

*IN-BIOS[9,5]000 2018*

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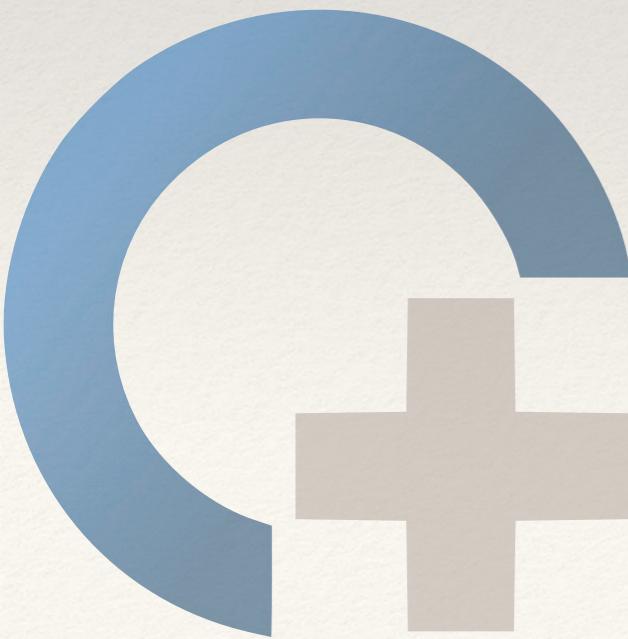
# Illumina Technology

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Oct 22, 2018

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OUS, Ullevål, Oslo

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# DNA sequencing

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- ❖ 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



Benchtop sequencers

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

Production-scale sequencers

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

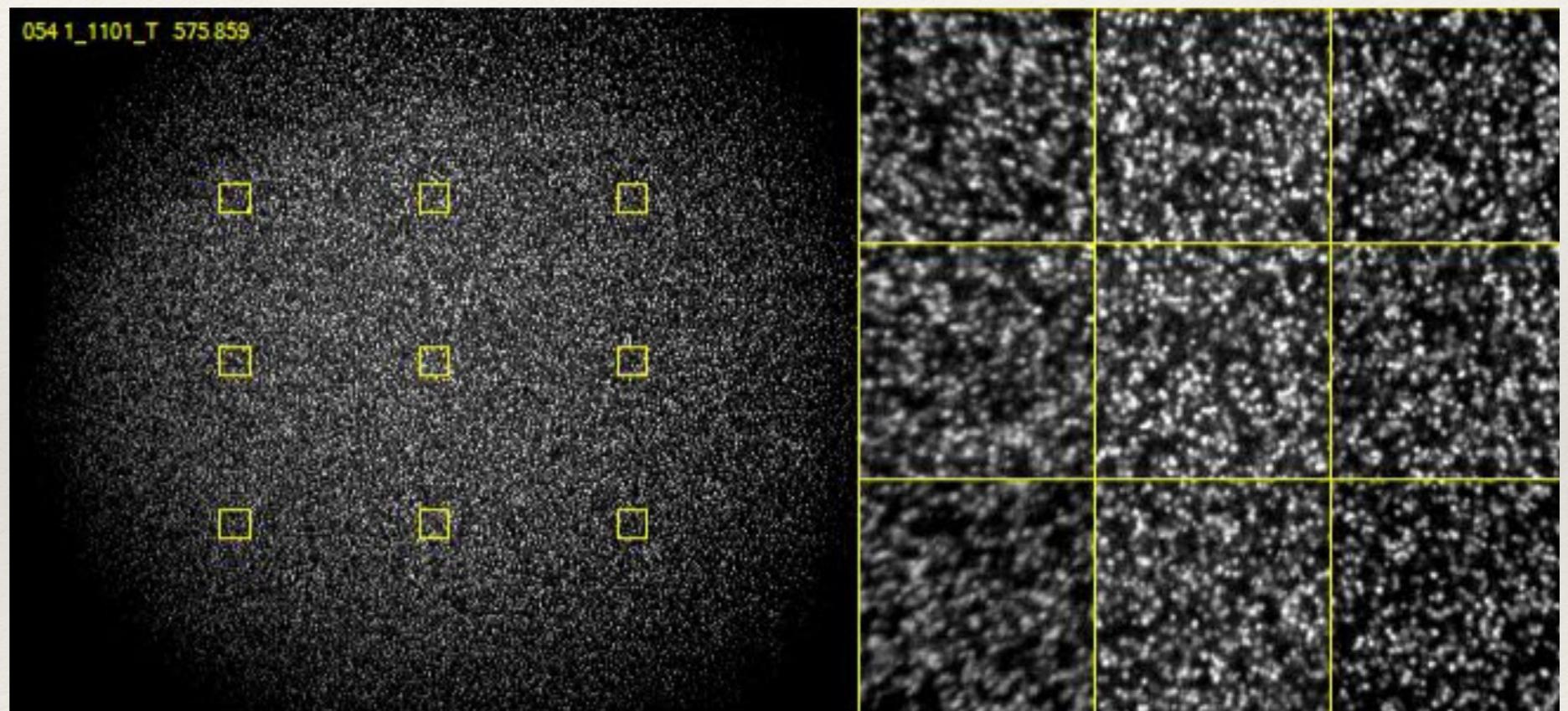
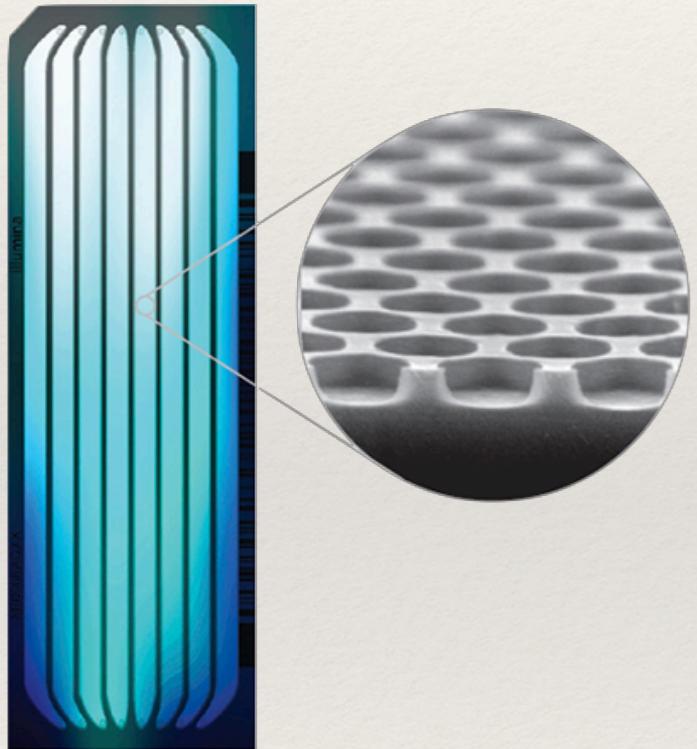
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# Illumina sequencers

