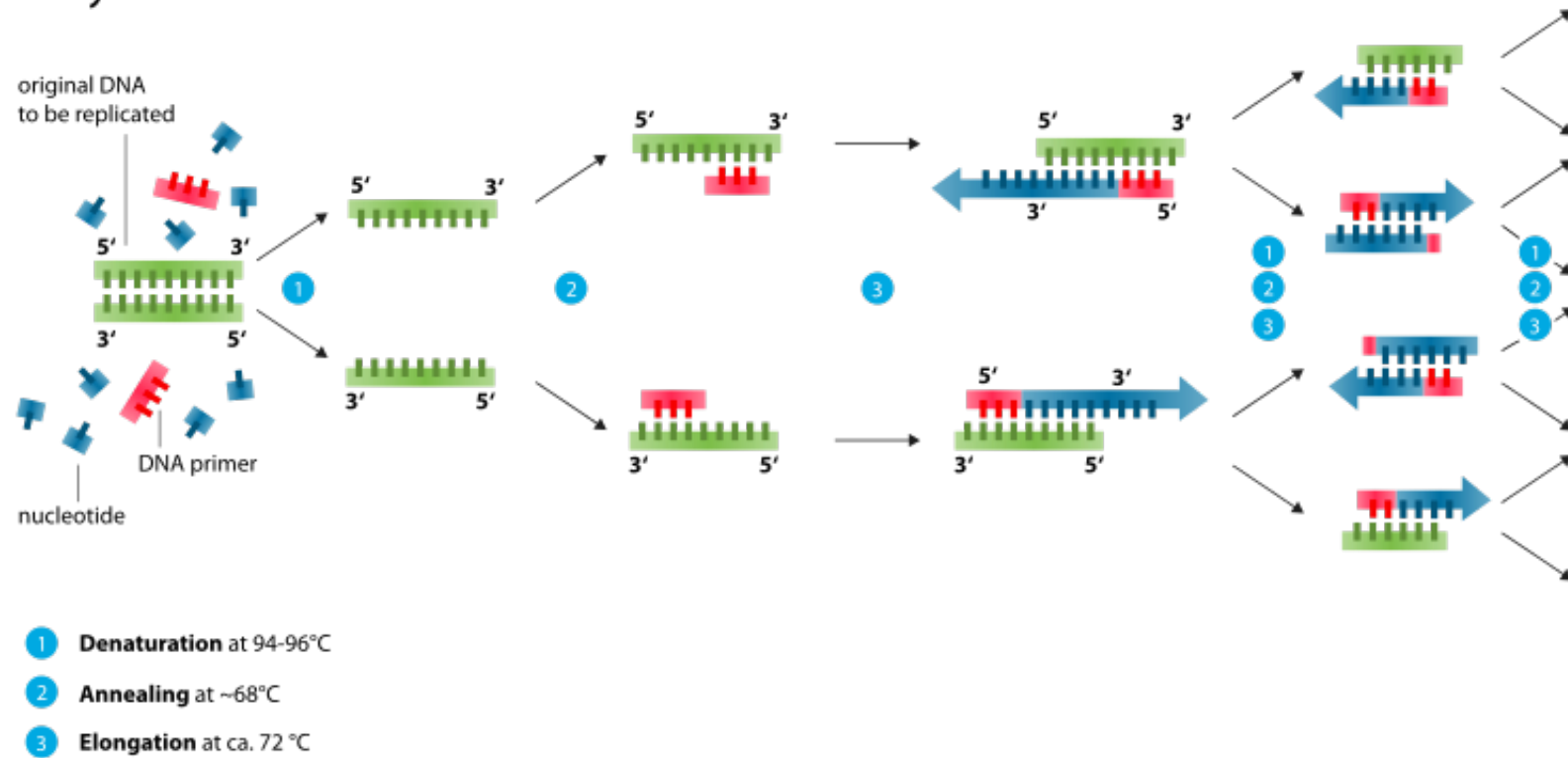
- Sanger (sorting by size)
- 454 (luminescence)
- Illumina (a different type of luminescence)
- Pacific Biosciences (trapped polymerase)
- Oxford Nanopore (nanopore)

