IN-BIOS[9,5]000 2020

Illumina Technology

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Oct 19, 2020

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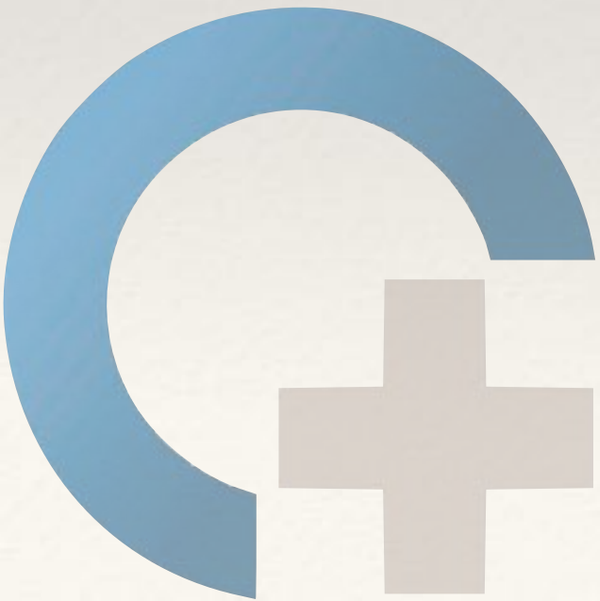


DNA sequencing

- First generation - past, present
  - Up to 1 kb; high quality data; multiplexed
  - SANGER; Highly automated (ABI Sanger 3730xl)
- Second generation - present
  - Shorter reads; Massive parallelisation and real high throughput
  - Illumina, BGISeq, Ion-torrent, [454, Solid]
  - RNA is reverse-transcribed to cDNA before sequencing
- Third generation - [present] future
  - Long-read sequencing; Single-molecule sequencing (without amplification)
  - PacBio, Oxford Nanopore, [more in development]
  - Potential to sequence RNA directly

High throughput sequencing

illumina®

MiniSeq
MiSeq
NextSeq
HiSeq 2500
HiSeq 3/4000
HiSeq X
NovoSeq

BGISEQ

Roche 454
SOLiD
Ion Torrent

PACBIO®

RS II
Sequel

Oxford NANOPORE Technologies®

MinION
Flongle
GridION
PromethION

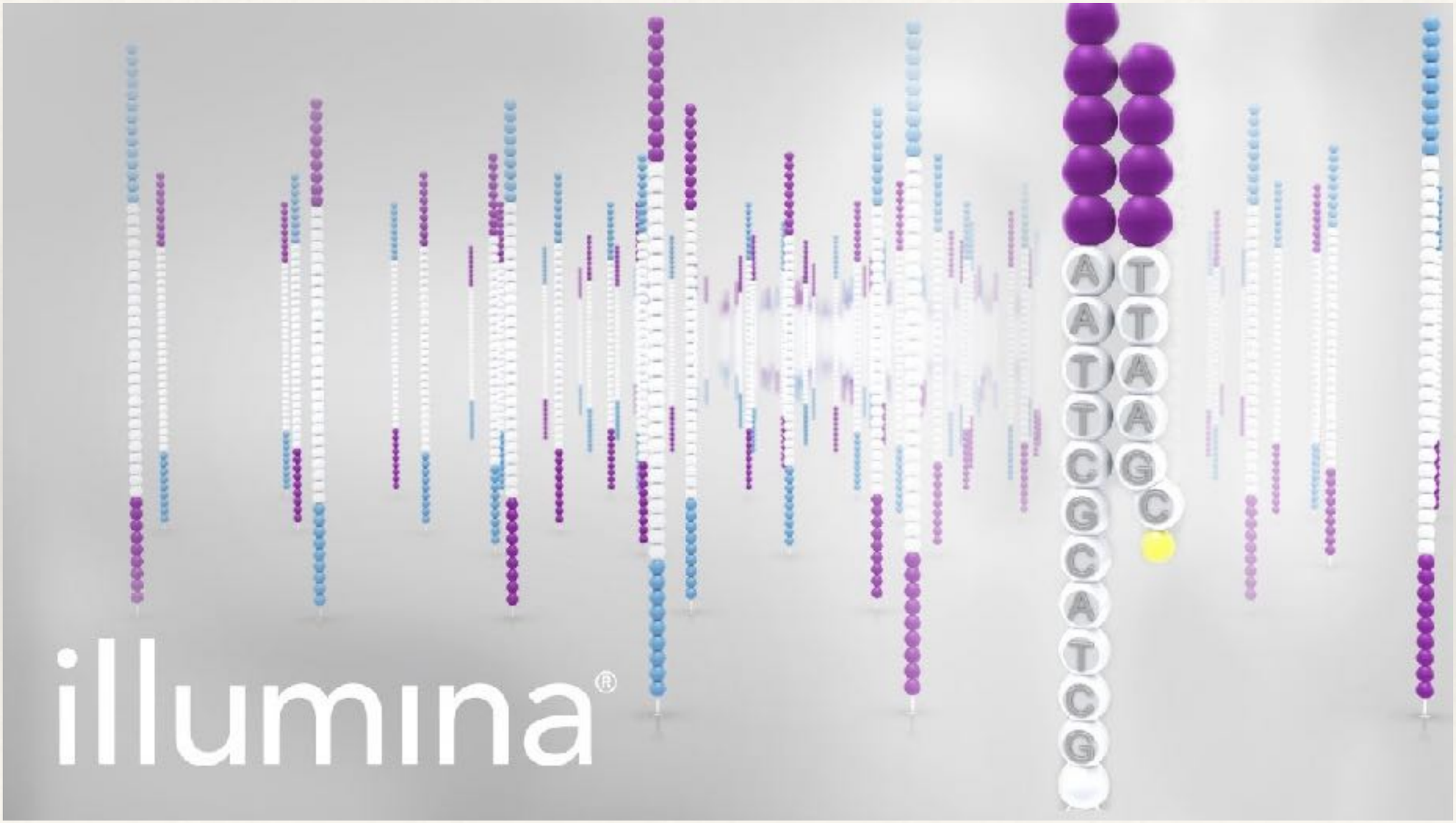
Illumina sequencers

	iSeq	MiniSeq	MiSeq	NextSeq	HiSeq 2500	HiSeq 3/4000	HiSeq X	NovaSeq
	Benchtop sequencers			Production-scale sequencers				
Data output:	144 Mb - 500 Gb			9 Gb - 2400 Gb				
Read length:	25 - 300 nt			50 - 250 nt				
Read type:	Single/Paired end			Single/Paired end				