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

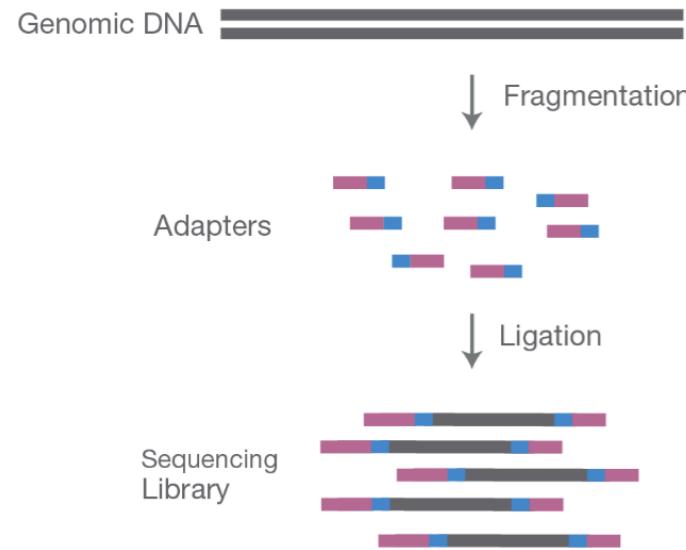
# Sequencing



# Library prep and sequencing

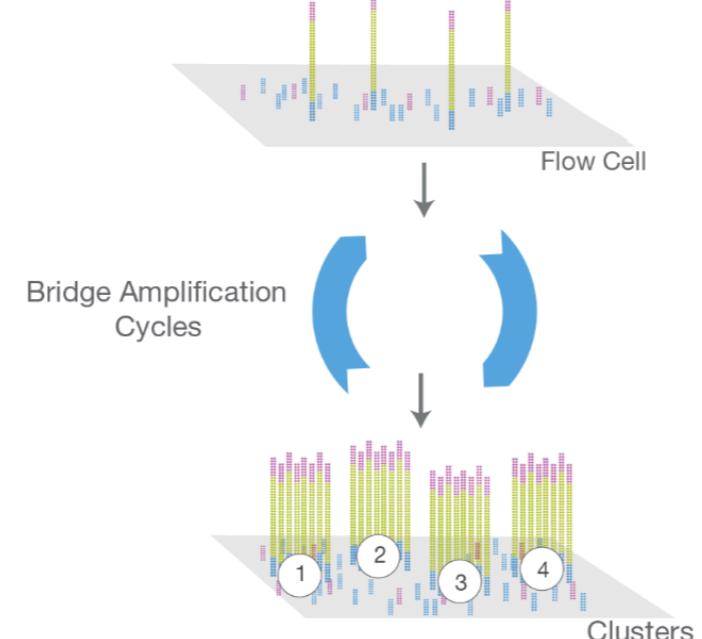
Fragment (DNA) sequenced: up to 800 bp

## A. Library Preparation



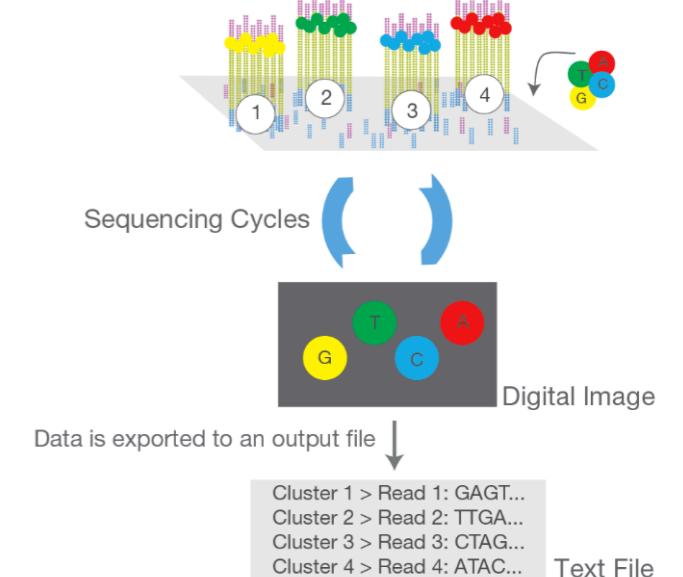
NGS library is prepared by fragmenting a gDNA sample and ligating specialized adapters to both fragment ends.

## 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



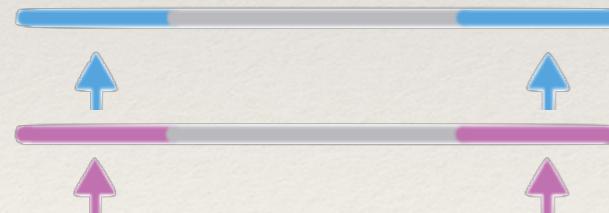
Sequencing reagents, including fluorescently labeled nucleotides, are added and the first base is incorporated. The flow cell is imaged and the emission from each cluster is recorded. The emission wavelength and intensity are used to identify the base. This cycle is repeated "n" times to create a read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

# Library prep and sequencing

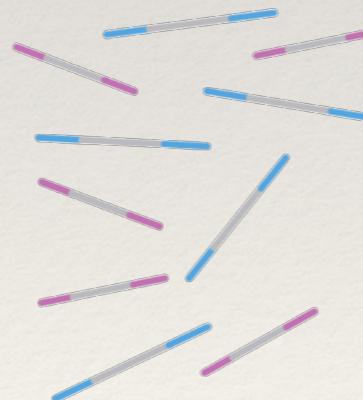
Fragment (DNA) sequenced: up to 800 bp

Add adapters during library preparation

Multiplexing: single/dual index



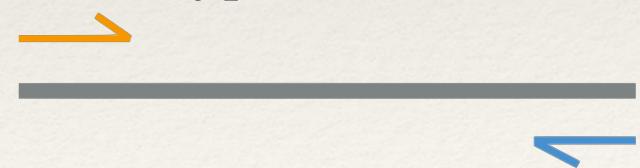
Multiplexing: pooling



Read type: Single end



Read type: Paired end



# Library prep

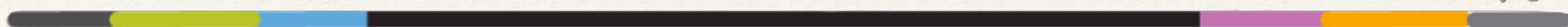
<https://www.illumina.com/techniques/sequencing/ngs-library-prep/library-prep-methods.html>

Try the “Sequencing Methods selector”

Check out 3 posters: DNA, RNA and single cell



P7 Index 1



Index 2 P5



# Single/dual indexed samples

MiSeq  
HiSeq 2500  
NovaSeq

Figure 1 Single-Indexed Sequencing

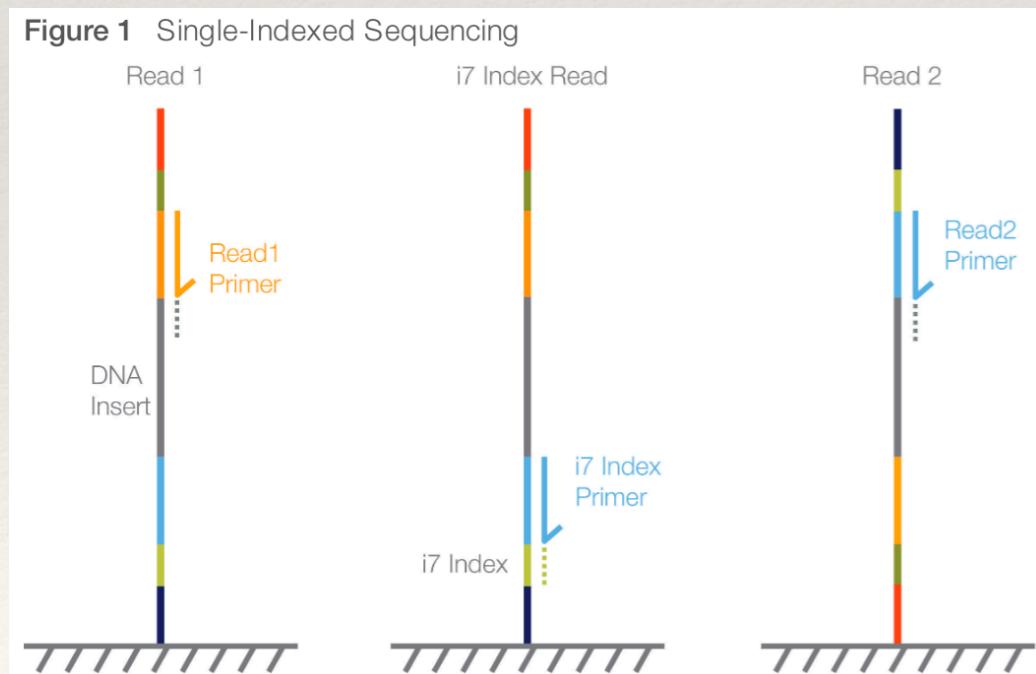


Figure 2 Dual-Indexed Sequencing on a Paired-End Flow Cell (Workflow A)