Sanger Sequencing: chain termination



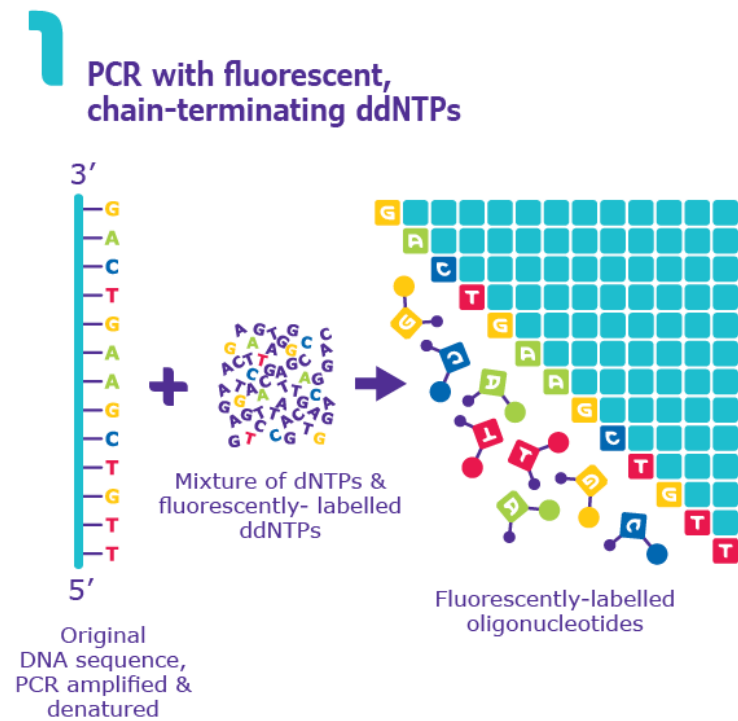
<https://www.sigmaaldrich.com/technical-documents/articles/biology/sanger-sequencing.html#:~:text=Sanger%20sequencing%2C%20also%20known%20as,the%20name%20the%20Sanger%20Sequence.>

Polymerase chain reaction - PCR

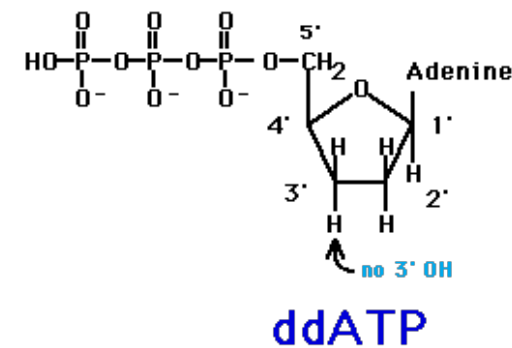
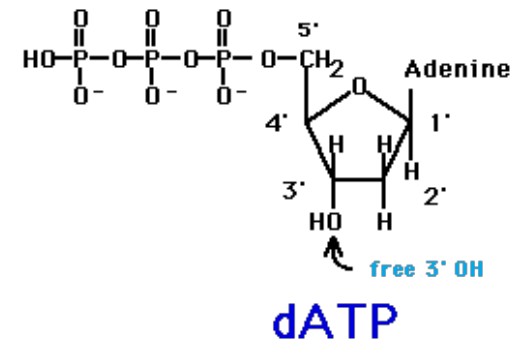


