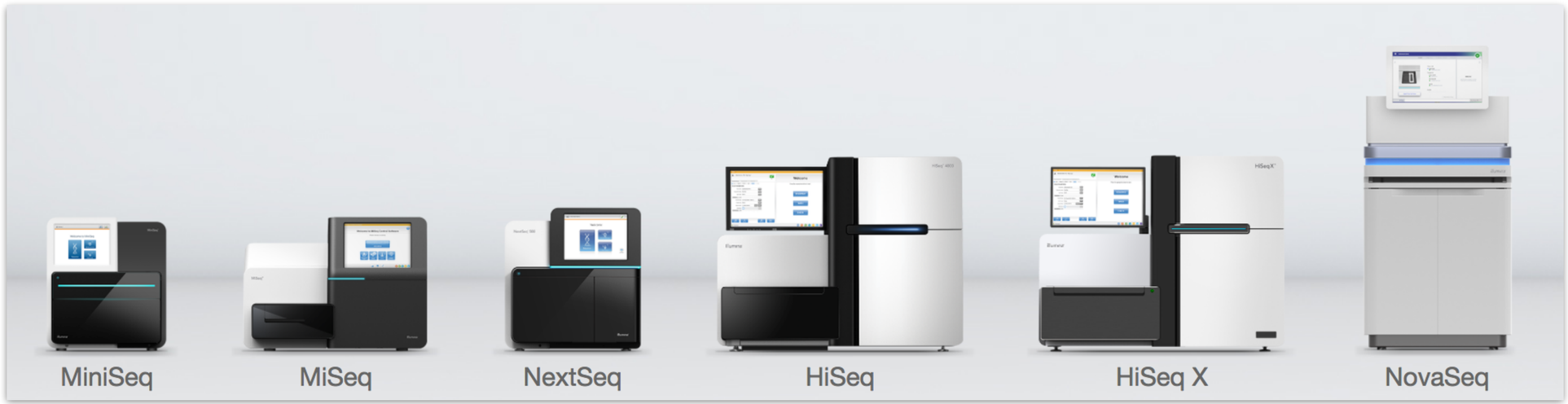
Sequencing Technologies



Illumina: Sequencing Platforms

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

Benchtop

iSeq 100 System

MiniSeq System

MiSeq Series ⊕

NextSeq Series ⊕

Run Time	9–17.5 hours	4–24 hours	4–55 hours	12–30 hours
Maximum Output	1.2 Gb	7.5 Gb	15 Gb	120 Gb
Maximum Reads Per Run	4 million	25 million	25 million †	400 million
Maximum Read Length	2 × 150 bp	2 × 150 bp	2 × 300 bp	2 × 150 bp

Production-Scale

NextSeq Series ⊕

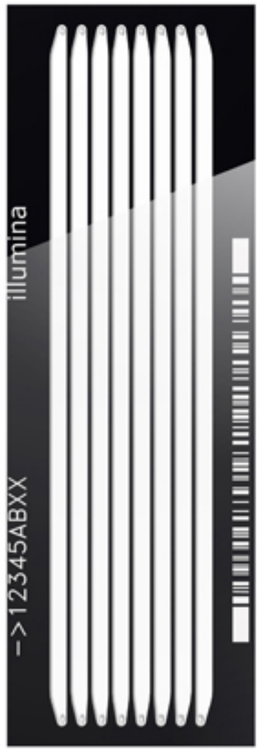
HiSeq Series ⊕

HiSeq X Series‡

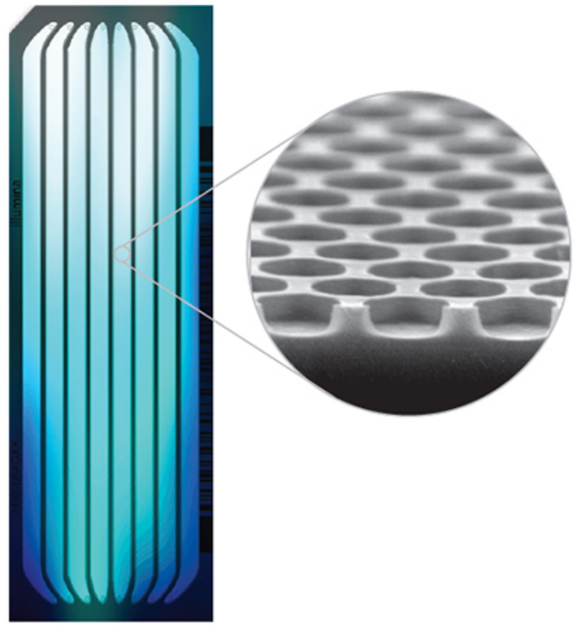
NovaSeq 6000 System

Run Time	12–30 hours	< 1–3.5 days (HiSeq 3000/HiSeq 4000) 7 hours–6 days (HiSeq 2500)	< 3 days	16–36 hours (Dual S2 flow cells) 44 hours (Dual S2 flow cells)
Maximum Output	120 Gb	1500 Gb	1800 Gb	6000 Gb
Maximum Reads Per Run	400 million	5 billion	6 billion	20 billion
Maximum Read Length	2 × 150 bp	2 × 150 bp	2 × 150 bp	2 × 150 bp

