

IN-BIOS[9,5]000 2022

illumina Technology

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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 series
NovaSeq
NovaSeq X

BGI **SEQ**

Roche 454
SOLiD
Ion Torrent



PACBIO[®]

RS II
Sequel



MinION
Flongle
GridION

PromethION P2/Solo
PromethION 24/48

Illumina sequencers



Benchtop sequencers

Production-scale sequencers

Data output: 144 Mb - 500 Gb
Read length: 25 - 300 nt
Read type: Single / Paired end

9 Gb - 2400 Gb
50 - 250 nt
Single / Paired end

Illumina sequencers - 2023



Benchtop sequencers

Production-scale sequencers

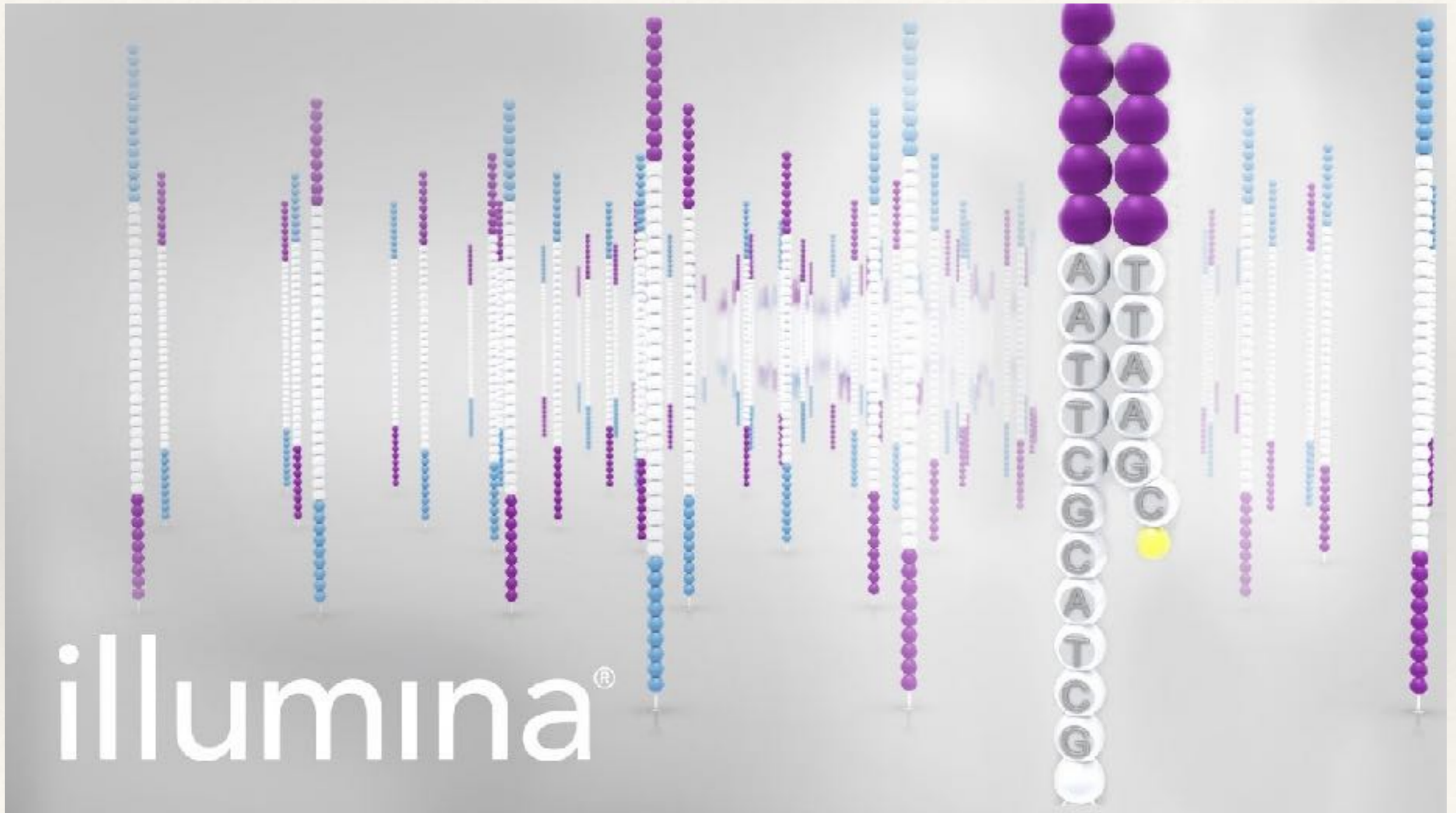
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9 Gb - 16000 Gb
50 - 250 nt
Single / Paired end

