Sequencing Technologies

Sequencing Platforms

- Illumina: https://www.illumina.com/
  - Short read, sequencing by synthesis.
- Oxford Nanopore (MinION): https://nanoporetech.com/
  - Long read, nanopore based sequencing reads out change in current at each base as single molecule moves through protein nanopore.
- Pacific Biosciences: http://www.pacb.com/
  - Long read, single molecule real-time (SMRT) sequencing reads signal on fluorescent labeled nucleotides.

Sequencing Library

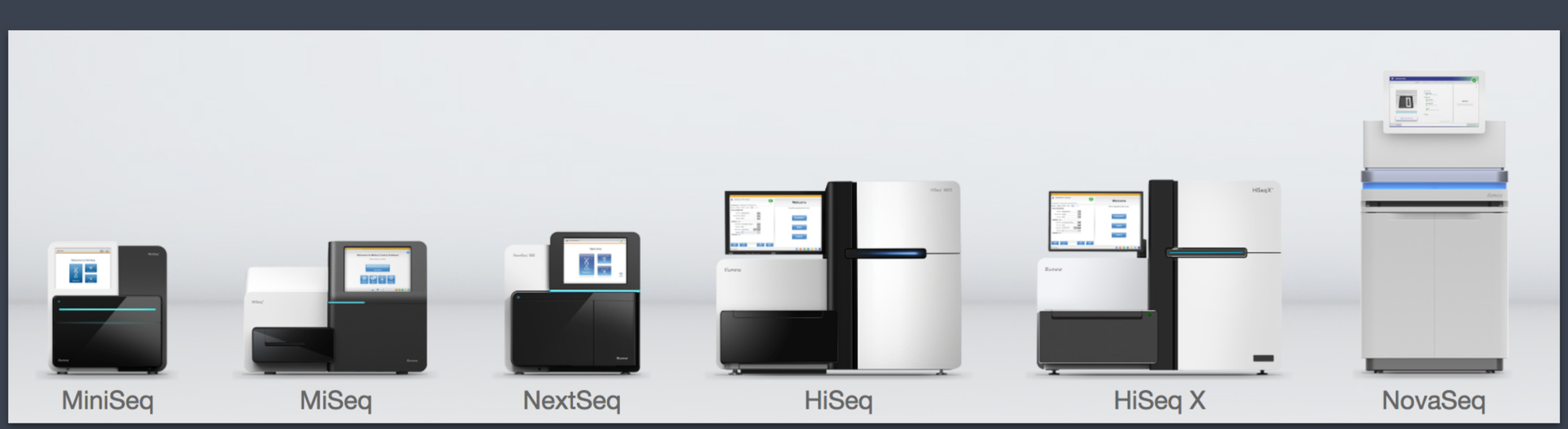
Piece of DNA or cDNA:

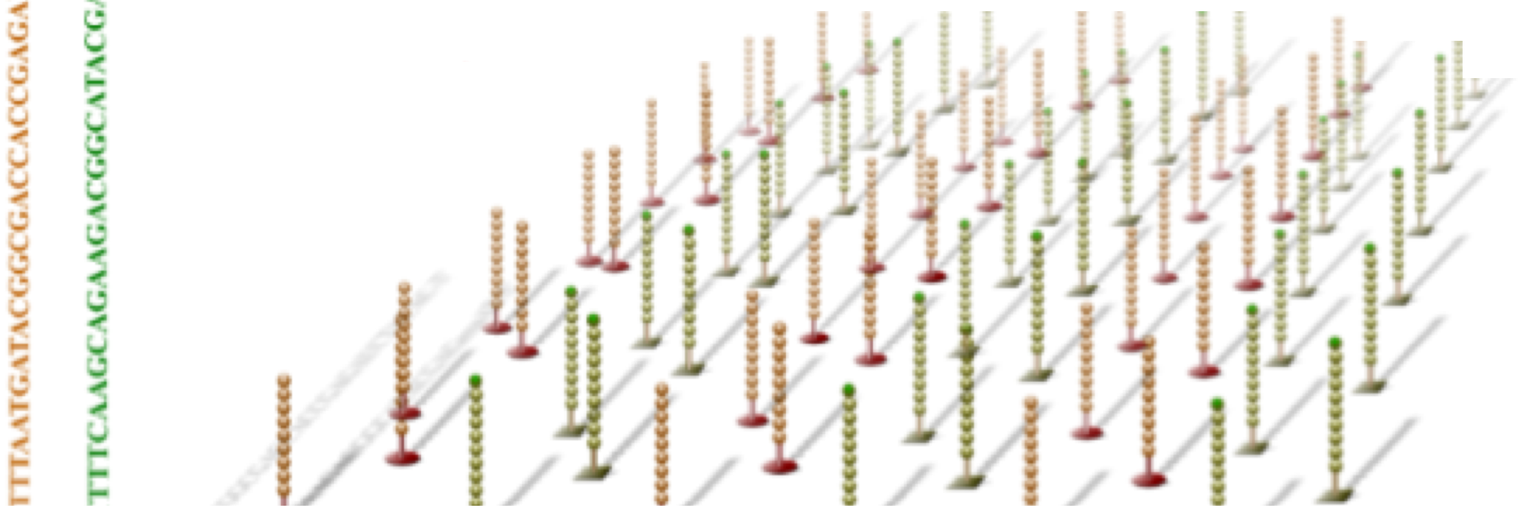
Insert

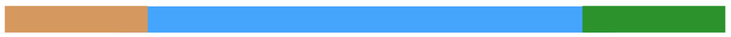
Insert + Sequencing Adapters:

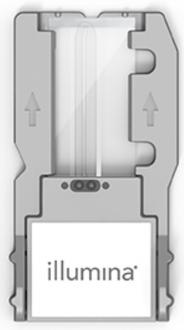
Library

Illumina: Sequencing by Synthesis



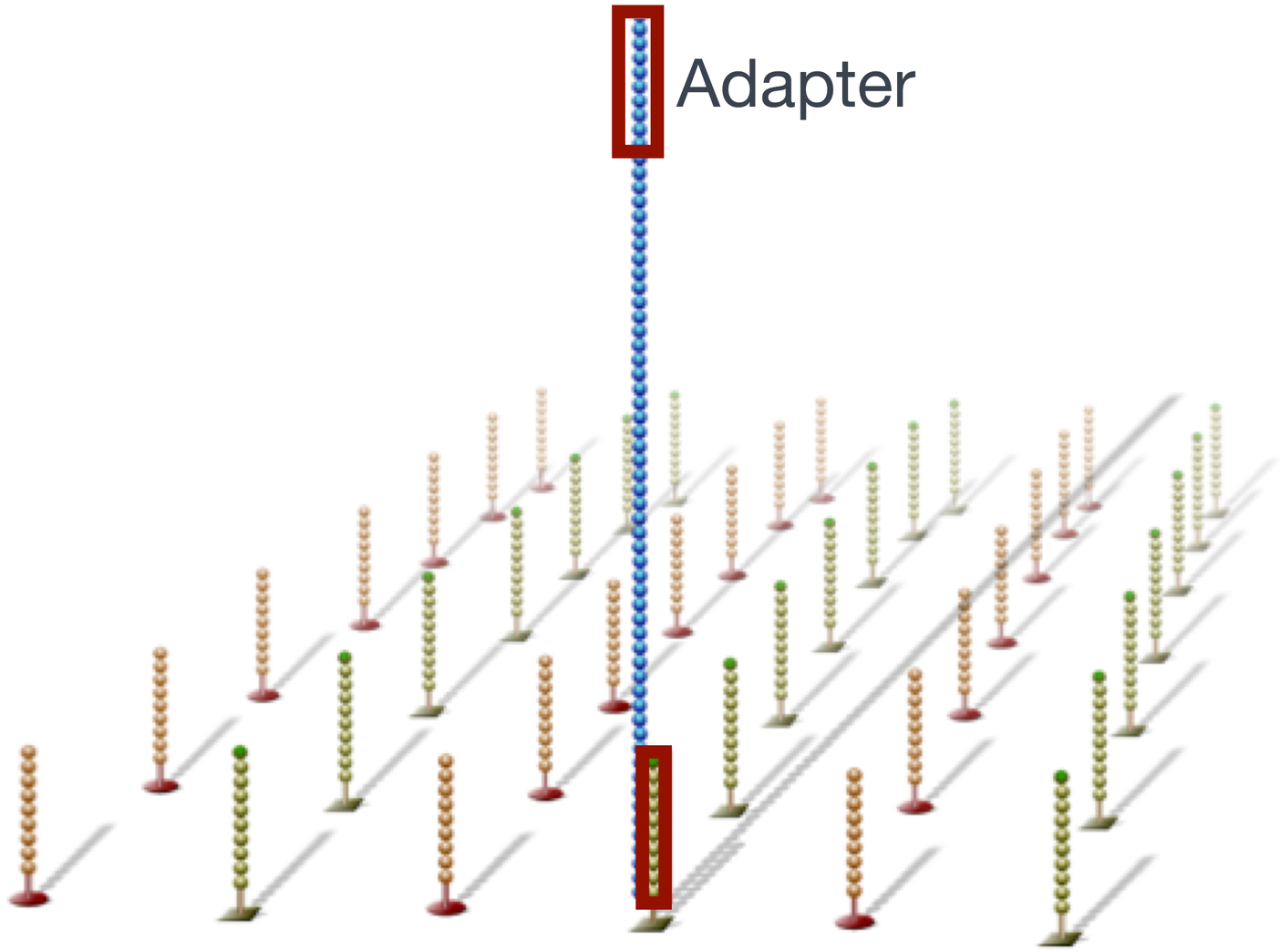






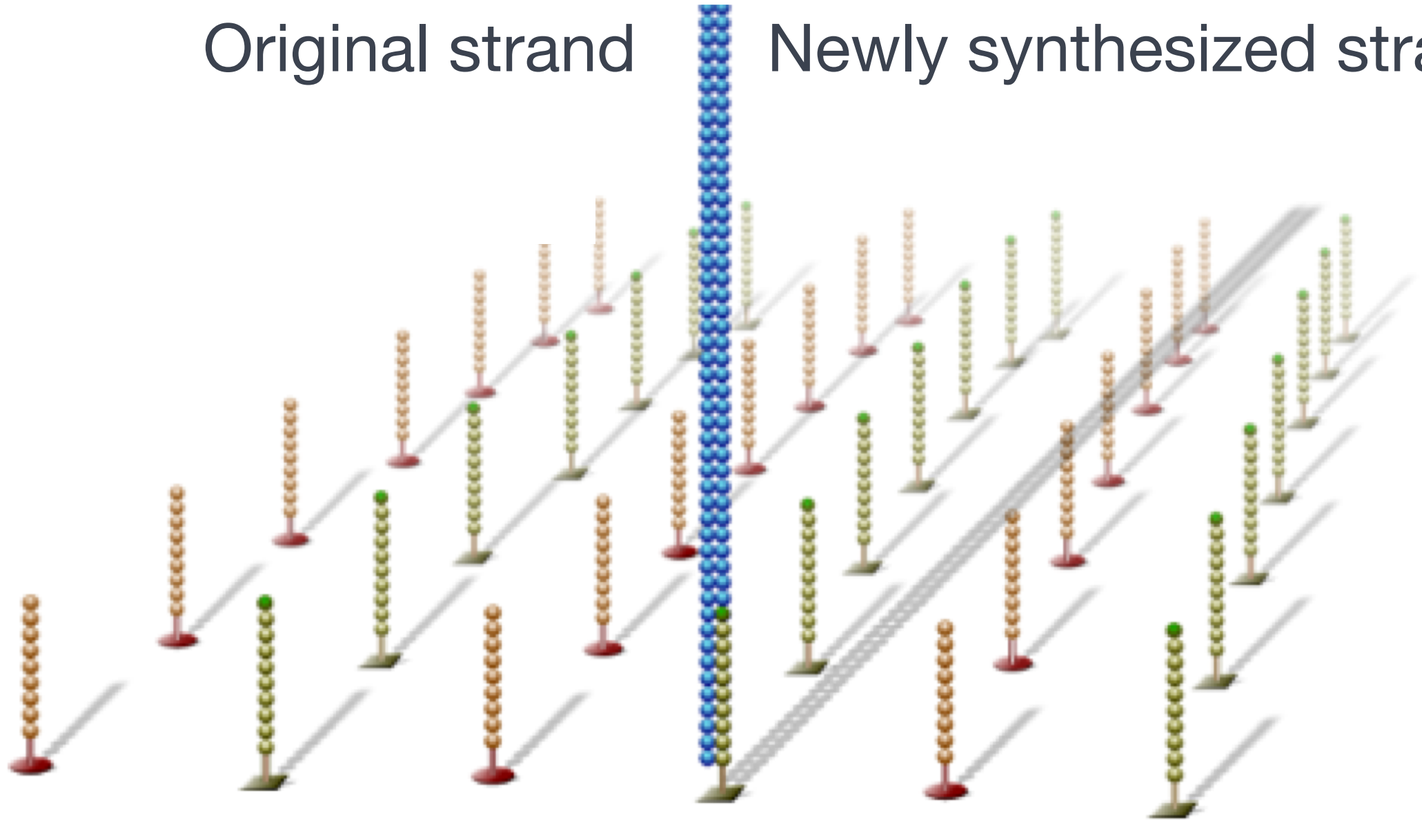
Illumina: flow cell

http://training.bioinformatics.ucdavis.edu/docs/2014/09/september-2014-workshop/Monday_JF_HTS_lecture.html



Illumina: cluster generation

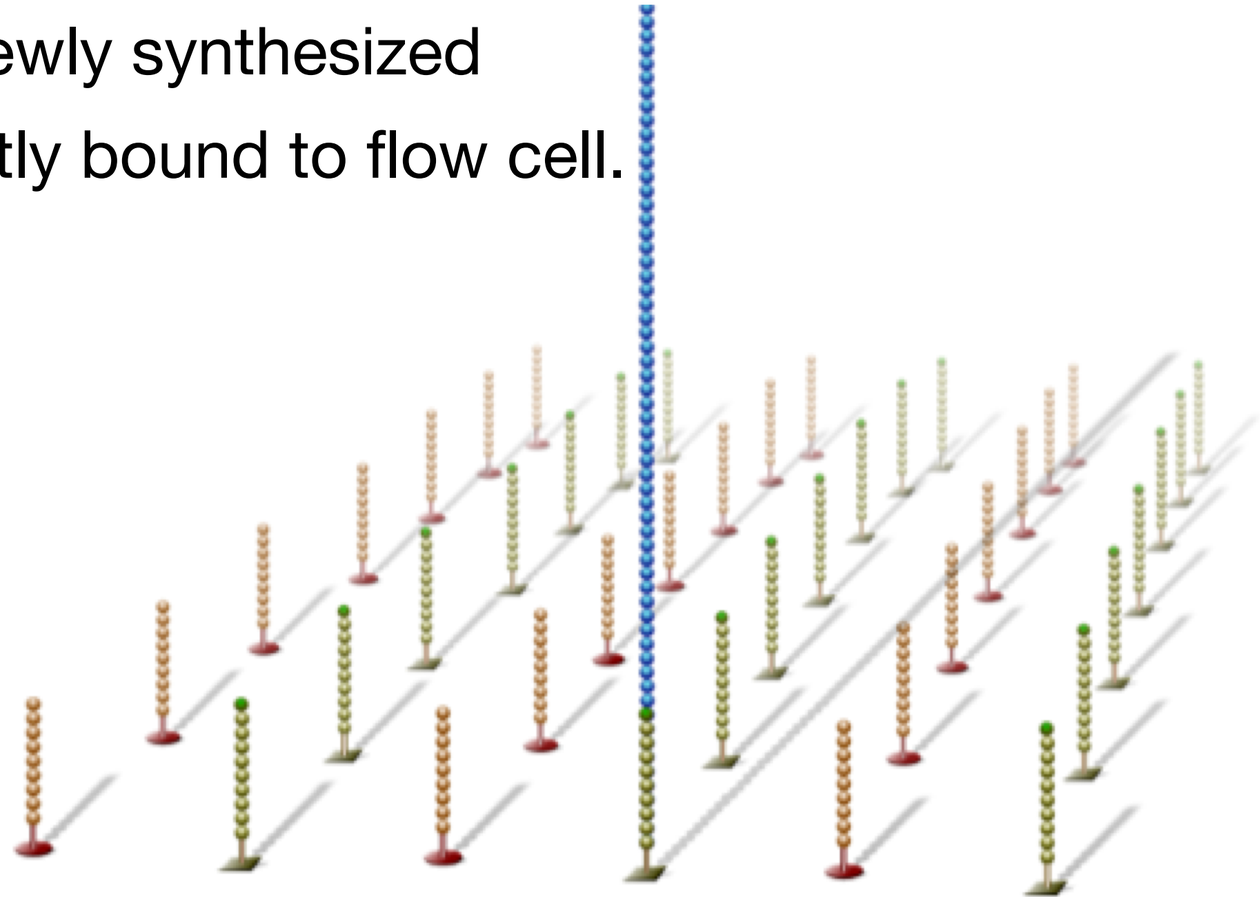
http://training.bioinformatics.ucdavis.edu/docs/2014/09/september-2014-workshop/Monday_JF_HTS_lecture.html



Illumina: cluster generation

http://training.bioinformatics.ucdavis.edu/docs/2014/09/september-2014-workshop/Monday_JF_HTS_lecture.html

dsDNA is denatured, original DNA washed away. Newly synthesized strand is covalently bound to flow cell.