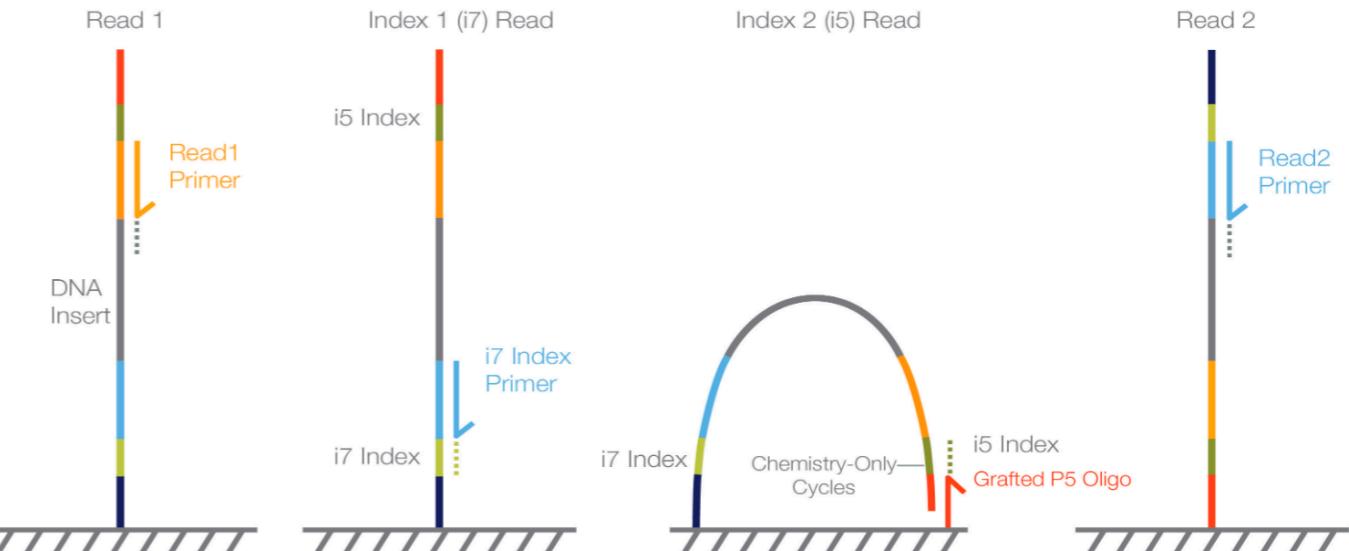
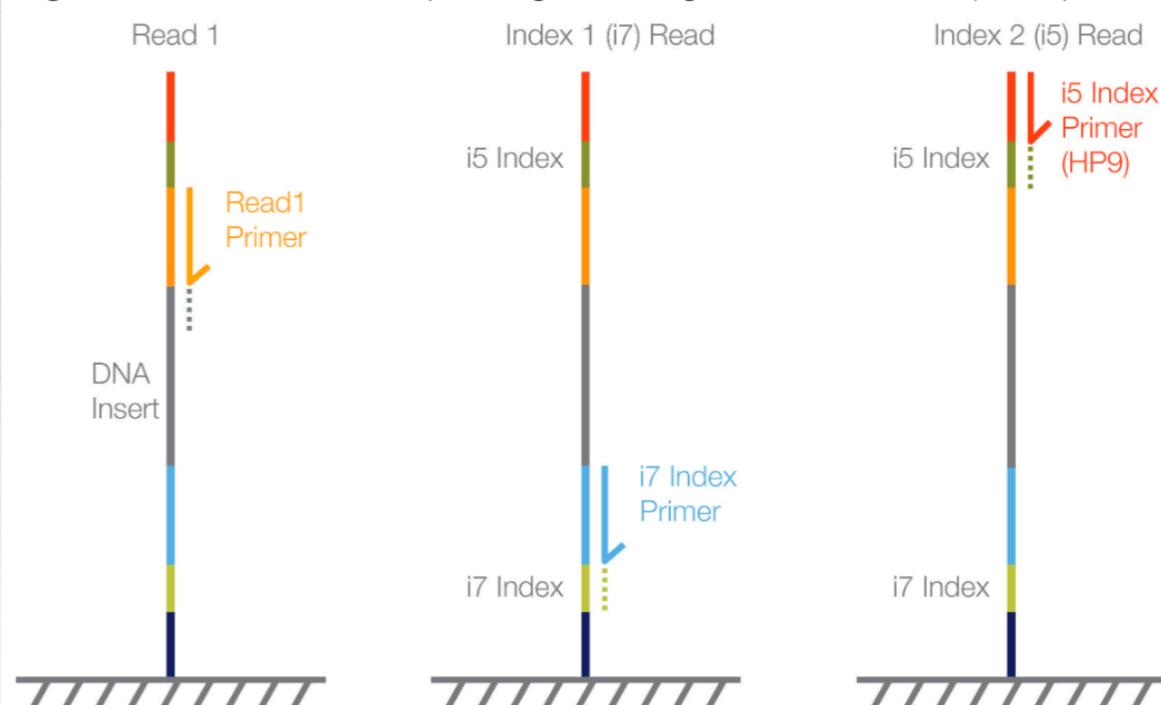


Figure 5 Dual-Indexed Sequencing on a Single-Read Flow Cell (HiSeq 2500 or HiSeq 2000)



