



HiSeq2000 / 2500



Ion torrent



Ion Proton

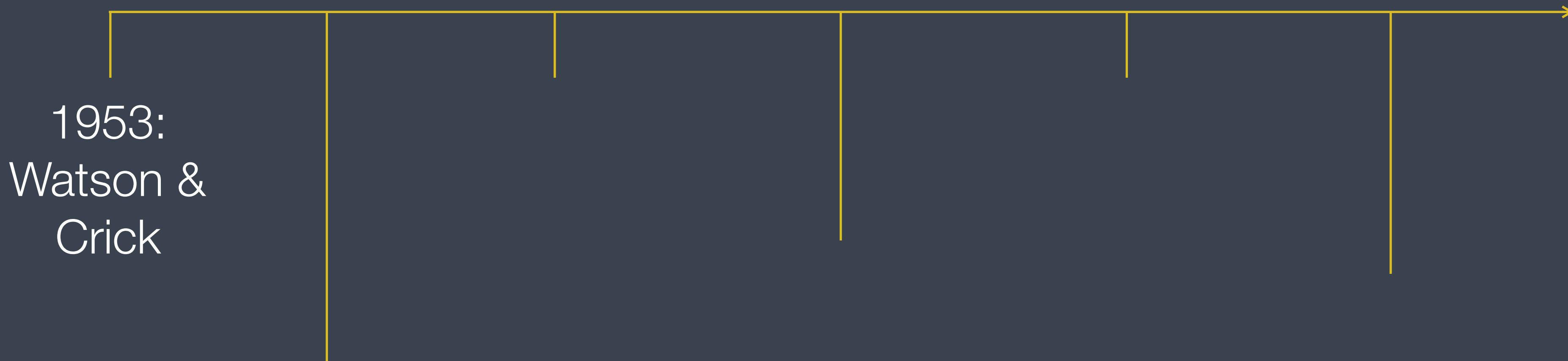


GS-FLX

MiSeq

Sequencing Technologies

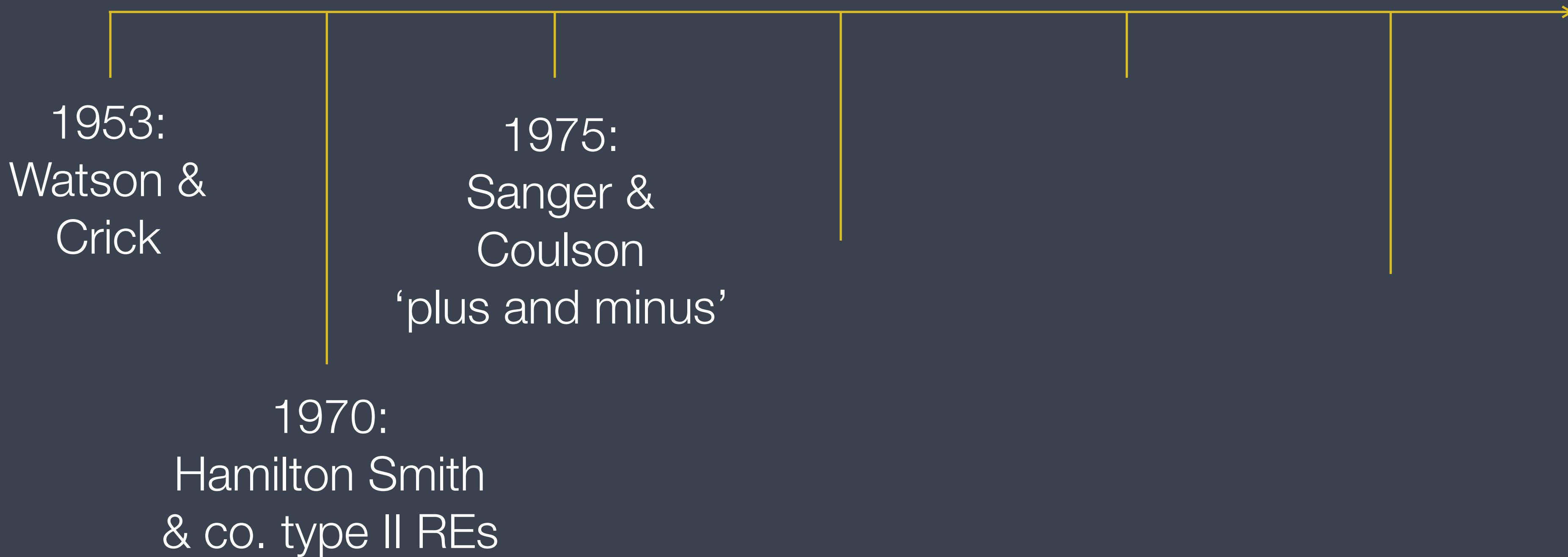
Unraveling DNA: The evolution of Sanger sequencing



Unraveling DNA: The evolution of Sanger sequencing



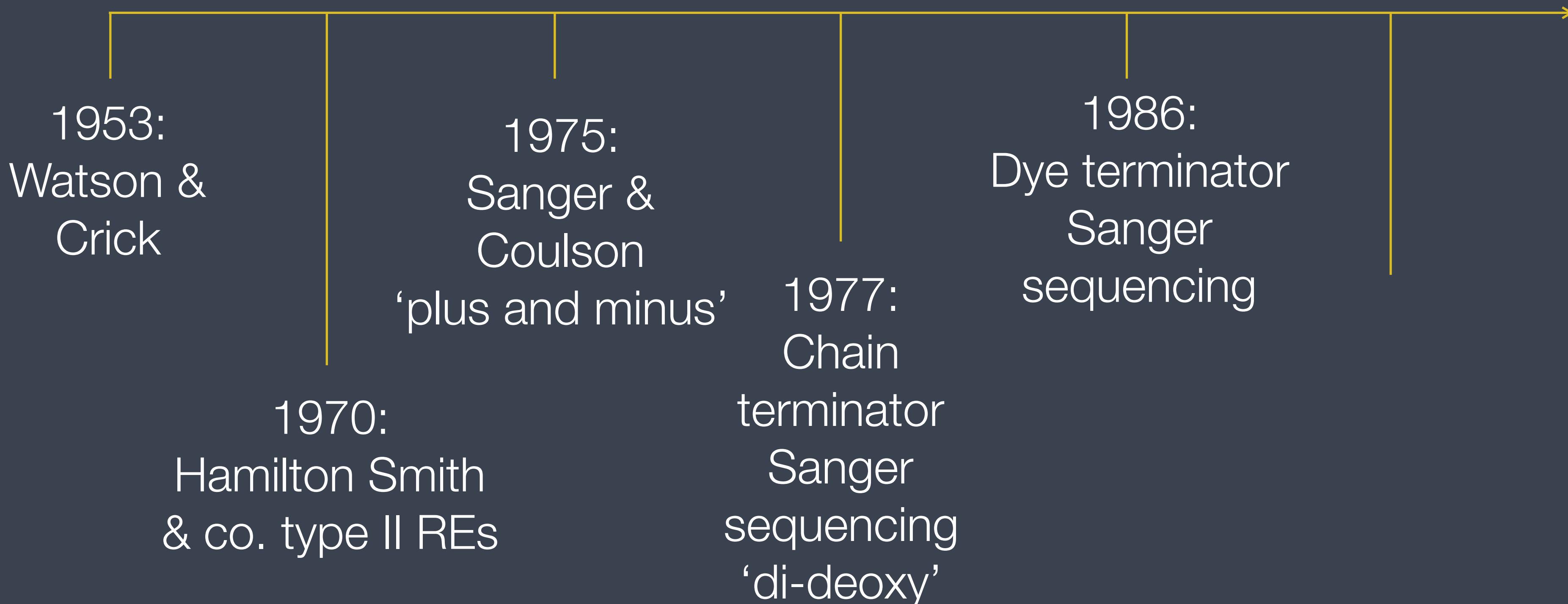
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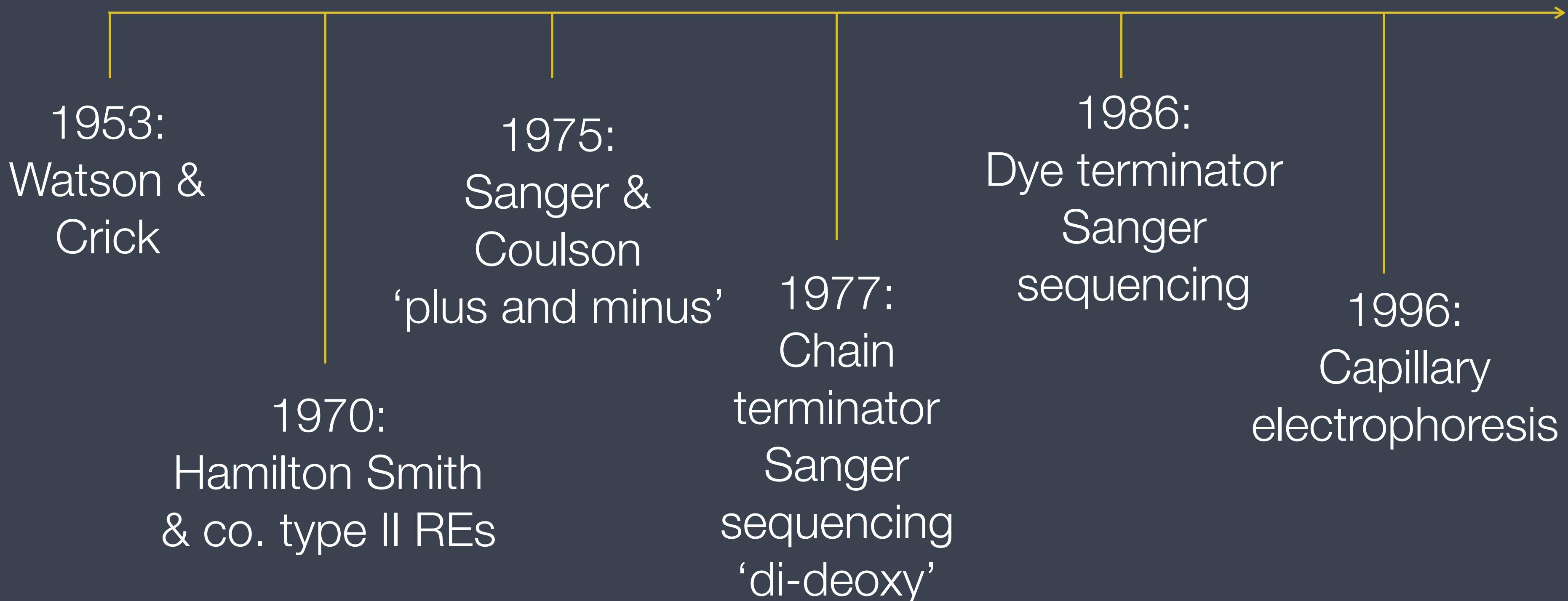
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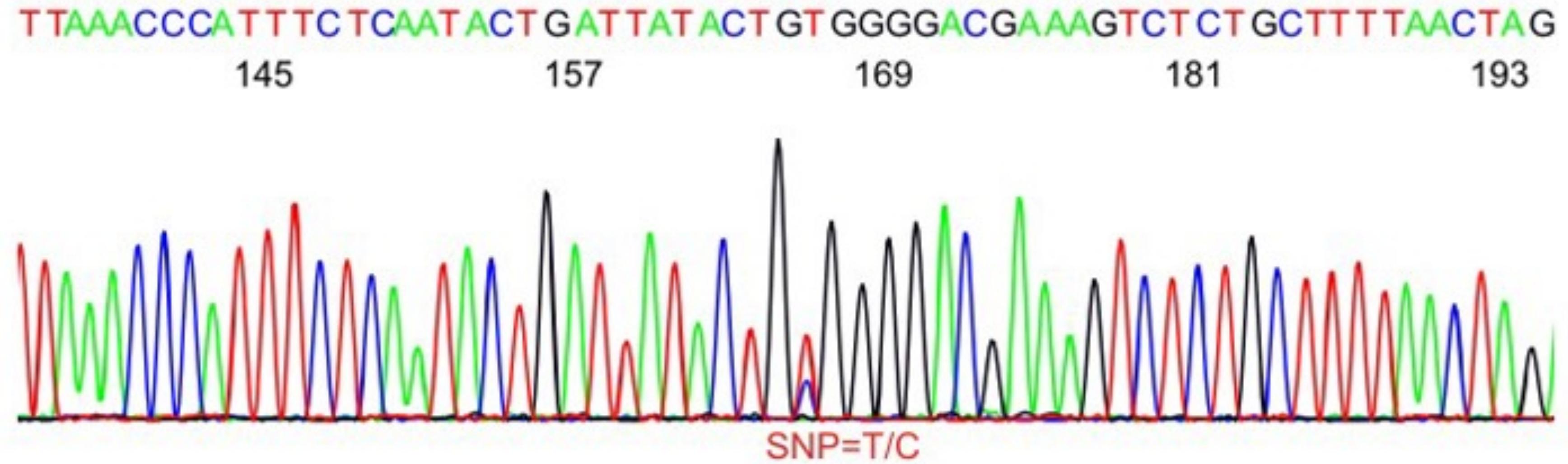