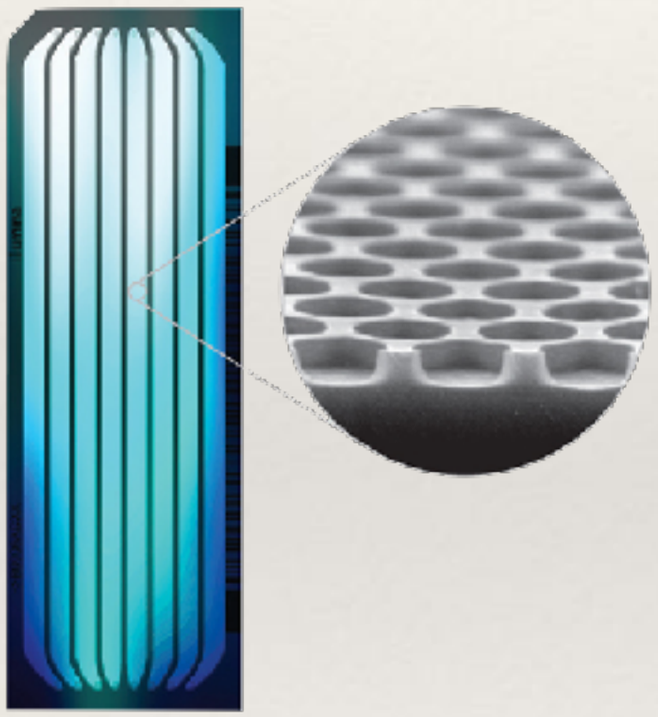
Illumina sequencers

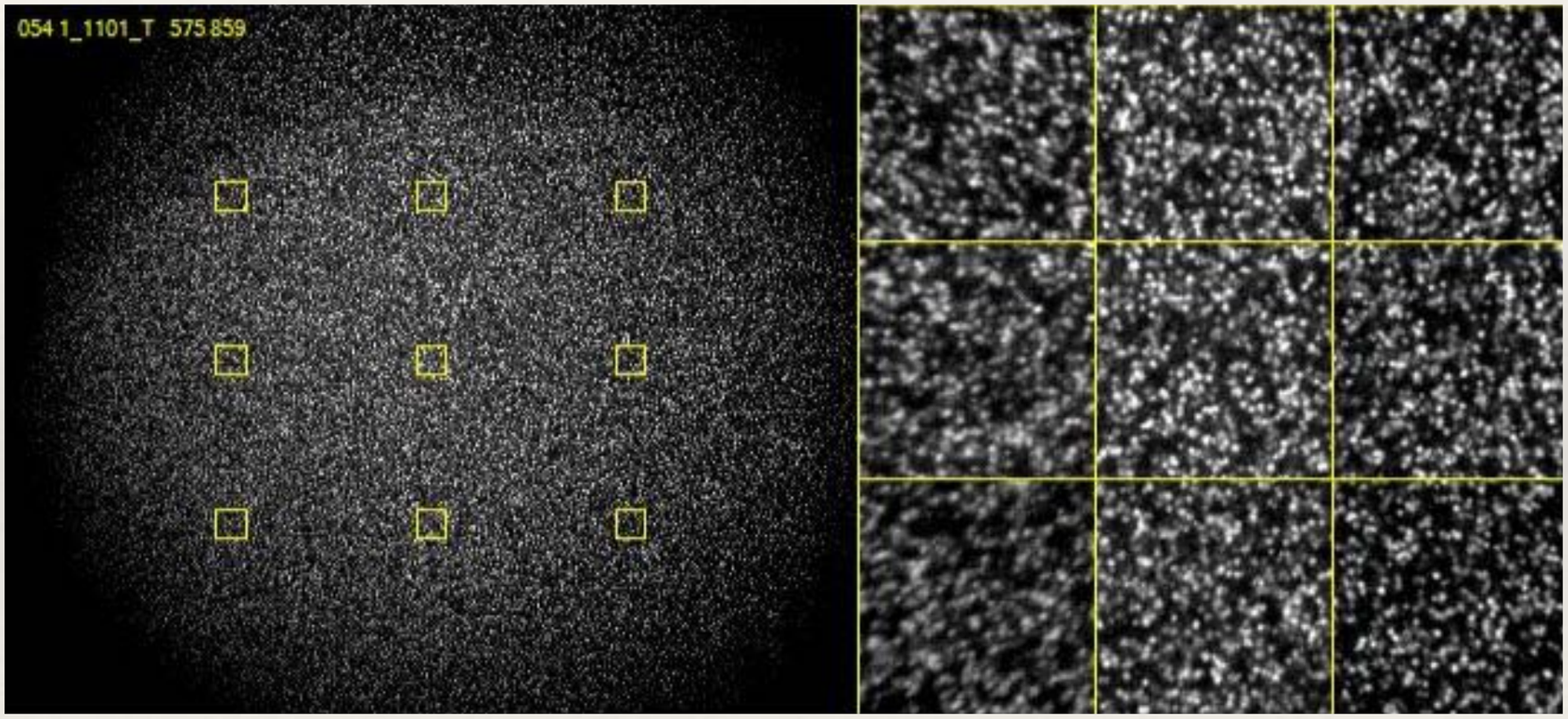
- Second generation sequencing technique
- Sequencing-by-synthesis aka SBS
  - https://www.youtube.com/watch?v=fCd6B5HRaZ8
- Mass parallelisation and real high throughput