# Single/dual indexed samples

iSeq  
MiniSeq  
NextSeq  
HiSeq 3/4000  
HiSeq X

Figure 1 Single-Indexed Sequencing

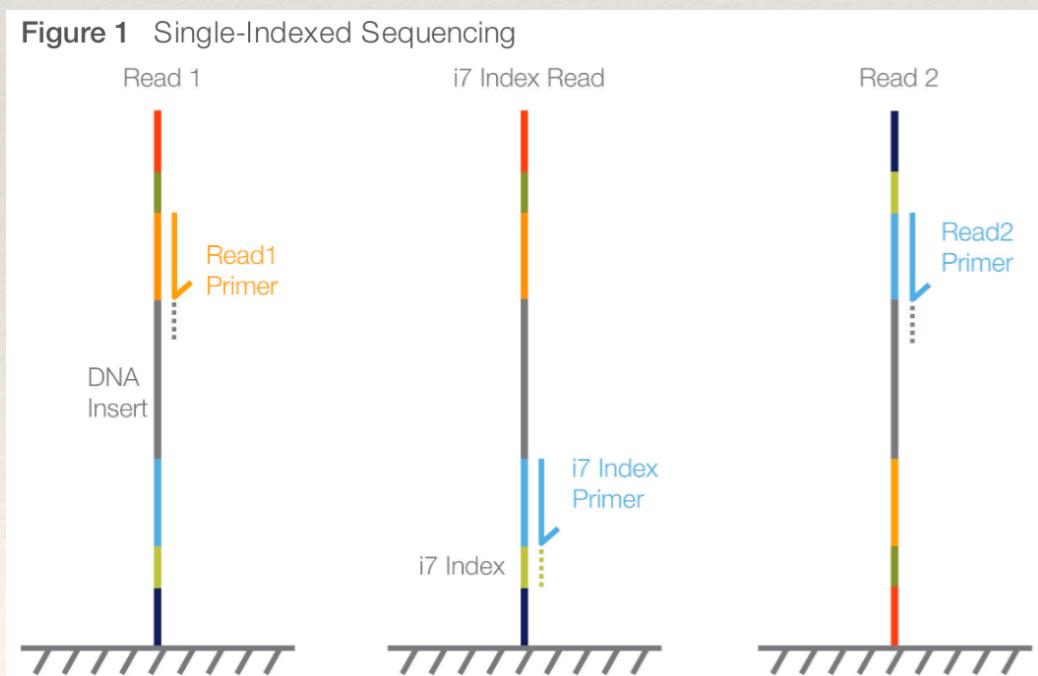


Figure 3 Dual-Indexed Sequencing on a Paired-End Flow Cell (Workflow B)

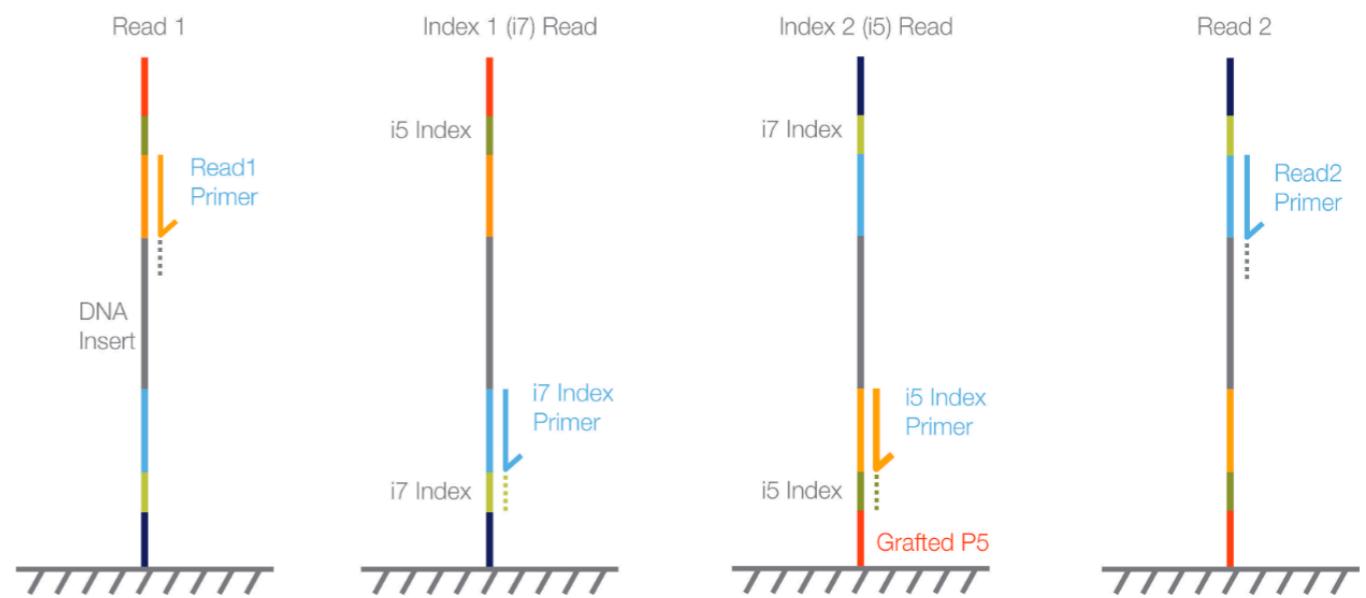
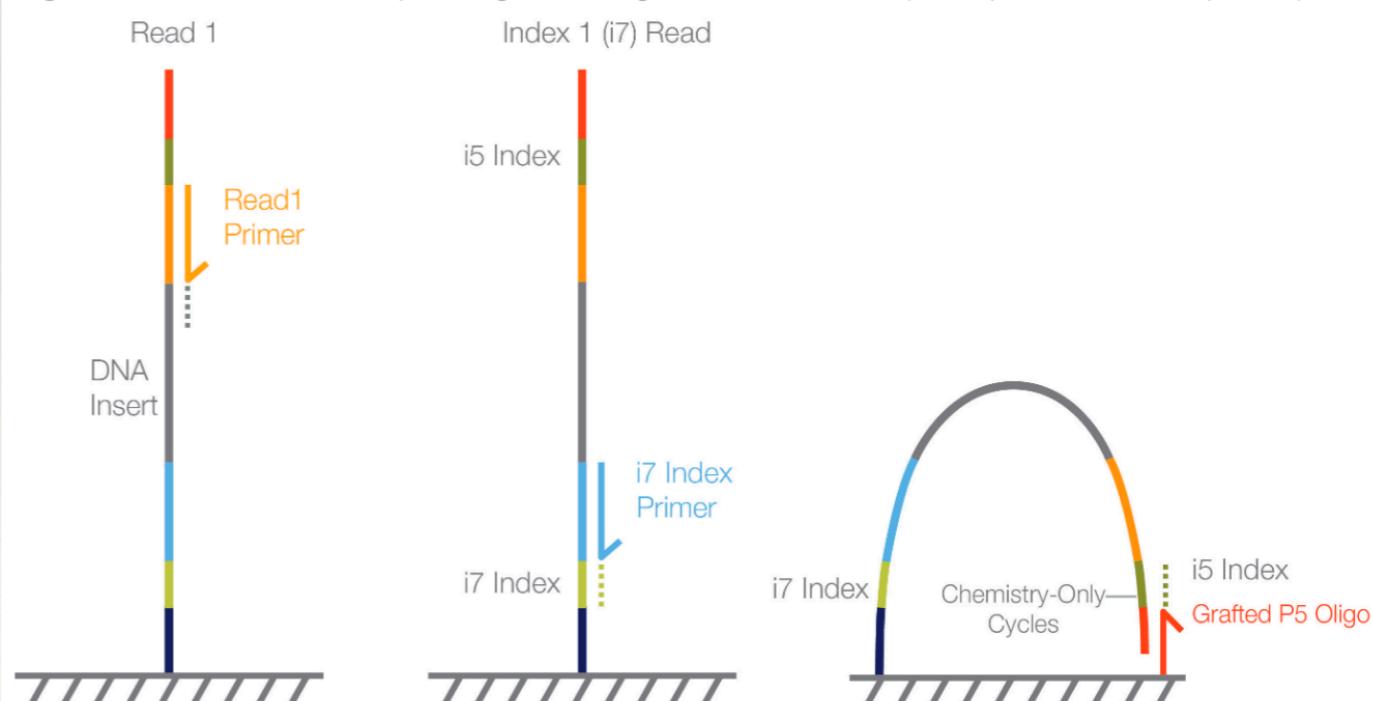
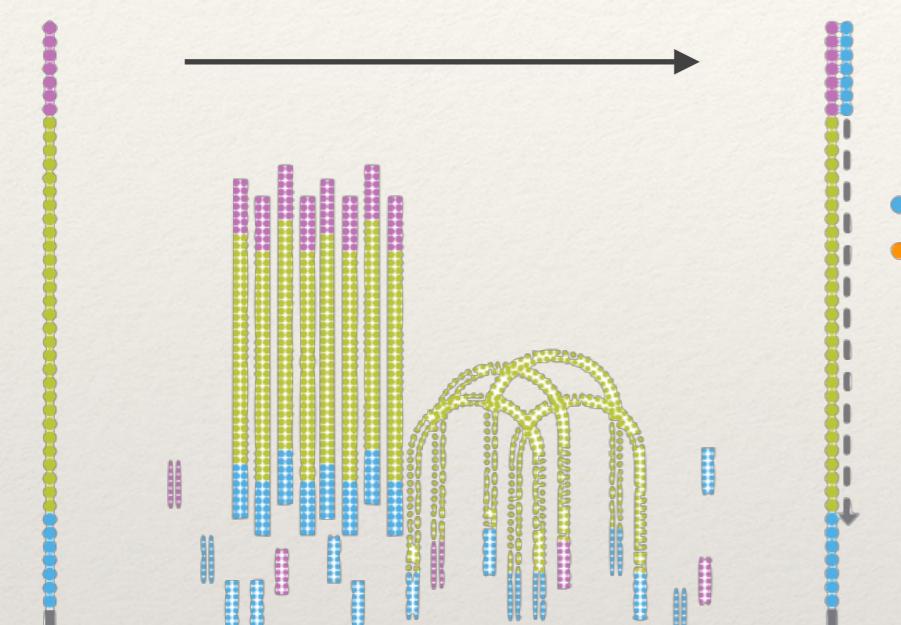