Unraveling DNA: The evolution of Sanger sequencing



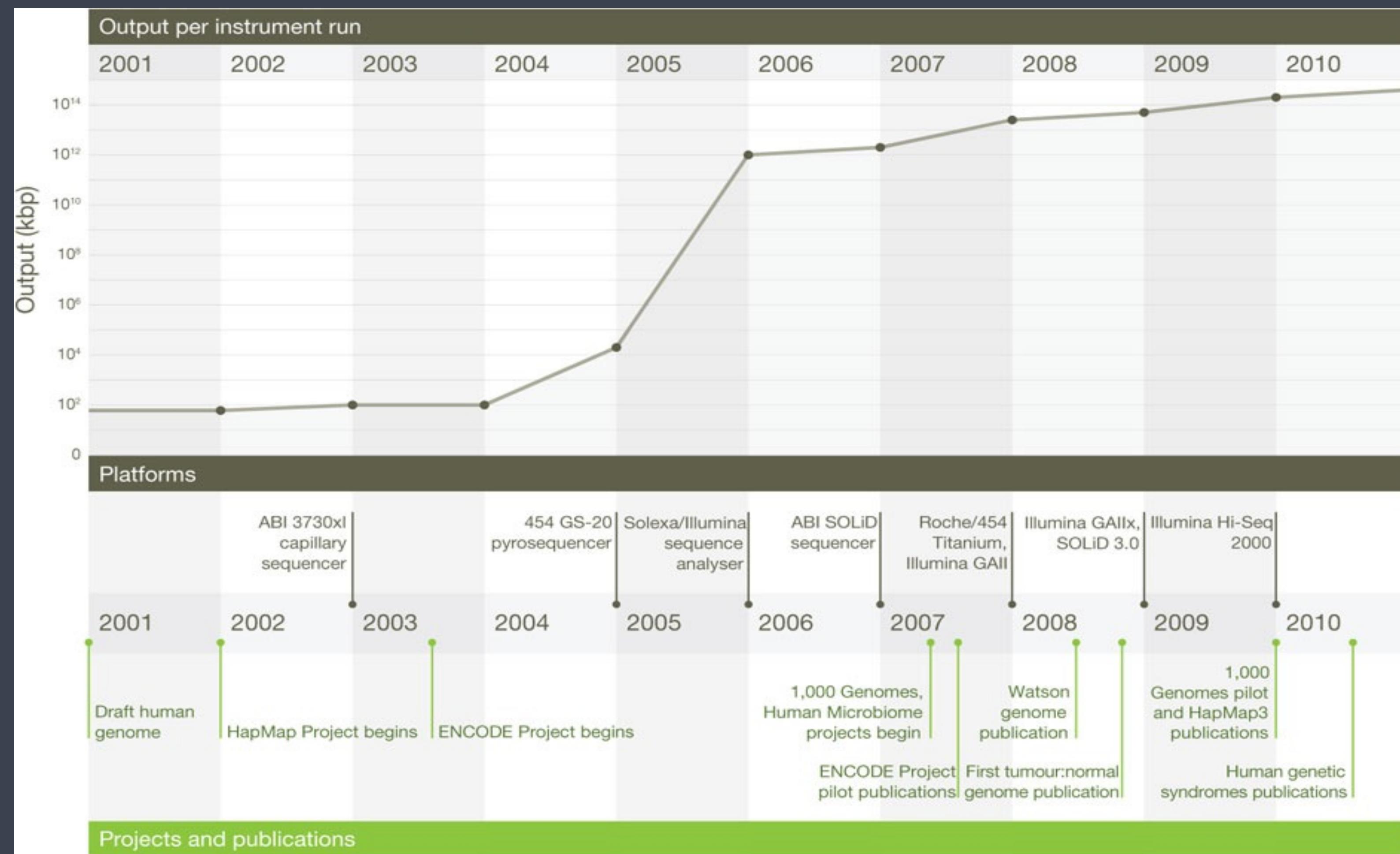
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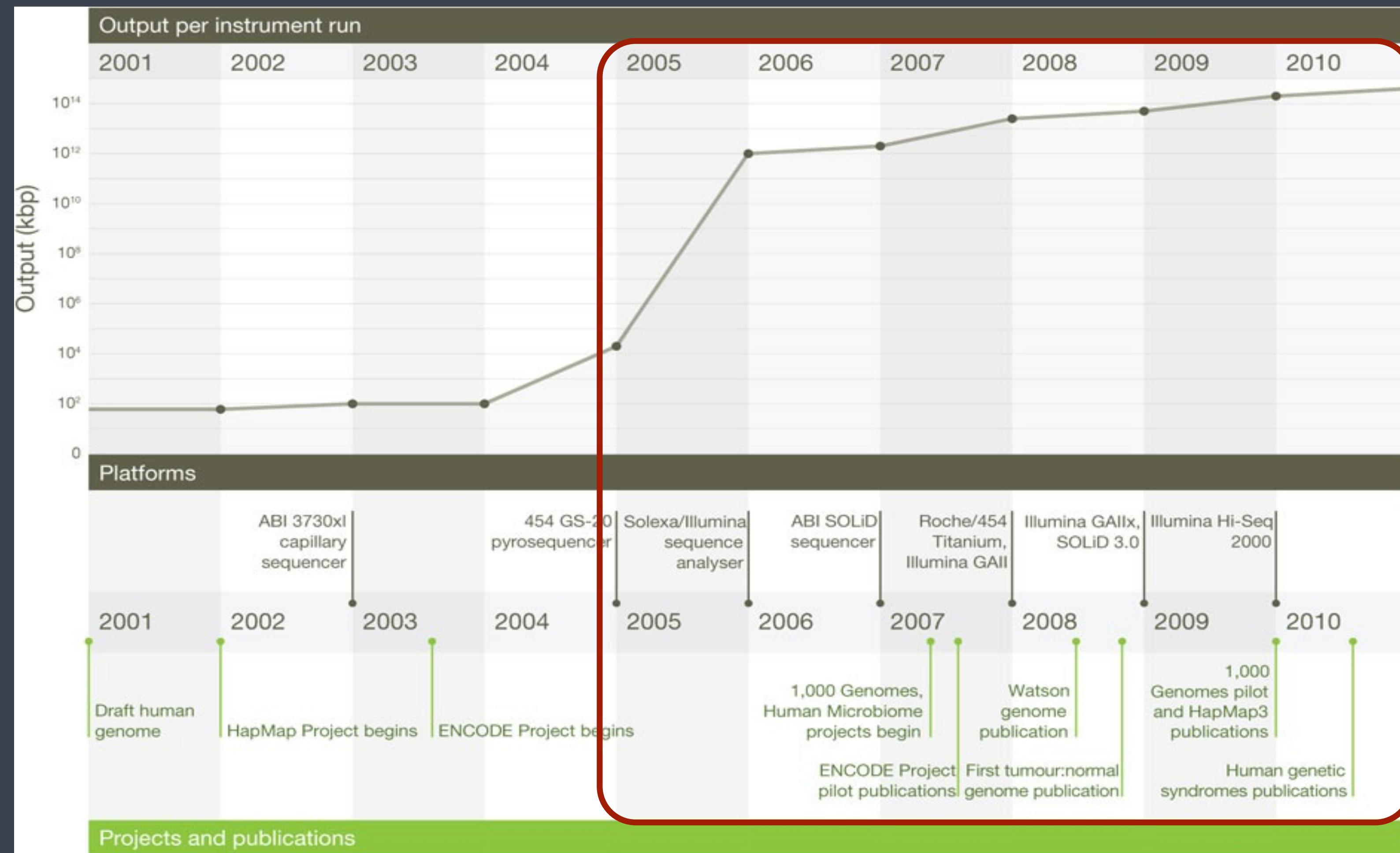




Interpreting sequencing data

<http://www.xcelrisgenomics.com/SS-SNPGenotypingbySequencingonABI3730xl.html>

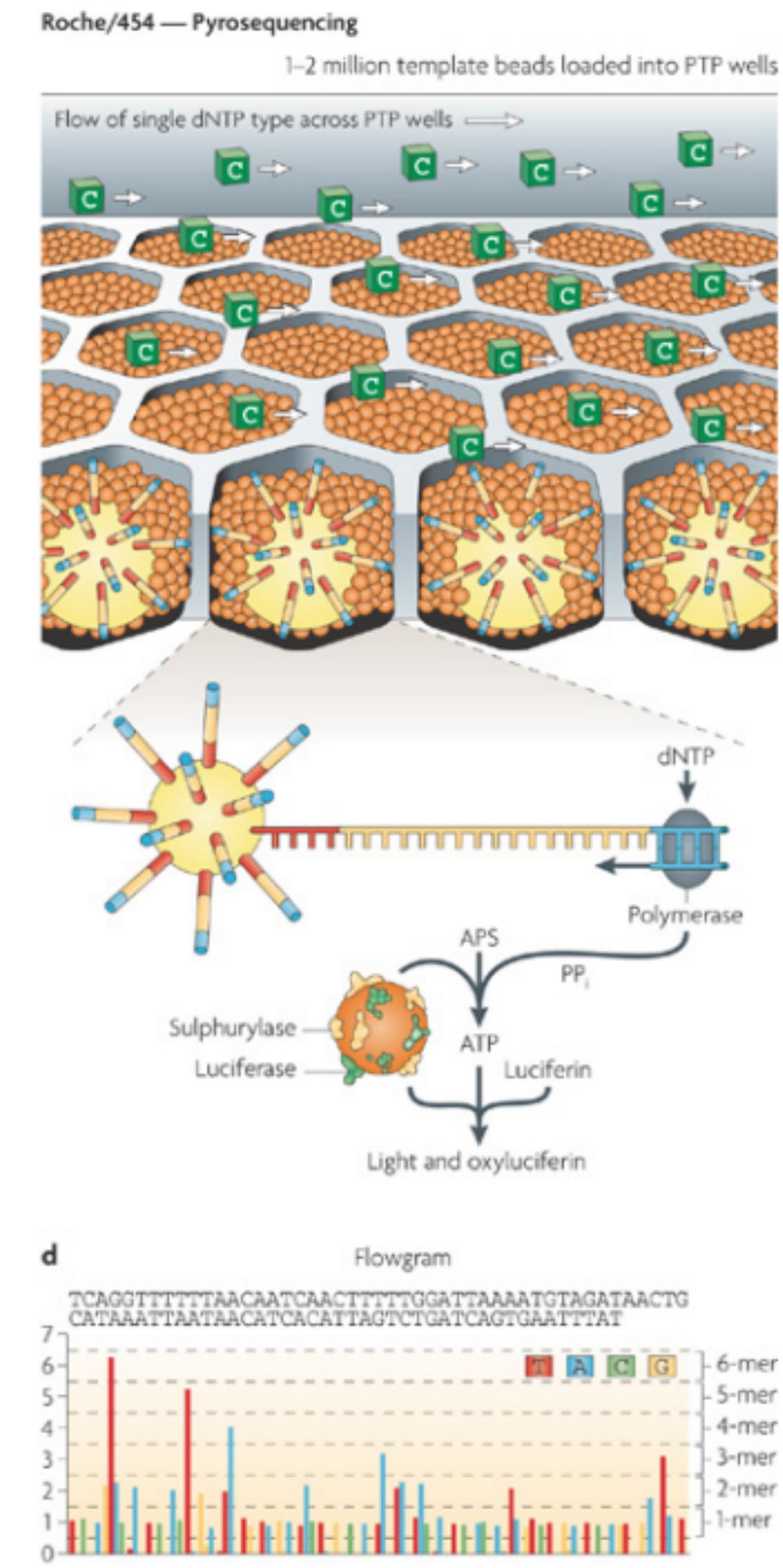


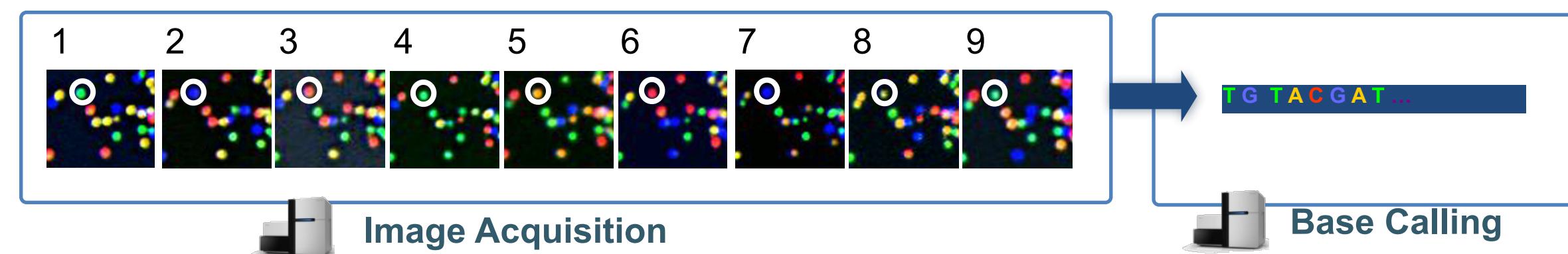
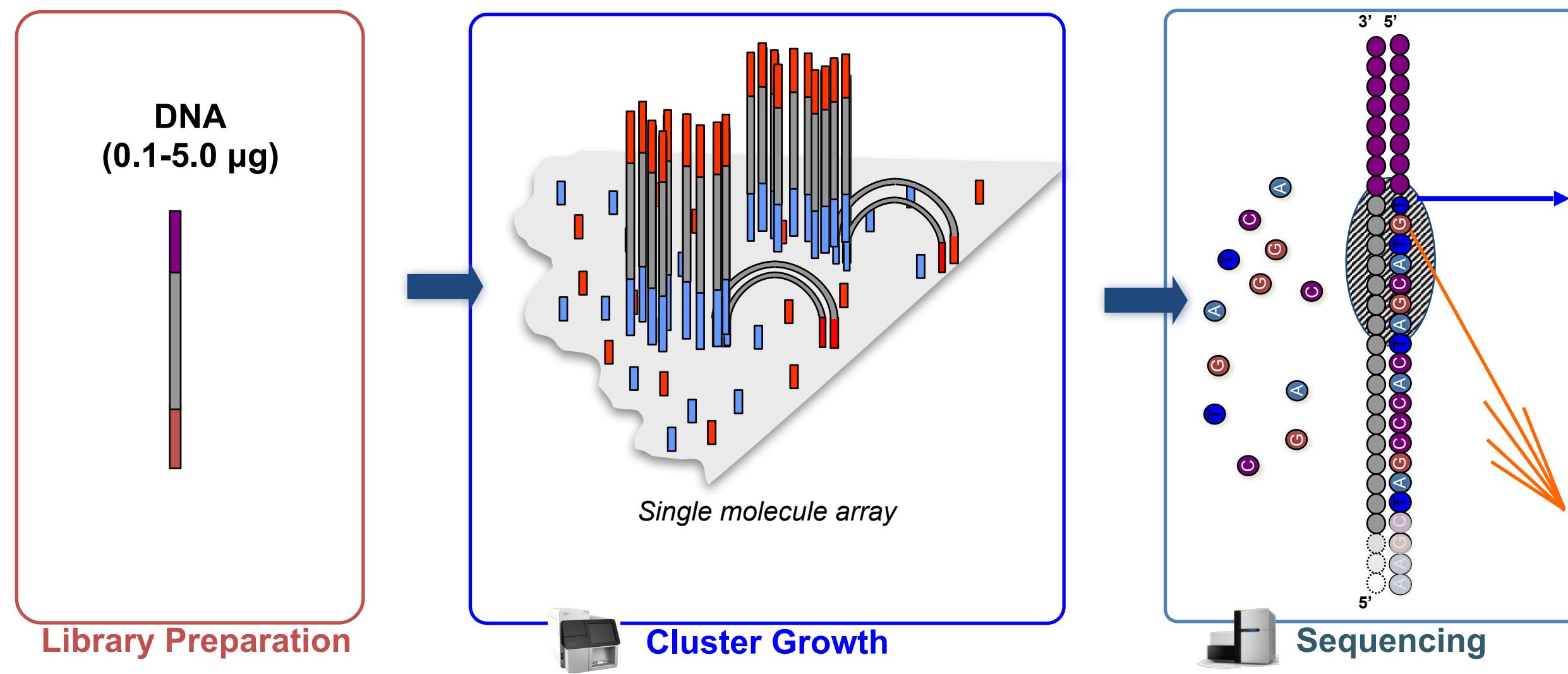


Next-Generation Sequencing Technologies

454 Pyrosequencing

- ▶ DNA bound to capture beads for emulsion PCR
- ▶ Beads loaded onto plate (one bead per well)
- ▶ Addition of complementary base results in ATP production > activates luciferin > light production > CCD
- ▶ ‘Flowgram’ generated for each well