Nextseq500



HiSeq2500

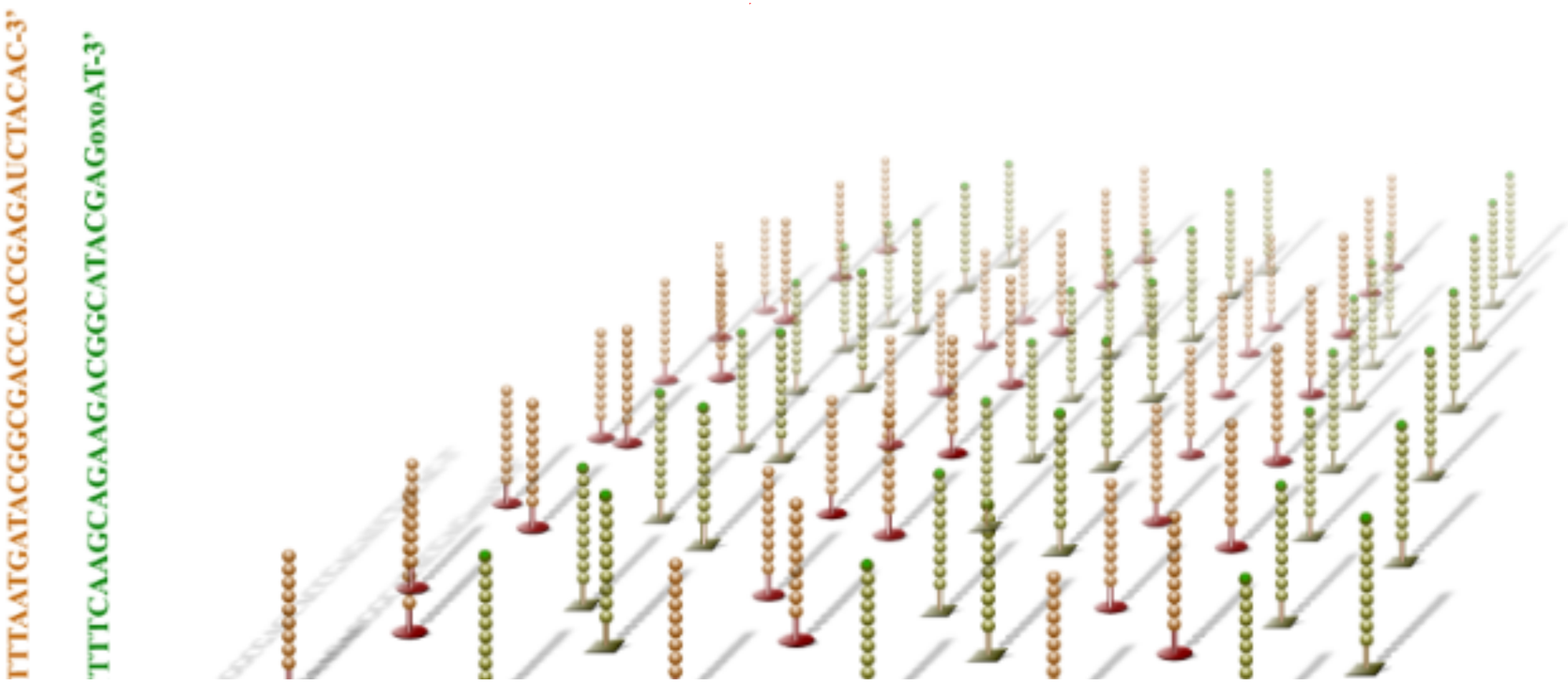


HiSeq3000/4000

Illumina: flow cell

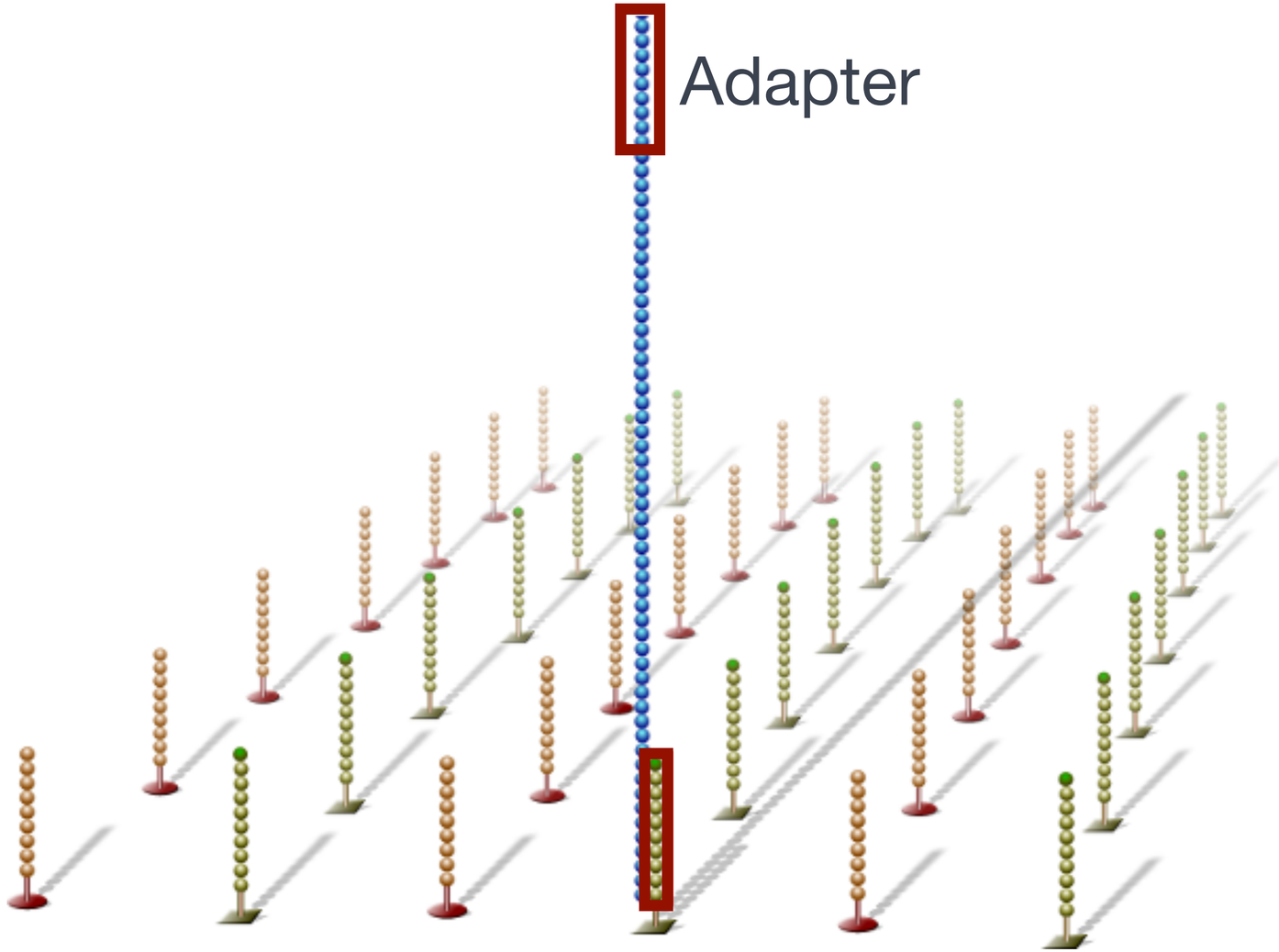
https://www.illumina.com/company/news-center/multimedia-images.html

Illumina Sequencing by Synthesis
How does it work?