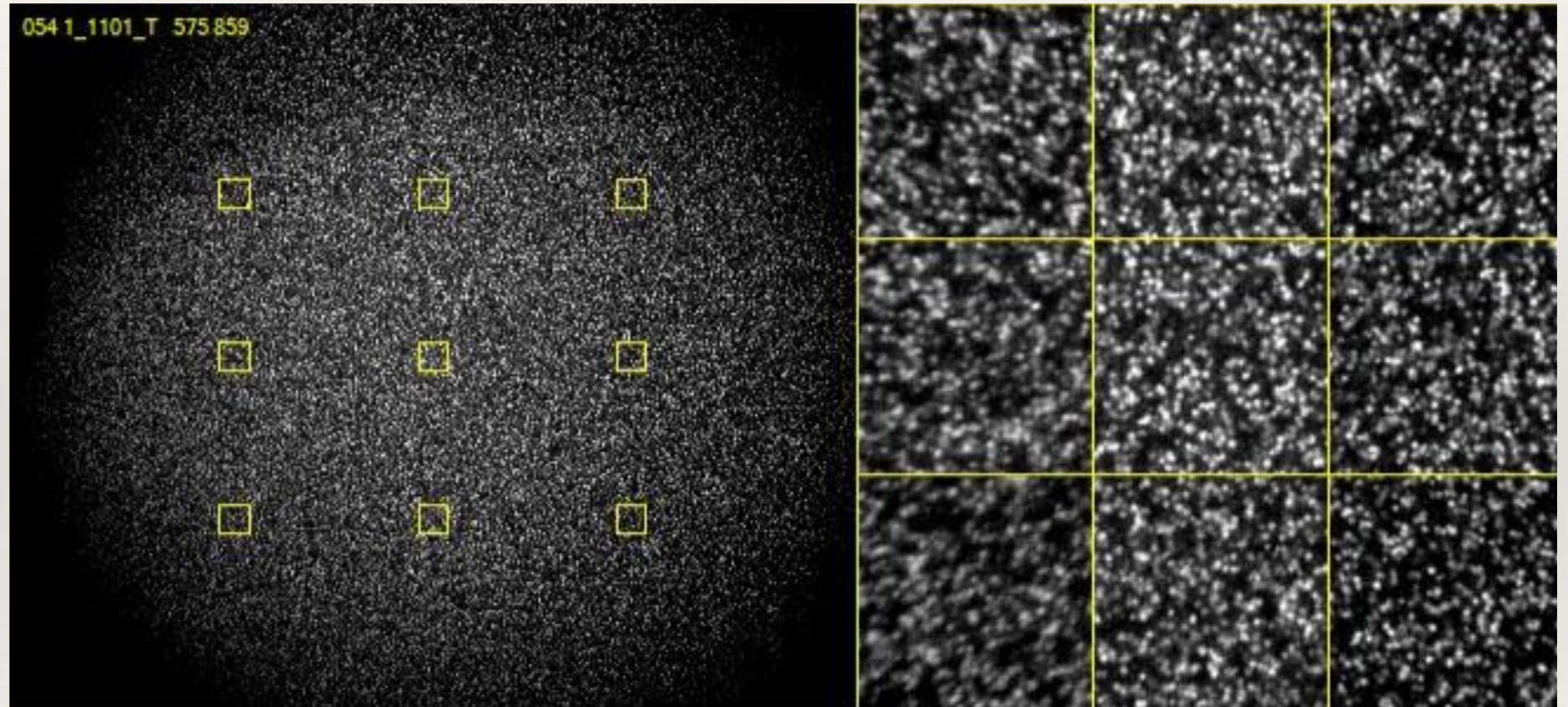
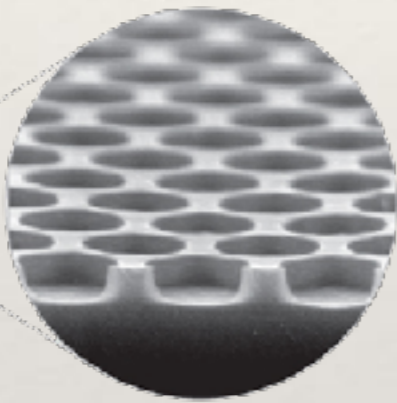
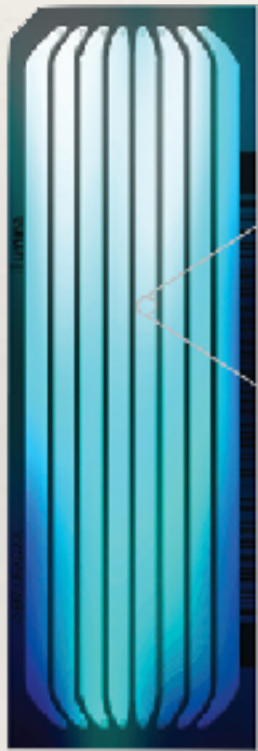
Illumina sequencers

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

Illumina sequencers



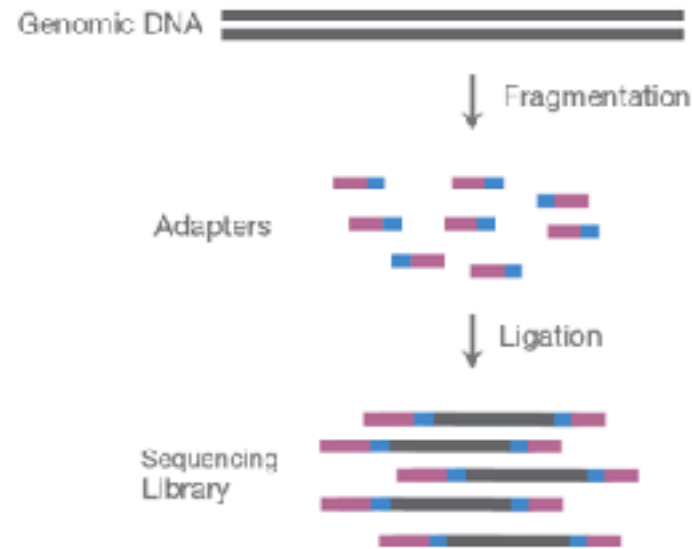
Sequencing



Library prep and sequencing

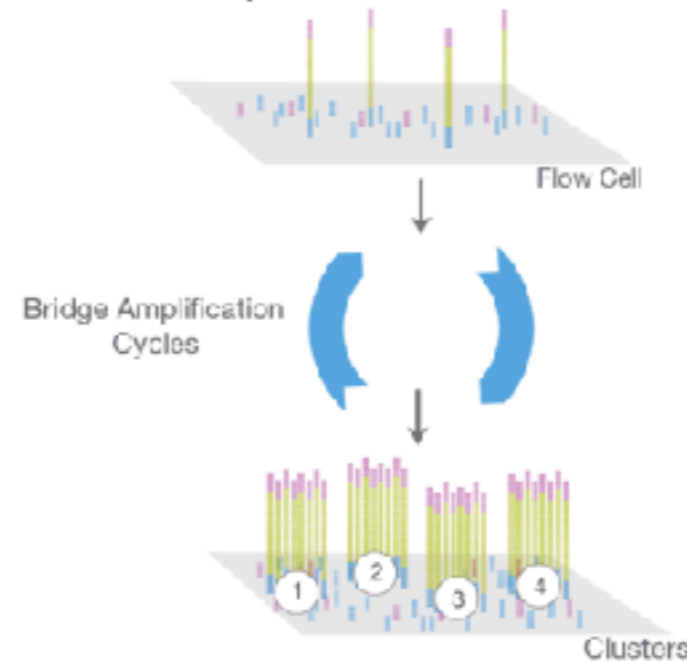
Fragment (DNA) sequenced: up to 800 bp

A. Library Preparation



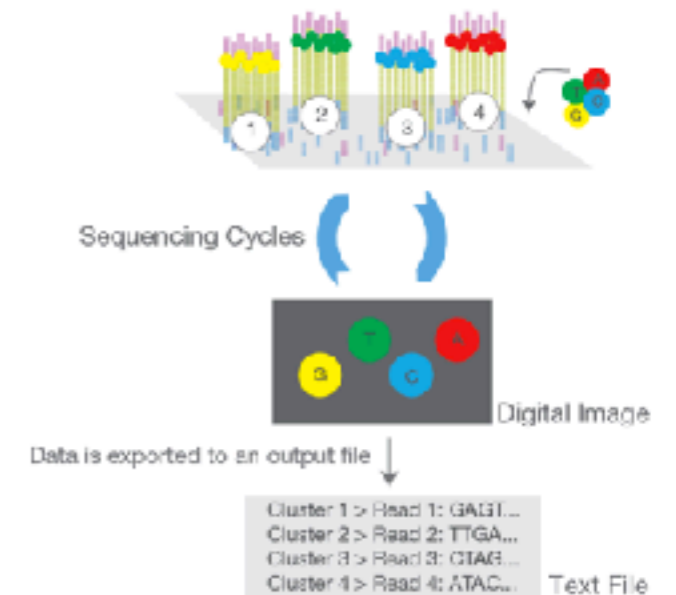
NGS library is prepared by fragmenting a gDNA sample and ligating specialized adapters to both fragment ends.