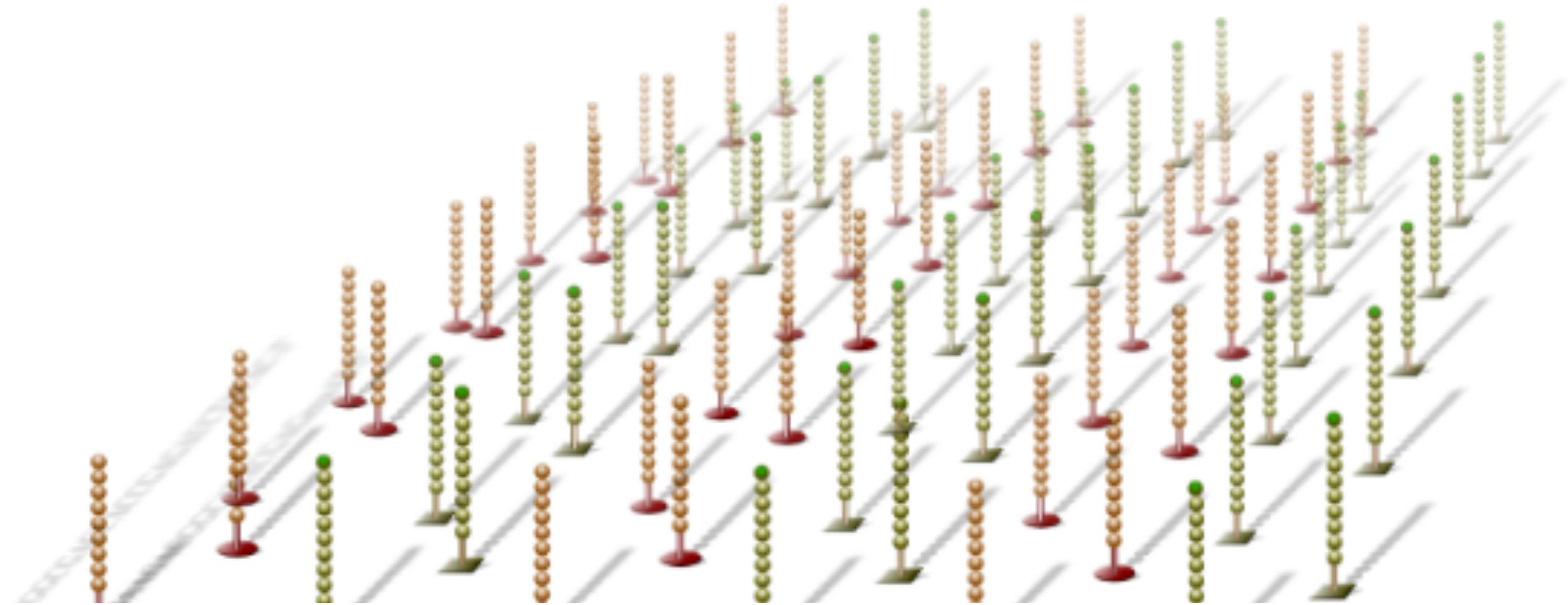


11



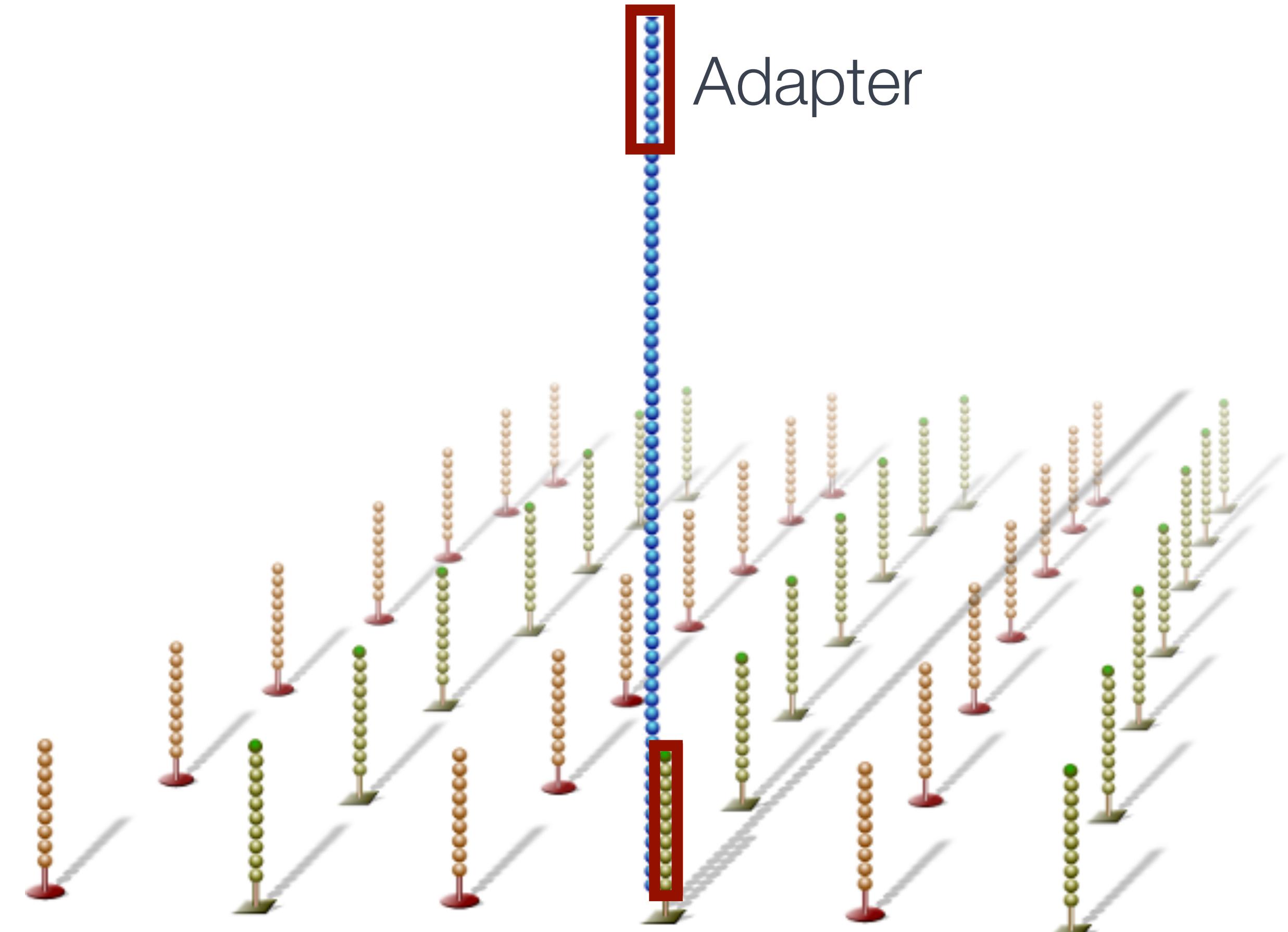
Sequencing by Synthesis

TTAATGATACGGCACCCGAGAUCTACAC-3'
TTCAAGCAGAAGACGGCATACGAGoxoAT-3'



