An overview of genomics and sequencing terminology and practices

Mark Stenglein, GDW

Genomics of Disease in Wildlife

Math undergrad → 7 years as a software engineer → PhD in virology / mol. biol. → Postdoc using microarrays, NGS, and bioinformatics → Assoc. Professor at CSU

1999, Bankgok, Thai Airways test facility

Department of MICROBIOLOGY, IMMUNOLOGY & PATHOLOGY
COLLEGE OF VETERINARY MEDICINE & BIOMEDICAL SCIENCES

CENTER FOR VECTOR-BORNE INFECTIOUS DISEASES

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advancing health worldwide™

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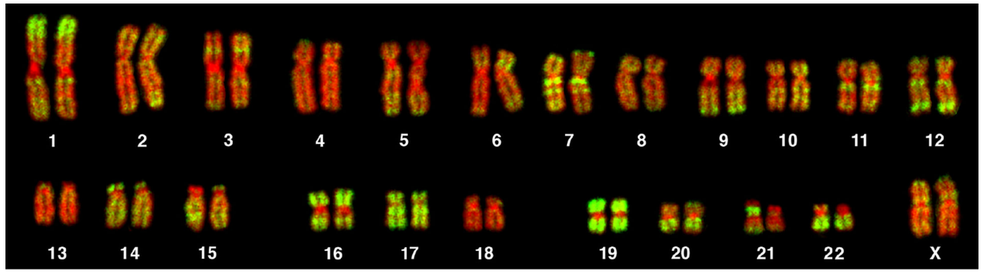
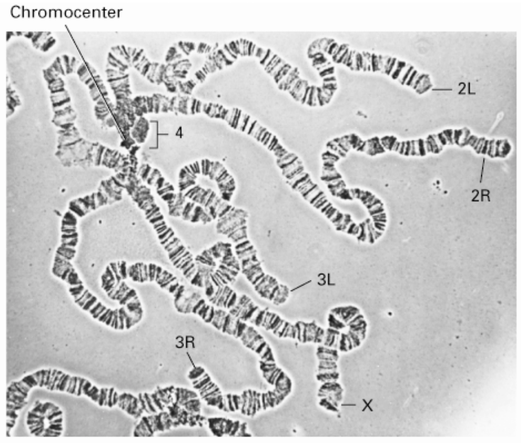
Non-sequencing genomic techniques