https://commons.wikimedia.org/wiki/File:Polymerase_chain_reaction.svg

Sanger Sequencing: chain termination

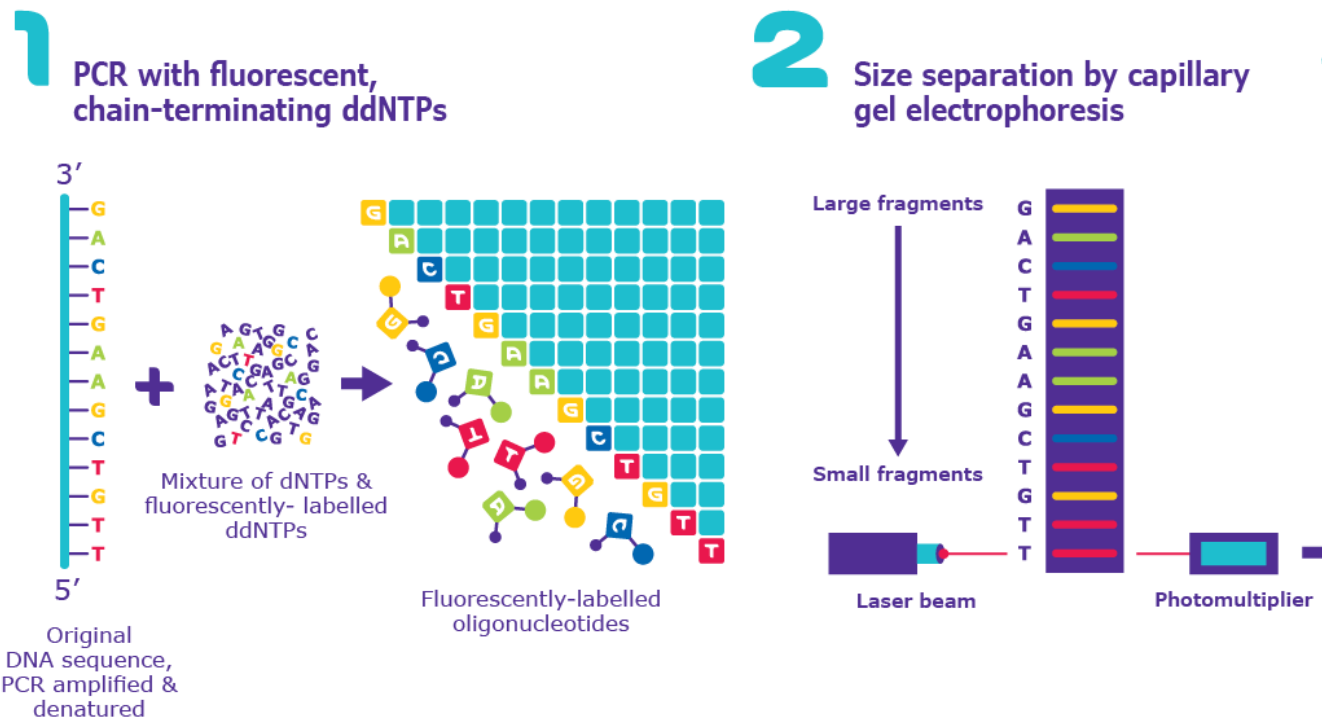