Illumina sequencers



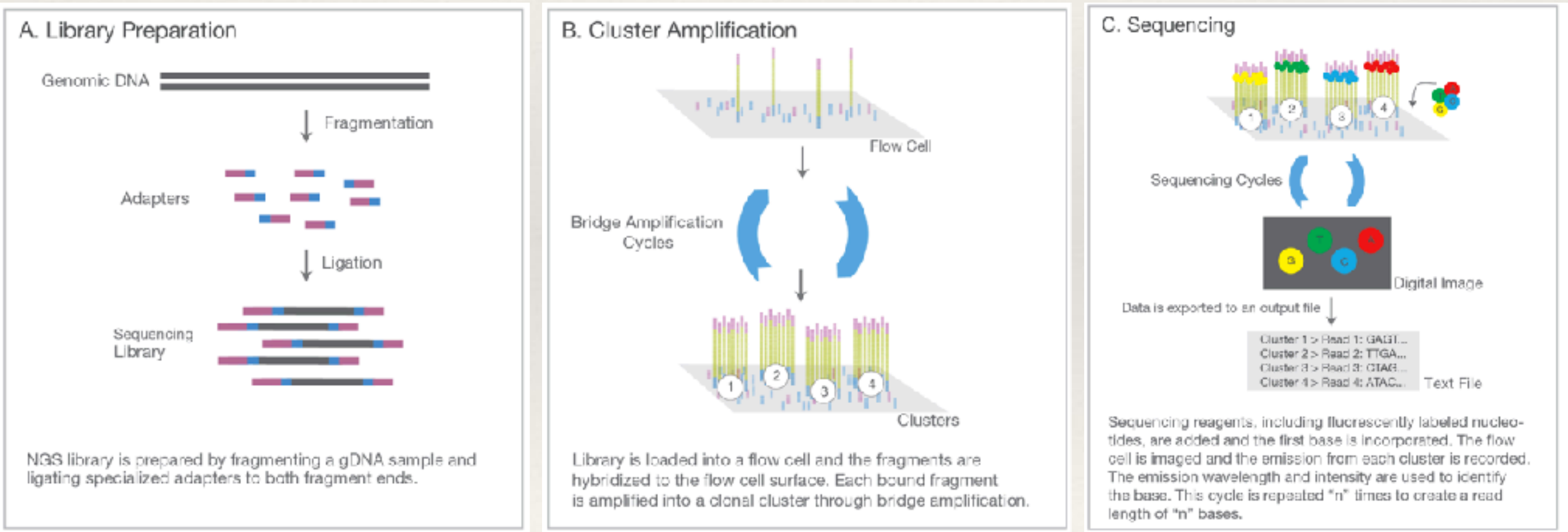
Sequencing





Library prep and sequencing

Fragment (DNA) sequenced: up to 800 bp



https://www.illumina.com/documents/products/illumina_sequencing_introduction.pdf

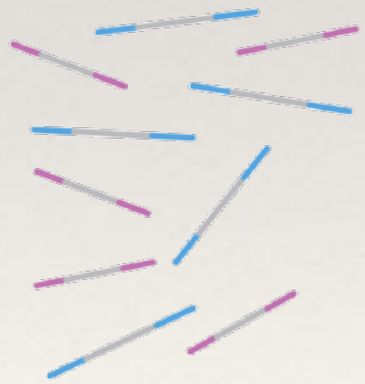
Library prep and sequencing

Fragment (DNA) sequenced: up to 800 bp

Add adapters during library preparation

Multiplexing: single/dual index

Multiplexing: pooling



Read type: Single end

Read type: Paired end

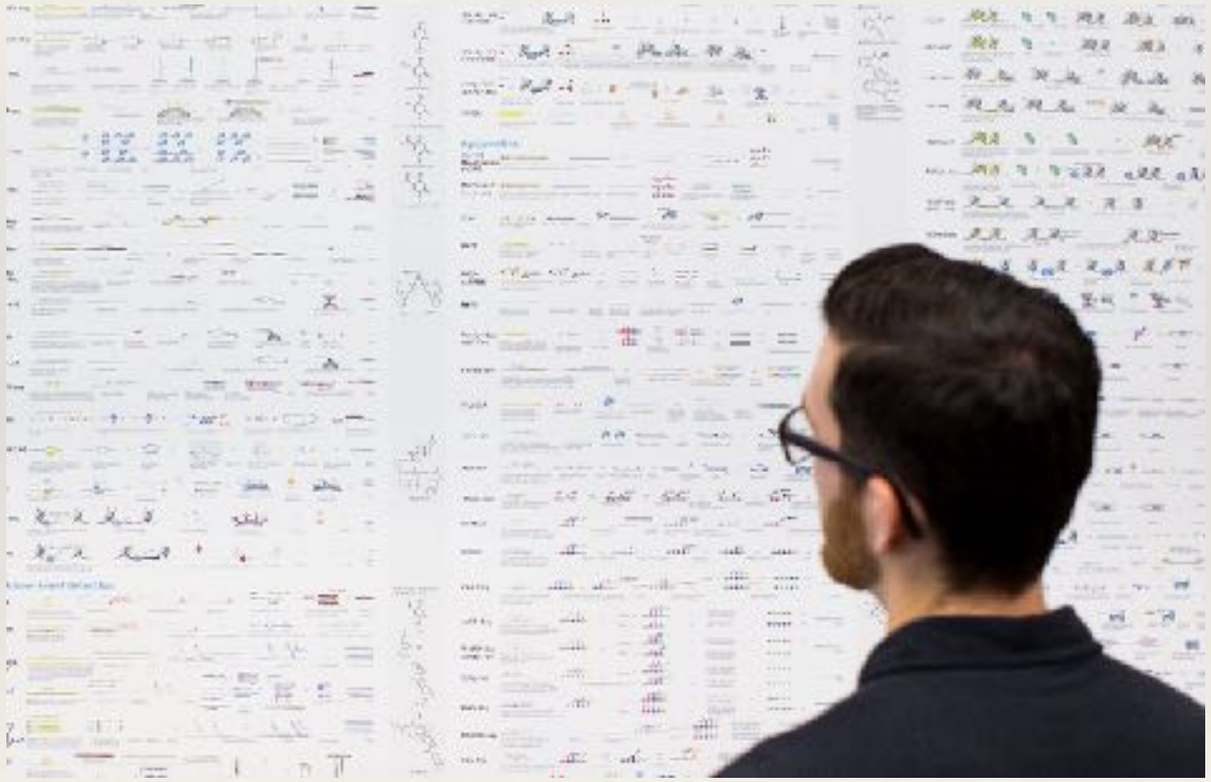
https://www.illumina.com/documents/products/illumina_sequencing_introduction.pdf

Library prep

https://www.illumina.com/techniques/sequencing/ngs-library-prep/library-prep-methods.html

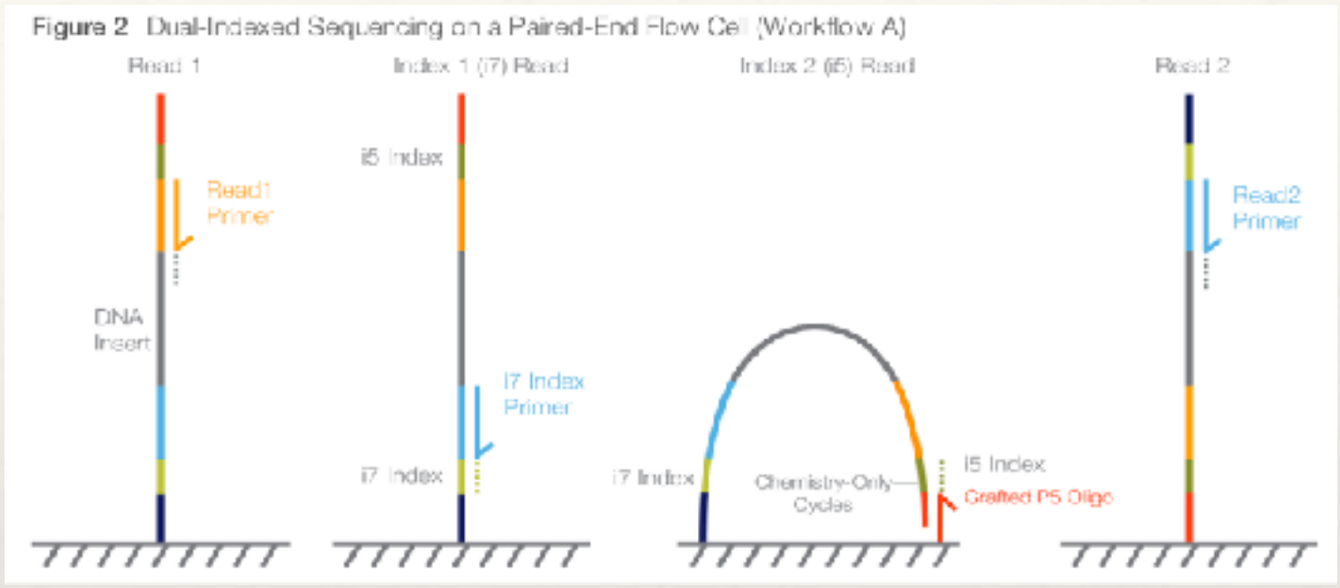
Try the “Sequencing Methods selector”