B. Cluster Amplification



Library is loaded into a flow cell and the fragments are hybridized to the flow cell surface. Each bound fragment is amplified into a clonal cluster through bridge amplification.

C. Sequencing



Sequencing reagents, including fluorescently labeled nucleotides, are added and the first base is incorporated. The flow cell is imaged and the emission from each cluster is recorded. The emission wavelength and intensity are used to identify the base. This cycle is repeated "n" times to create a read length of "n" bases.

Library prep and sequencing

Fragment (DNA) sequenced: up to 800 bp



Add adapters during library preparation

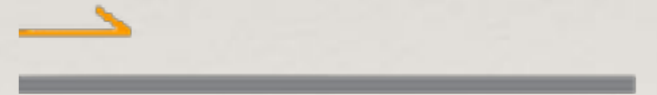
Multiplexing: single / dual index



Multiplexing: pooling



Read type: Single end

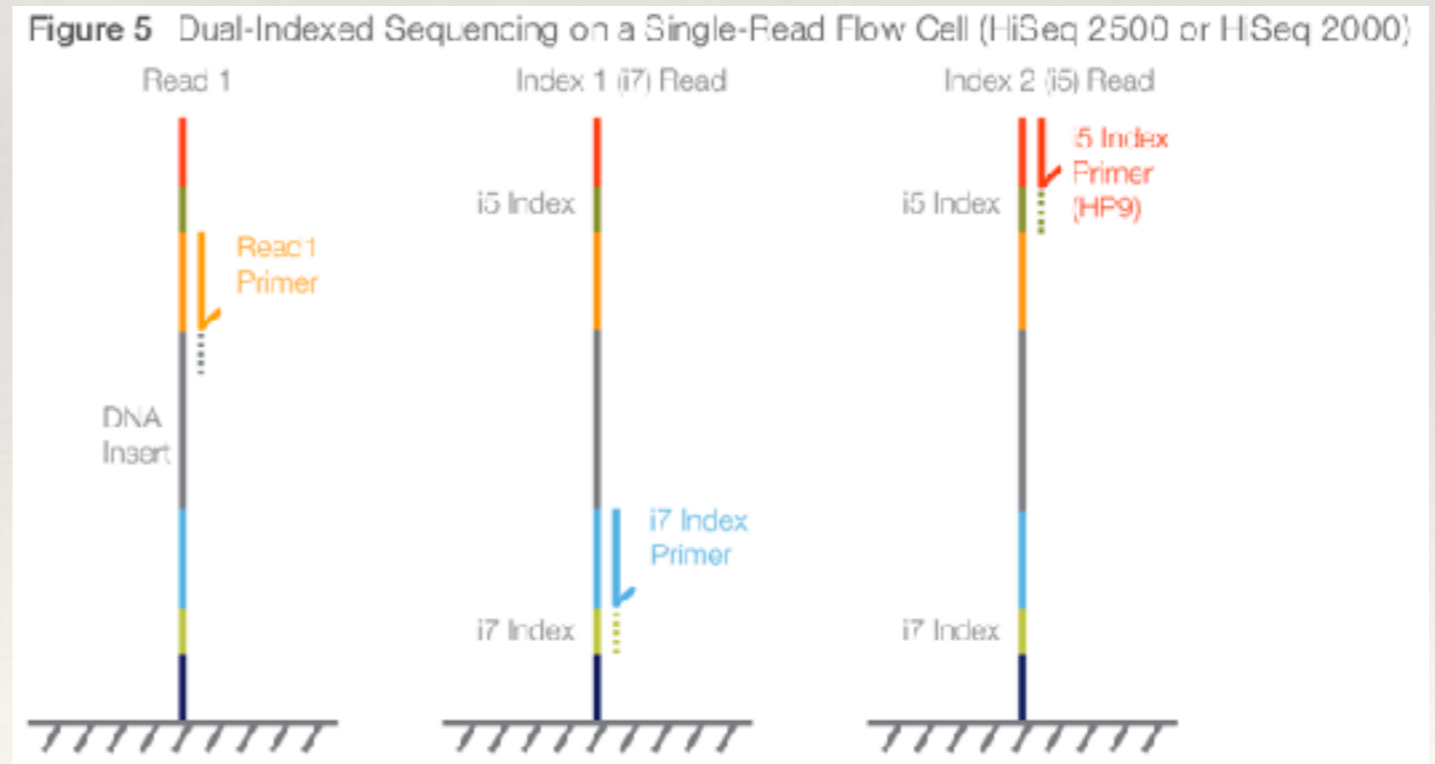
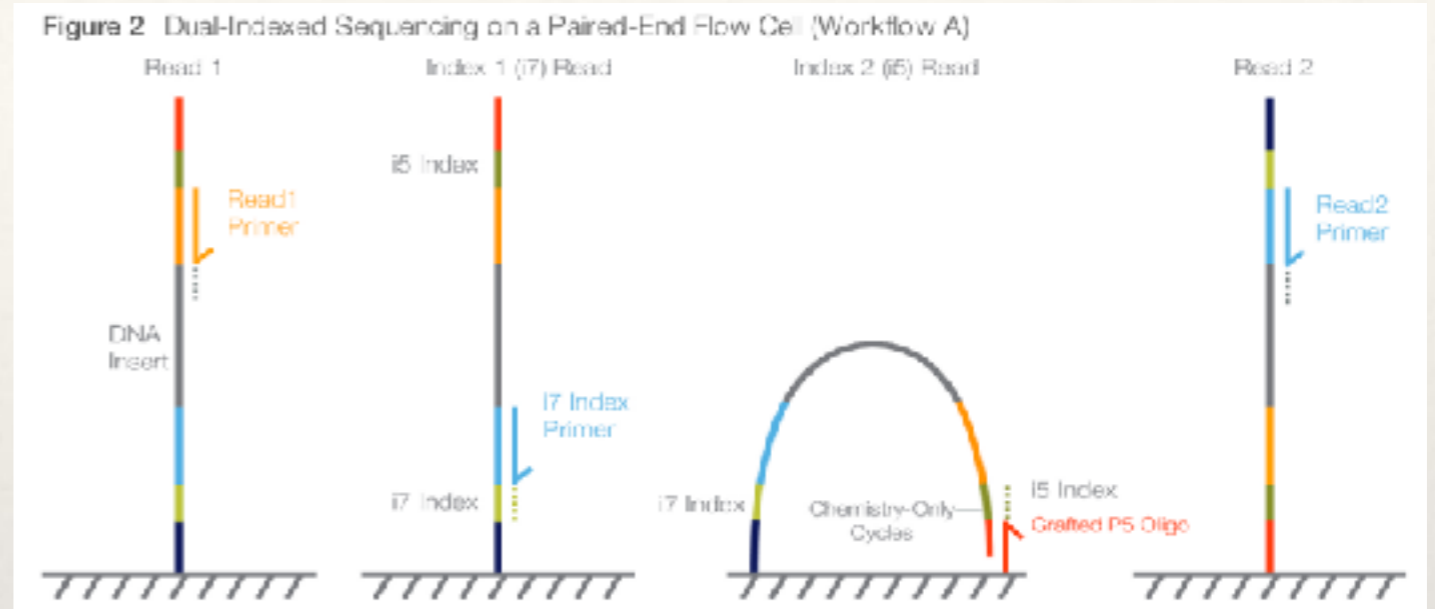
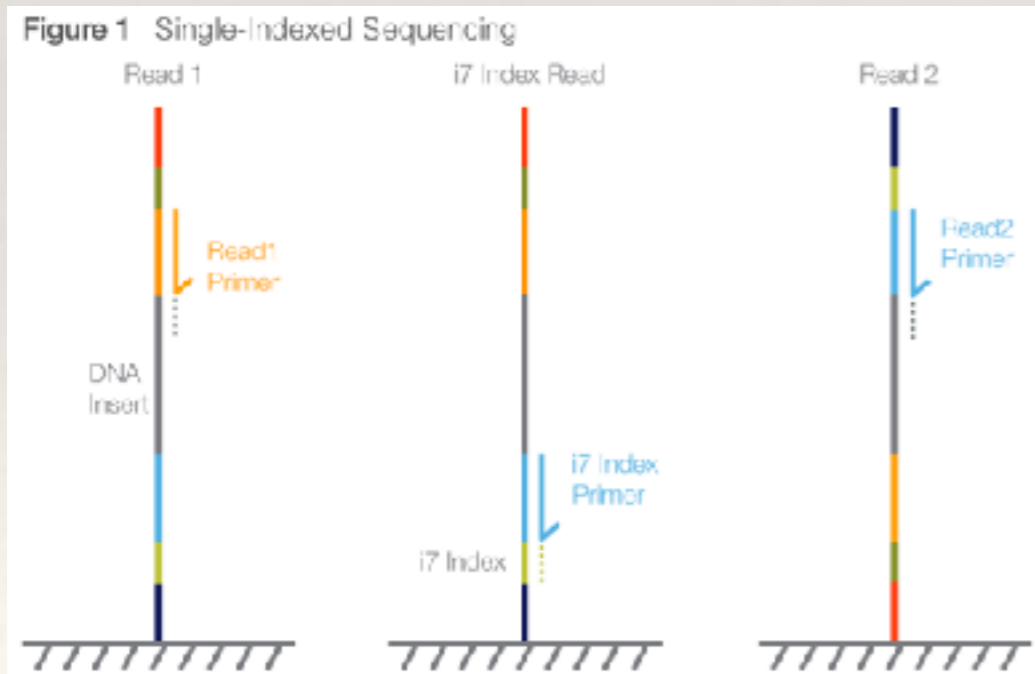