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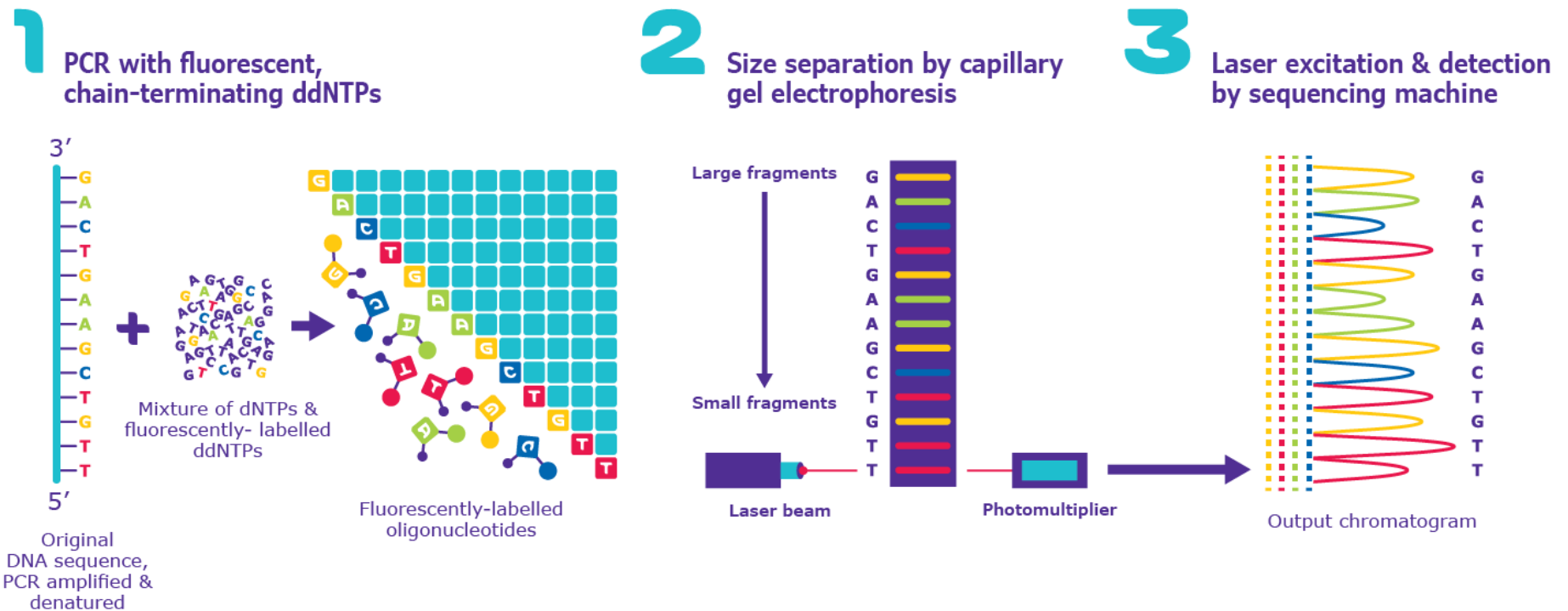
<https://biology.reachingfordreams.com/molecular-genetics/methods-in-molecular-genetics/21-dna-sequencing>