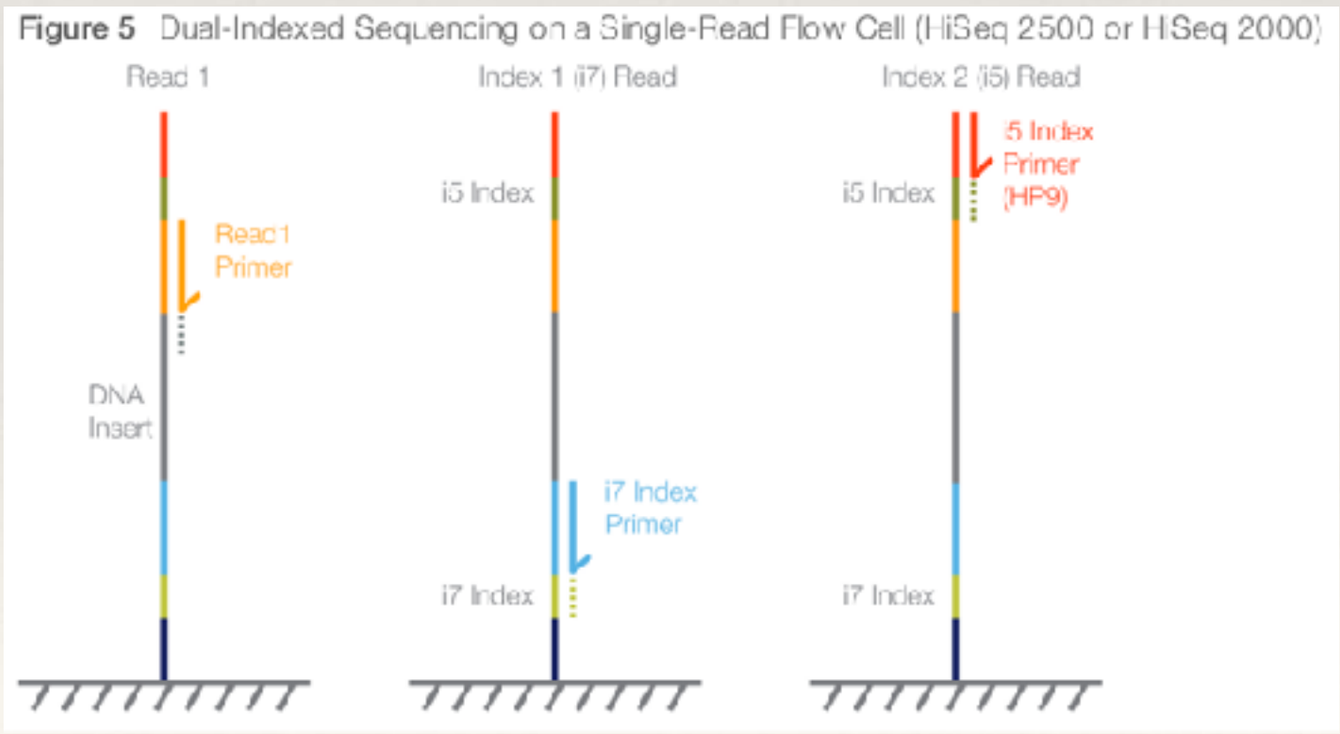
Check out 3 posters: DNA, RNA and single cell