Read type: Paired end



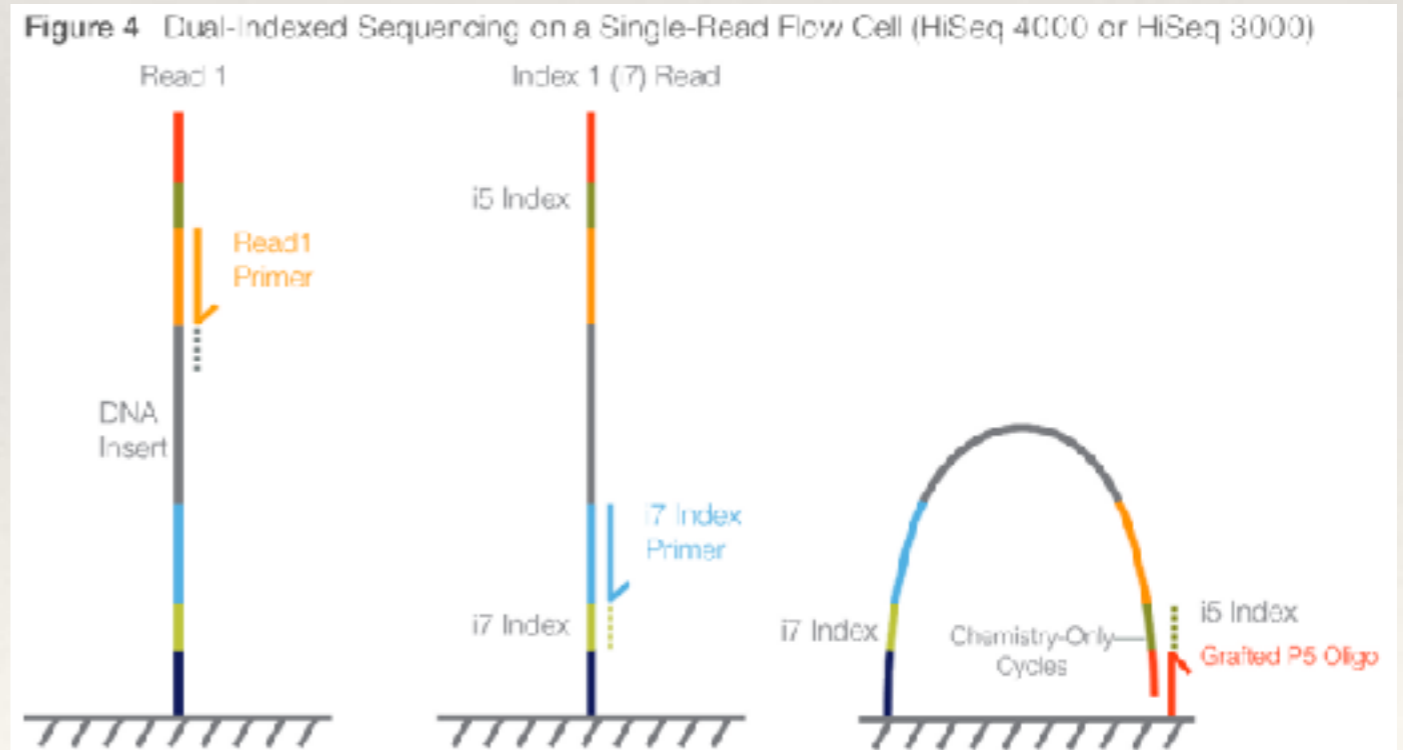
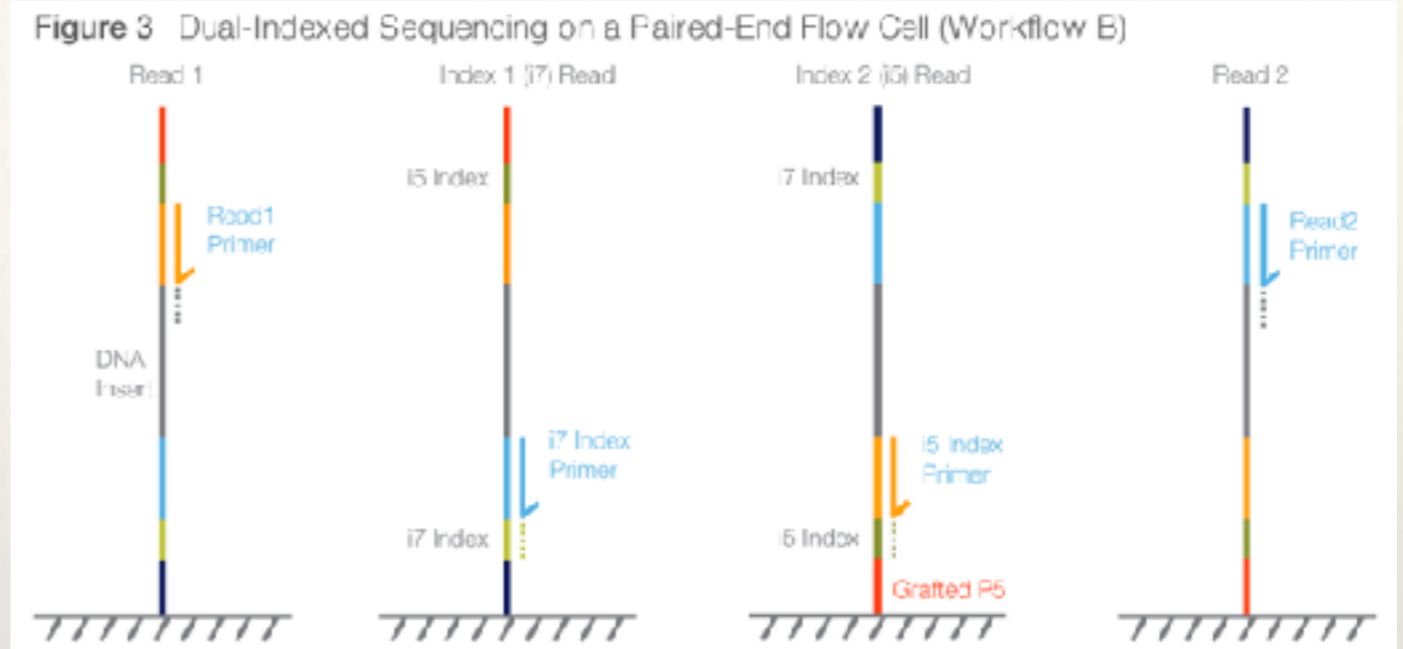
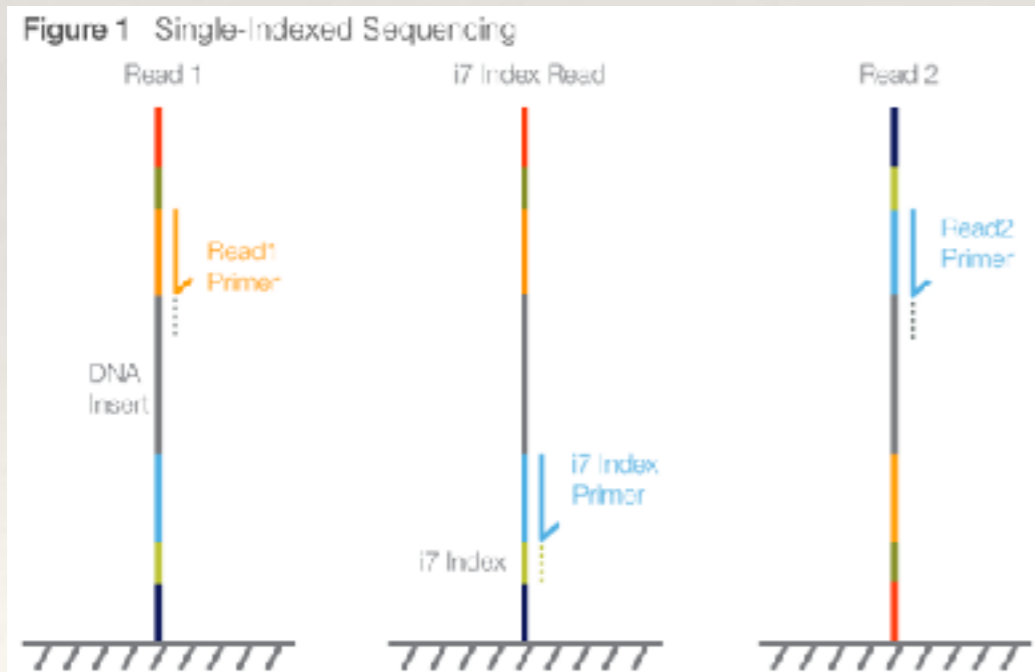
Single/dual indexed samples