FISH

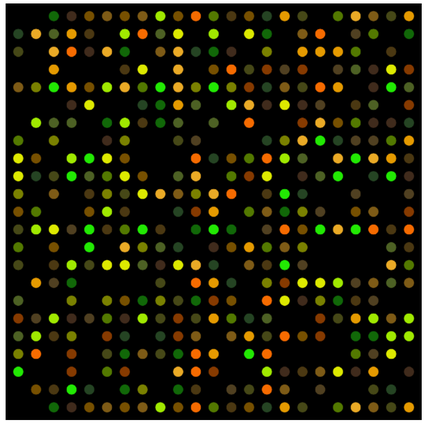


Bolzer et al (2005) PLoS Biol

Polytene chromosomes

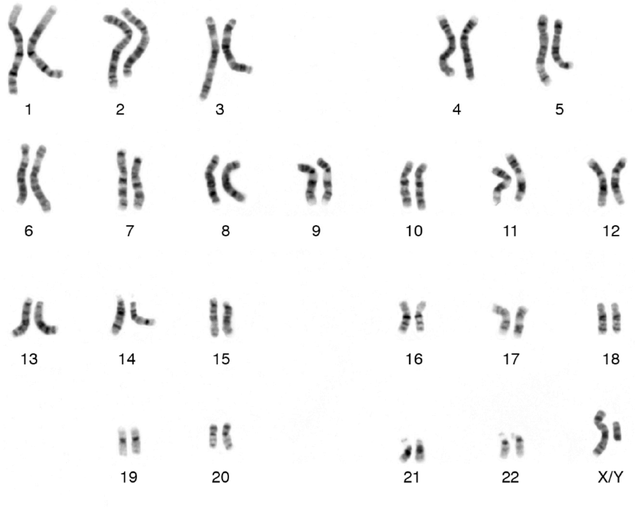


Microarray

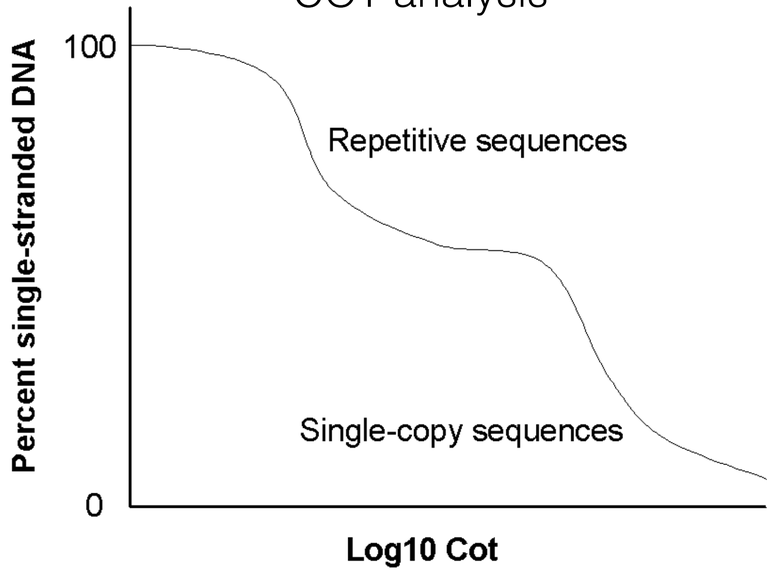


Wikimedia commons

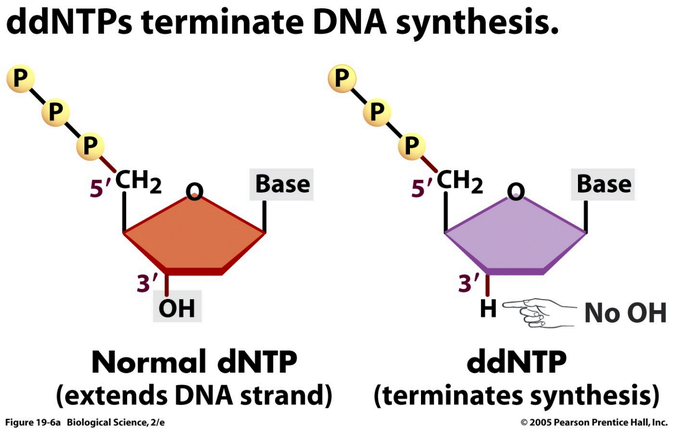
Karyotype

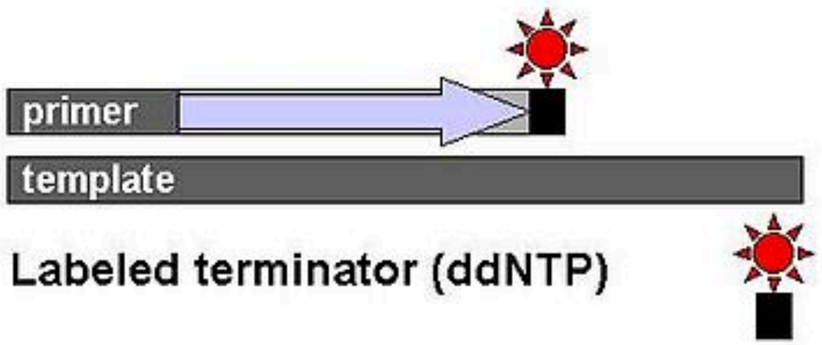


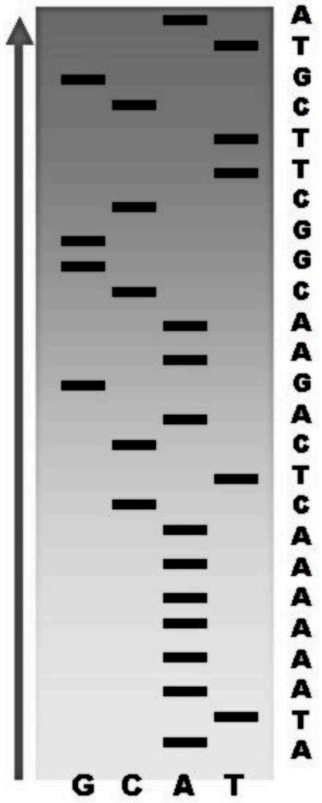
COT analysis

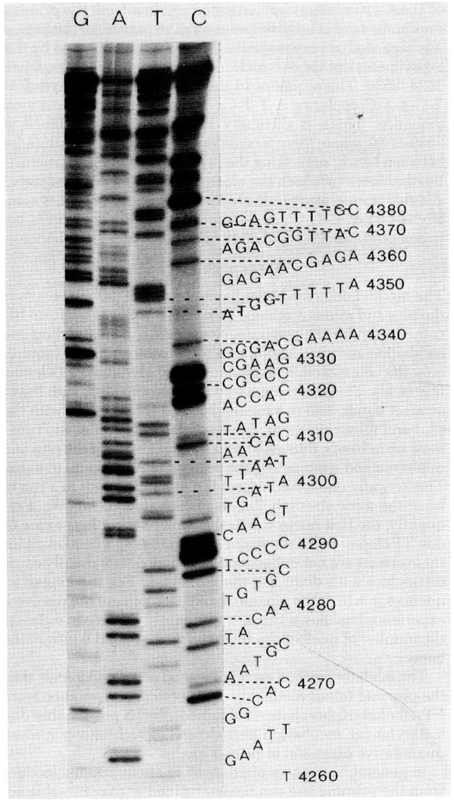


Sanger Sequencing (1977): sequencing 1 target at a time









Slide courtesy Dan Sloan. Image credits: Sanger et al (1977) and Wikipedia

Next generation sequencing (NGS) ~ deep sequencing ~ high throughput sequencing (HTS)

All simultaneously sequence **many molecules in parallel**

Short read sequencing (Illumina)

- Millions of reads
- Relatively short: ~50-300 nt (Illumina)
- Relative low error rates
- Cheaper per base pair of data generated

MiSeq

$100,000-$1,000,000

Long read sequencing

- Fewer, longer reads
- >1 kb (PacBio), up to 100s of kb (Oxford Nanopore)
- Relative high error rates