Illumina: cluster generation

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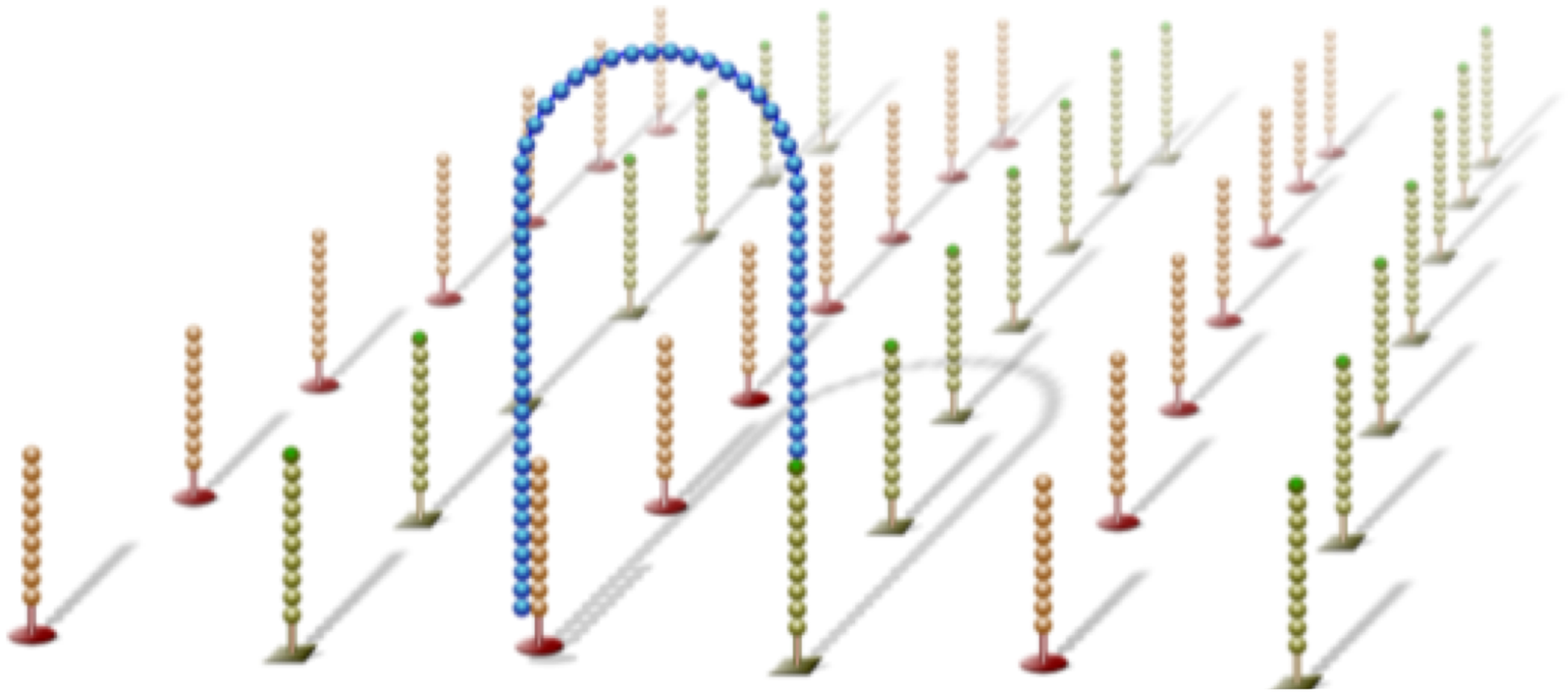
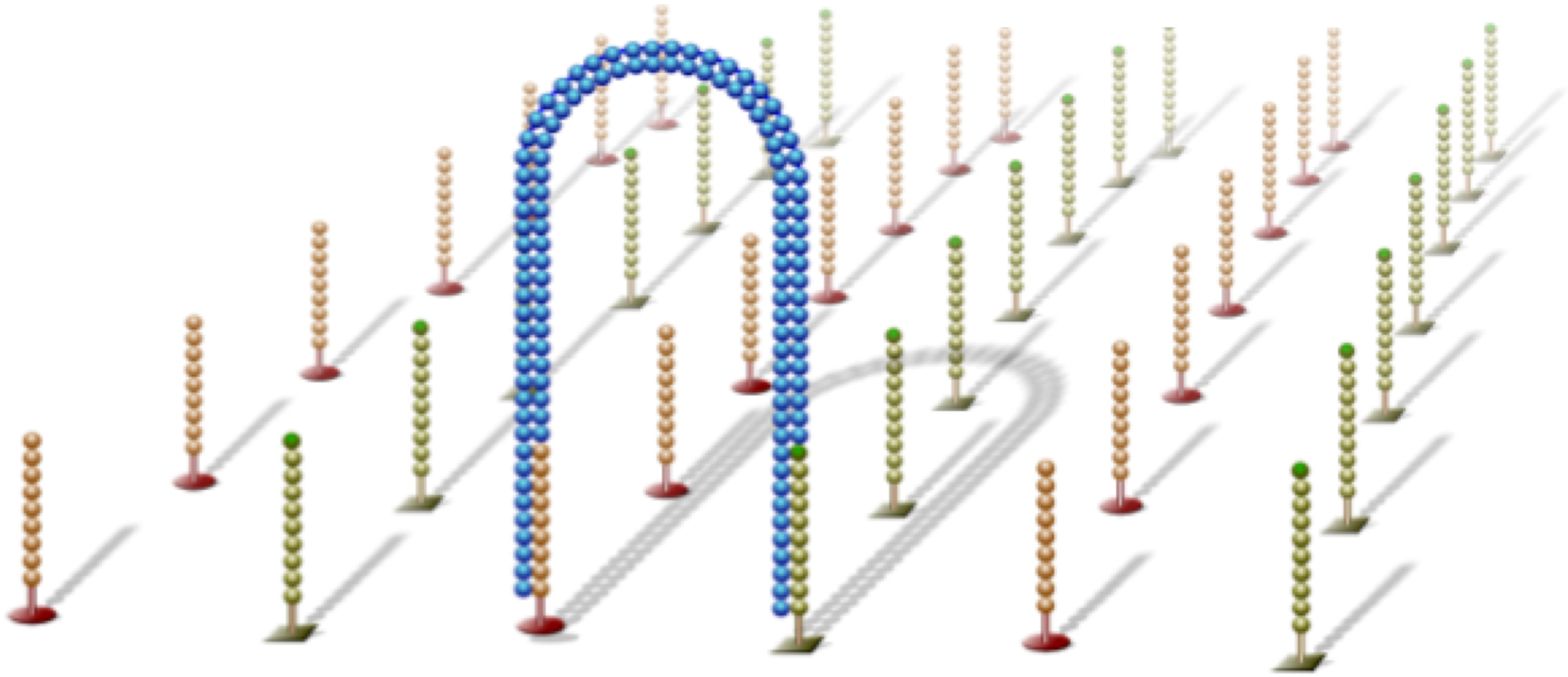


Illumina: bridge amplification

http://training.bioinformatics.ucdavis.edu/docs/2014/09/september-2014-workshop/Monday_JF_HTS_lecture.html