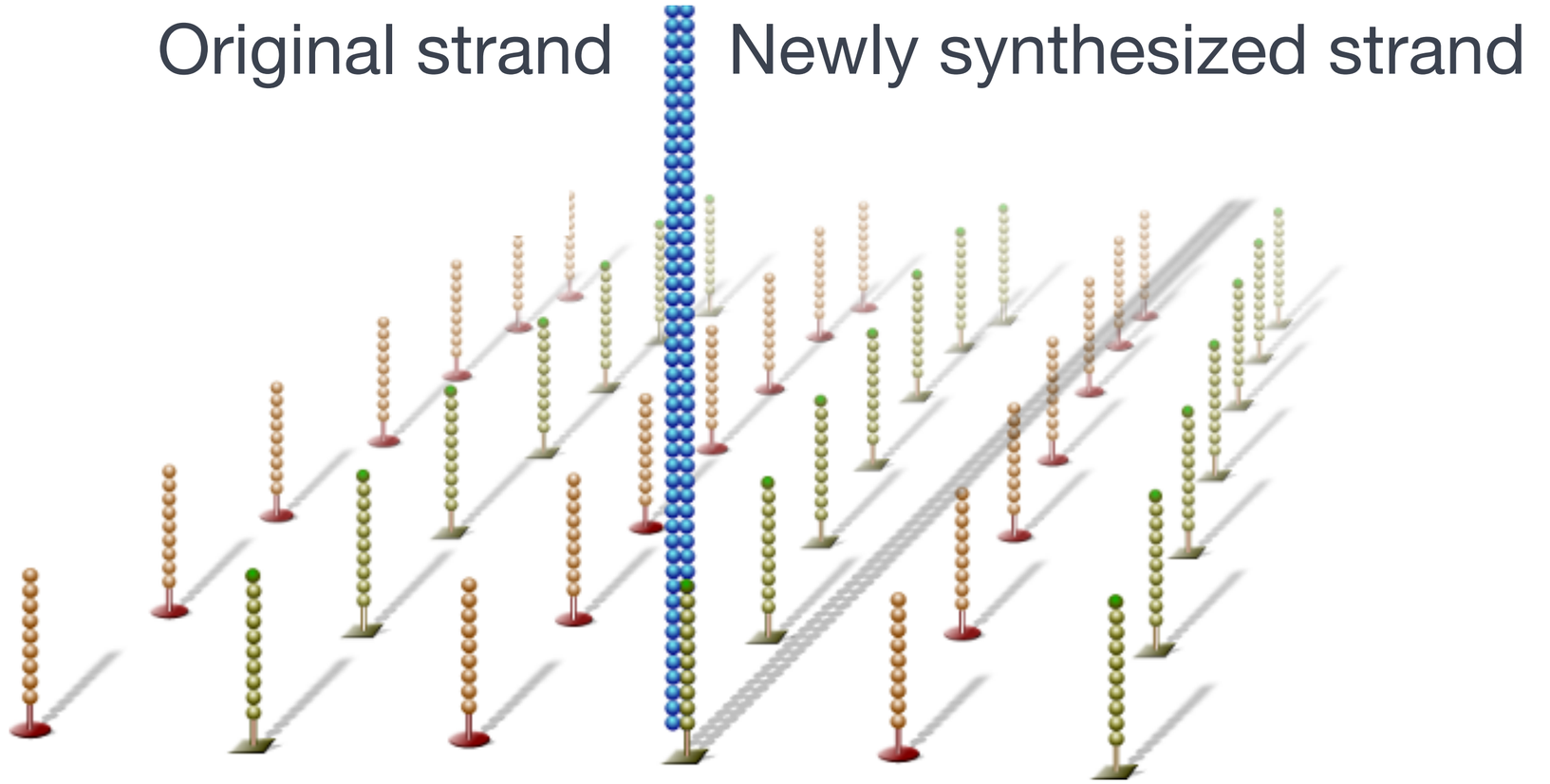
Illumina: flow cell

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



Illumina: cluster generation

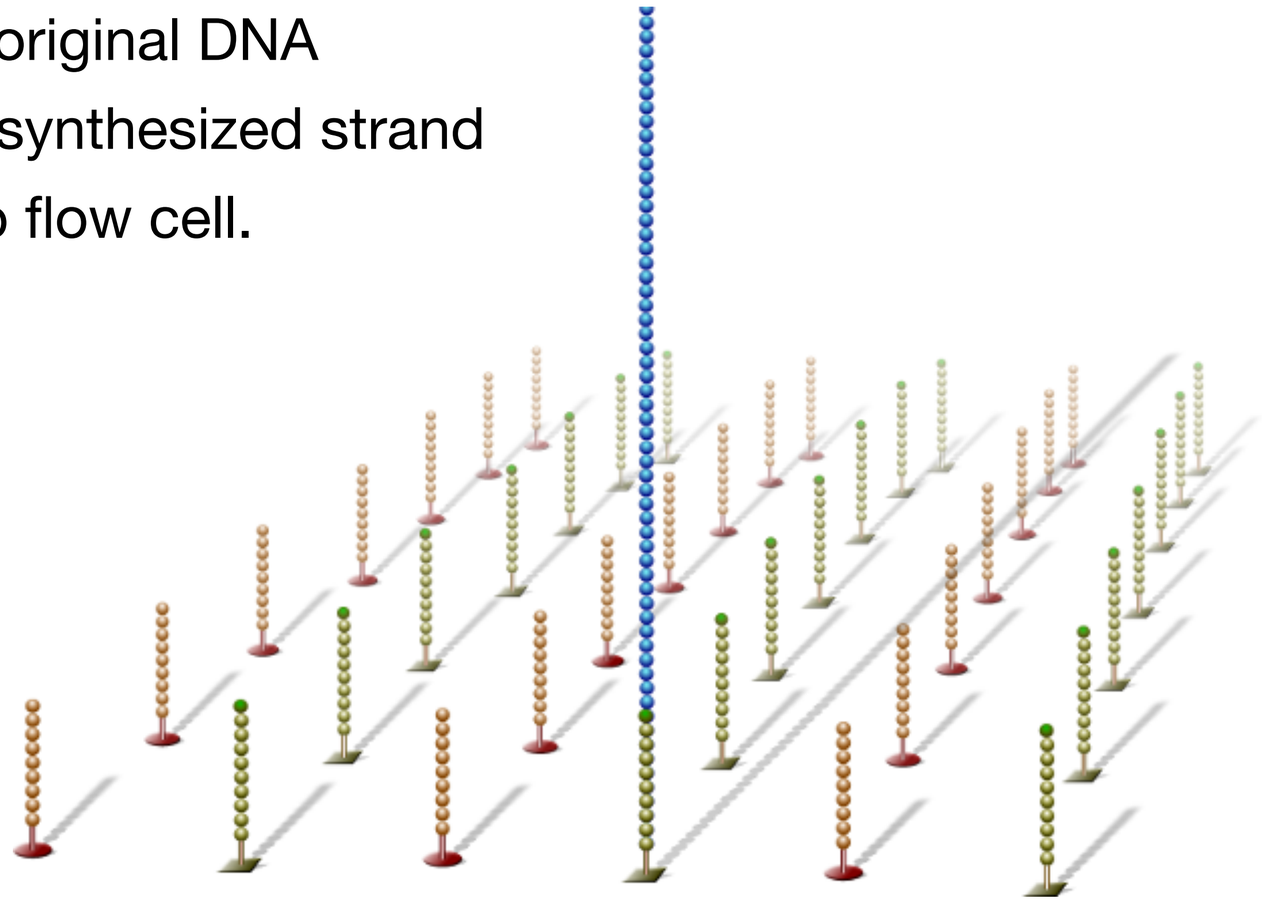
http://training.bioinformatics.ucdavis.edu/docs/2014/09/september-2014-workshop/Monday_JF_HTS_lecture.html