Oxford Nanopore MinION

$1000

PacBio RS-II

Illumina sequencing happens on flow cells

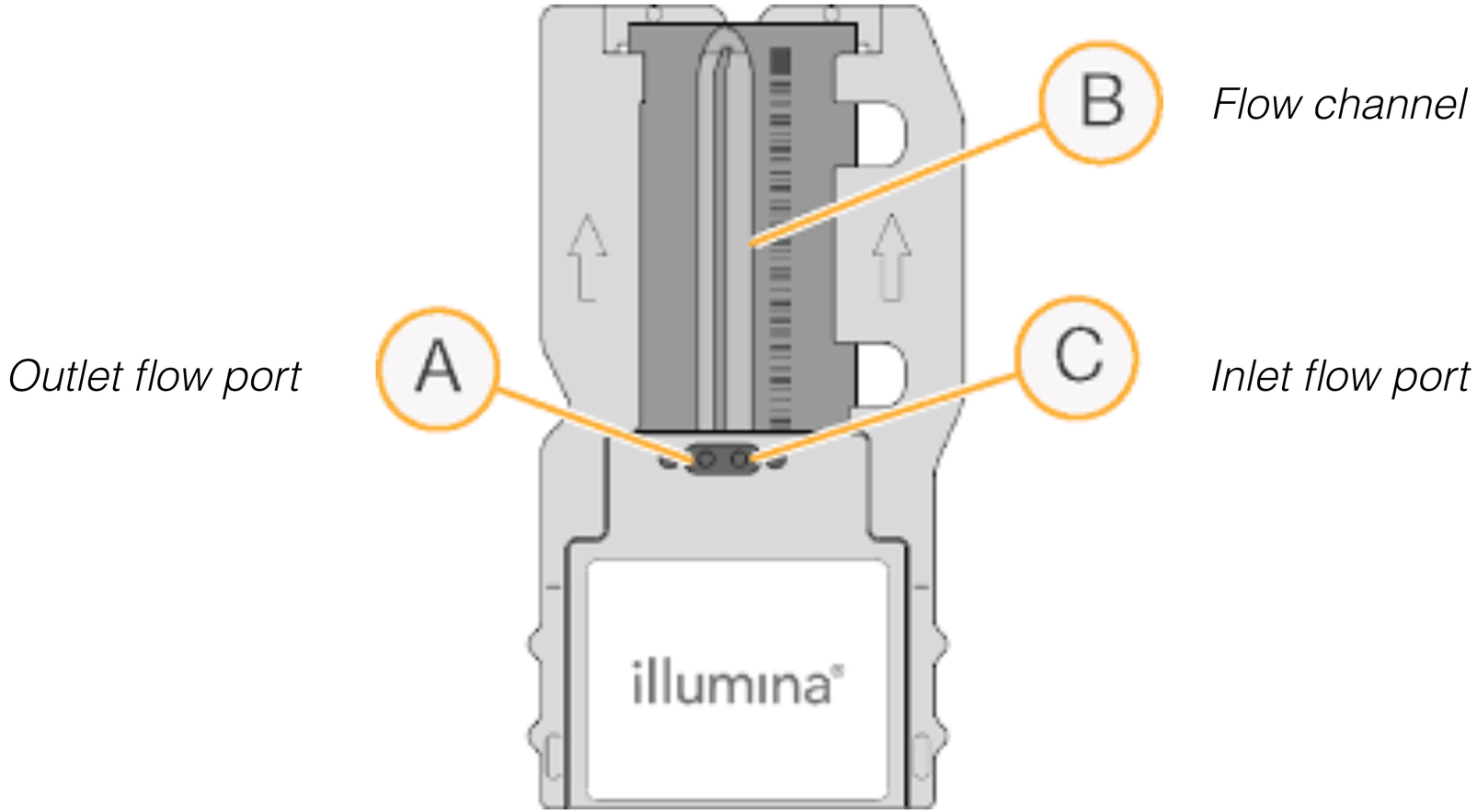
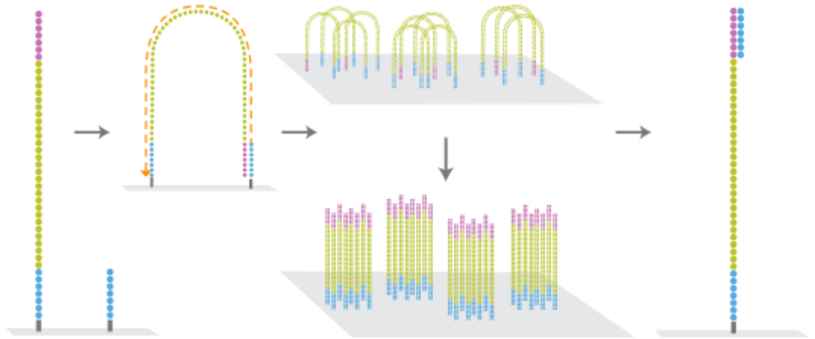
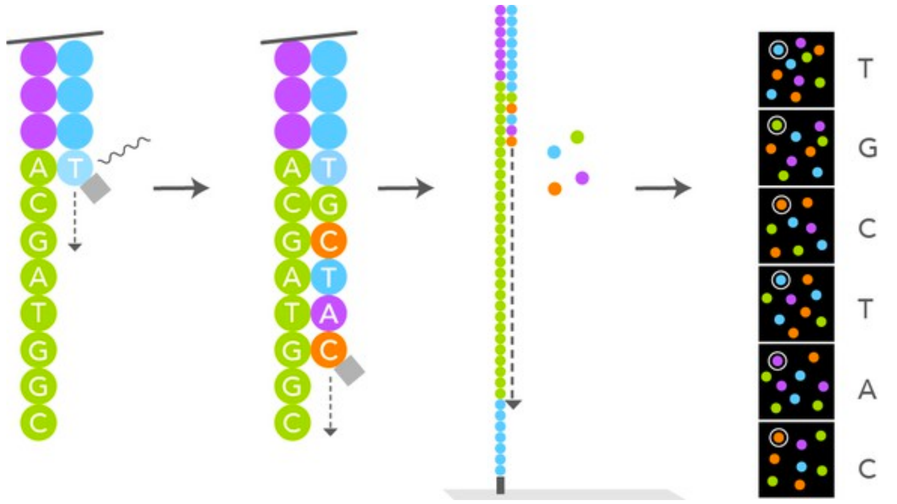


Image credit: Illumina

Illumina instruments use sequencing by synthesis (SBS)



Millions of clusters per flow cell
Each cluster contains 1000s of clonal copies of a library molecule



Library molecules are sequenced by primer extension reactions that incorporate chain-terminated, fluorescent nucleotides