Illumina: bridge amplification

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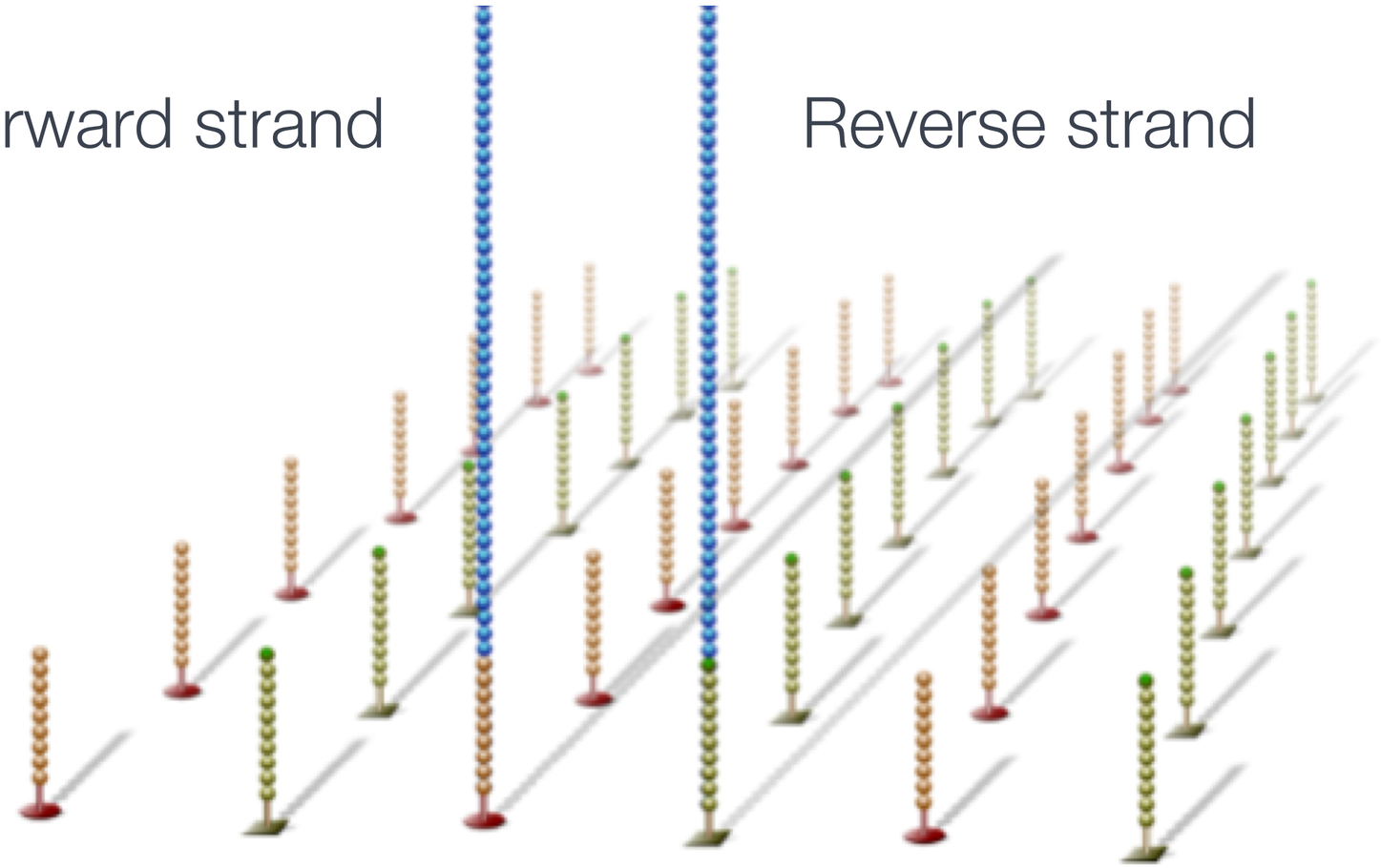

Illumina: bridge amplification

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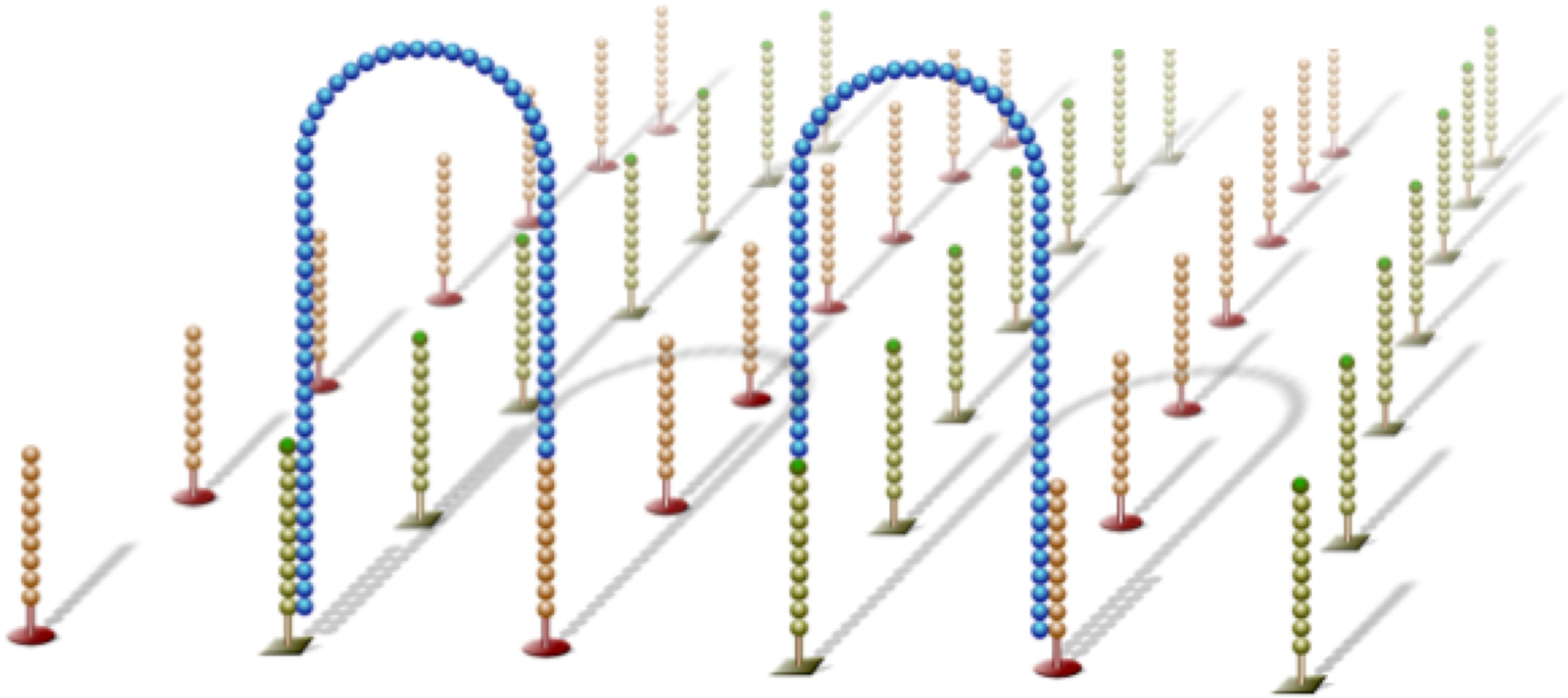