Illumina: flow cell

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

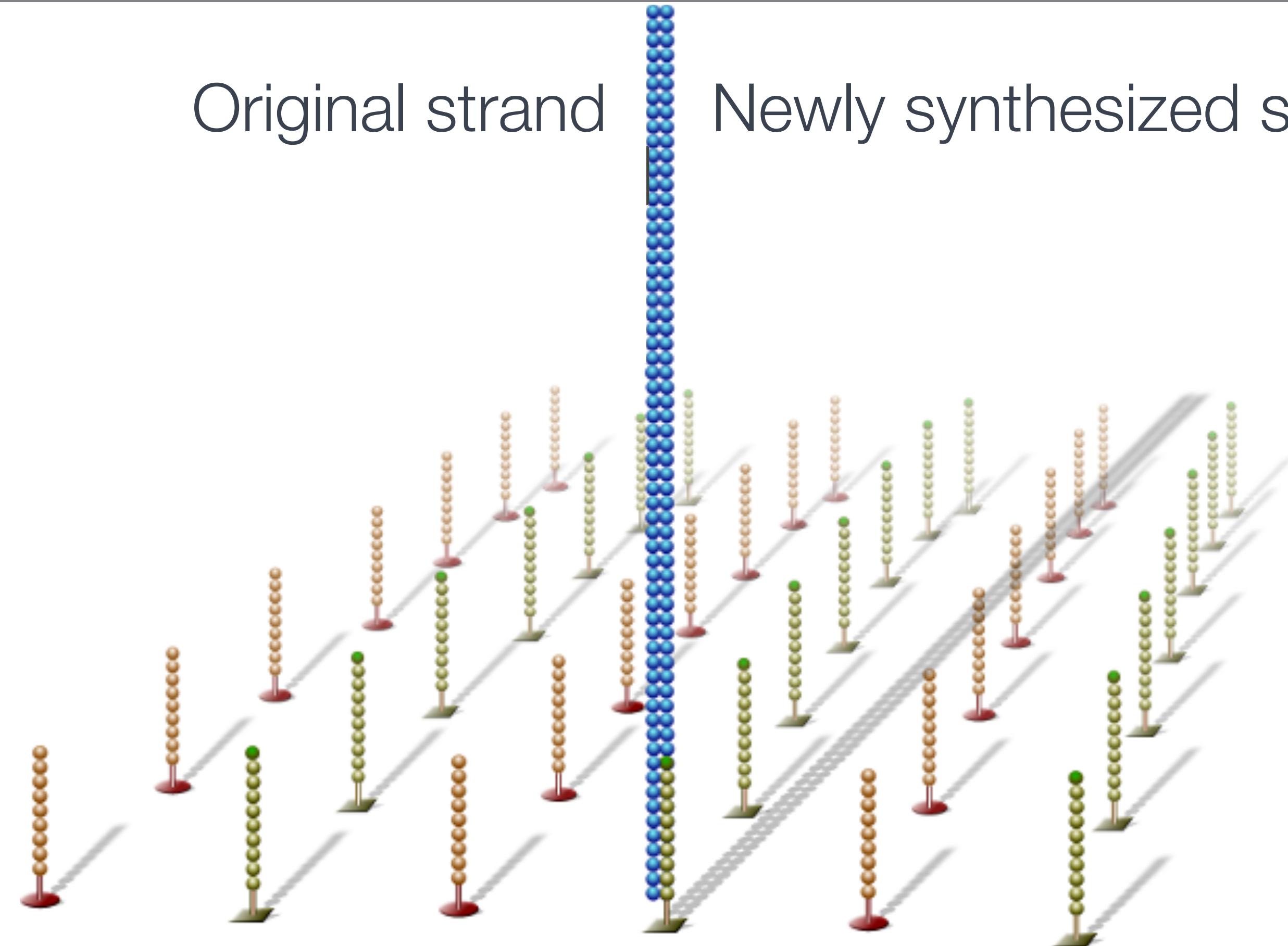


Illumina: cluster generation

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

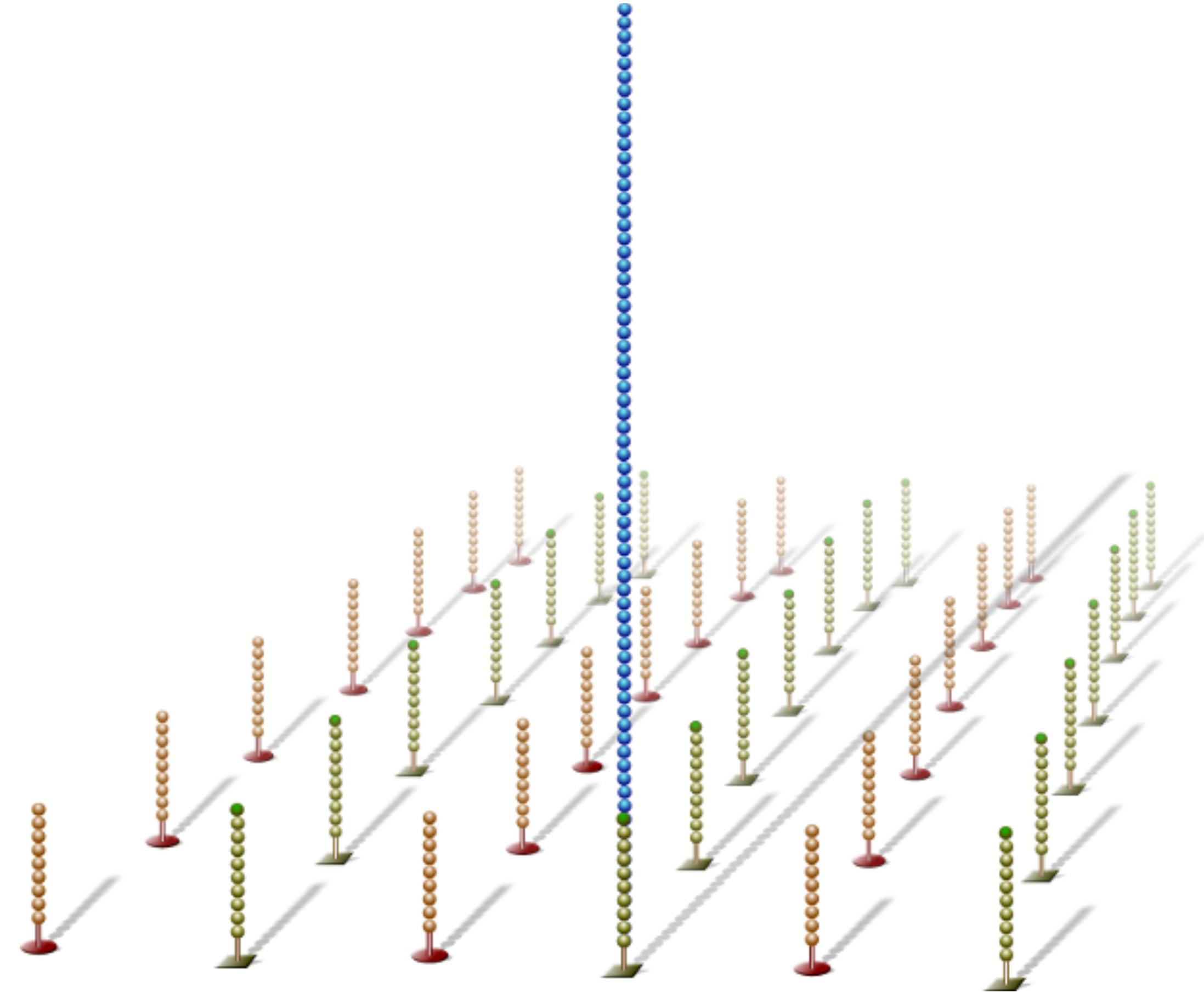
Original strand

Newly synthesized strand



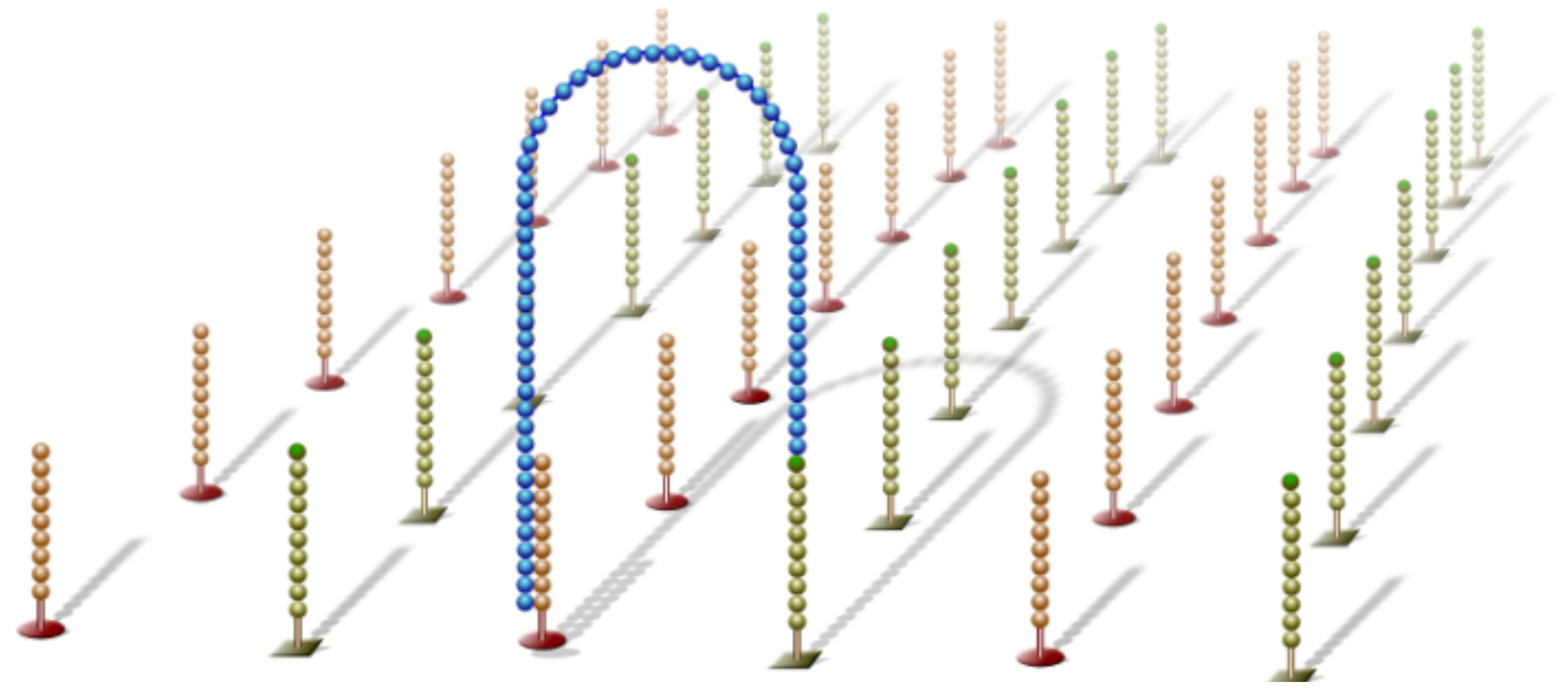
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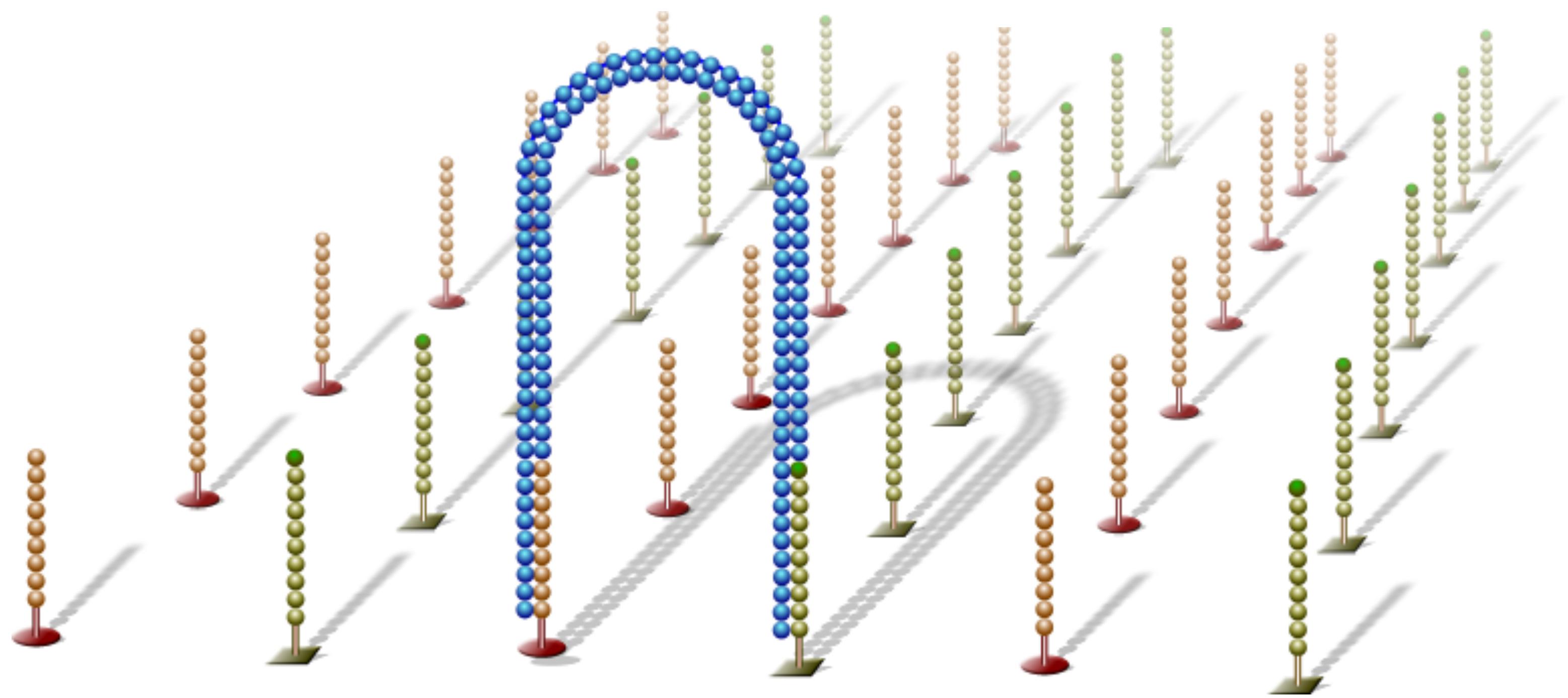
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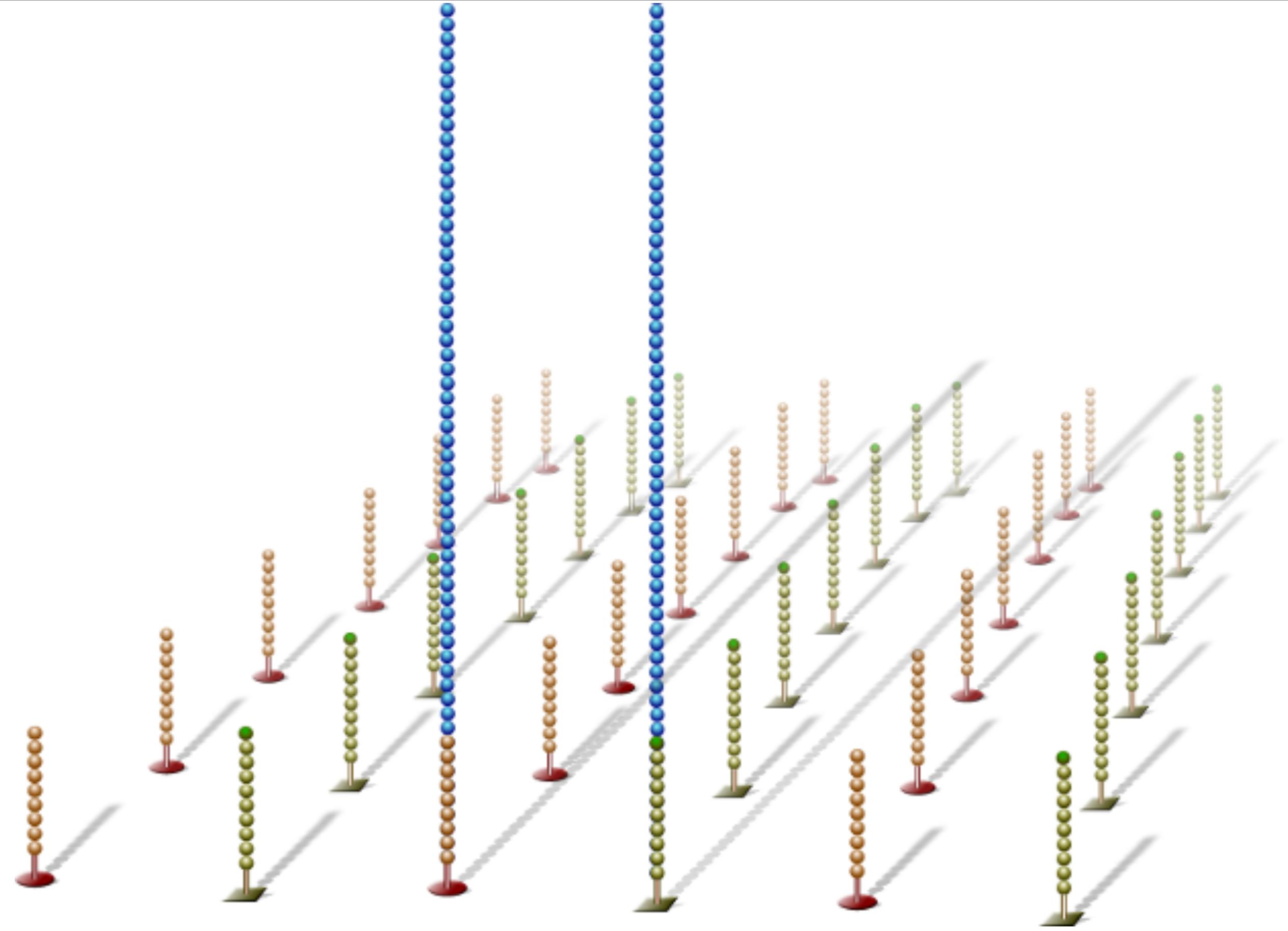
Illumina: bridge amplification