MiSeq
HiSeq
NovaSeq

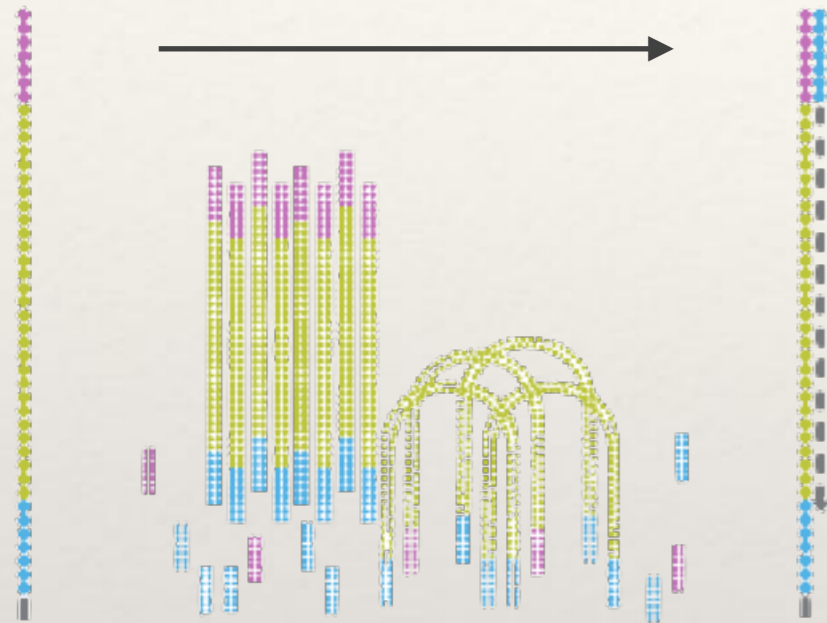


Single/dual indexed samples

iSeq
MiniSeq
NextSeq

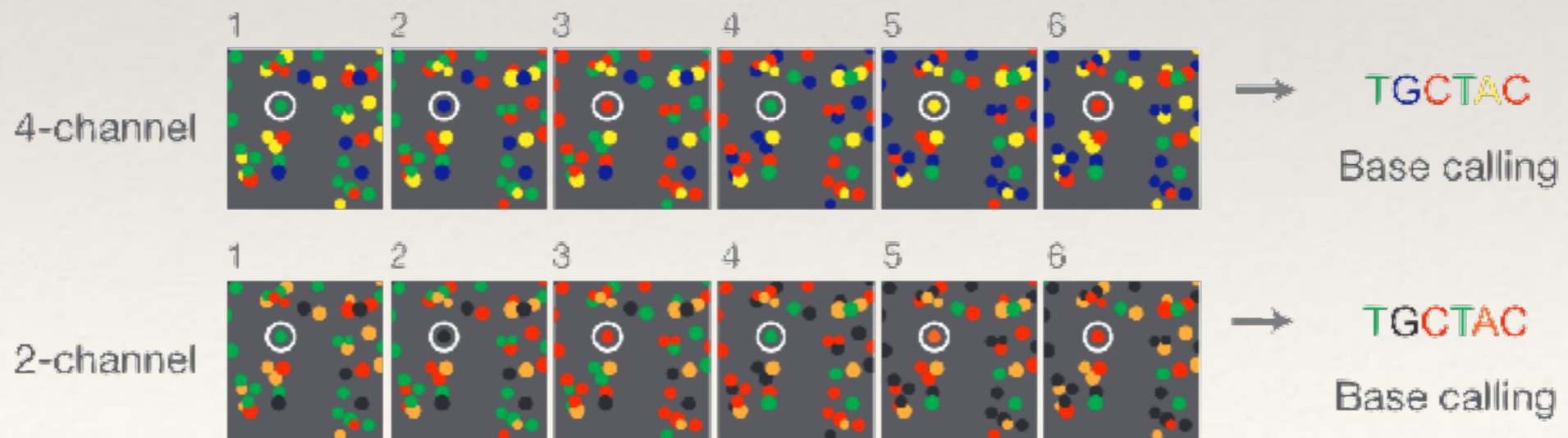
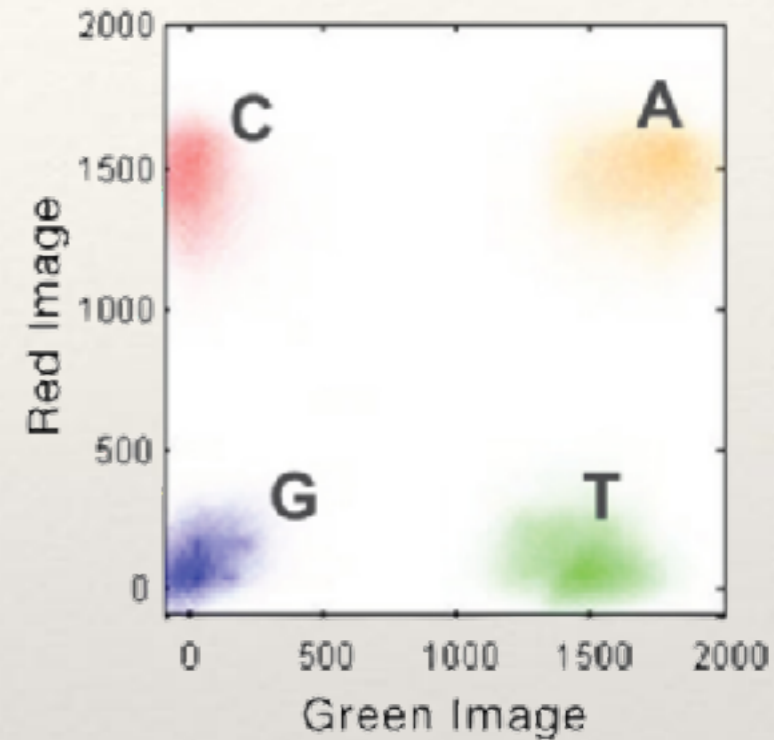