Illumina: bridge amplification

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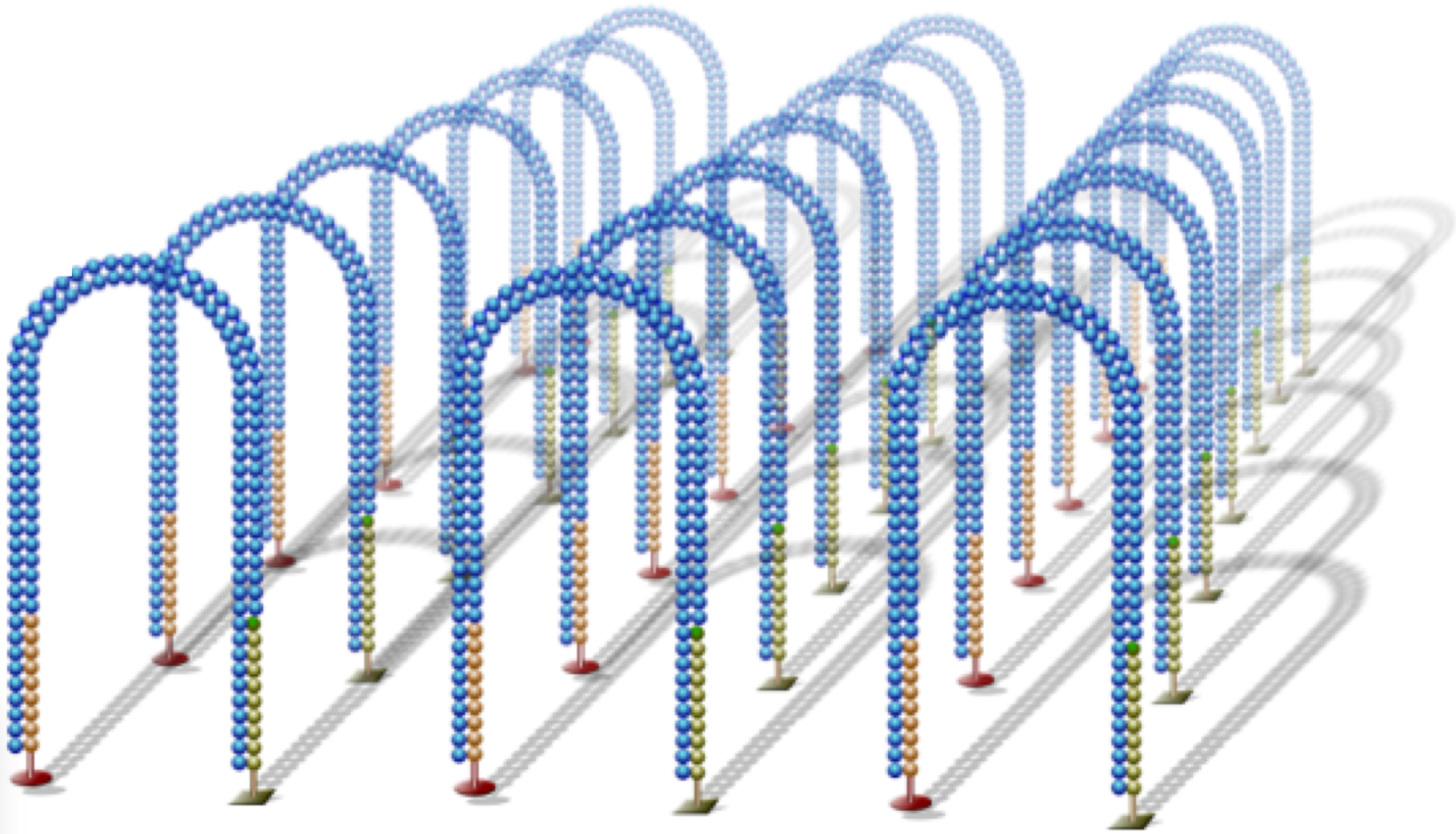


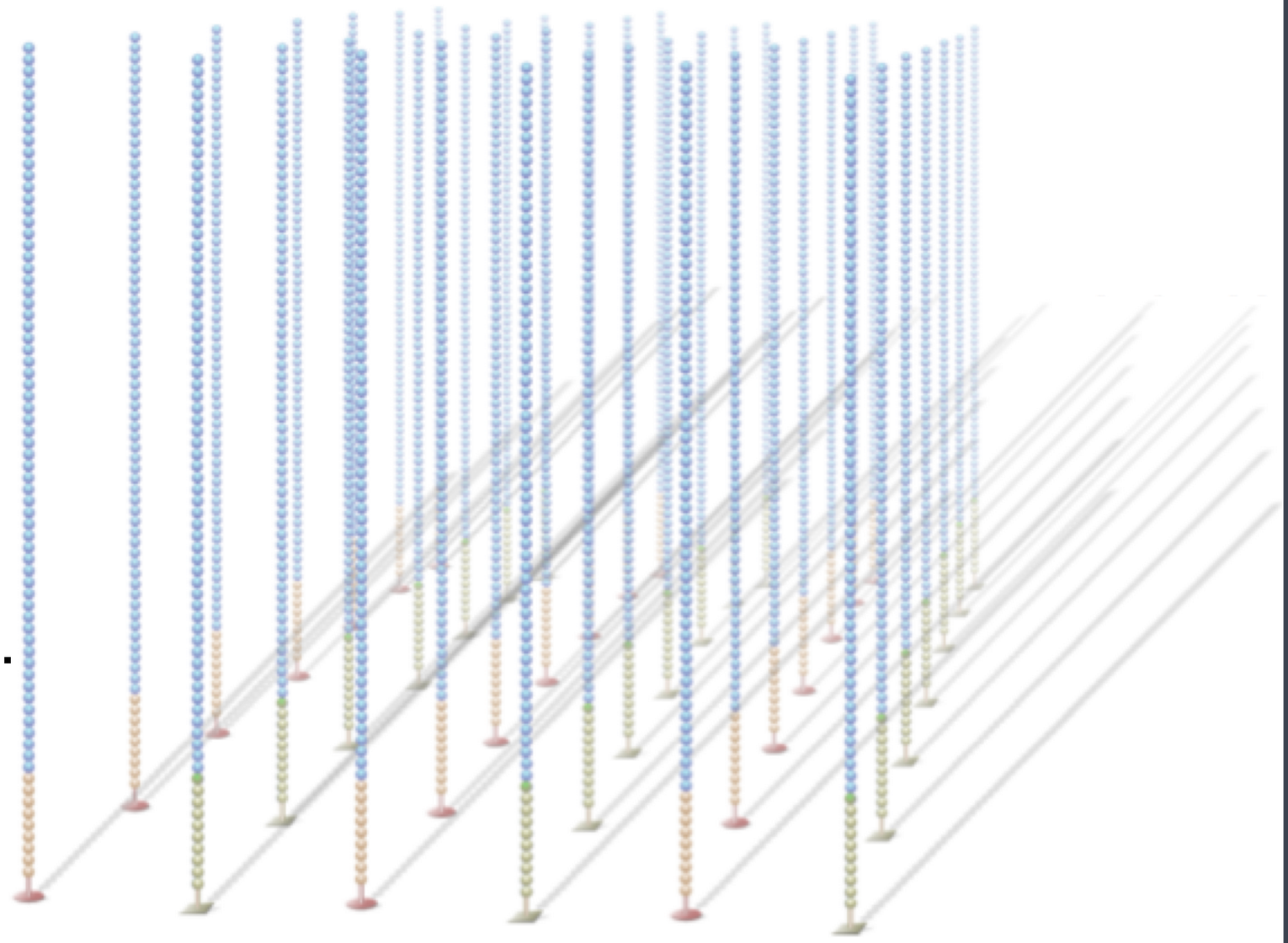
Illumina: bridge amplification

http://training.bioinformatics.ucdavis.edu/docs/2014/09/september-2014-workshop/Monday_JF_HTS_lecture.html

Illumina: cluster generation

Reverse strands are cleaved and washed off.

3’ ends are blocked to prevent unwanted priming.