Single/dual indexed samples

MiSeq
HiSeq 2500
NovaSeq

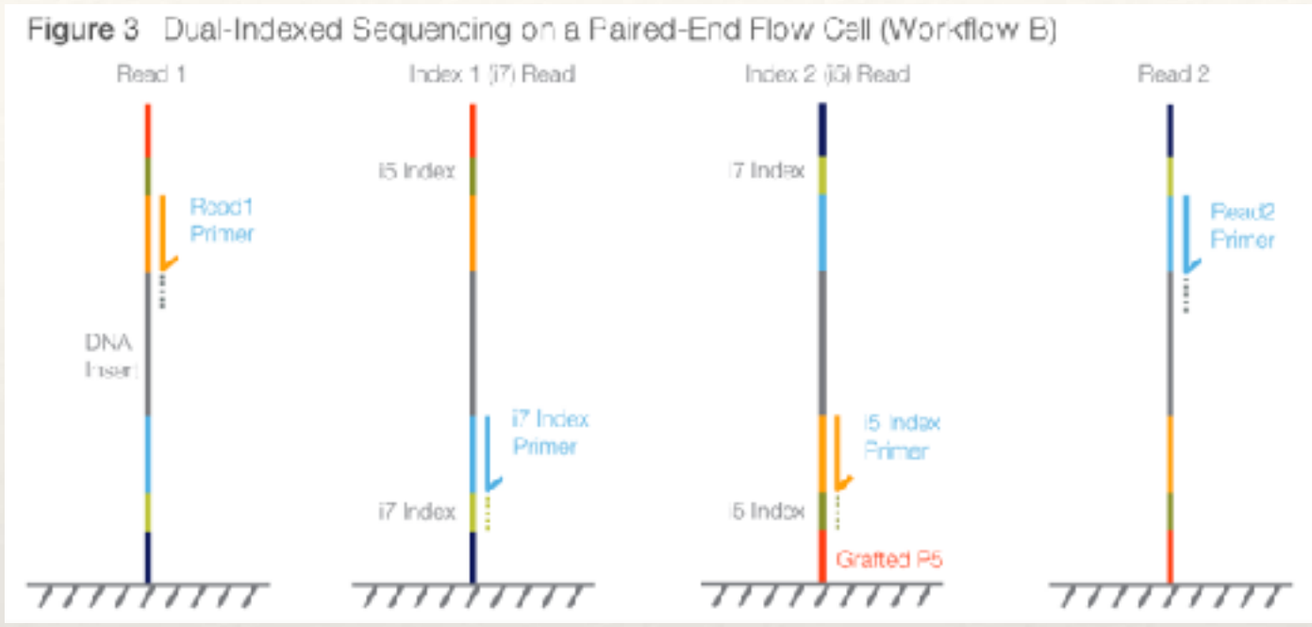


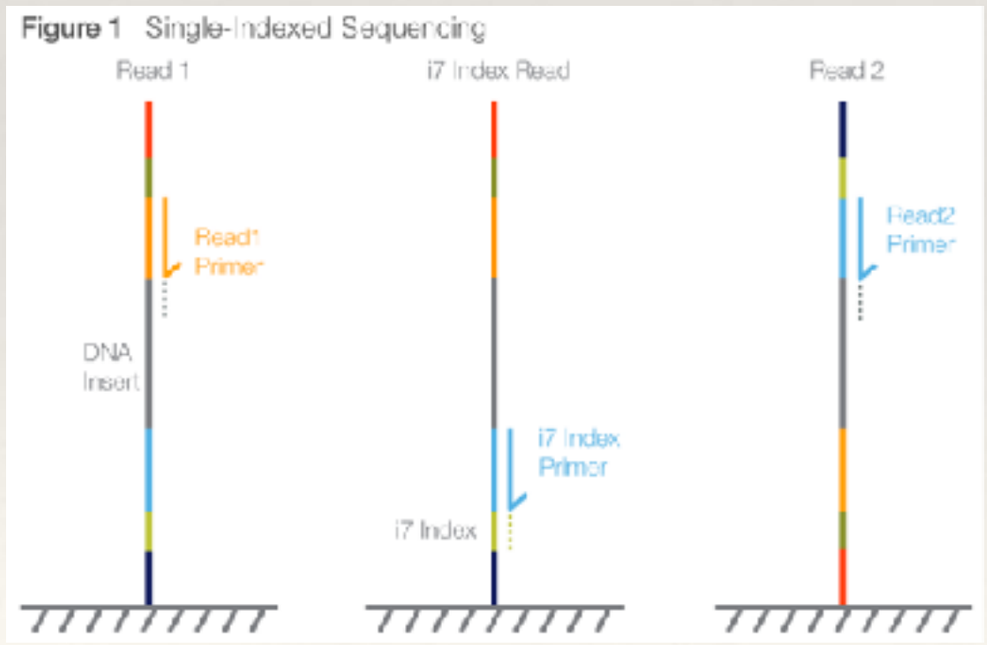


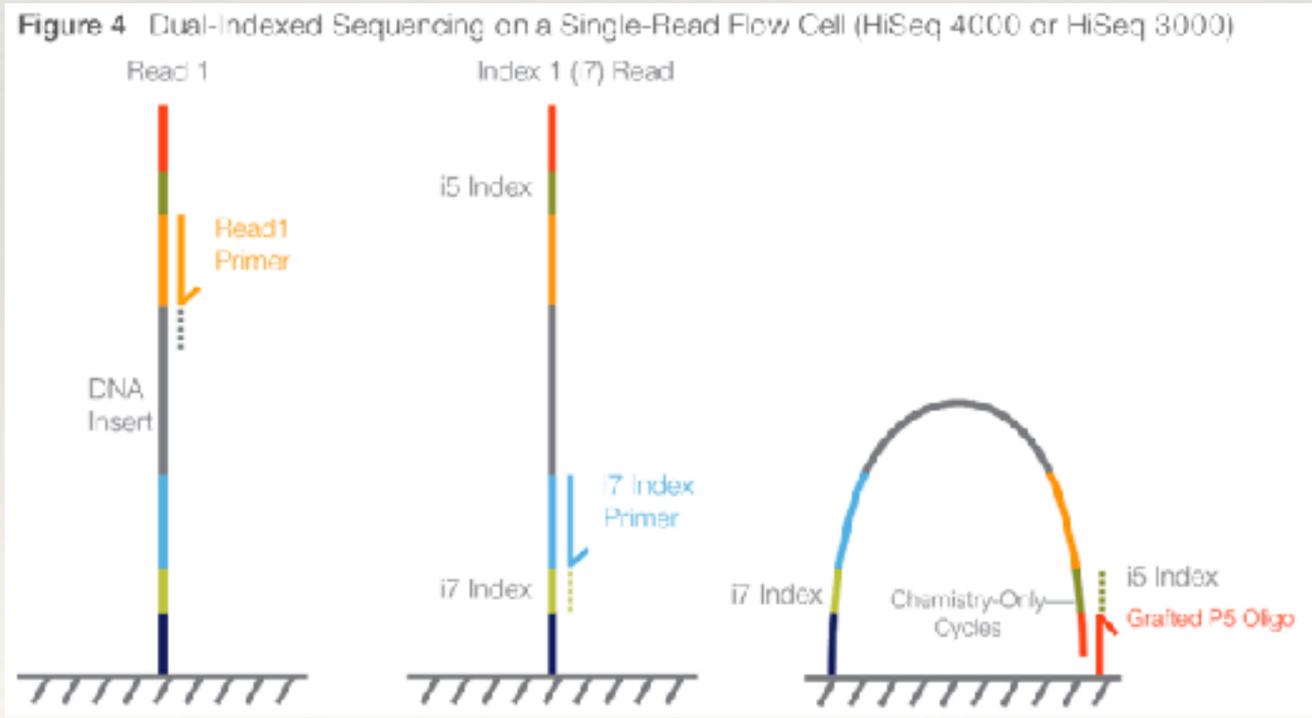
https://support.illumina.com/content/dam/illumina-support/documents/documentation/system_documentation/miseq/indexed-sequencing-overview-guide-15057455-04.pdf