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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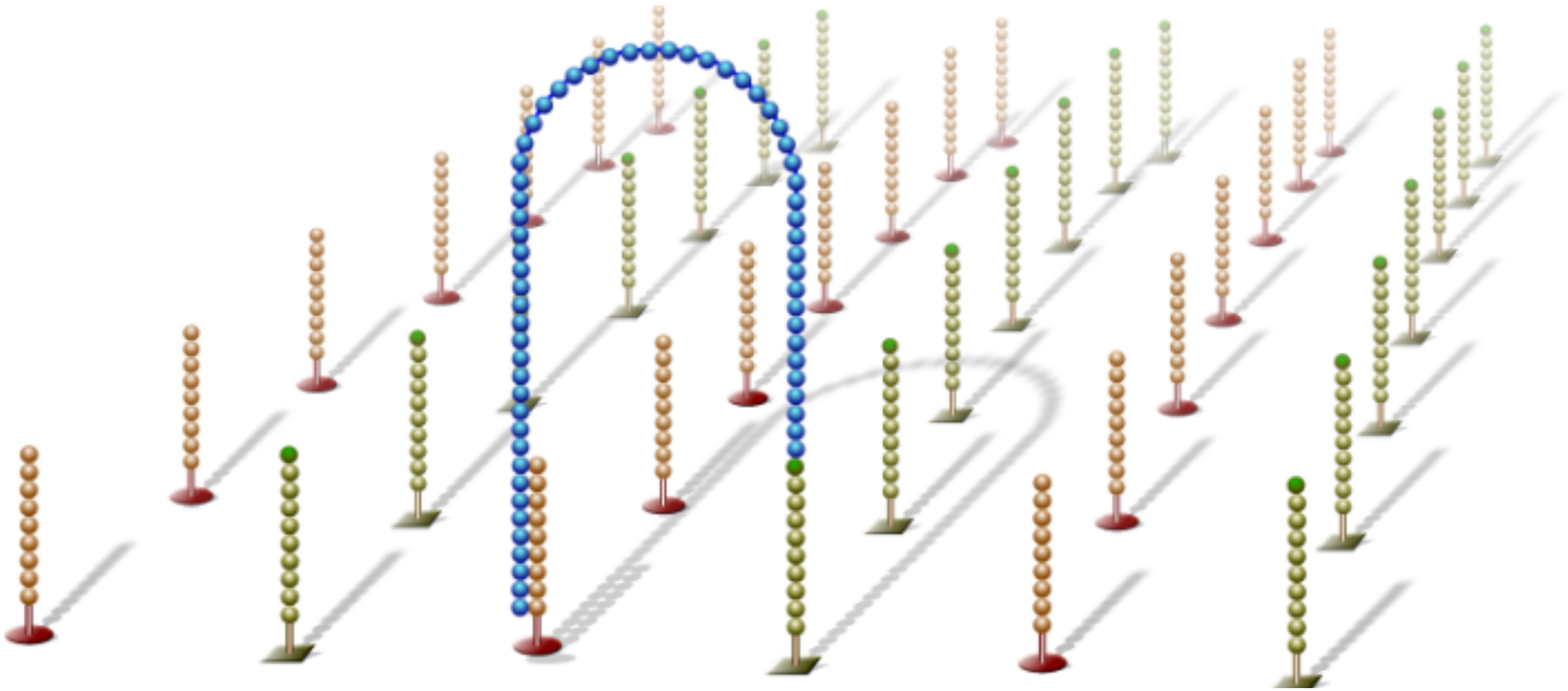
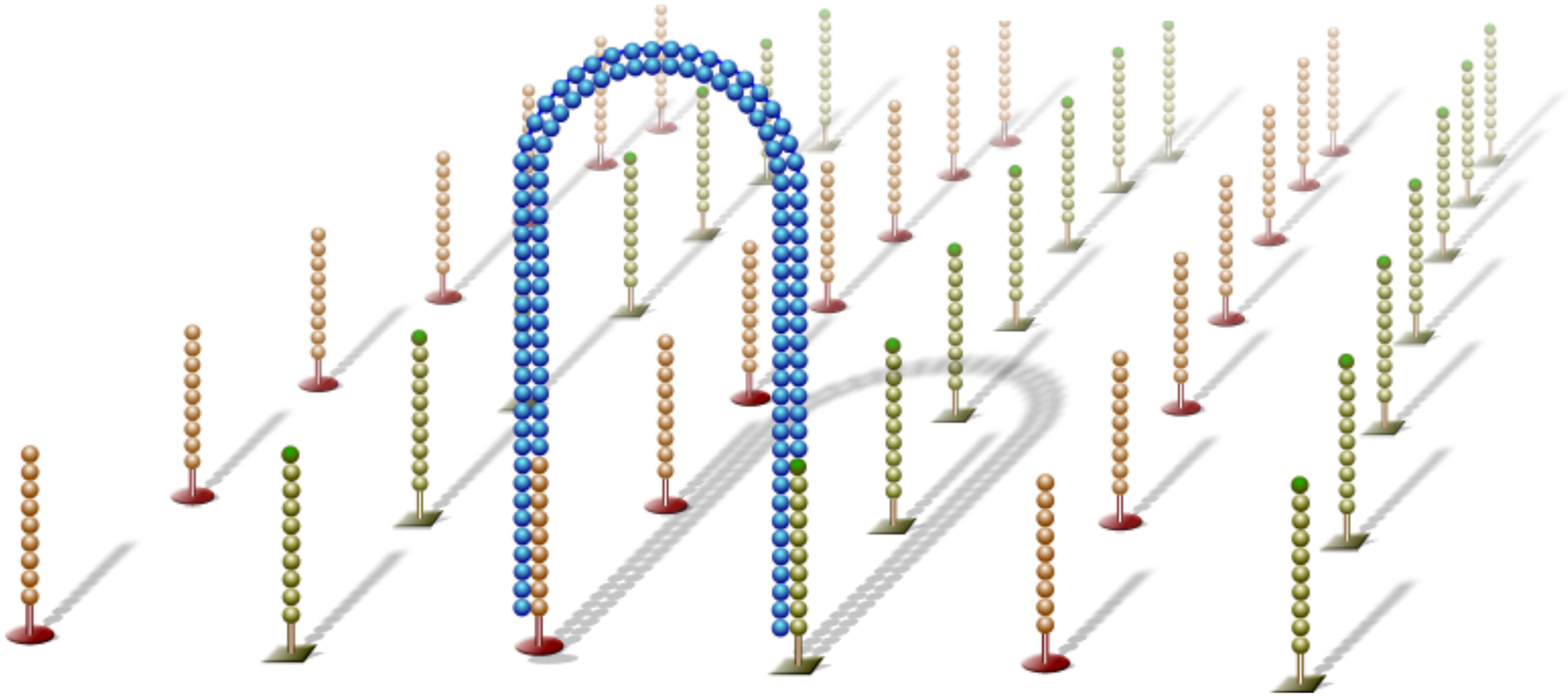