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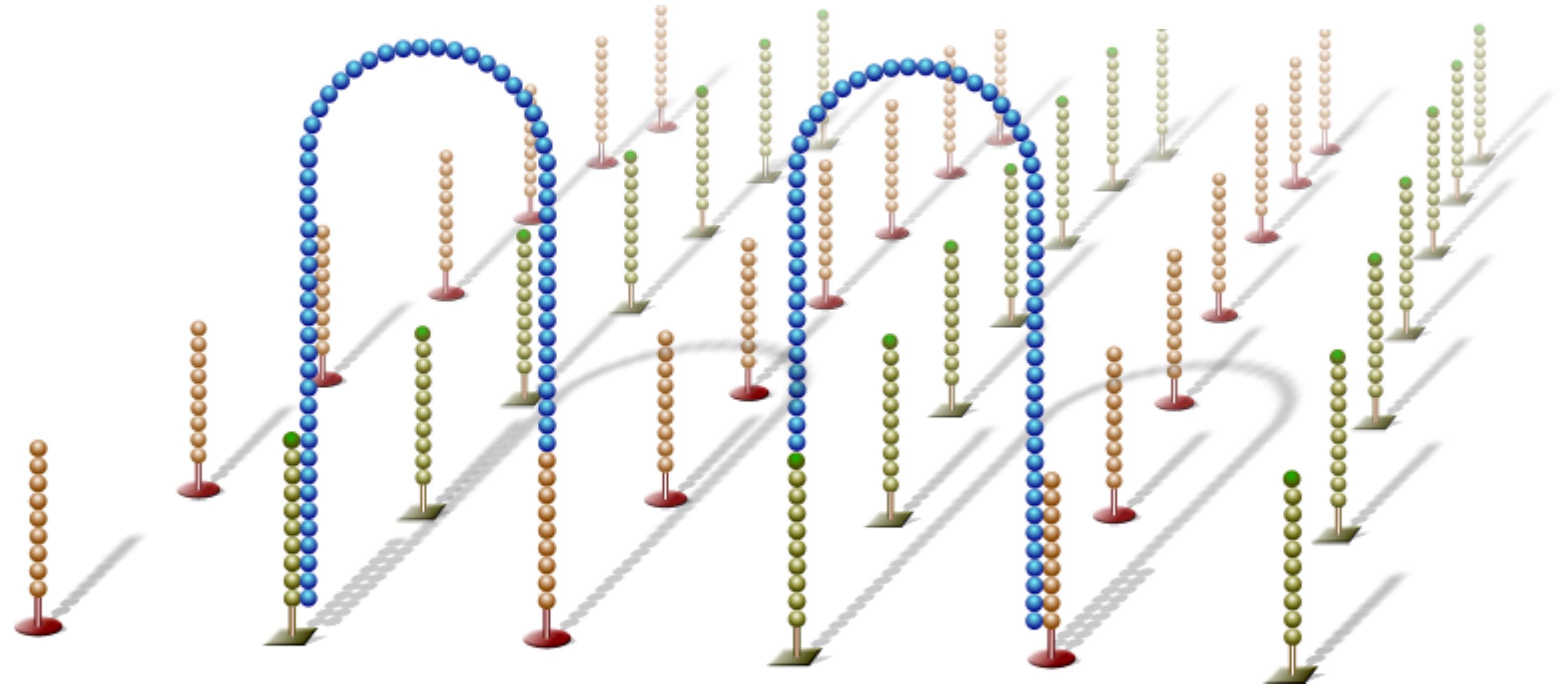
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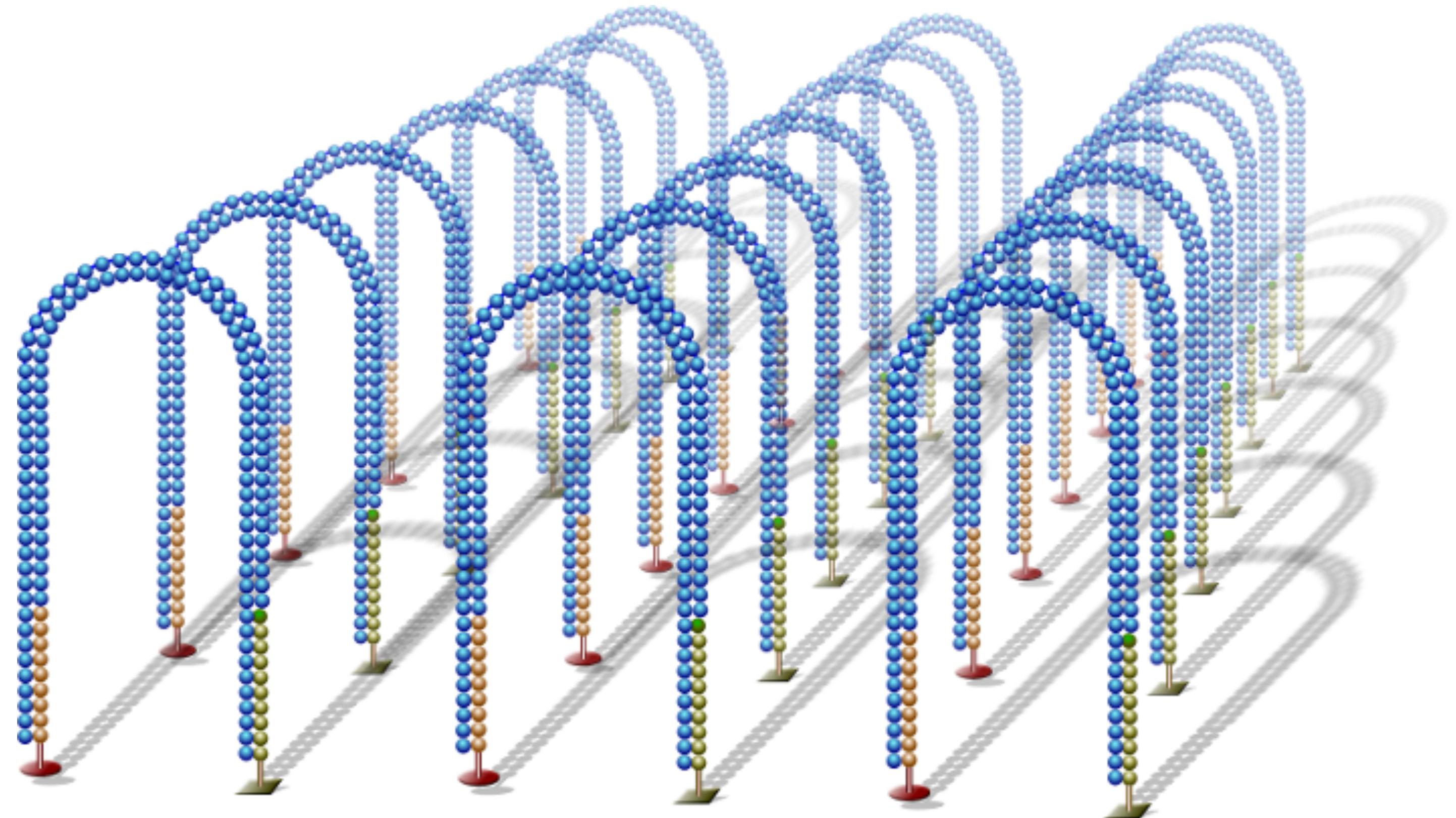
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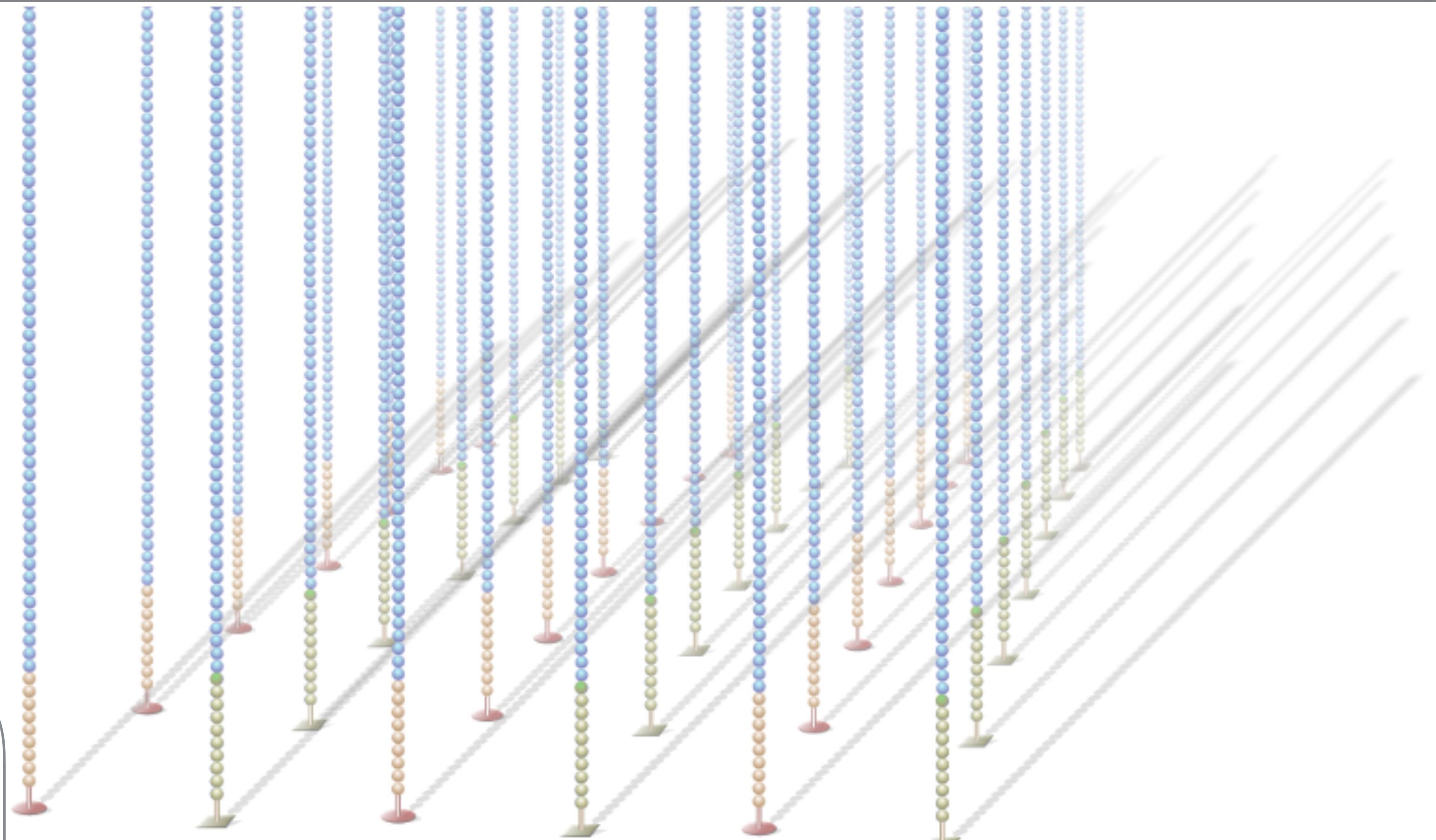
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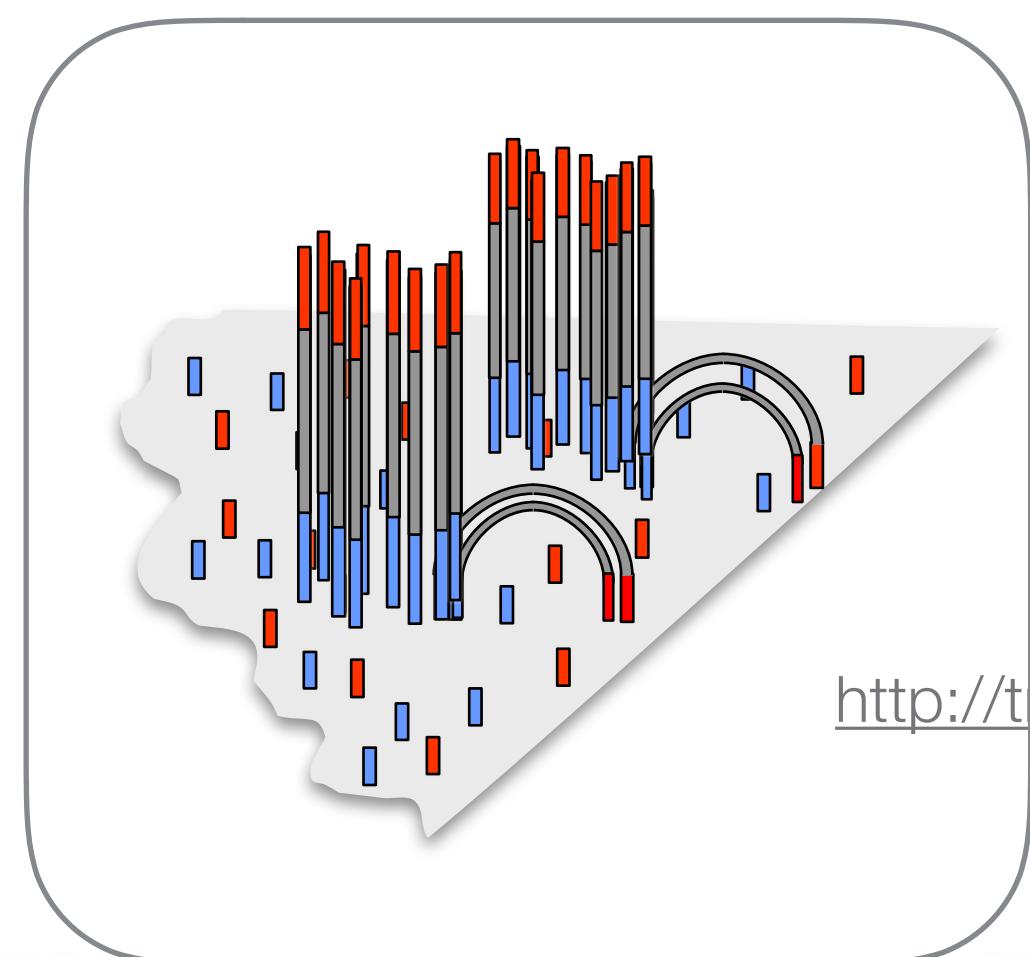
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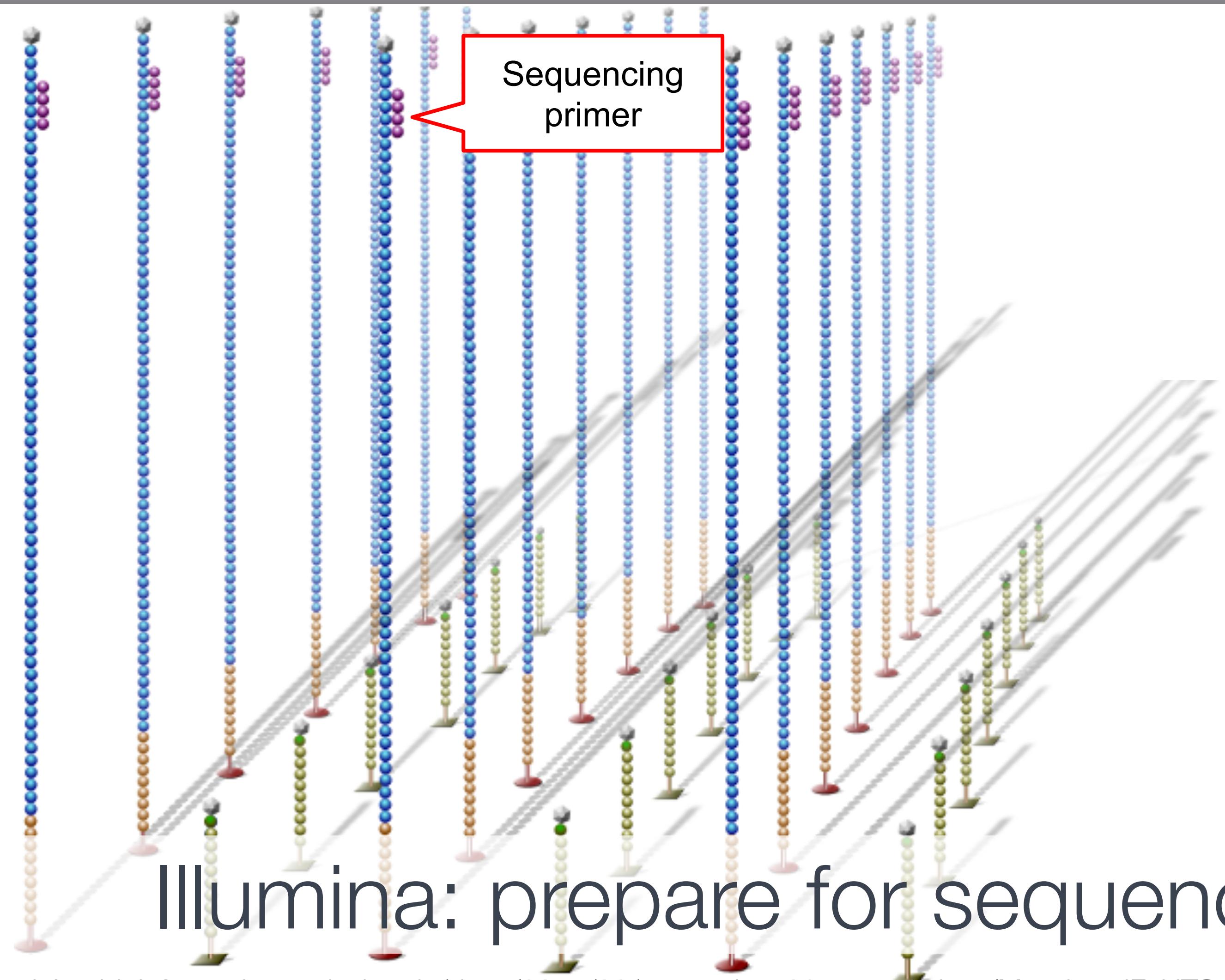
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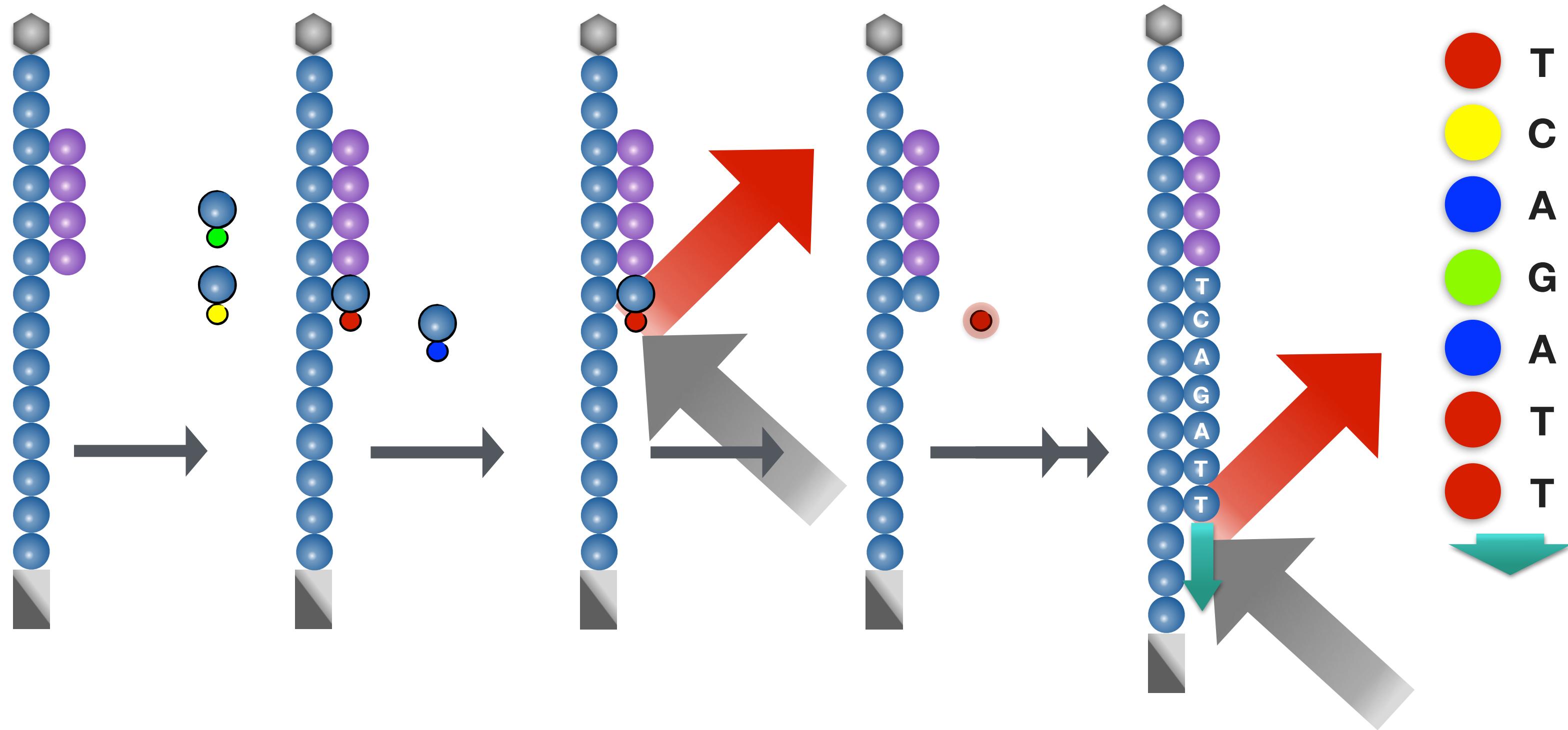
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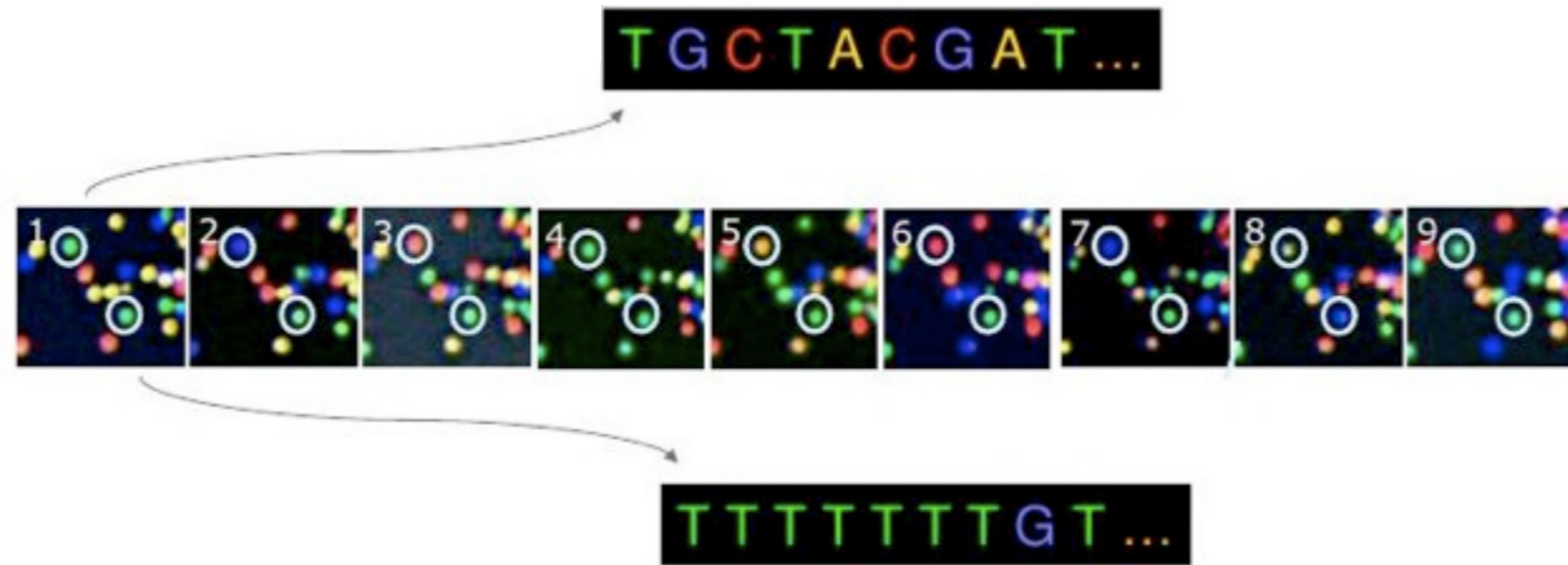