Figure 4 Dual-Indexed Sequencing on a Single-Read Flow Cell (HiSeq 4000 or HiSeq 3000)

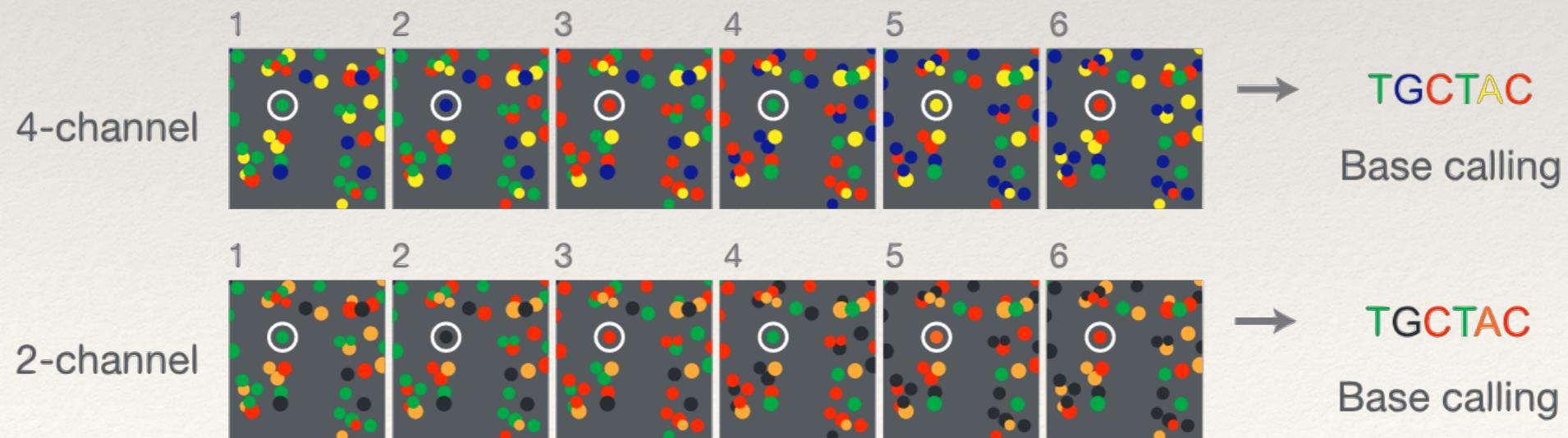
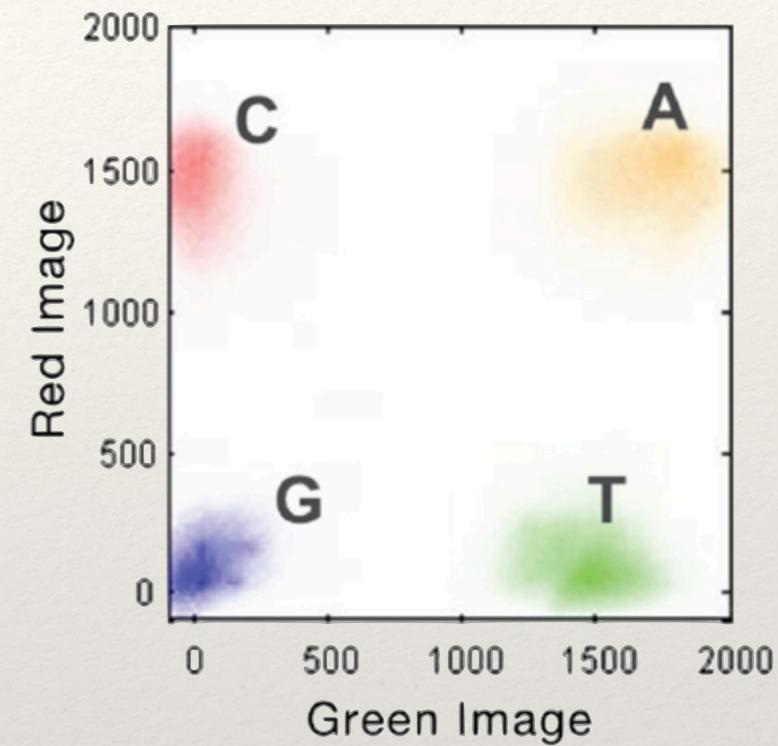