Sanger Sequencing: chain termination



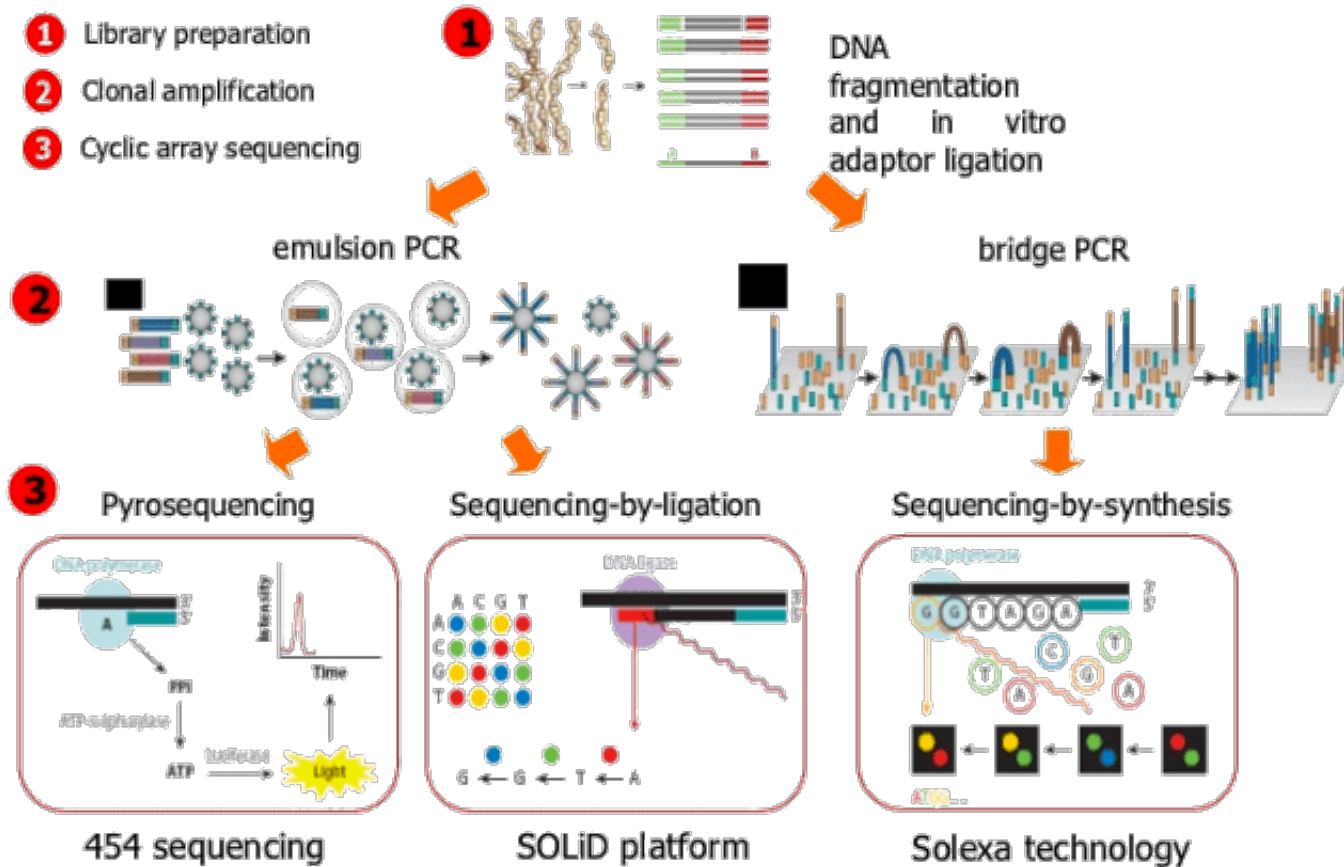
<https://www.sigmaaldrich.com/technical-documents/articles/biology/sanger-sequencing.html#:~:text=Sanger%20sequencing%2C%20also%20known%20as,the%20name%20the%20Sanger%20Sequence.>

Sanger Sequencing: chain termination



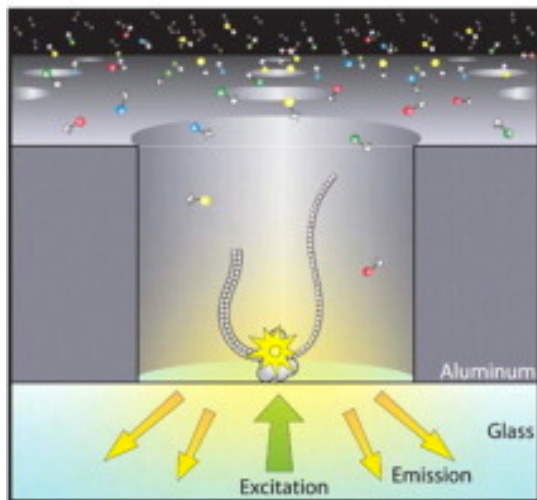
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Next-generation DNA sequencing