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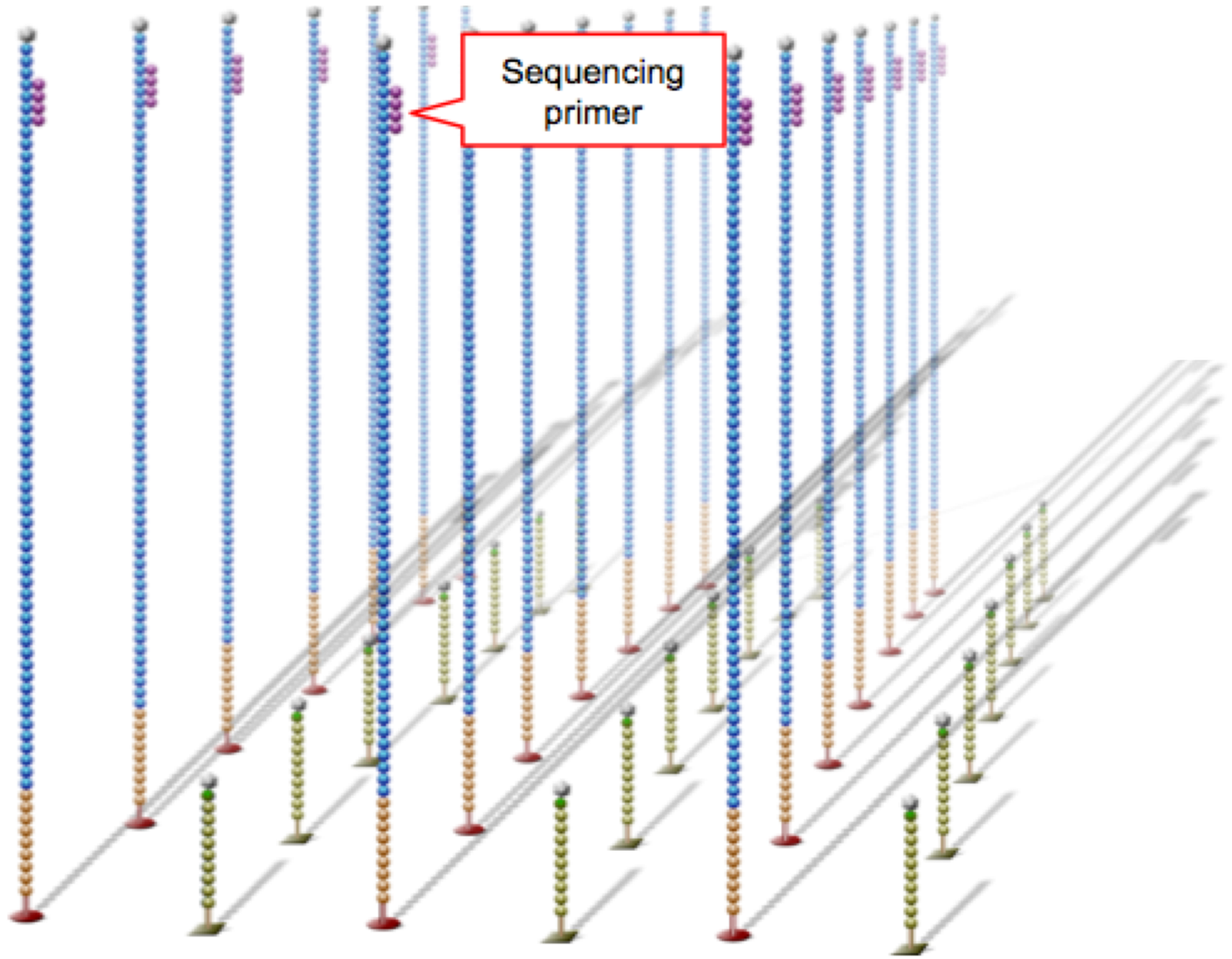
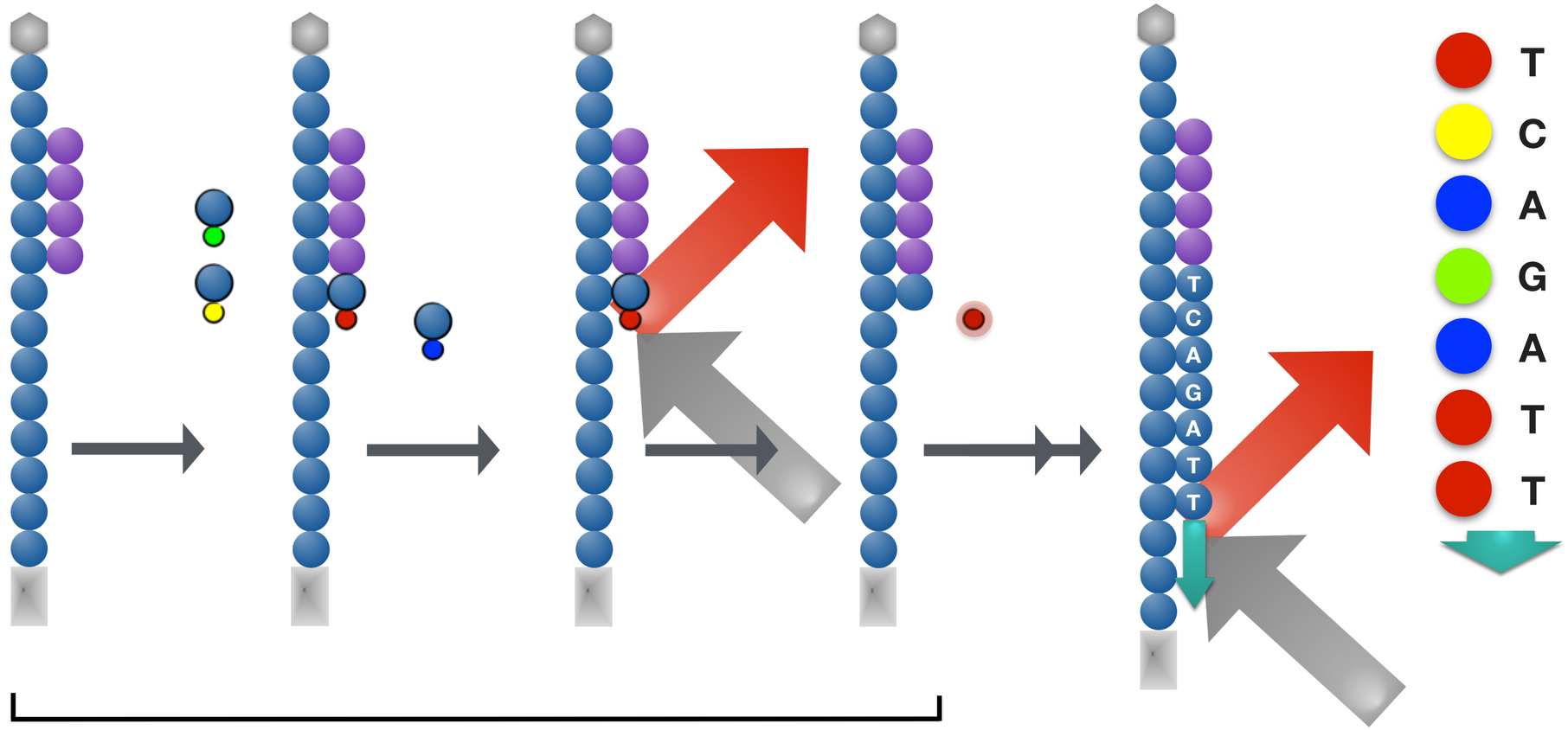


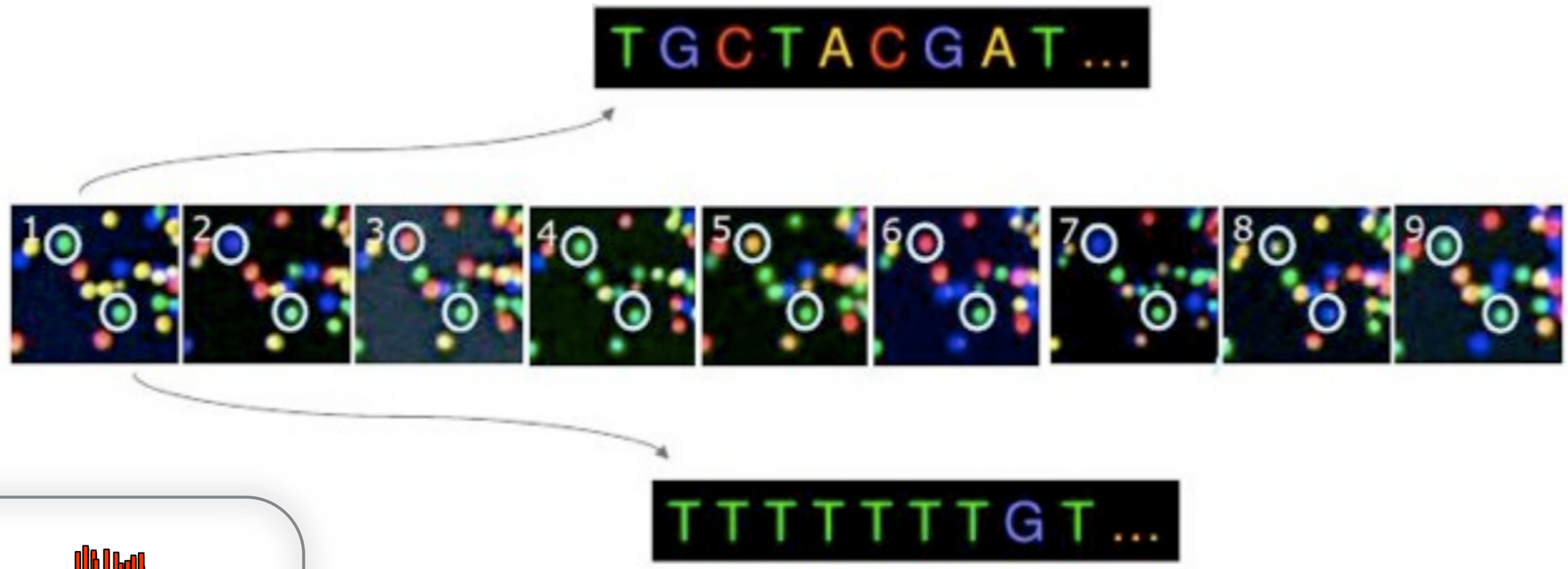
Illumina: prepare for sequencing

http://training.bioinformatics.ucdavis.edu/docs/2014/09/september-2014-workshop/Monday_JF_HTS_lecture.html