# Four vs two colour chemistry



Old machines use 4 colours  
New machines use 2 colours

Sequencing is almost  
twice as fast



# Illumina sequencers



Benchtop sequencers

Read length:  
Single end

36  
50  
75

Read length:  
Paired end

25  
50  
75

100  
125  
150

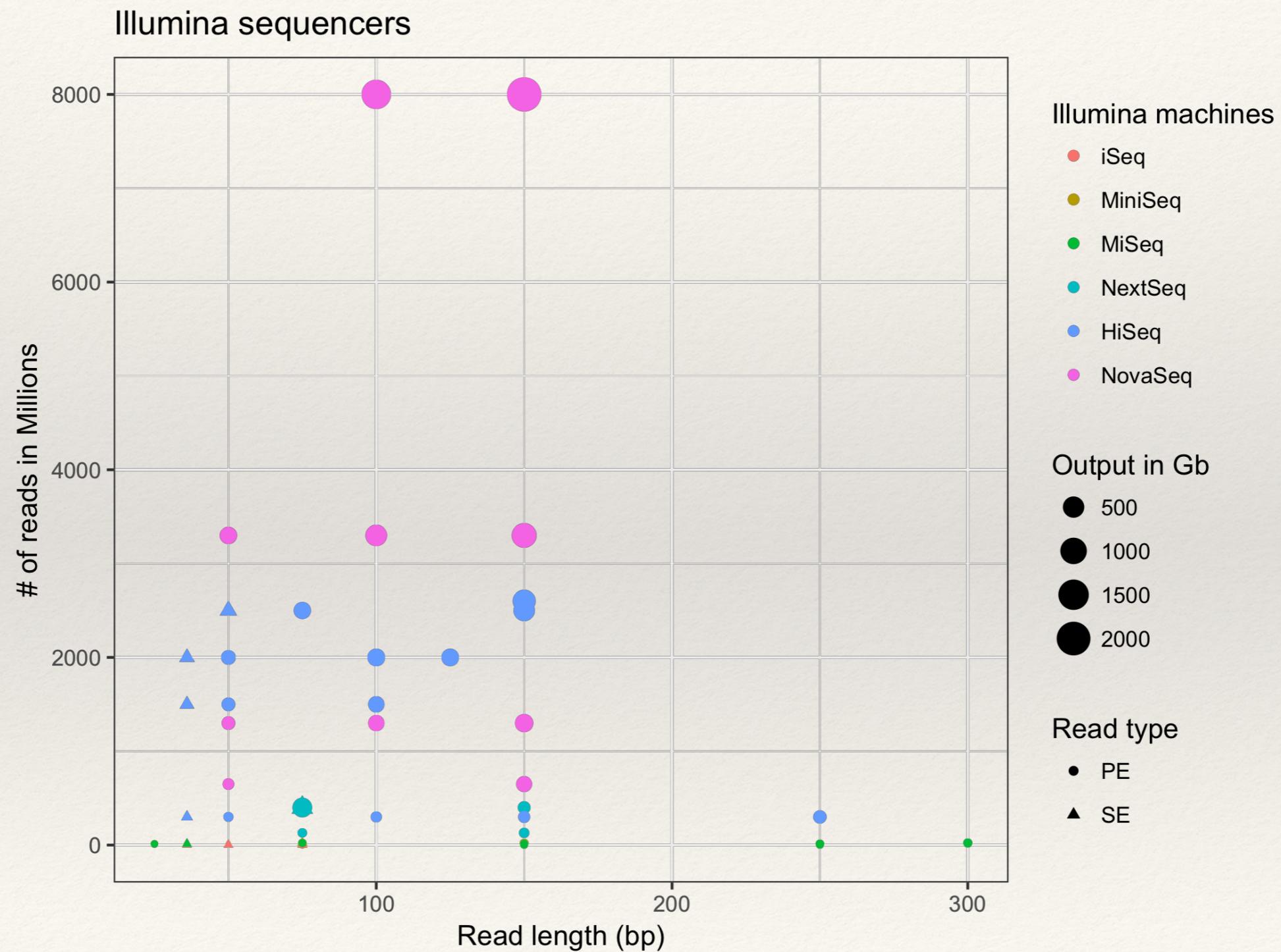