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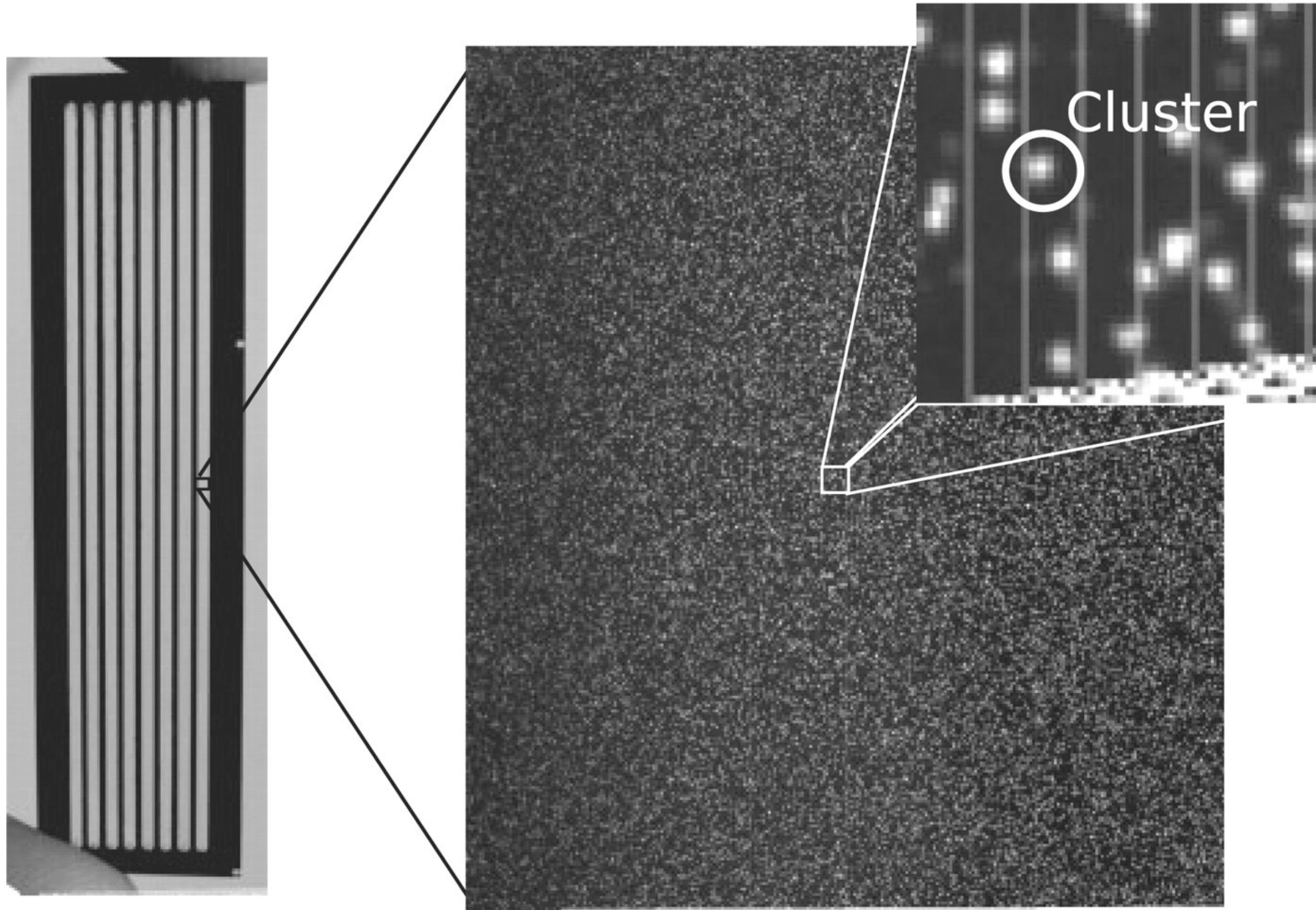


Illumina: sequencing by synthesis



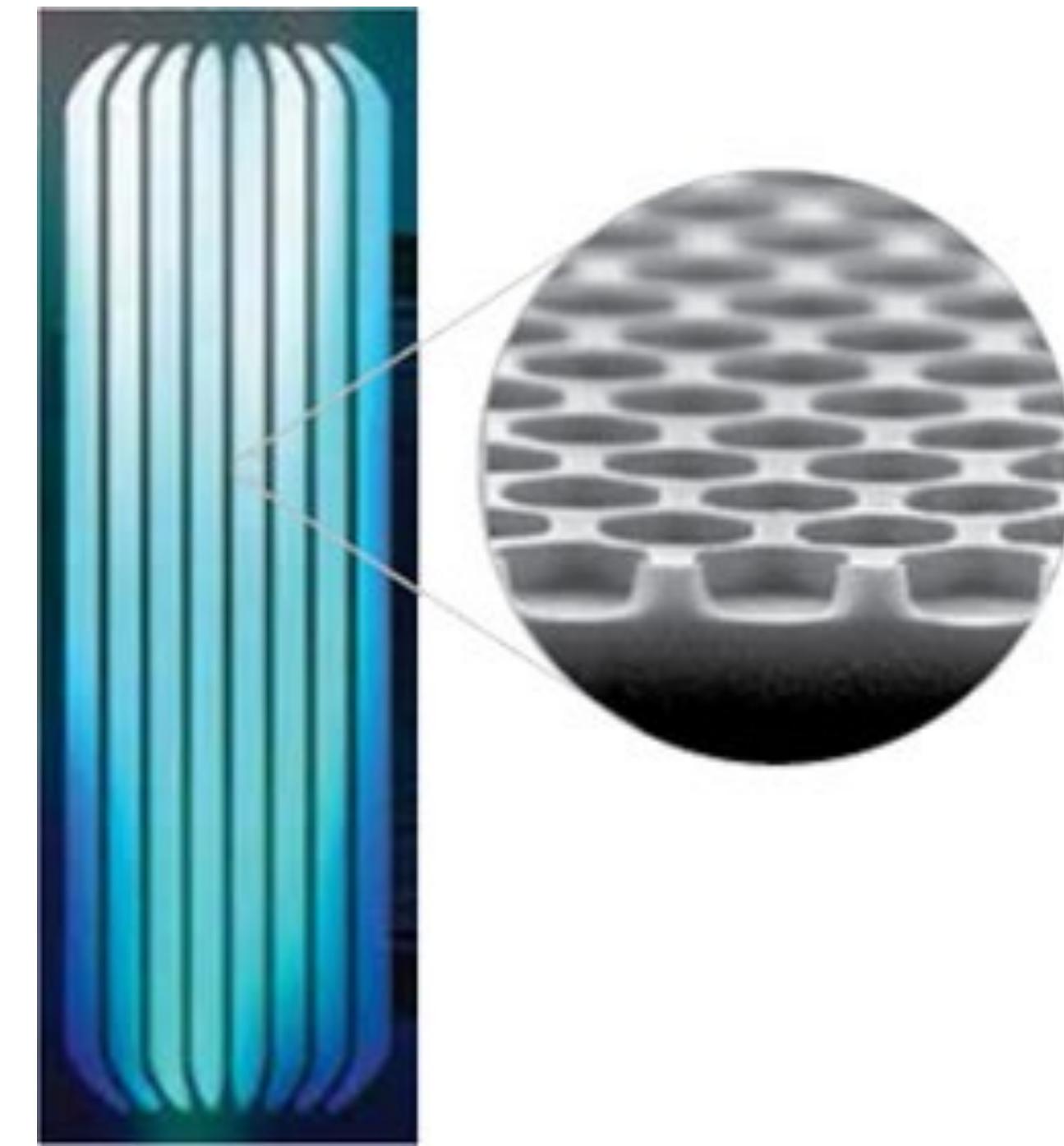
Sequencing by synthesis

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



Whiteford et al, Swift: primary data analysis for the Illumina Solexa sequencing platform, Bioinformatics 2009

Illumina: Flowcell (GAIIx, HiSeq 2000)