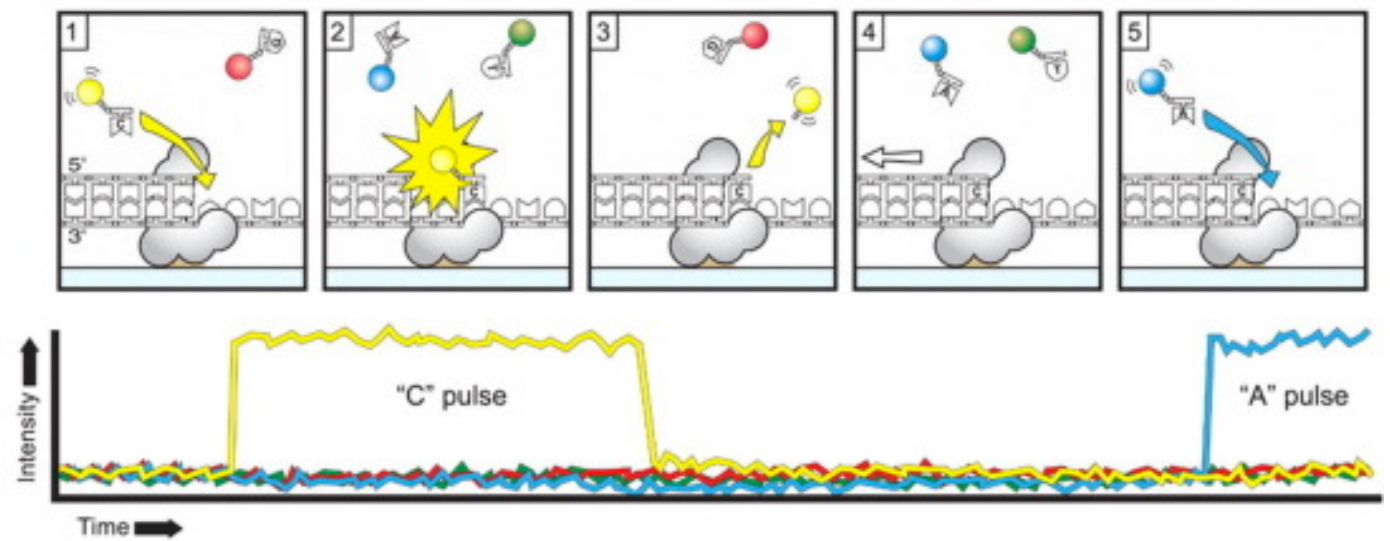


Pacific Biosciences

A



B



Oxford Nanopore

SEQUENCE

Nanopore devices perform DNA/RNA sequencing directly and in real time.
The technology is scalable from miniature devices to high-throughput installations.

Which device is best for you?