Four vs two colour chemistry



Old machines use 4 colours
New machines use 2 colours

Sequencing is almost
twice as fast



Illumina sequencers



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

-

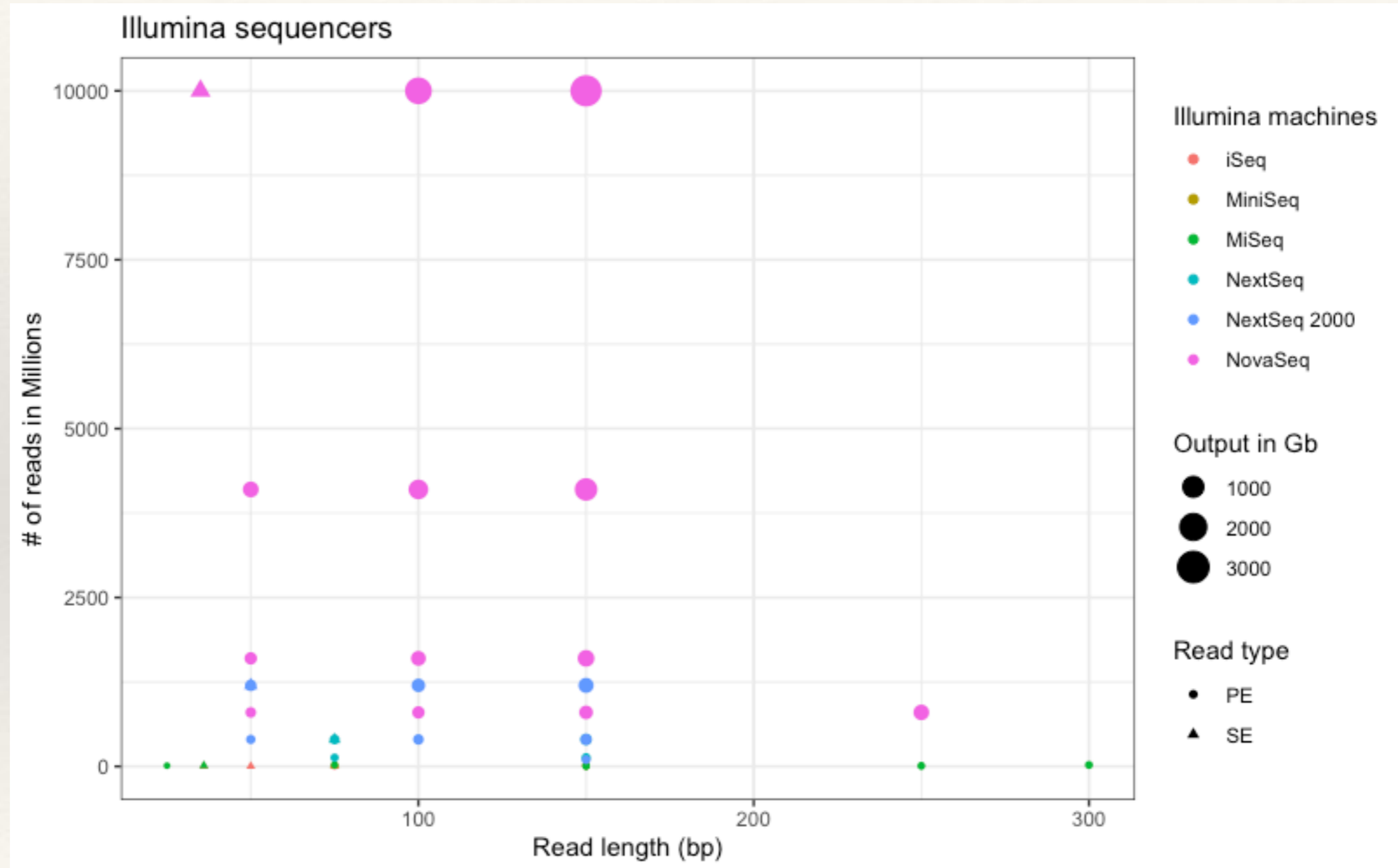
75

75