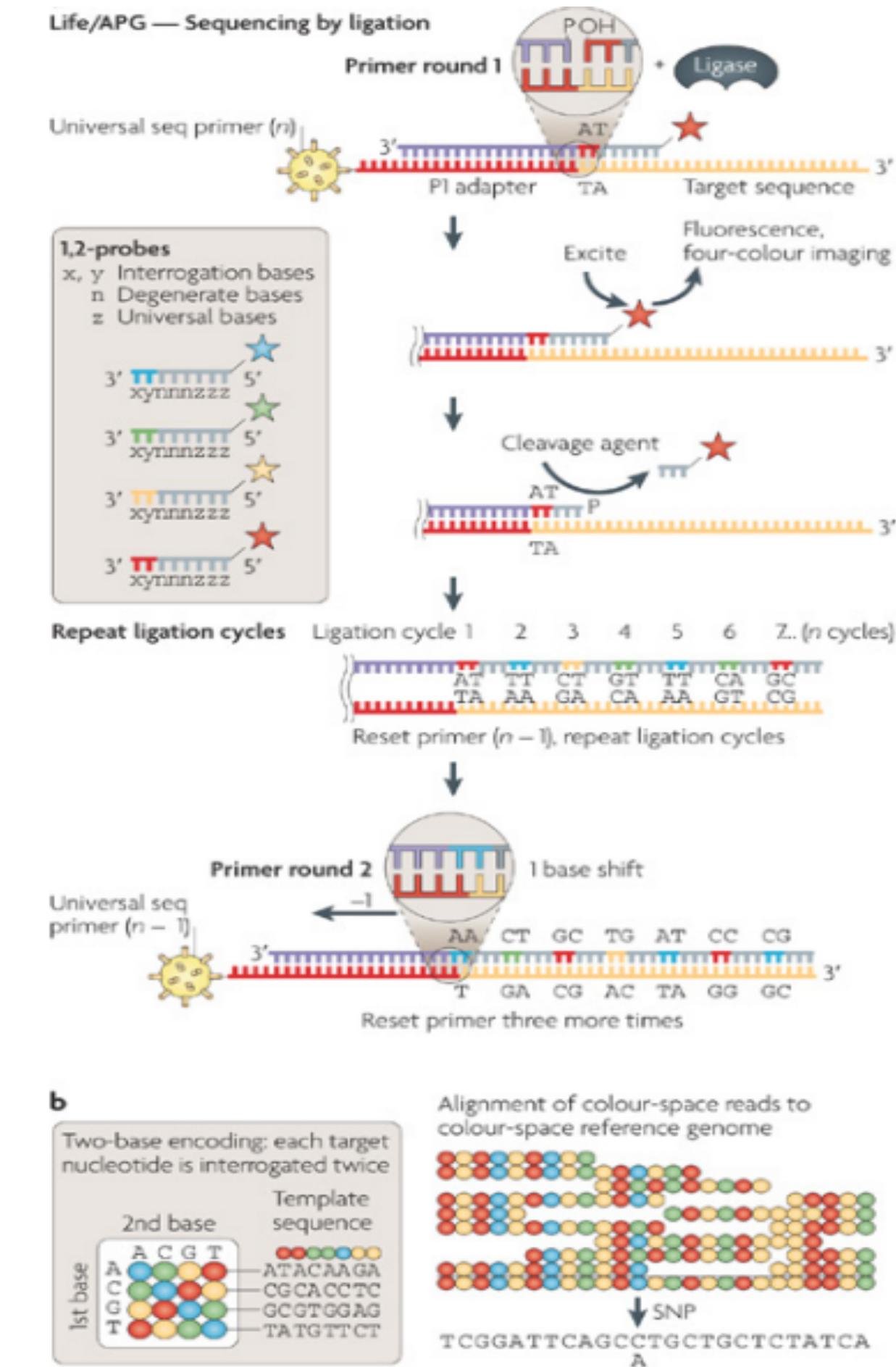
<http://www.illumina.com/systems/hiseq-3000-4000/technology.html>

Illumina: Flowcell (HiSeq 3000)

SOLID:

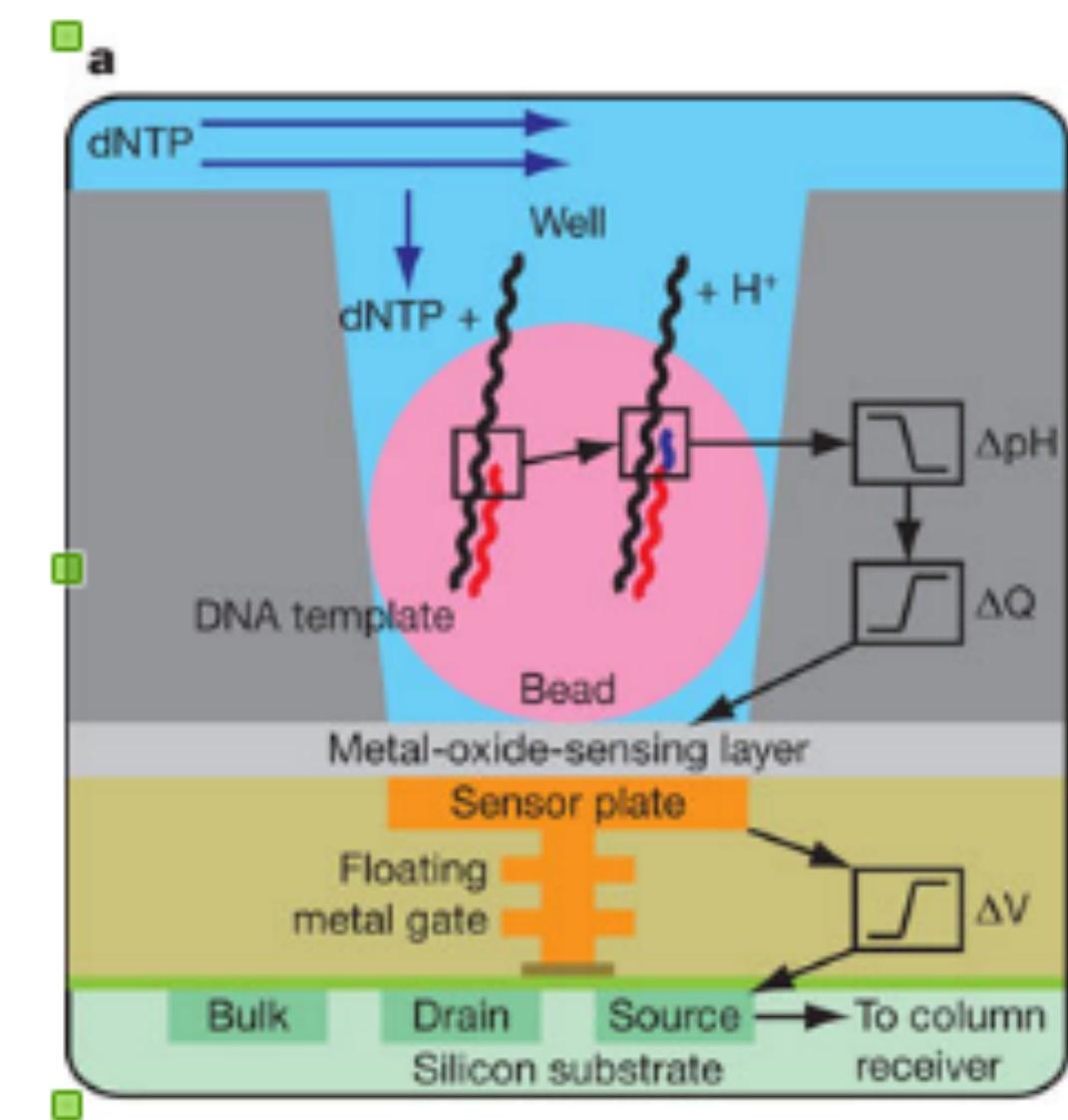
Sequencing by Oligonucleotide Ligation and Detection

- ▶ Emulsion PCR on beads which are attached to flow cell
- ▶ '2-base encoding' via oligonucleotides with degenerate sequence and fluorophore on 5' end
- ▶ Sequence extension by ligation
- ▶ Nine sets of primers, subsequently starting at n - 1,2,3....9
- ▶ All possible sequencing combinations need to be encoded



IonTorrent

- ▶ Similar to 454 but uses semiconducting chip to detect dNTP incorporation
- ▶ Microwells with ion (pH) sensors
- ▶ Nucleotides flow sequentially over ion semiconductor → detecting hydrogen ions during polymerization
- ▶ IonProton and Ion PGM



JM Rothberg *et al.* *Nature* **475**, 348-352 (2011) doi:10.1038/nature10242

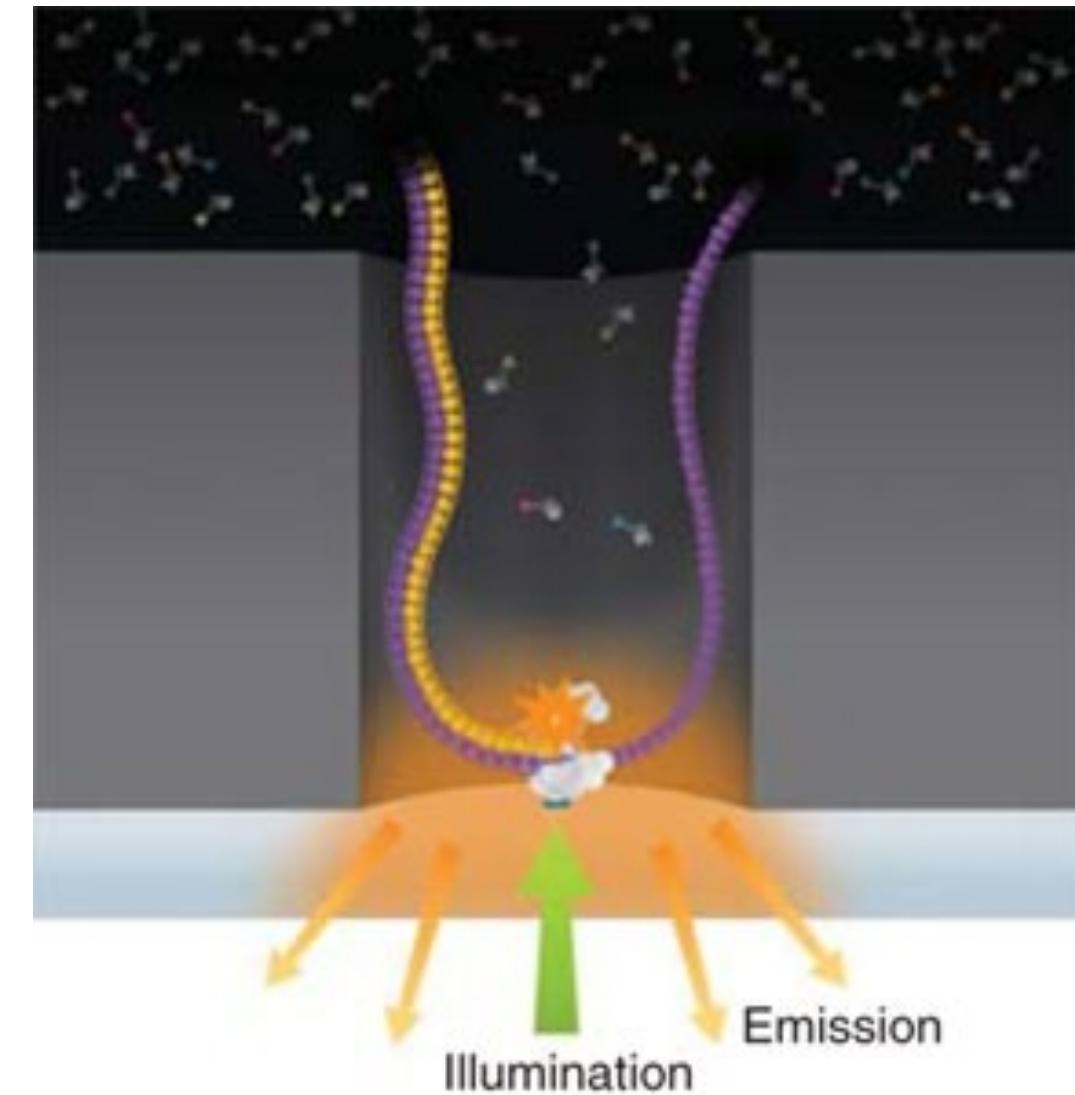
life
technologies™

Third Generation Sequencing Technologies

PacBio:

Single molecule real-time sequencing (SMRT)

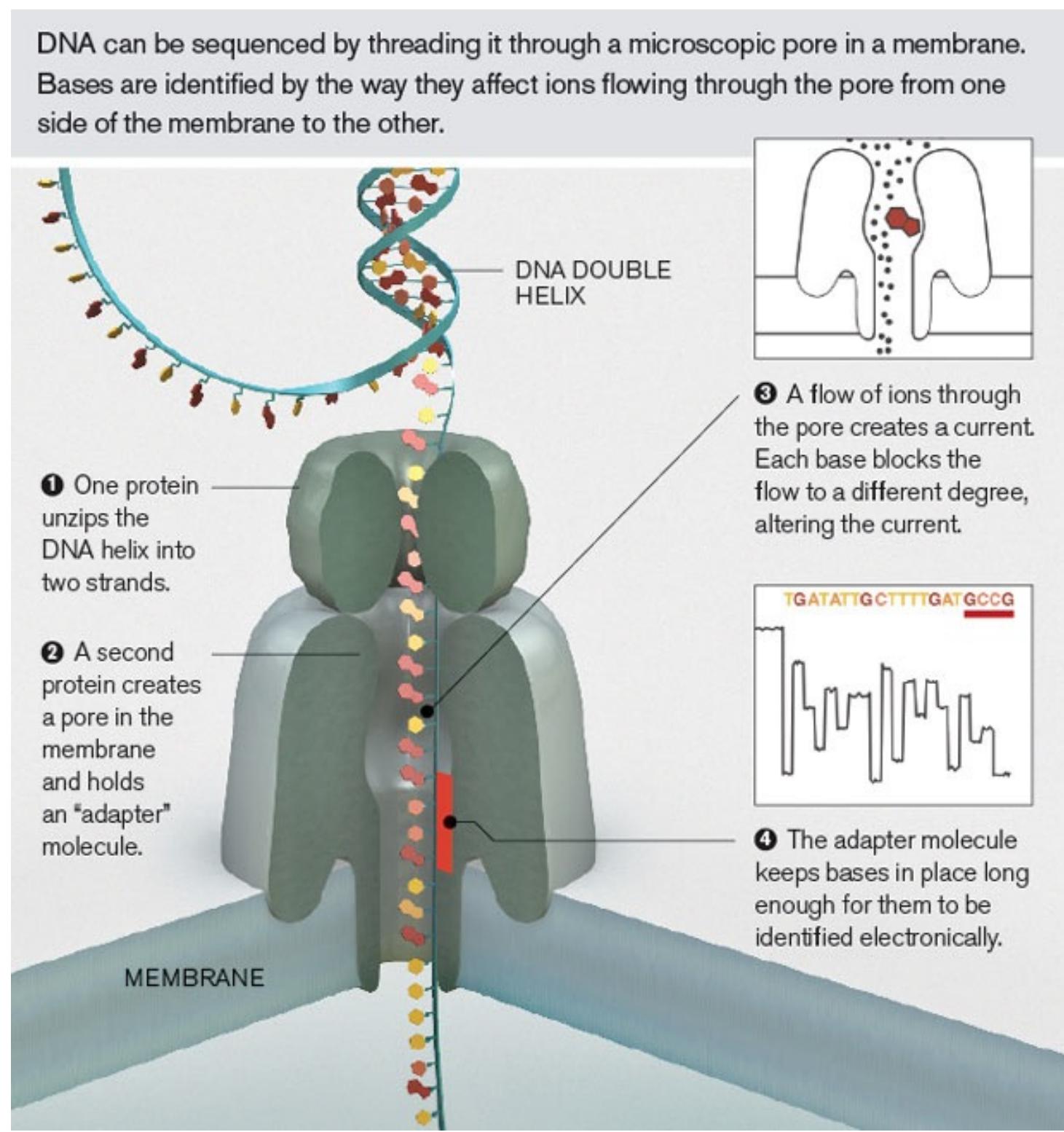
- ▶ DNA synthesis detected on a single strand and never halted
- ▶ Polymerase is affixed to the bottom of a tiny hole (~70 nm) in ZMW
- ▶ Only bottom portion of hole is illuminated allowing for detection of single dye-labeled nucleotide
- ▶ No amplification bias; least GC bias