Single/dual indexed samples

iSeq
MiniSeq
NextSeq
HiSeq 3/4000
HiSeq X

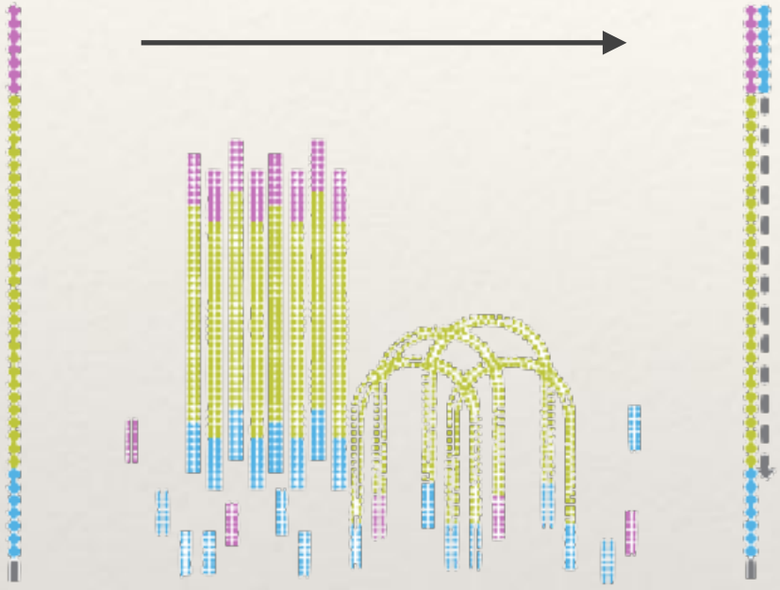






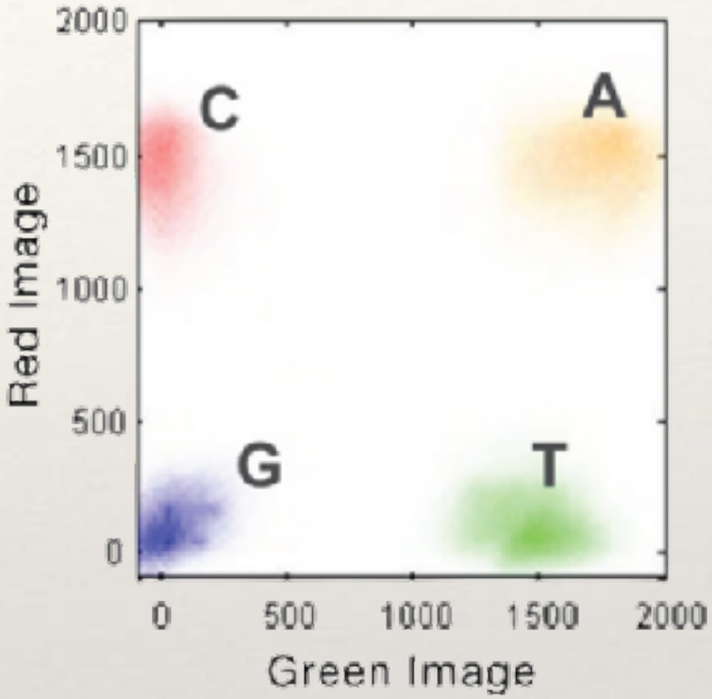
https://support.illumina.com/content/dam/illumina-support/documents/documentation/system_documentation/miseq/indexed-sequencing-overview-guide-15057455-04.pdf