Flongle



MiniON



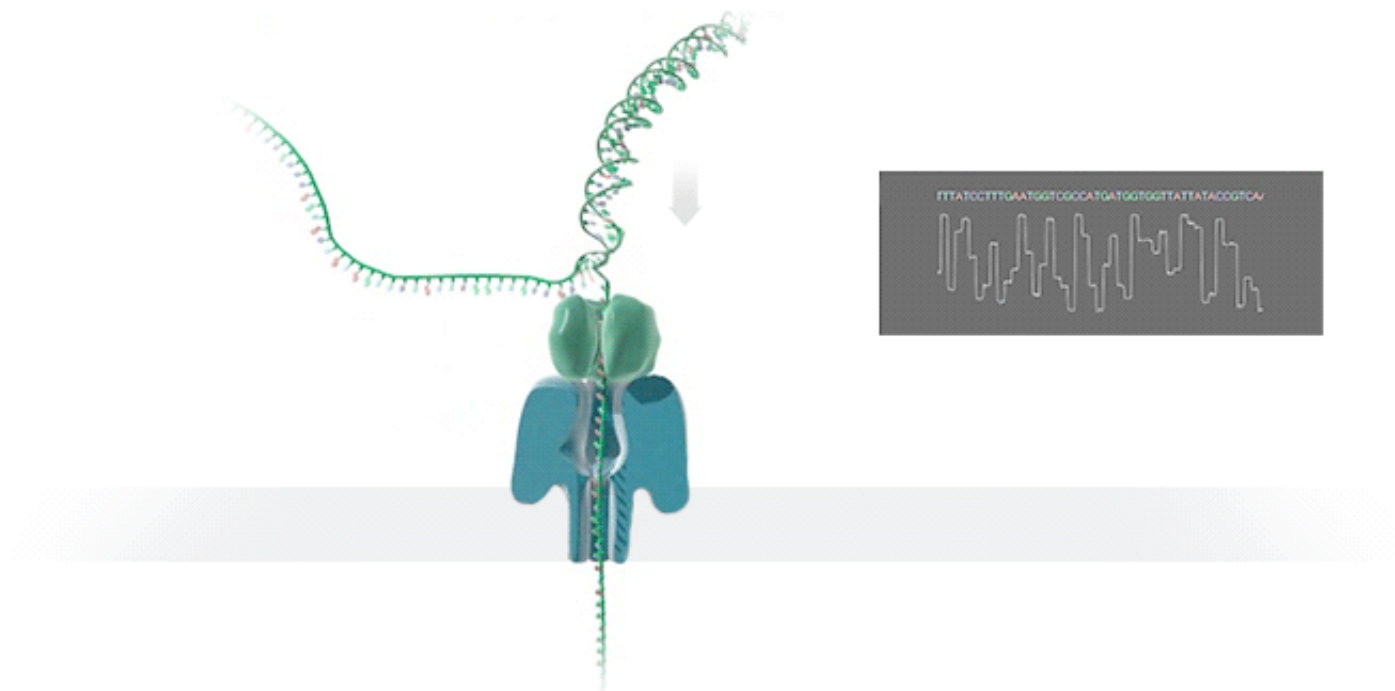
GridION



PromethION

<https://nanoporetech.com/products>

Oxford Nanopore



<https://nanoporetech.com/how-it-works#:~:text=A%20nanopore%20is%20a%20very%20small%20hole&text=In%20its%20devices%2C%20Oxford%20Nanopore,used%20to%20identify%20that%20molecule.>



and Think

What are some considerations when choosing a sequencing platform?



and Think

What are some considerations when choosing a sequencing platform?

- Biological Question
- Cost
- Read length

Representing sequences computationally

- **FASTA: two lines per sequence**

> Seq1 header line

ATCGTAGACGGTCGGATAGACTTA

- **FASTQ: four lines per sequence, encodes quality information**

@seqid header info

ATCGTAGACGGTCGGATAGACTTA

+seqid other info (**optional**)

!+30qr-130!@+-@@@+--+@@@

Table 1 ASCII Characters Encoding Q-scores 0–40

Symbol	ASCII Code	Q-Score	Symbol	ASCII Code	Q-Score	Symbol	ASCII Code	Q-Score
!	33	0	/	47	14	=	61	28
"	34	1	0	48	15	>	62	29
#	35	2	1	49	16	?	63	30
\$	36	3	2	50	17	@	64	31
%	37	4	3	51	18	A	65	32
&	38	5	4	52	19	B	66	33
'	39	6	5	53	20	C	67	34
(40	7	6	54	21	D	68	35
)	41	8	7	55	22	E	69	36
*	42	9	8	56	23	F	70	37
+	43	10	9	57	24	G	71	38
,	44	11	:	58	25	H	72	39
-	45	12	;	59	26	I	73	40
.	46	13	<	60	27			