PacBio: RSII

- ▶ RS II released 2013; costly (~\$800K) and a behemoth of a machine
- ▶ Certified PacBio providers undergo rigorous training; many worldwide locations
- ▶ Long reads with reasonably high accuracy; ideal for whole genome assembly
- ▶ Low input sample amounts (10ng to 1 μ g)
- ▶ Up to 16 SMRT cells in one run





Nanopore

- ▶ Single stranded DNA is passed through a protein pore created in a membrane (nanopore)
- ▶ Ions flow through the pore; measure the current as DNA goes through
- ▶ Bases are identified by the way they affect ions flowing through the pore (~6bp at a time)
- ▶ MinION, PromethION, GridION

Credit: John MacNeil

<http://www2.technologyreview.com/article/427677/nanopore-sequencing/>



Nanopore: MinION

- ▶ Portable, real-time biological analyses; limited release launched May 2014
- ▶ Long(er) reads, with accuracies as good or better than PacBio with 2D reads
- ▶ Reliably sequence small genomes of bacteria and viruses for cheap (\$1K access fee)

LETTER

doi:10.1038/nature16996

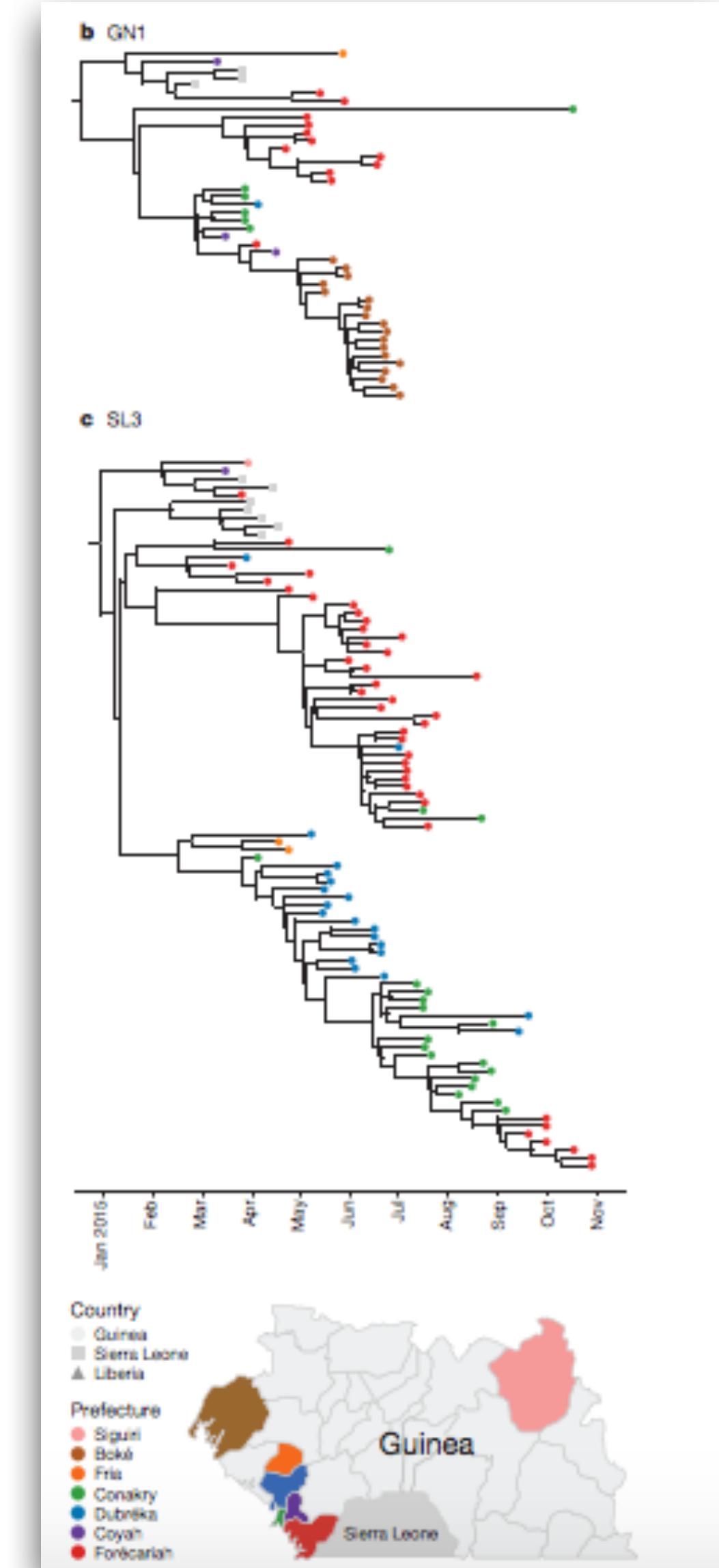
Real-time, portable genome sequencing for Ebola surveillance

Joshua Quick^{1*}, Nicholas J. Loman^{1*}, Sophie Duraffour^{2,3*}, Jared T. Simpson^{4,5*}, Ettore Severi^{6*}, Lauren Cowley^{7*}, Joseph Akoi Bore², Raymond Koundouno², Gytis Dudas⁸, Amy Mikhail⁷, Nobila Ouédraogo⁹, Babak Afrough^{2,10}, Amadou Bah^{2,11}, Jonathan H. J. Baum^{2,3}, Beate Becker-Ziaja^{2,3}, Jan Peter Boettcher^{2,12}, Mar Cabeza-Cabrero^{2,3}, Álvaro Camino-Sánchez², Lisa L. Carter^{2,13}, Juliane Doerrbecker^{2,3}, Theresa Enkirch^{2,14}, Isabel García-Dorival^{2,15}, Nicole Hetzelt^{2,12}, Julia Hinzmman^{2,12}, Tobias Holm^{2,3}, Liana Eleni Kafetzopoulou^{2,16}, Michel Koropogui^{2,17}, Abigael Kosgey^{2,18}, Eeva Kuismä^{2,10}, Christopher H. Logue^{2,10}, Antonio Mazzarelli^{2,19}, Sarah Meisel^{2,3}, Marc Mertens^{2,20}, Janine Michel^{2,12}, Didier Ngabo^{2,10}, Katja Nitzsche^{2,3}, Elisa Pallasch^{2,3}, Livia Victoria Patrono^{2,3}, Jasmine Portmann^{2,21}, Johanna Gabriella Repits^{2,22}, Natasha Y. Rickett^{2,15,23}, Andreas Sachse^{2,12}, Katrien Singethan^{2,24}, Inês Vitoriano^{2,10}, Rahel L. Yemanaberhan^{2,3}, Elsa G. Zekeng^{2,15,23}, Trina Racine²⁵, Alexander Bello²⁵, Amadou Alpha Sall²⁶, Ousmane Faye²⁶, Oumar Faye²⁶, N'Faly Magassouba²⁷, Cecelia V. Williams^{28,29}, Victoria Amburgey^{28,29}, Linda Winona^{28,29}, Emily Davis^{29,30}, Jon Gerlach^{29,30}, Frank Washington^{29,30}, Vanessa Montell³¹, Marine Joudain³¹, Marion Bererd³¹, Alimou Camara³¹, Hermann Somlare³¹, Abdoulaye Camara³¹, Marianne Gerard³¹, Guillaume Bado³¹, Bernard Bailler³¹, Déborah Delaune^{32,33}, Koumpingnin Yacouba Nebie³⁴, Abdoulaye Diarra³⁴, Yacouba Savane³⁴, Raymond Bernard Pallawo³⁴, Giovanna Jaramillo Gutierrez³⁵, Natacha Milhano^{6,36}, Isabelle Roger³⁴, Christopher J. Williams^{6,37}, Facinet Yattara¹⁷, Kuiama Lewandowski¹⁰, James Taylor³⁸, Phillip Rachwal³⁸, Daniel J. Turner³⁹, Georgios Pollakis^{15,23}, Julian A. Hiscox^{15,23}, David A. Matthews⁴⁰, Matthew K. O'Shea⁴¹, Andrew McD. Johnston⁴¹, Duncan Wilson⁴¹, Emma Hutley⁴², Erasmus Smit⁴³, Antonino Di Caro^{2,19}, Roman Wölfel^{2,44}, Kilian Stoecker^{2,44}, Erna Fleischmann^{2,44}, Martin Gabriel^{2,3}, Simon A. Weller³⁸, Lamine Koivogui⁴⁵, Boubacar Diallo³, Sakoba Keïta¹⁷, Andrew Rambaut^{8,46,47}, Pierre Formenty³⁴, Stephan Günther^{2,3} & Miles W. Carroll^{2,10,48,49}

- Sequence data and analysis of 142 EBOV samples
- Generate results < 24h after receiving sample, with sequencing complete between 15-60 minutes



Figure 1 | Deployment of the portable genome surveillance system in Guinea. **a**, We were able to pack all instruments, reagents and disposable consumables within aircraft baggage. **b**, We initially established the genomic surveillance laboratory in Donka Hospital, Conakry, Guinea. **c**, Later we moved the laboratory to a dedicated sequencing laboratory in Coyah prefecture. **d**, Within this laboratory we separated the sequencing instruments (on the left) from the PCR bench (to the right). An uninterrupted power supply can be seen in the middle that provides power to the thermocycler. (Photographs taken by J.Q. and S.D.)

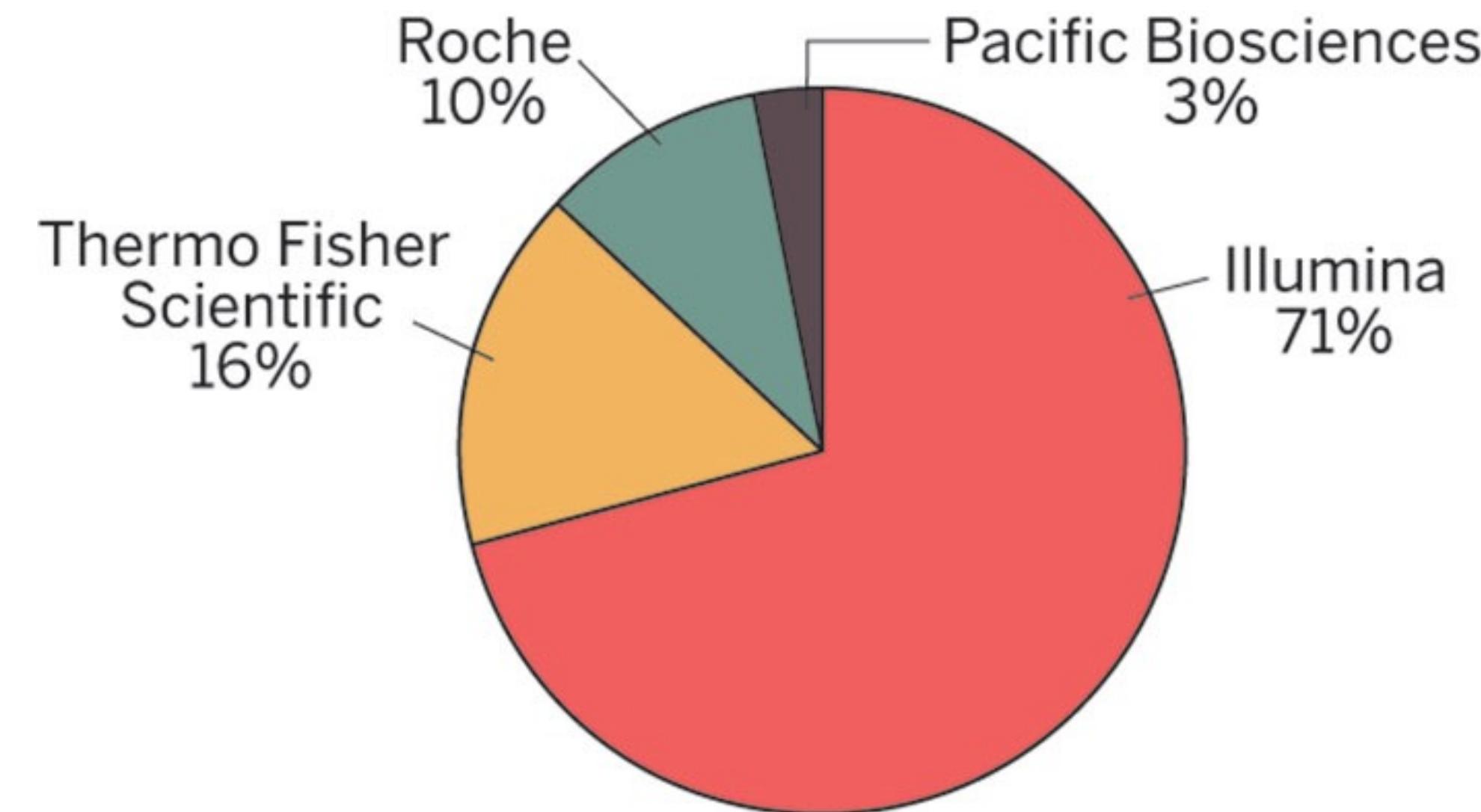


Comparison of NGS technologies

	Max Read Length	Runtime	Output per run	Error rates
Illumina HiSeq2500	2x125 bp	6 days	450-500 Gb	0.26%
IonTorrent PGM	~200 bp	2 hours	20-50 Mb on 314 100-200 Mb on 316 1Gb on 318 chip	1.71 %
PacBio RS II	Avg 10 Kb up to 30 Kb	2 hours	375 Mb	~10%*
MinION	Avg 10 Kb up to 50 Kb	no fixed run-time	200 - 300 Mb (varies)	~15%*

*prior to error correction methods

Illumina: the current ‘rulers’ of the throne



World market in 2013 = \$1.3 billion

SOURCES: Mizuho Securities USA, Frost & Sullivan

A rapidly evolving market

- ▶ Illumina's HiSeq 3000 and HiSeq 4000; an intermediate between the HiSeq X Ten and the well-loved HiSeq 2500

A rapidly evolving market

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- ▶ PacBio's new Sequel System; a more price effective version of RSII, a third the size and almost seven times more powerful

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- ▶ PacBio's new Sequel System; a more price effective version of RSII, a third the size and almost seven times more powerful
- ▶ Oxford Nanopore released fast-mode early access of MinION (Jan 2016); DNA transit speeds substantially increased with sequence quality preserved (550 bp/sec), Rapid 1D prep using transposase like Nextera kit

These materials have been developed by members of the teaching team at the [Harvard Chan Bioinformatics Core \(HBC\)](#). These are open access materials distributed under the terms of the [Creative Commons Attribution license \(CC BY 4.0\)](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