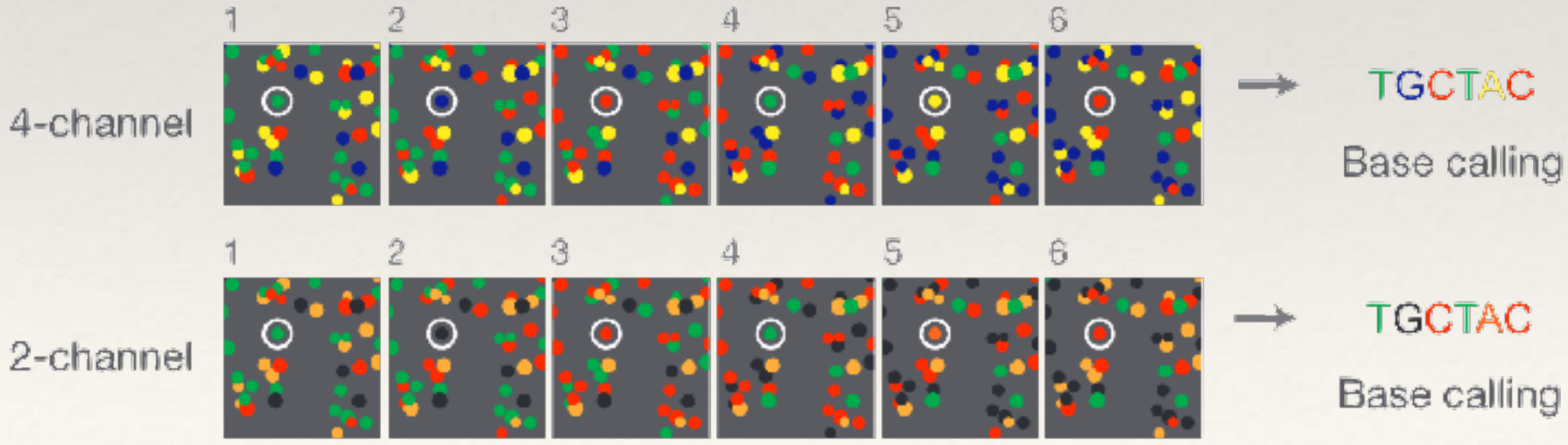
Four vs two colour chemistry



Old machines use 4 colours
New machines use 2 colours

Sequencing is almost
twice as fast





Illumina sequencers

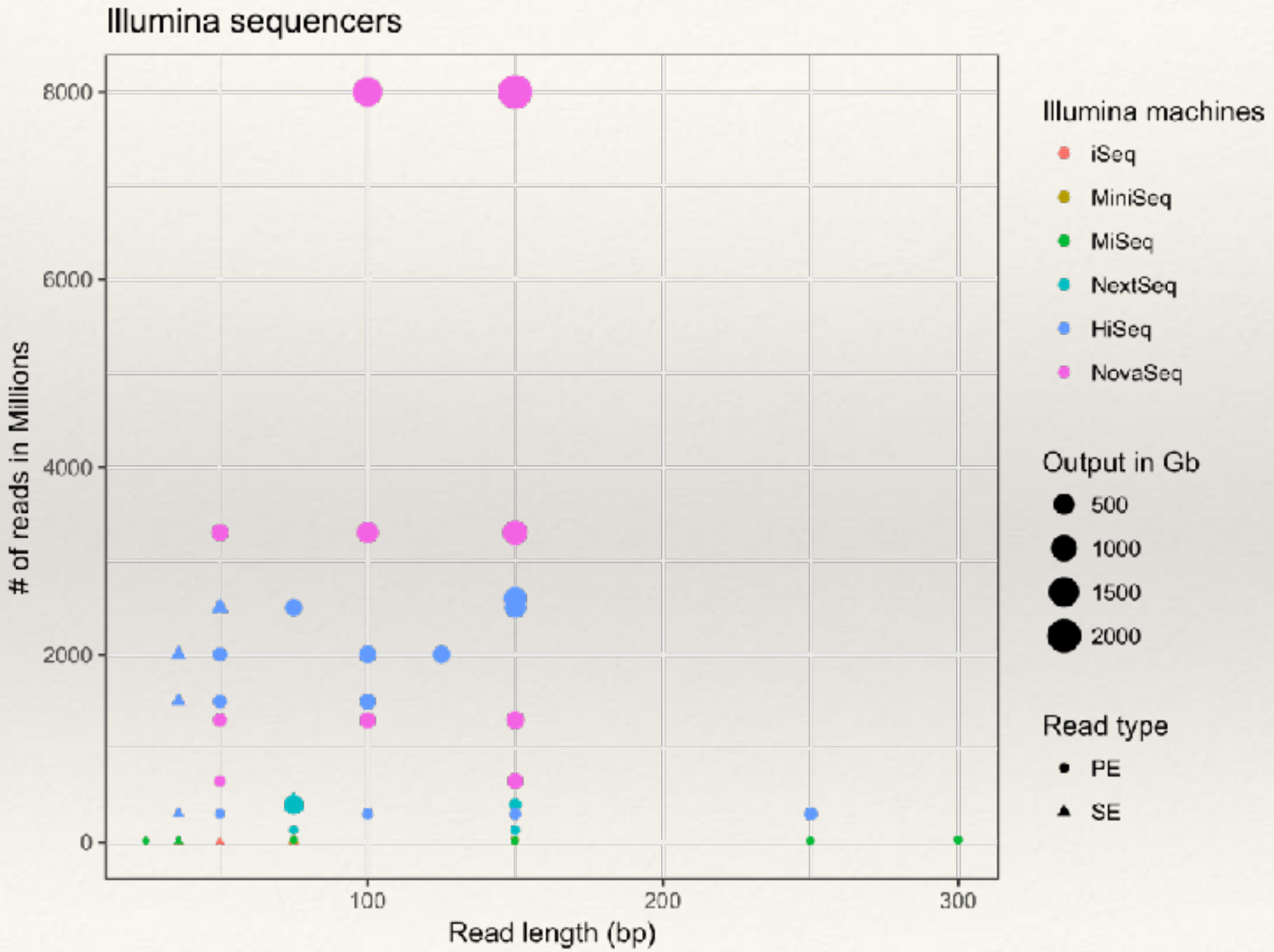
iSeq MiniSeq MiSeq NextSeq HiSeq HiSeq NovaSeq

3 / 4000 2500 X

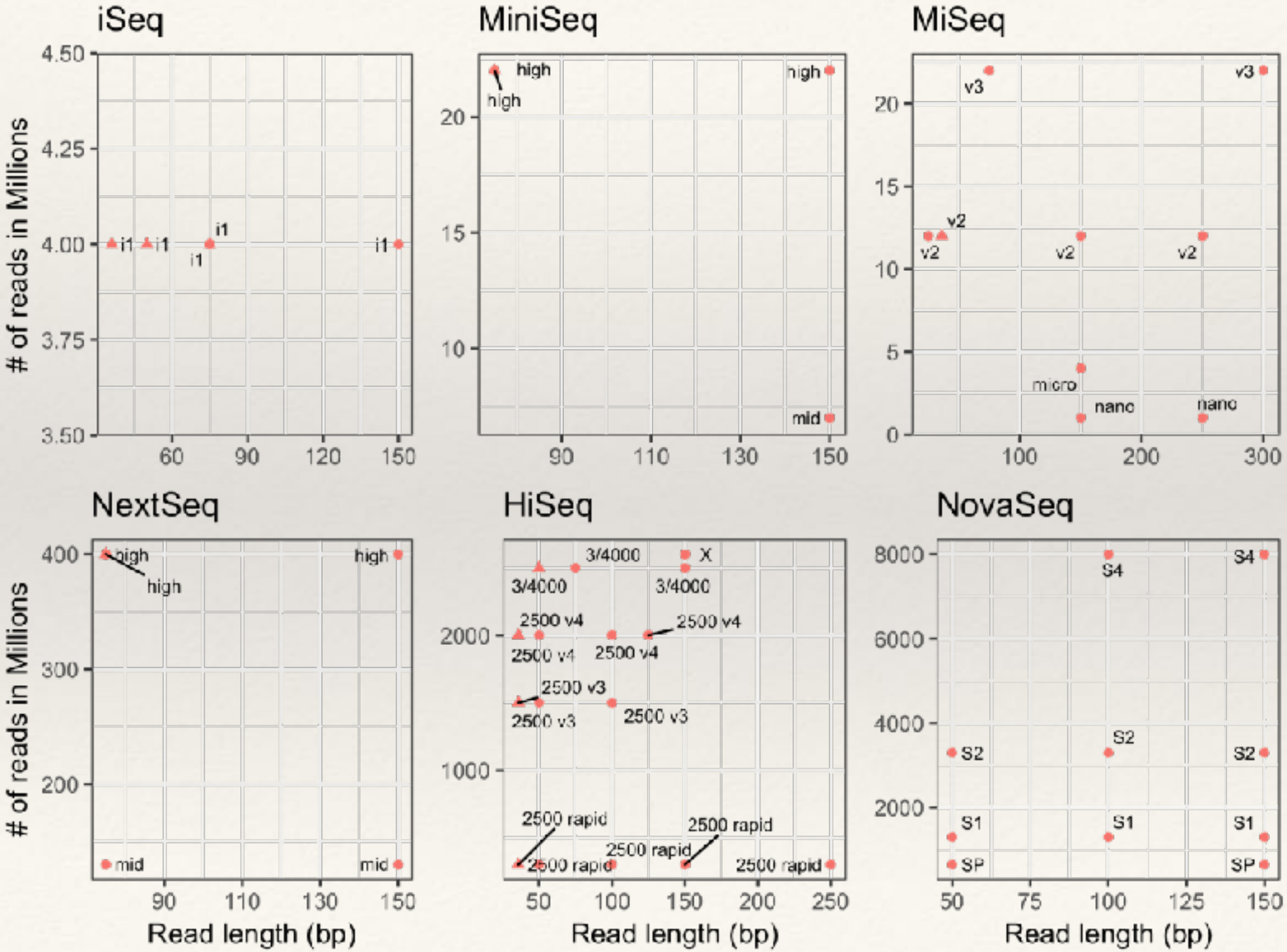
Benchtop sequencers | Production-scale sequencers

Read length: Single end	Read length: Paired end			Data output:
36	25	100	250	144 Mb
50	50	125	300	-
75	75	150		2400 Gb