Illumina: bridge amplification

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



Illumina: bridge amplification

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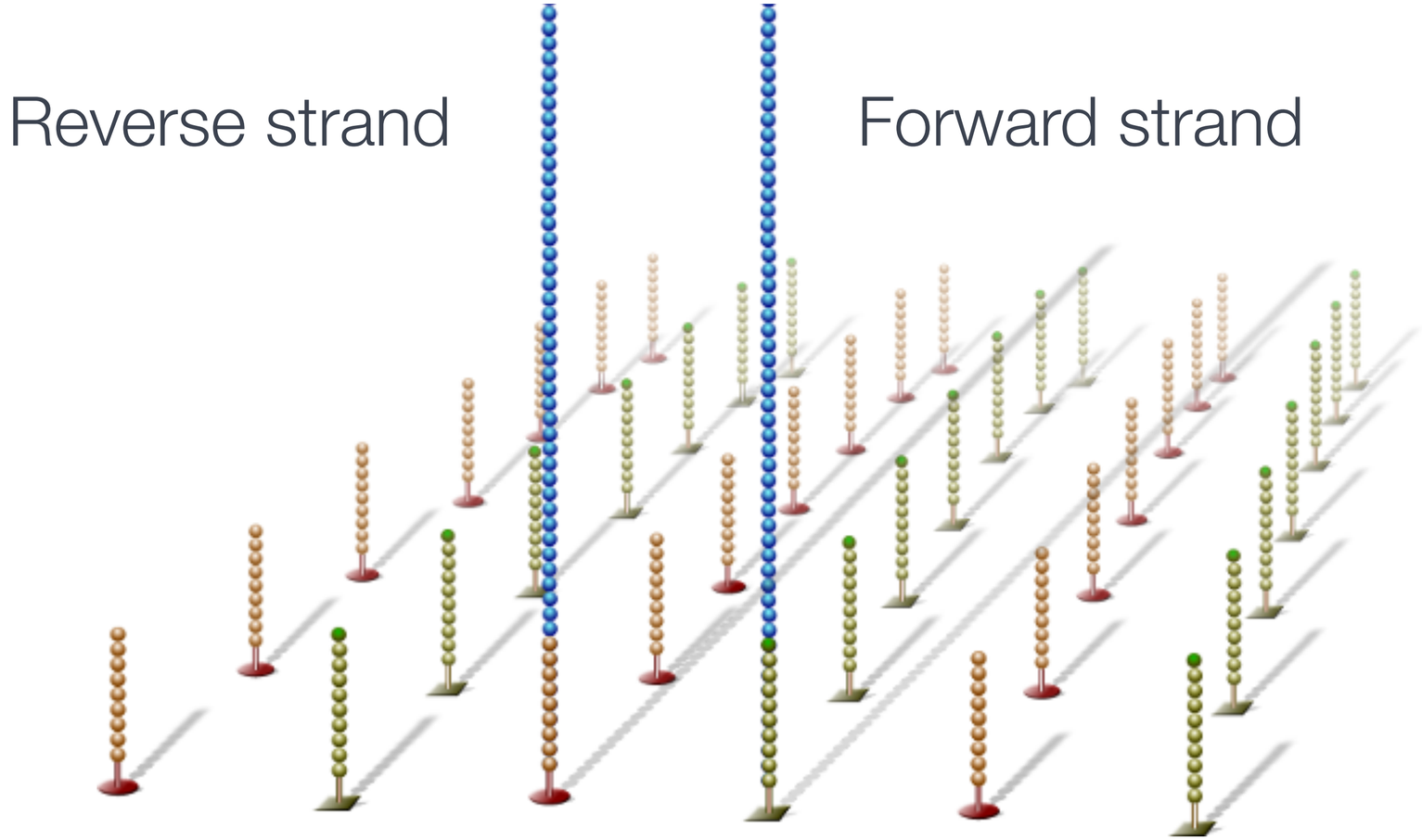