Illumina: sequencing by synthesis

http://training.bioinformatics.ucdavis.edu/docs/2014/09/september-2014-workshop/Monday_JF_HTS_lecture.html



Illumina: base calling

http://www.slideshare.net/CRS4/chris-jones-crs4-staff-meeting-24032010

Color Chemistry

4 color chemistry

2 color excitation / 4 color emission

Base	G filter	A filter	T filter	C filter
G	✓	x	x	x
A	x	✓	x	x
T	x	x	✓	x
C	x	x	x	✓
N	x	x	x	x

Poor quality reads show as NNN

2 color chemistry

2 color excitation / 2 color emission

Base	Green filter	Red filter
G	x	x
A	✓	✓
T	✓	x
C	x	✓
N	x	x

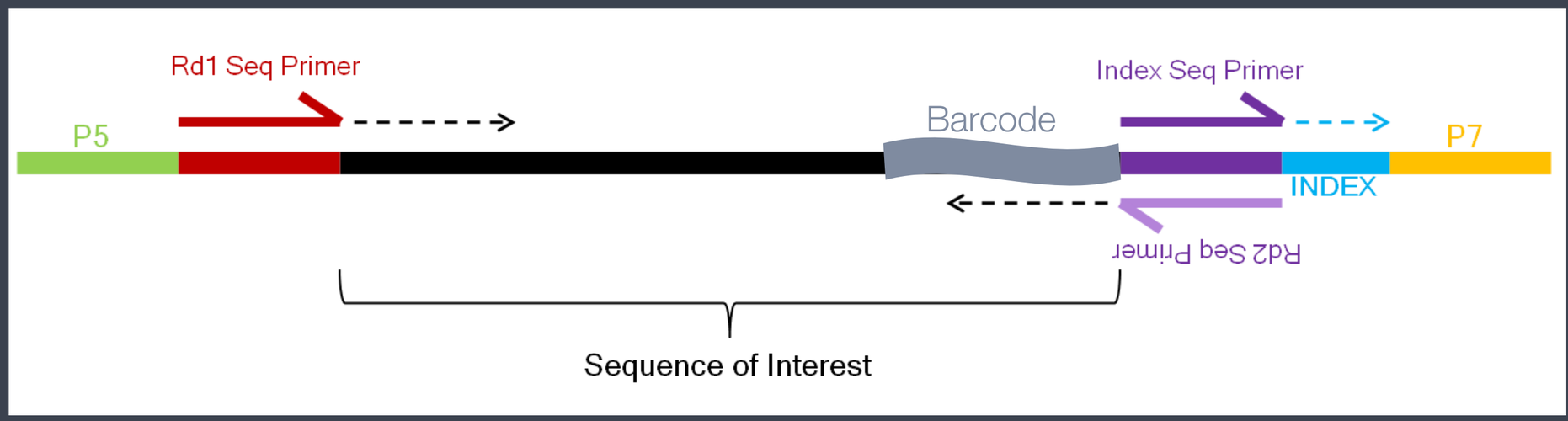
Poor quality reads show as GGG

https://www.slideshare.net/tallphil/scilifelab-ngi-novaseq-seminar/2

Sequencing Terminology

- Number of clusters = Number of reads
- Number of sequencing cycles = Length of reads
- Single End (SE) sequencing reads the forward strand of DNA formed at cluster generation.
- Paired End (PE) sequencing reads the forward and reverse strand of DNA.

Library Structure & Read Order



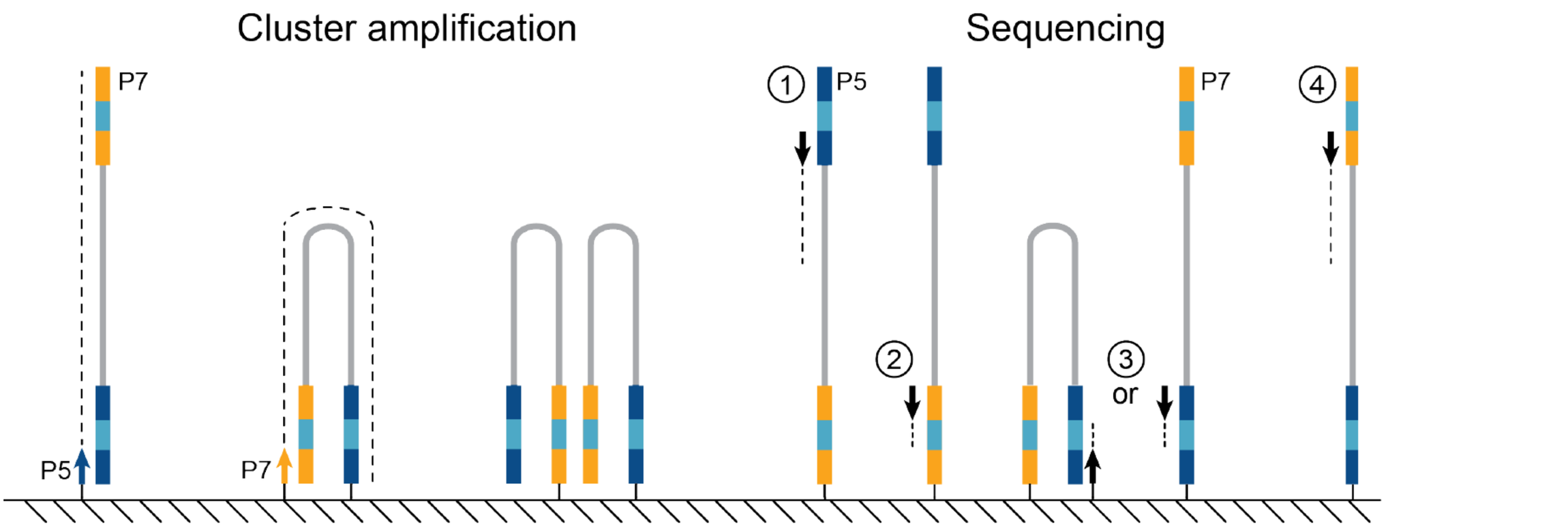
1. Read 1
2. Index Read 1 (i7)
3. Index Read 2 (i5)
4. Read 2

HiSeq/MiSeq (4 color)

A&C read on one camera

G&T read on other camera

NextSeq/NovaSeq (2 color)



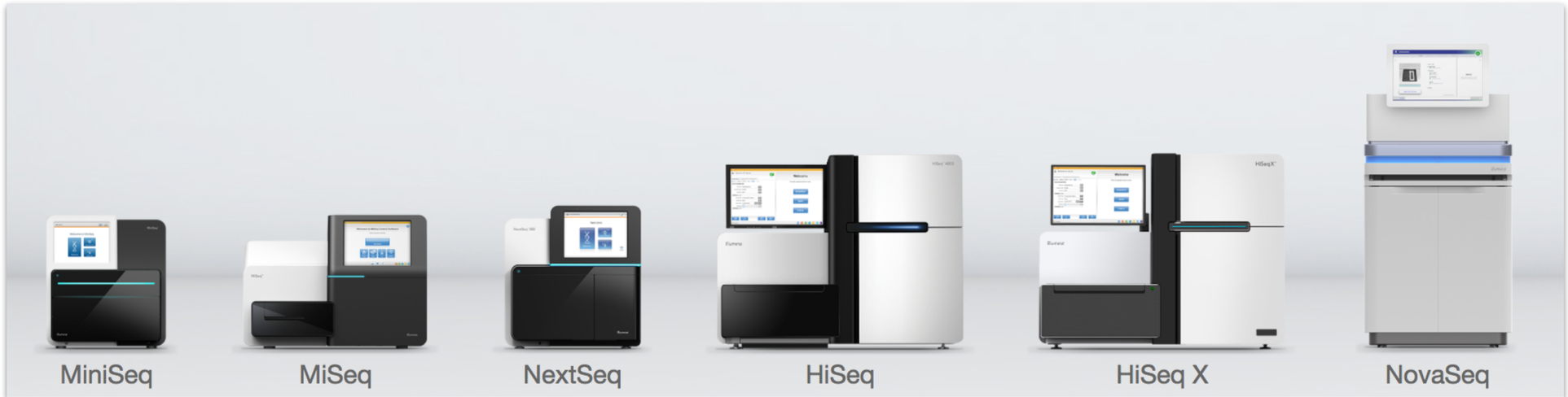
IDT
INTEGRATED DNA TECHNOLOGIES

Illumina: paired-end sequencing

https://www.slideshare.net/idtdna/unique-dualmatched-adapters-mitigate-index-hopping-between-ngs-samples