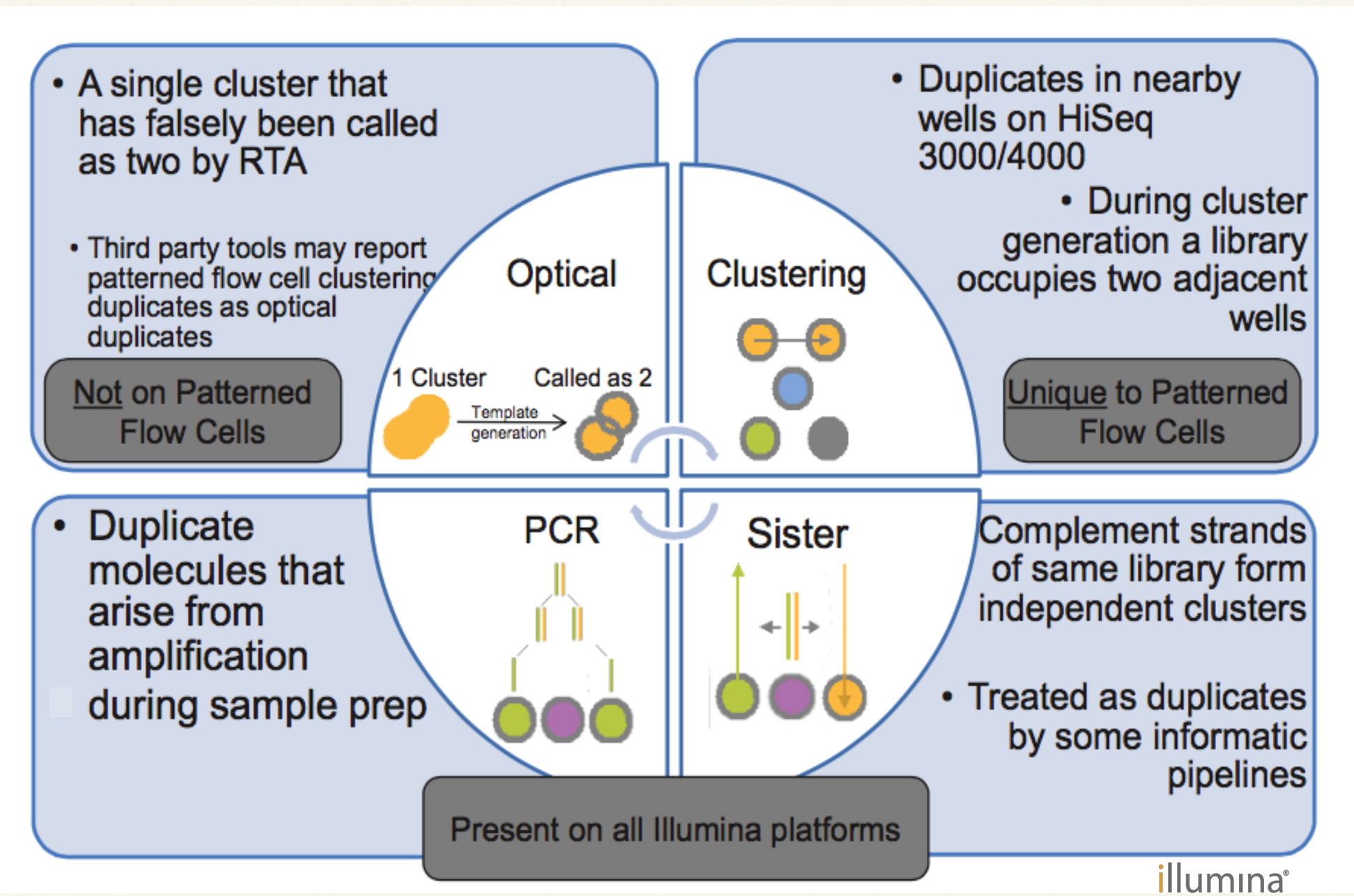
Data output


Illumina sequencers
8000
6000
4000
2000
0
# of reads in Millions
100
200
300
Read length (bp)
Illumina machines
iSeq
MiniSeq
MiSeq
NextSeq
HiSeq
NovaSeq
Output in Gb
500
1000
1500
2000
Read type
PE
SE

Data output

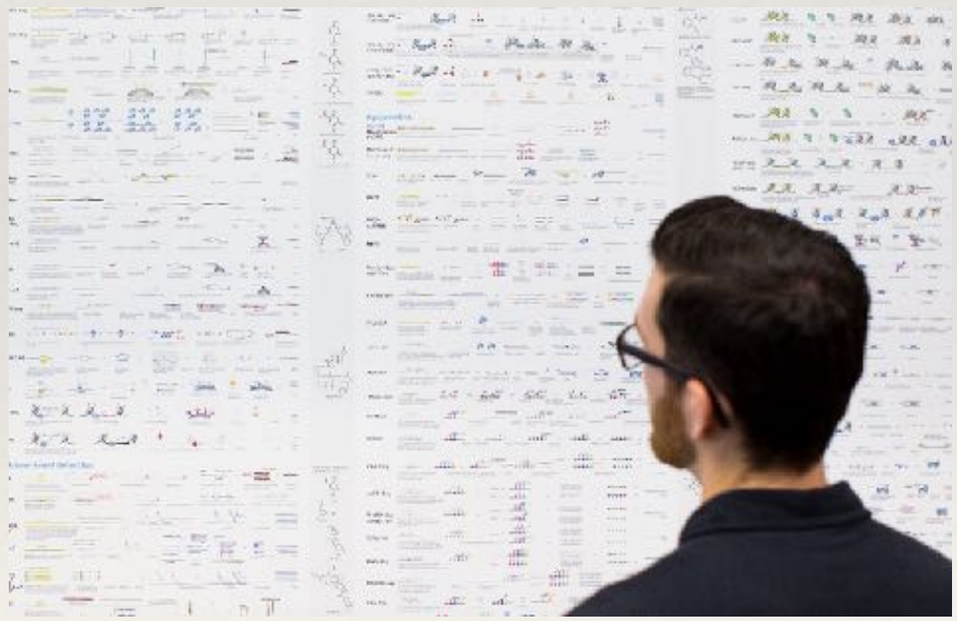

iSeq
MiniSeq
MiSeq
NextSeq
HiSeq
NovaSeq
# of reads in Millions
Read length (bp)
i1
high
mid
v3
v2
micro
nano
3/4000
X
2500 v4
2500 v3
2500 rapid
S4
S2
S1
SP

Known issues



What can you sequence using Illumina

- DNA studies
  - Whole genome sequencing - short reads are a pitfall
  - Genome re-sequencing
  - Exomes and target re-sequencing…
  - ChiP seq and more…
- RNA studies
- modification studies
  - Methylation and more…



https://www.illumina.com/techniques/sequencing/ngs-library-prep/library-prep-methods.html