Illumina: bridge amplification

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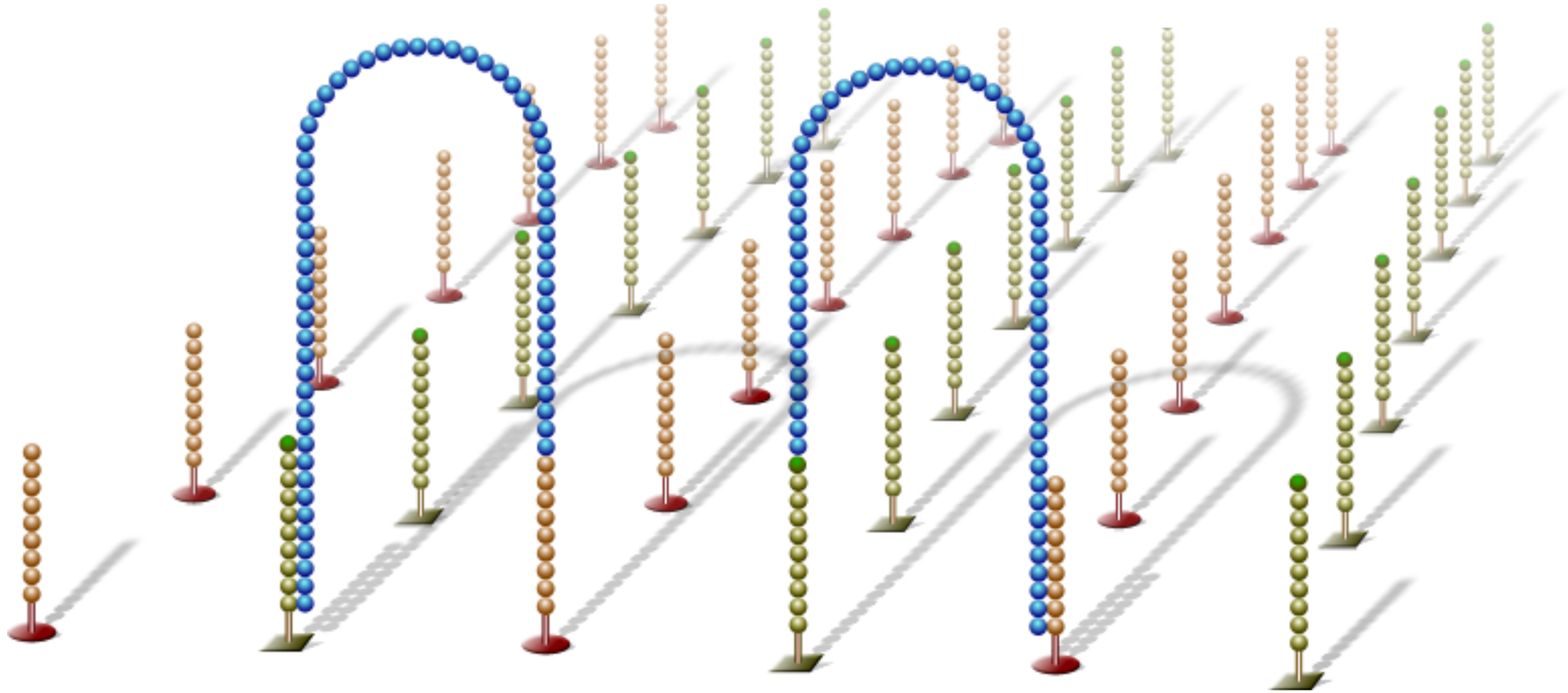
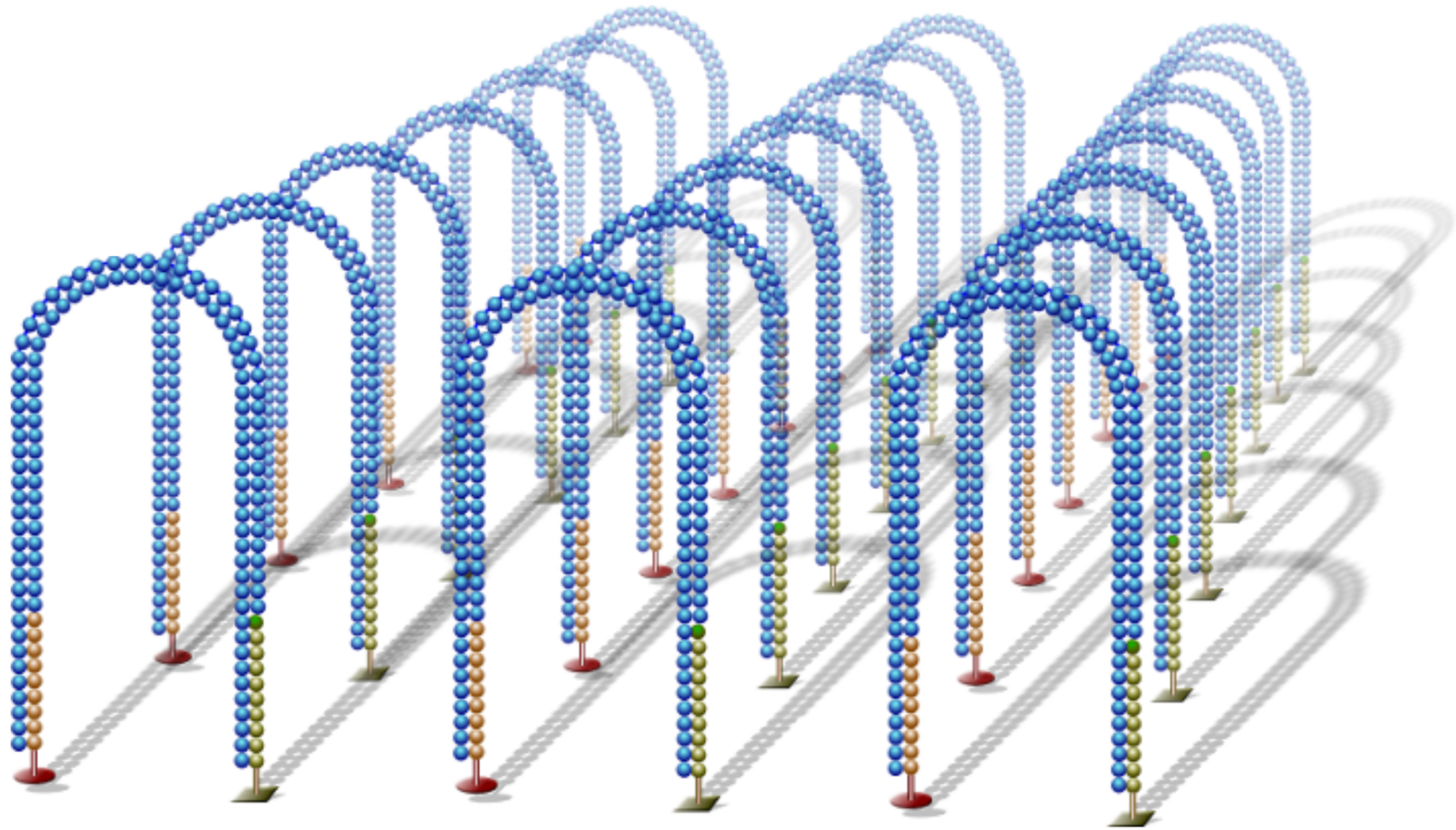