Paired End Sequencing

- After read 1 the product is washed away.
- After the index 1 read the product is washed away.
- Attached oligo bends over and binds the other adapter on the flow cell to perform 1 cycle of bridge amplification.
- 3’ ends are blocked and the forward strand is cleaved off.
- Sequencing of the reverse strand during read 2 proceeds via sequencing by synthesis.

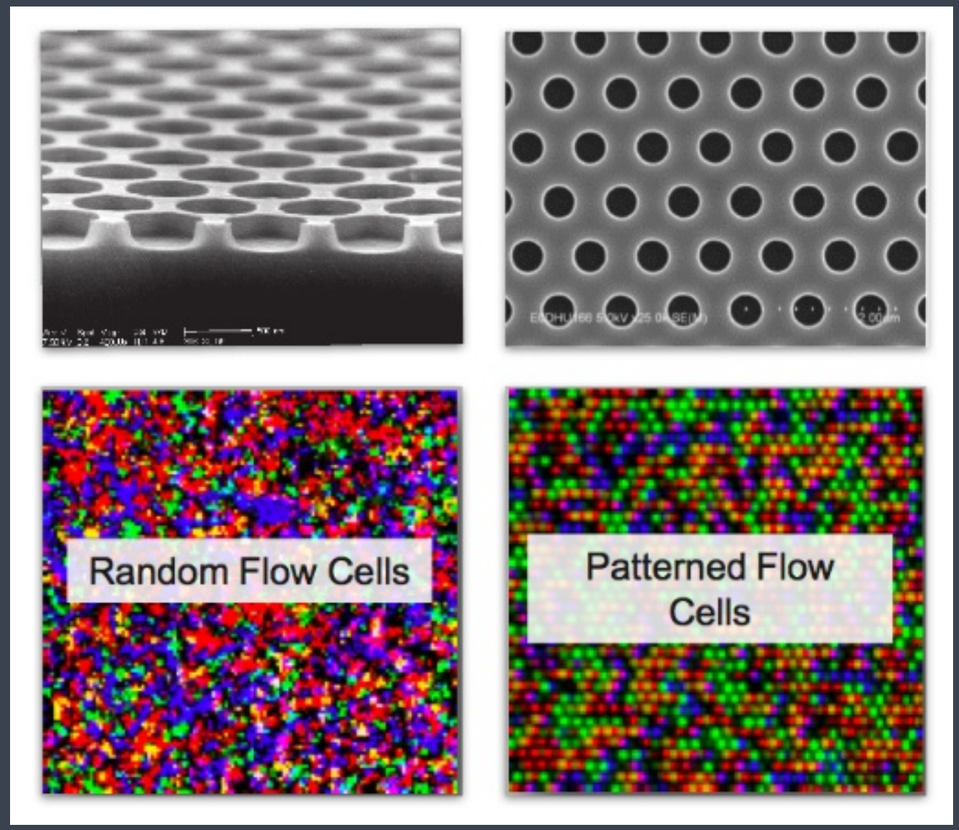


Illumina: sequencing platforms

https://www.illumina.com/systems/sequencing-platforms.html

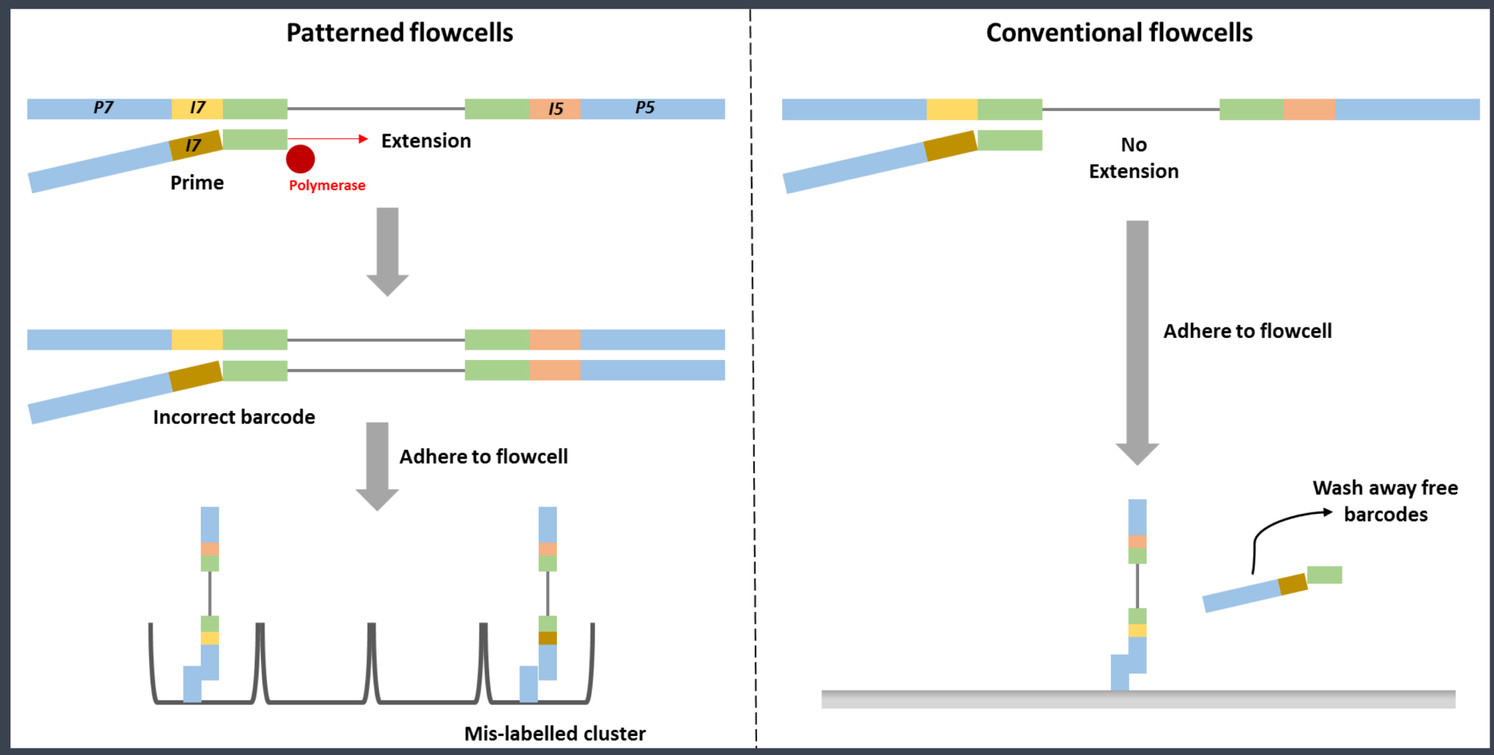
Patterned Flow Cells

- HiSeq 4000, HiSeq X, NovaSeq
- Single sequence per well
  - Higher density, more data
- ExAmp Chemistry
- Different side effects
  - Index hopping
  - Duplicate reads
  - Intolerance to variable insert size



https://www.slideshare.net/tallphil/scilifelab-ngi-novaseq-seminar/2

Index Hopping



- Free index primers in library can prime fragments and generate miss-indexed or miss-barcoded samples.
- Dual indexing greatly reduces index hopping issues.

https://sequencing.qcfail.com/articles/the-latest-illumina-sequencers-muddle-samples/