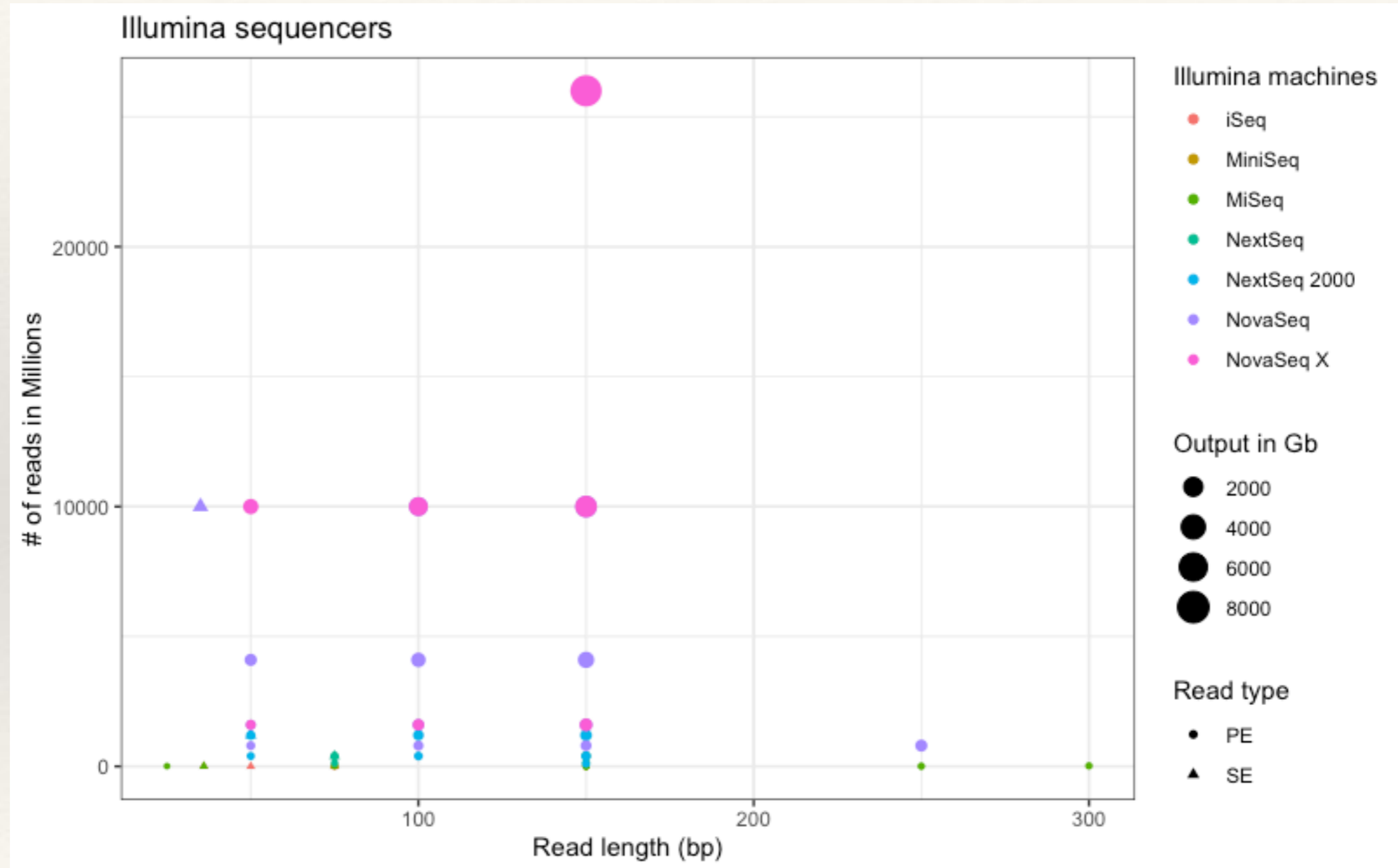
150

16000 Gb

Data output

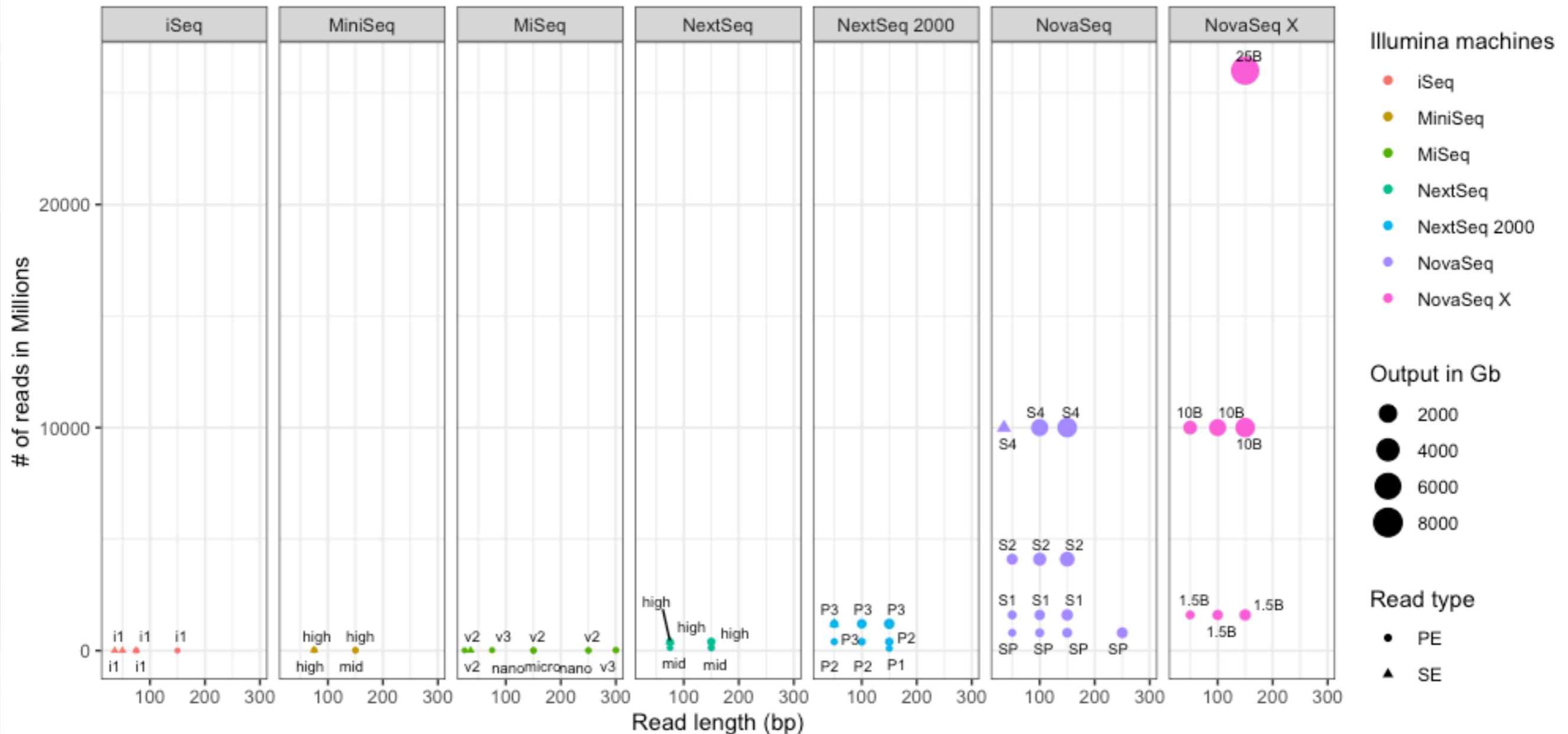


Data output - 2023

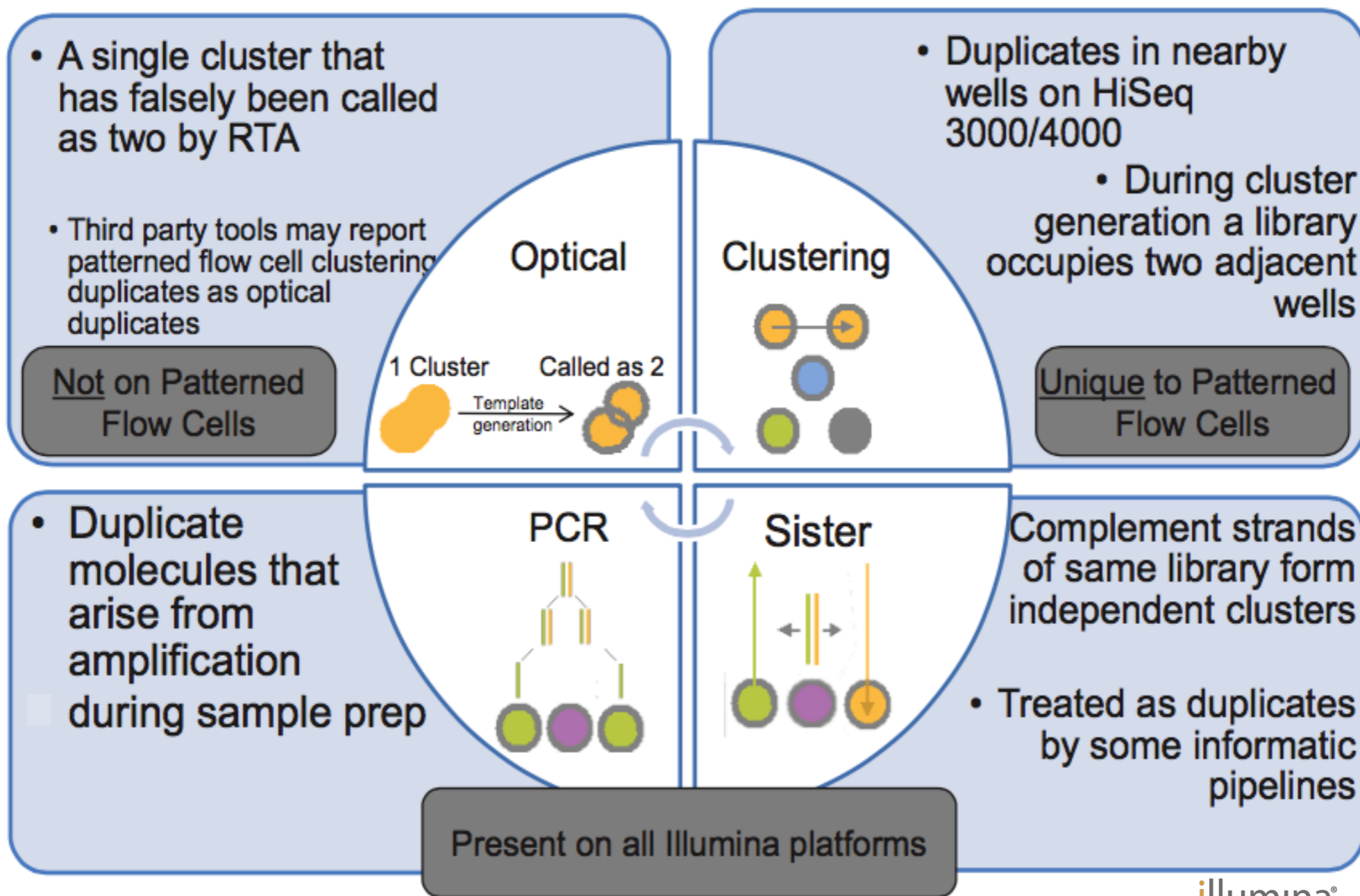


Data output

Illumina sequencers



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...