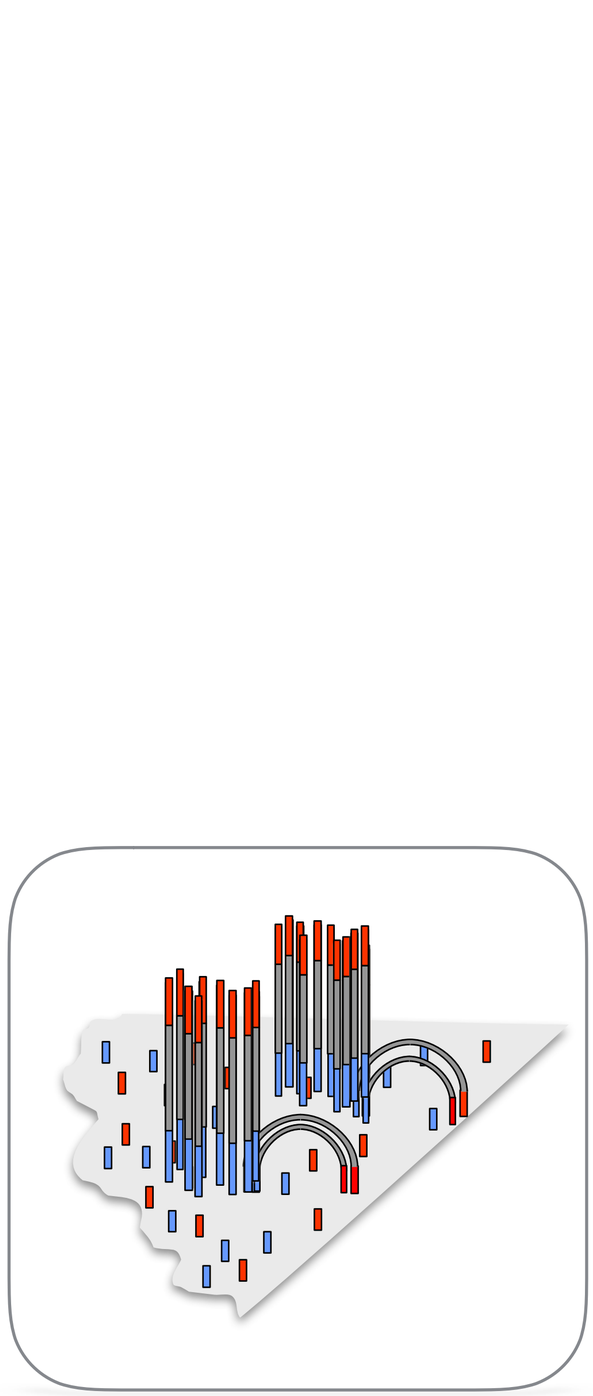
Illumina: bridge amplification

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

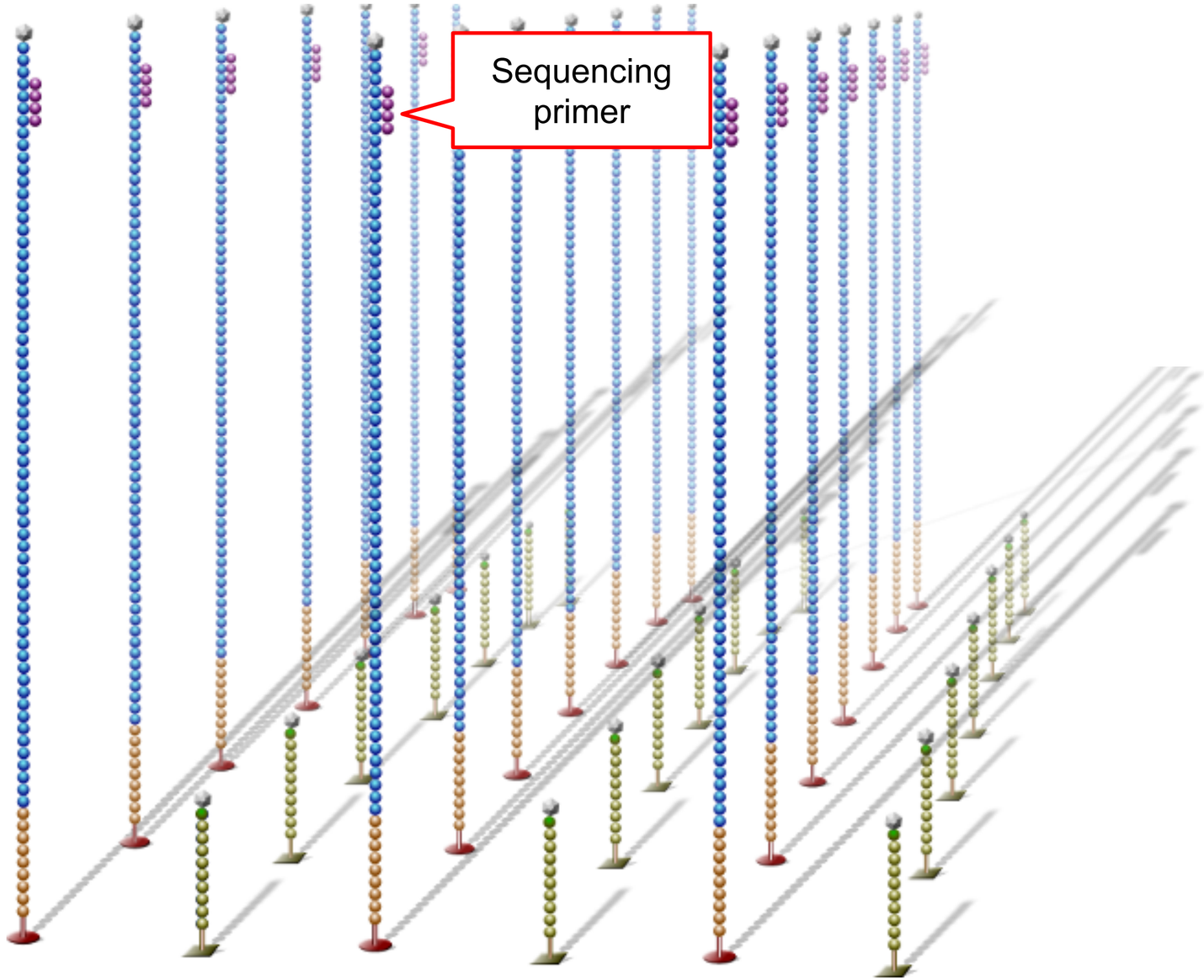


Illumina: bridge amplification

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

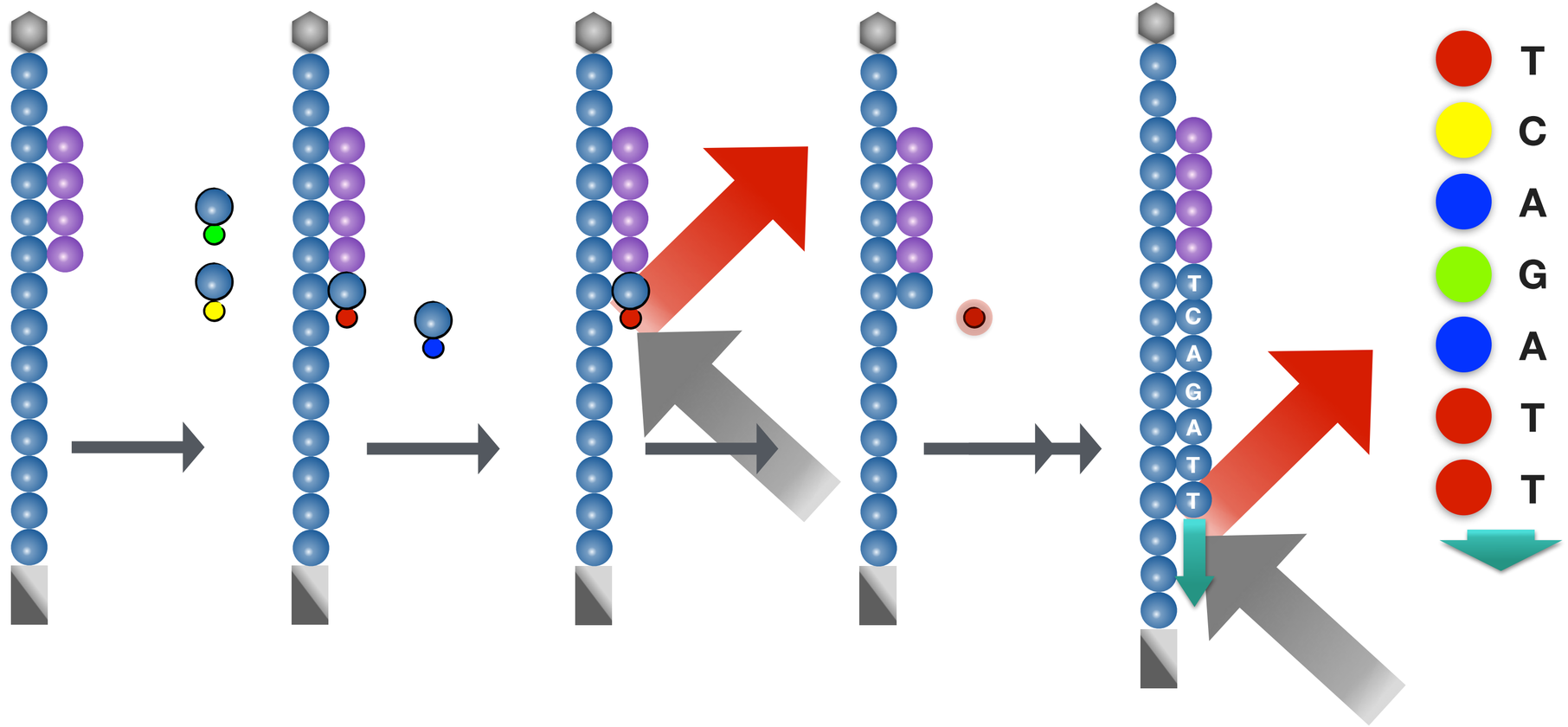


Illumina: cluster generation

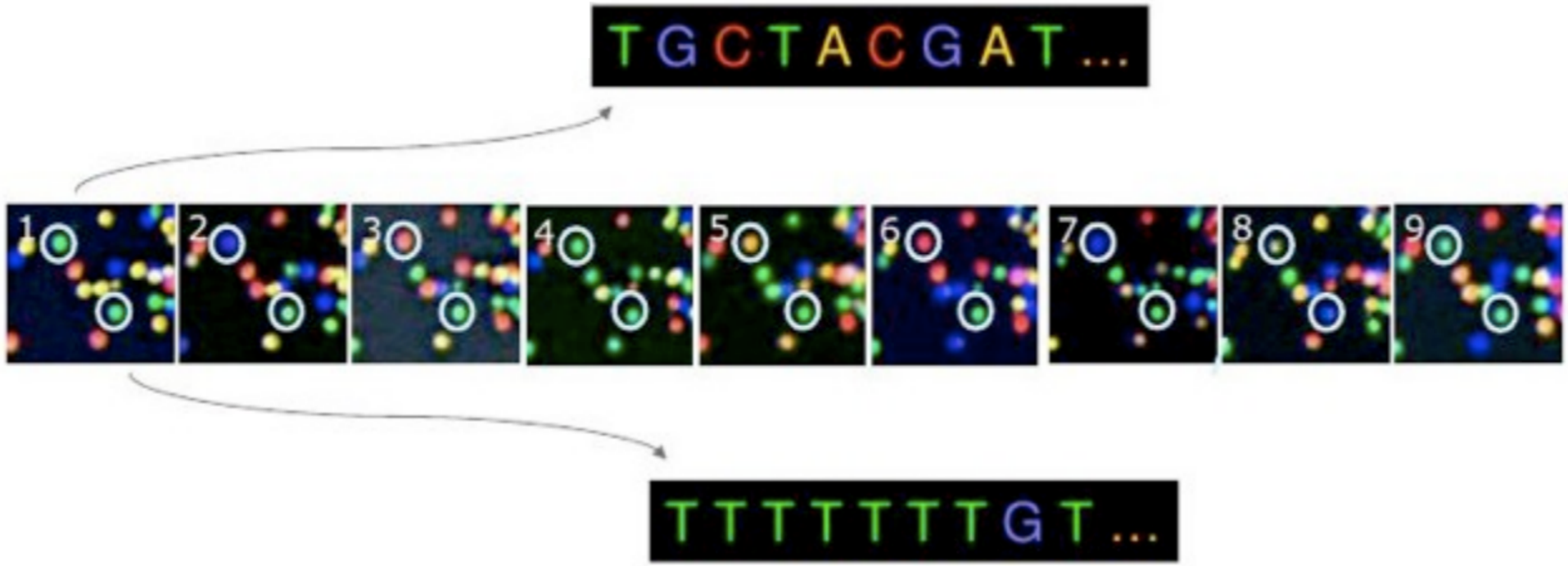


Illumina: Prepare for sequencing

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



Illumina: sequencing by synthesis



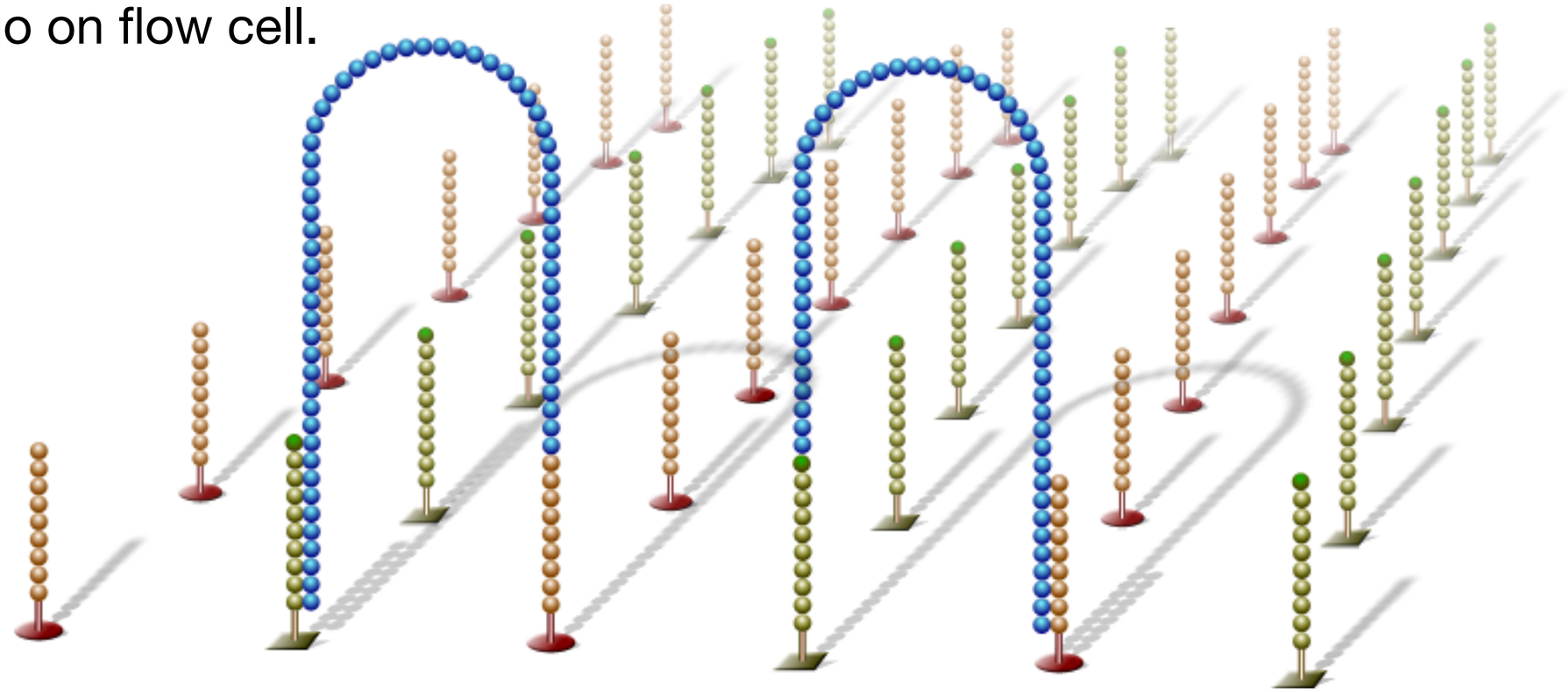
Illumina: base calling

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

Number of clusters ~= Number of reads

Number of sequencing cycles ~= Length of reads

dsDNA is denatured, and 3’ ends are de-protected. Template folds over and binds second oligo on flow cell.



Illumina: paired-end sequencing

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

Pacific Biosciences: http://www.pacb.com/

Oxford Nanopore (MinION): https://nanoporetech.com/

10X Genomics: https://www.10xgenomics.com/

Other Sequencing Platforms

	Advantages	Disadvantages
Pacific Biosciences	Iso-Seq protocol for transcripts up to 10Kb, high base calling accuracy	High cost, large machines
Oxford Nanopore	Various kits (direct RNA, direct cDNA, cDNA-PCR), short transcripts (< 700bp), portable, high yield	High errors rate affects assembling de novo transcripts, higher amount of cDNA input
10X Genomics	Low cost (integrated with short-read technology), barcoding for accurate isoform detection, low error rates	Extra preparation step (barcode), extra computational step

Transcriptomics with long read technologies

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