250  
300

Production-scale sequencers

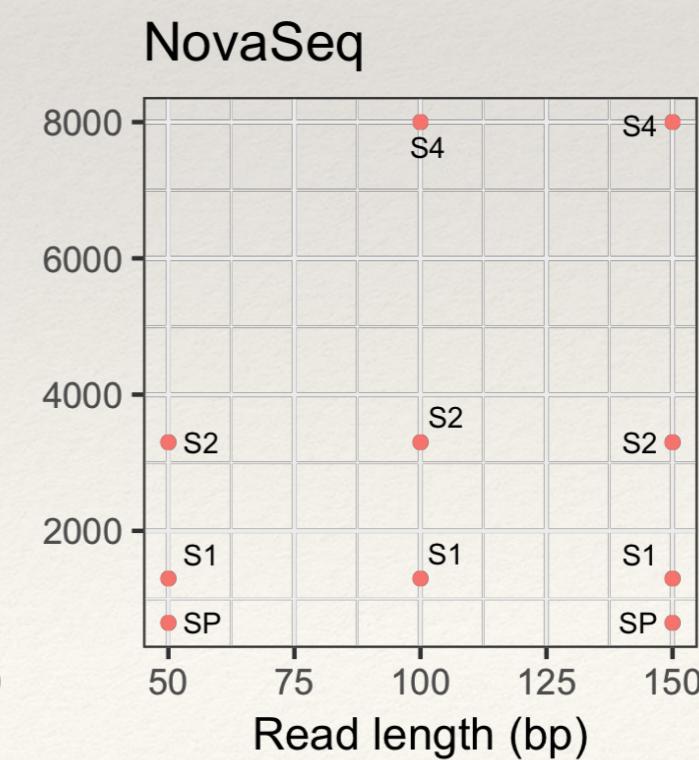
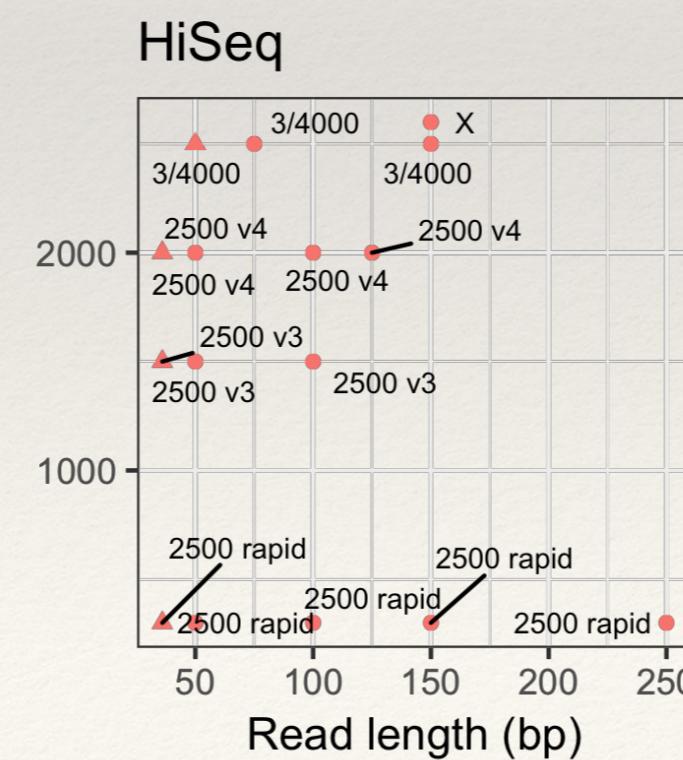
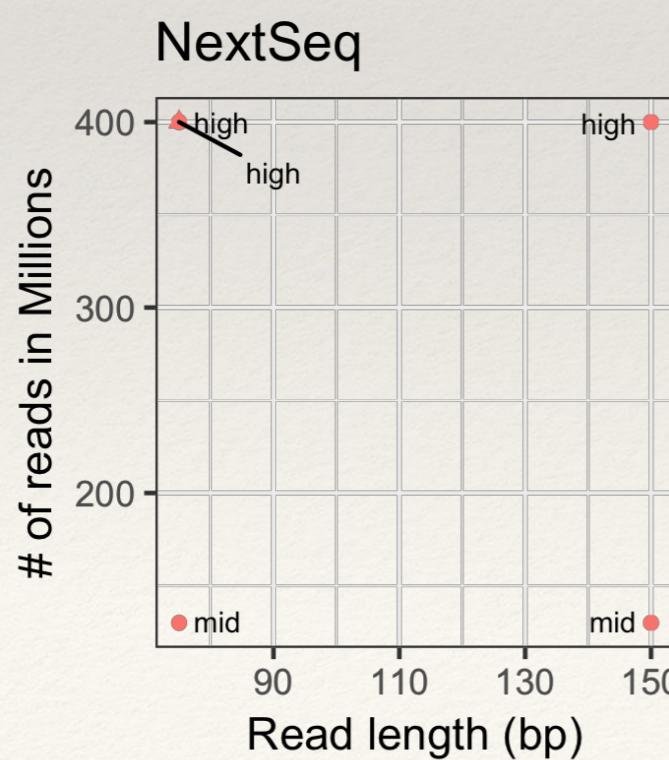
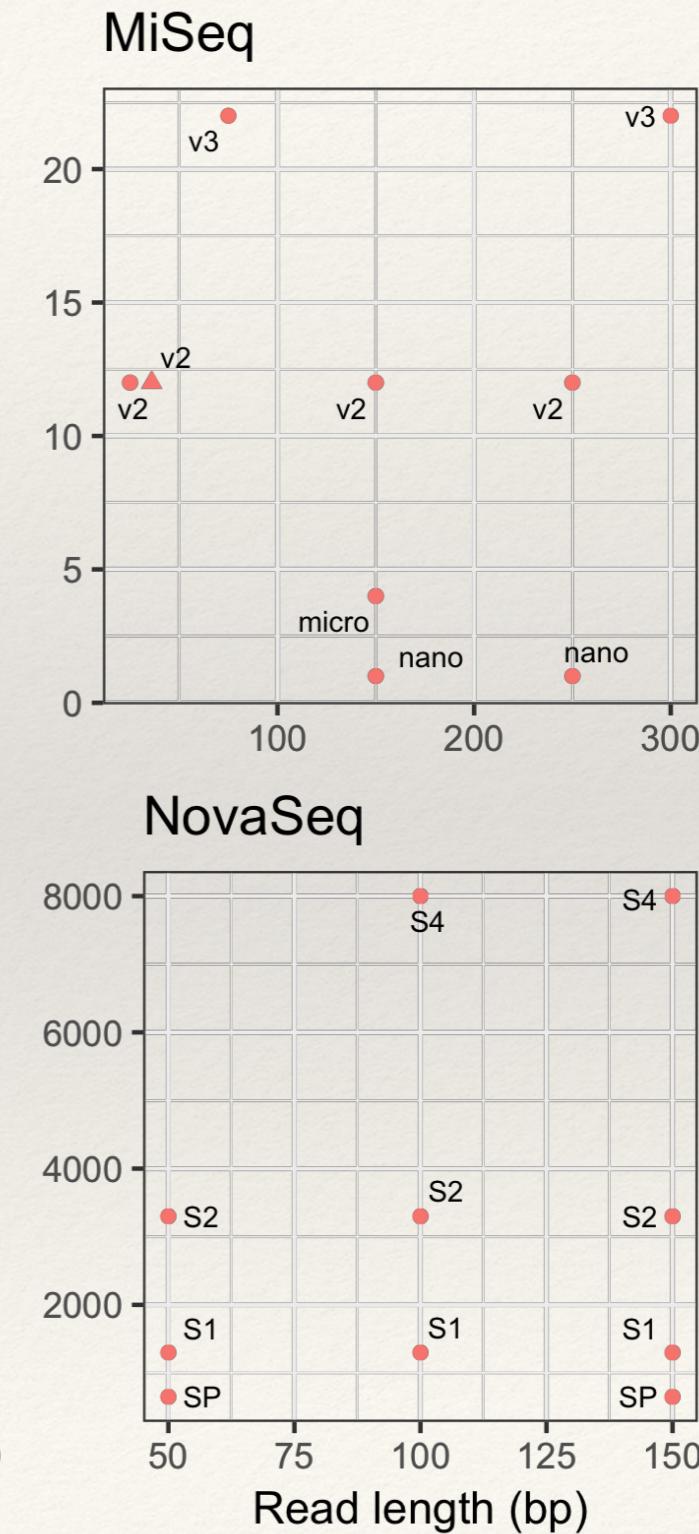
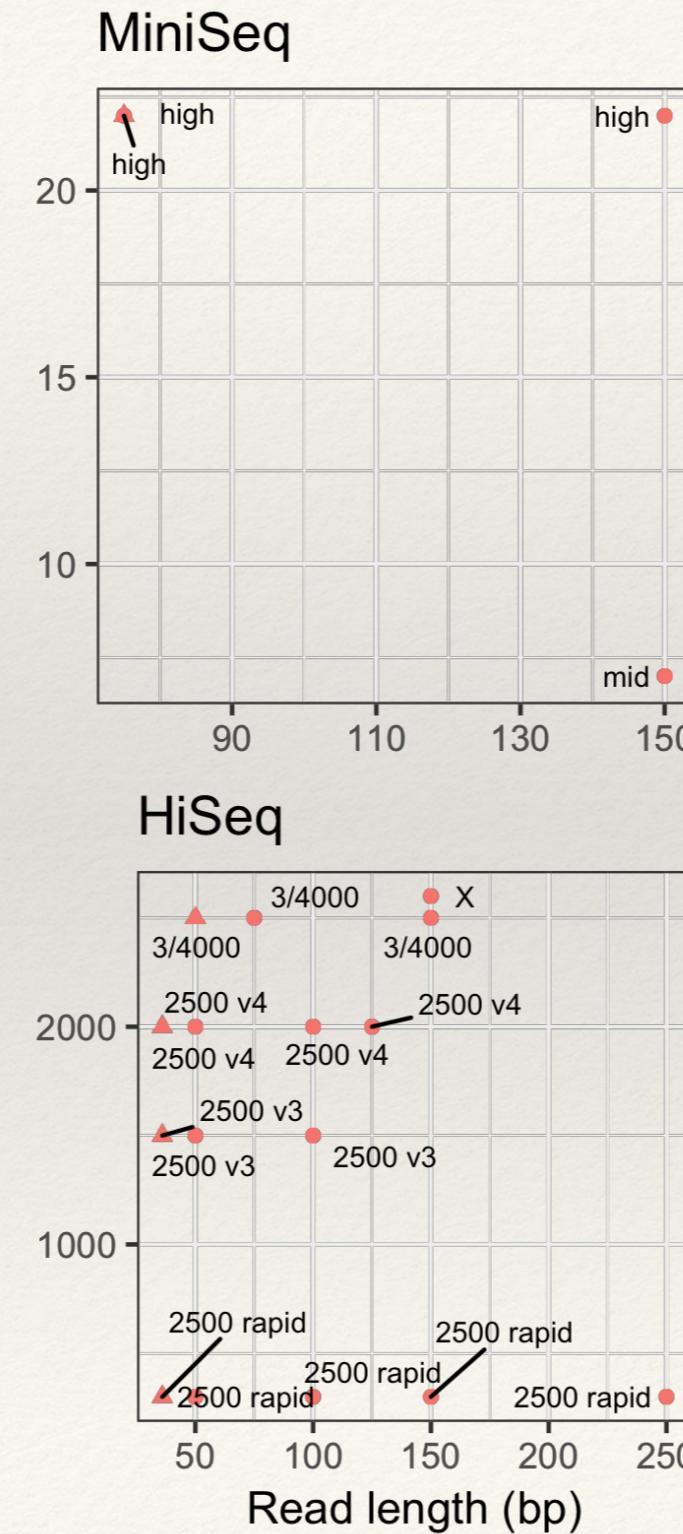
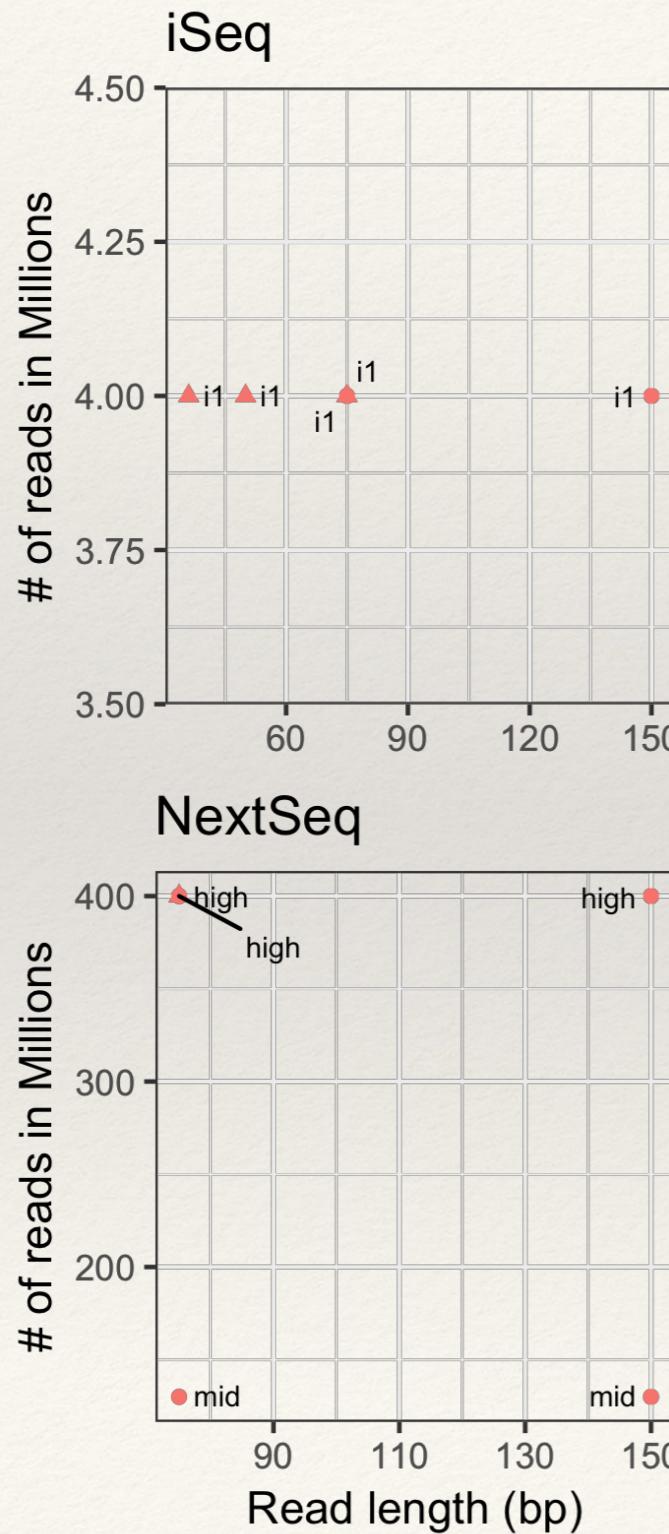
Data output:

144 Mb  
-  
2400 Gb

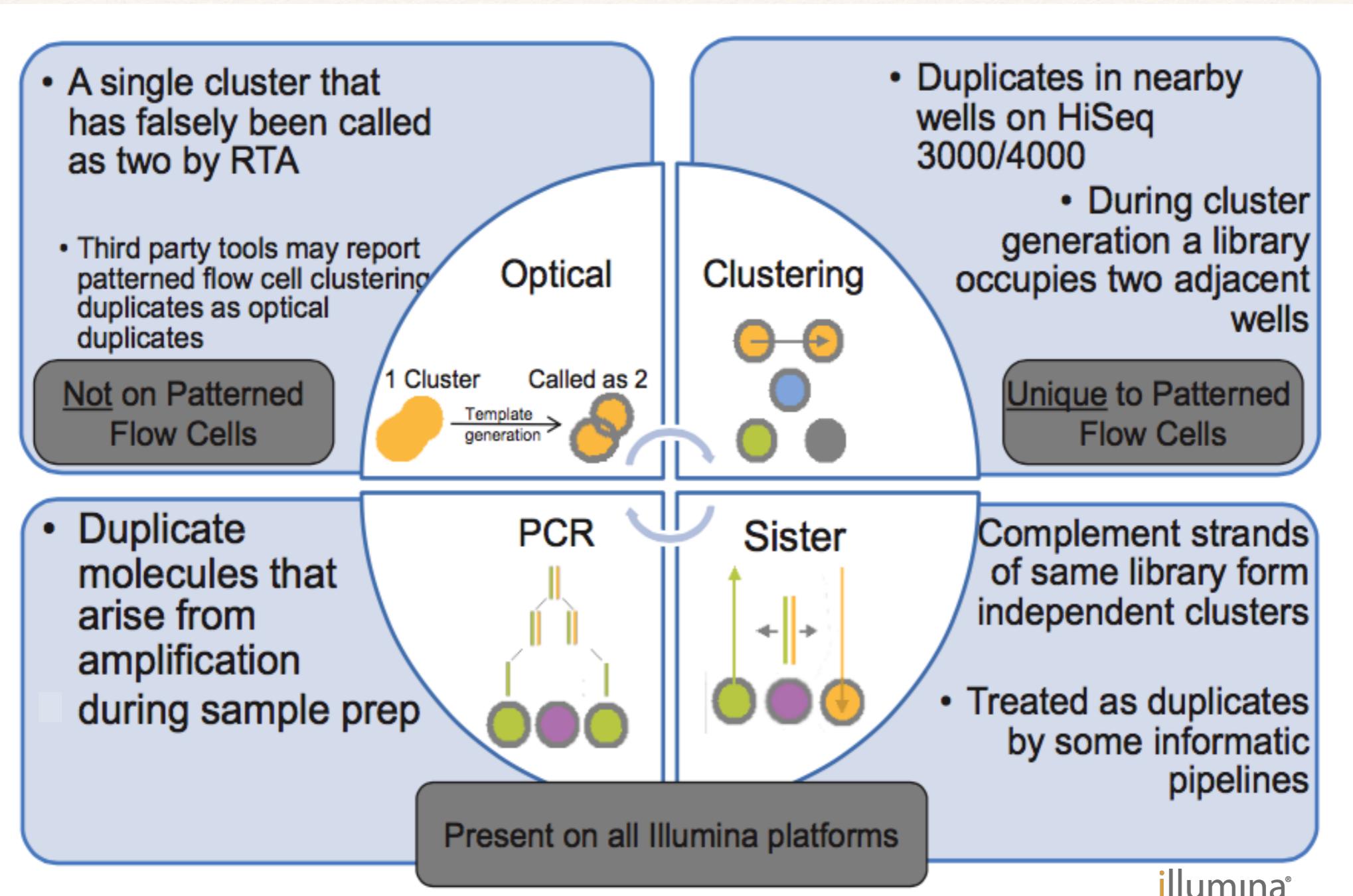
# Data output



# Data output



# Known issues



illumina®

# 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>