and Think

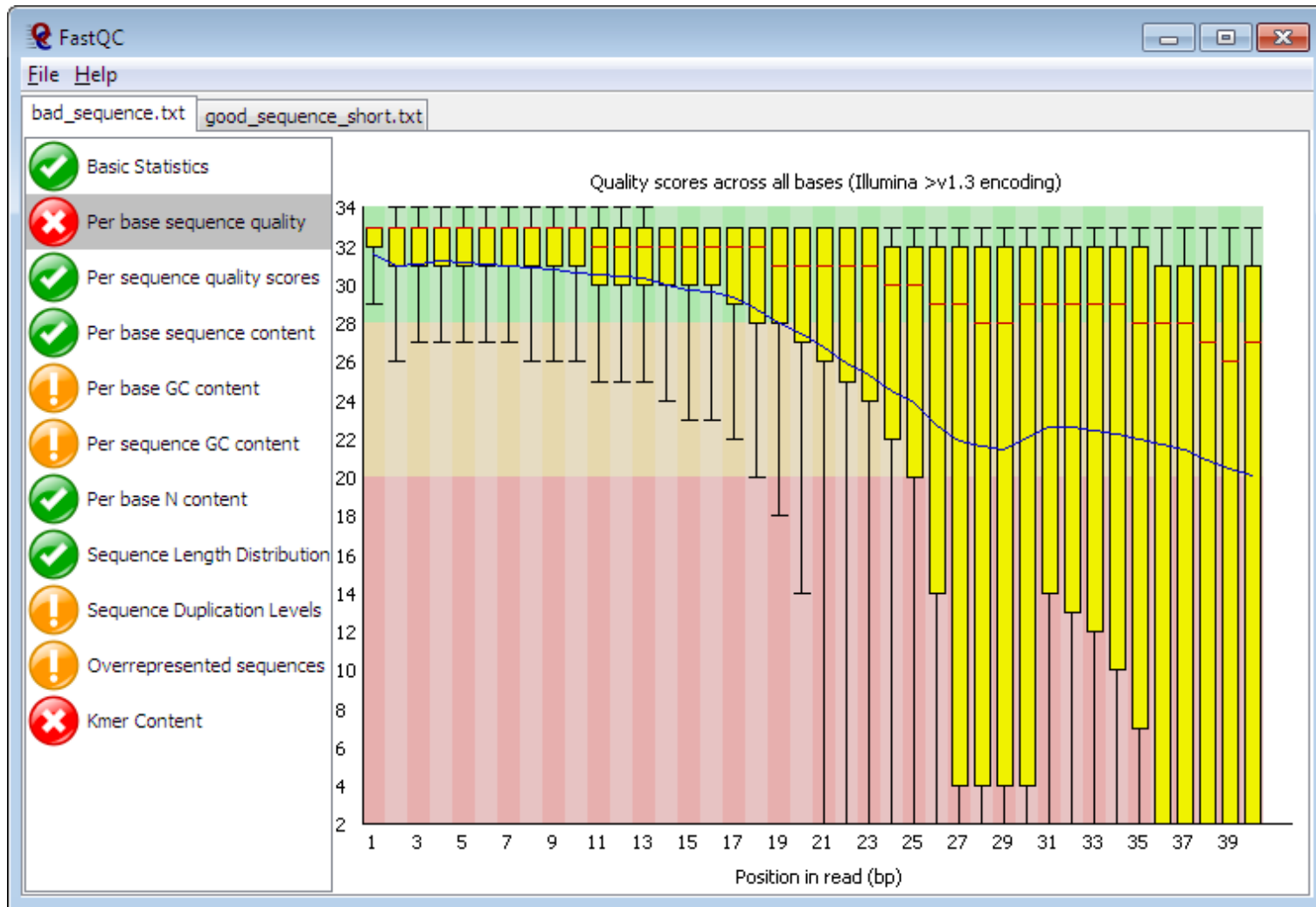
What are some potential reasons that a sequencing machine would provide an erroneous base call?



and Think

What are some potential reasons that a sequencing machine would provide an erroneous base call?

- Amplification errors
- Polluted light signal



<https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>

Summary

- Sequencing allows us to determine the order of the four nucleotide bases in DNA strands
- Sanger sequencing is based on the incorporation of chain-terminating dideoxynucleotides and can produce sequencing reads > 500 bp
- Next-generation sequencing platforms are high-throughput and can generate millions of sequencing reads in a short time period
- PacBio and Oxford Nanopore platforms generate reads that are tens of thousands of bases long