This technology is very similar to Sanger sequencing in principle

real raw Illumina sequencing data

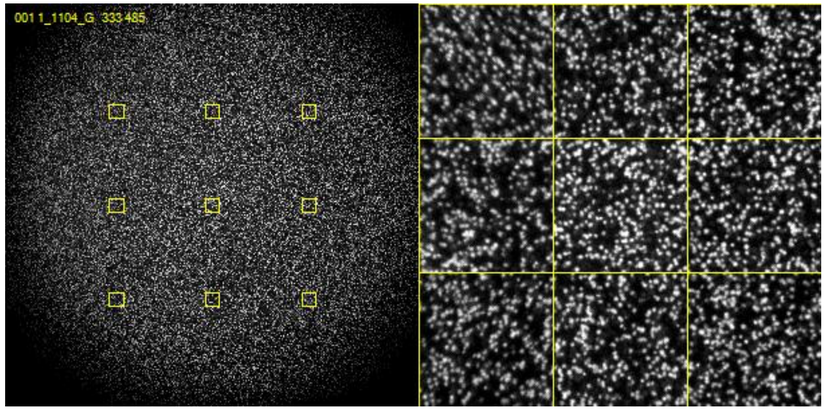
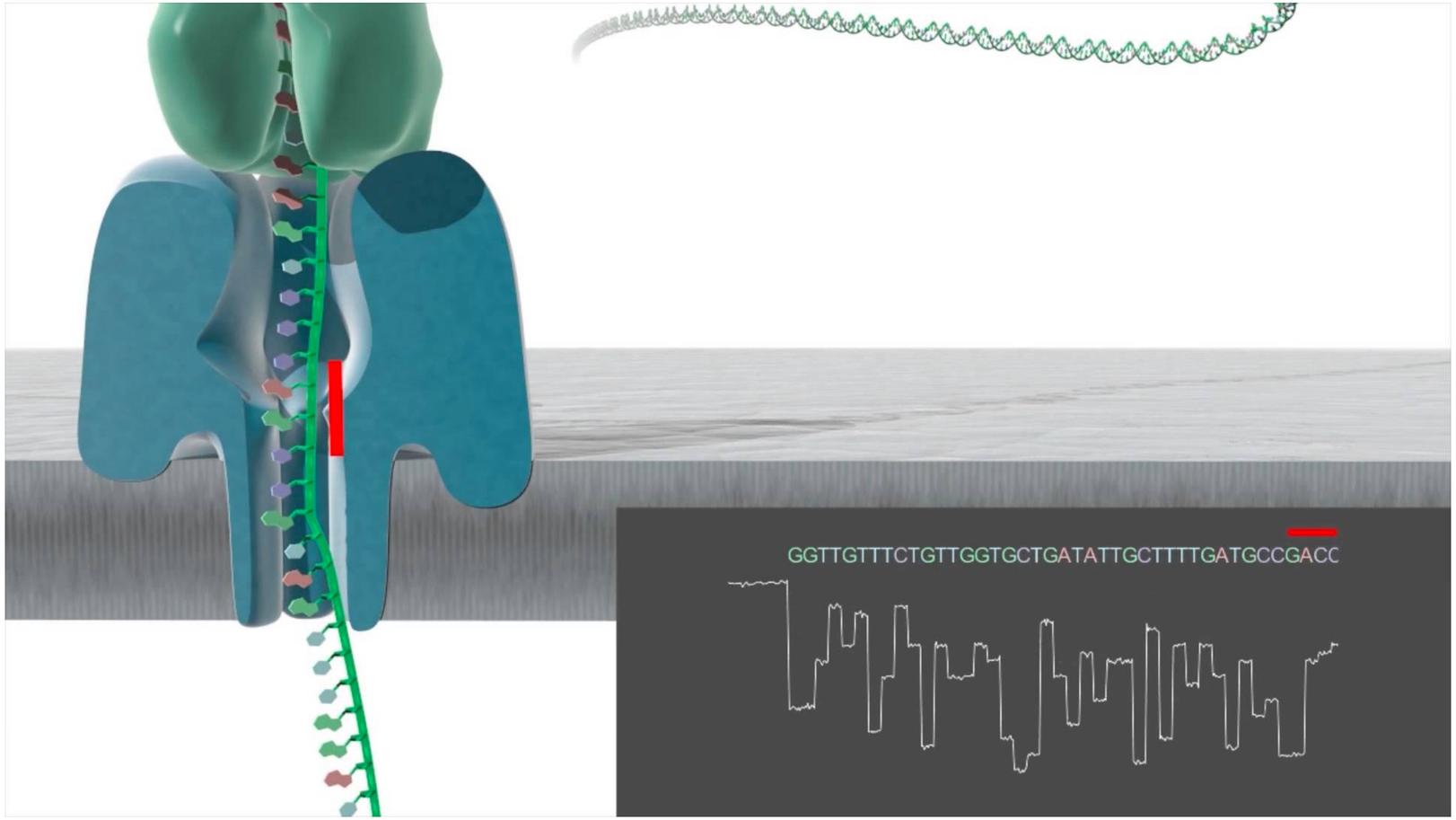


Image credit: Illumina

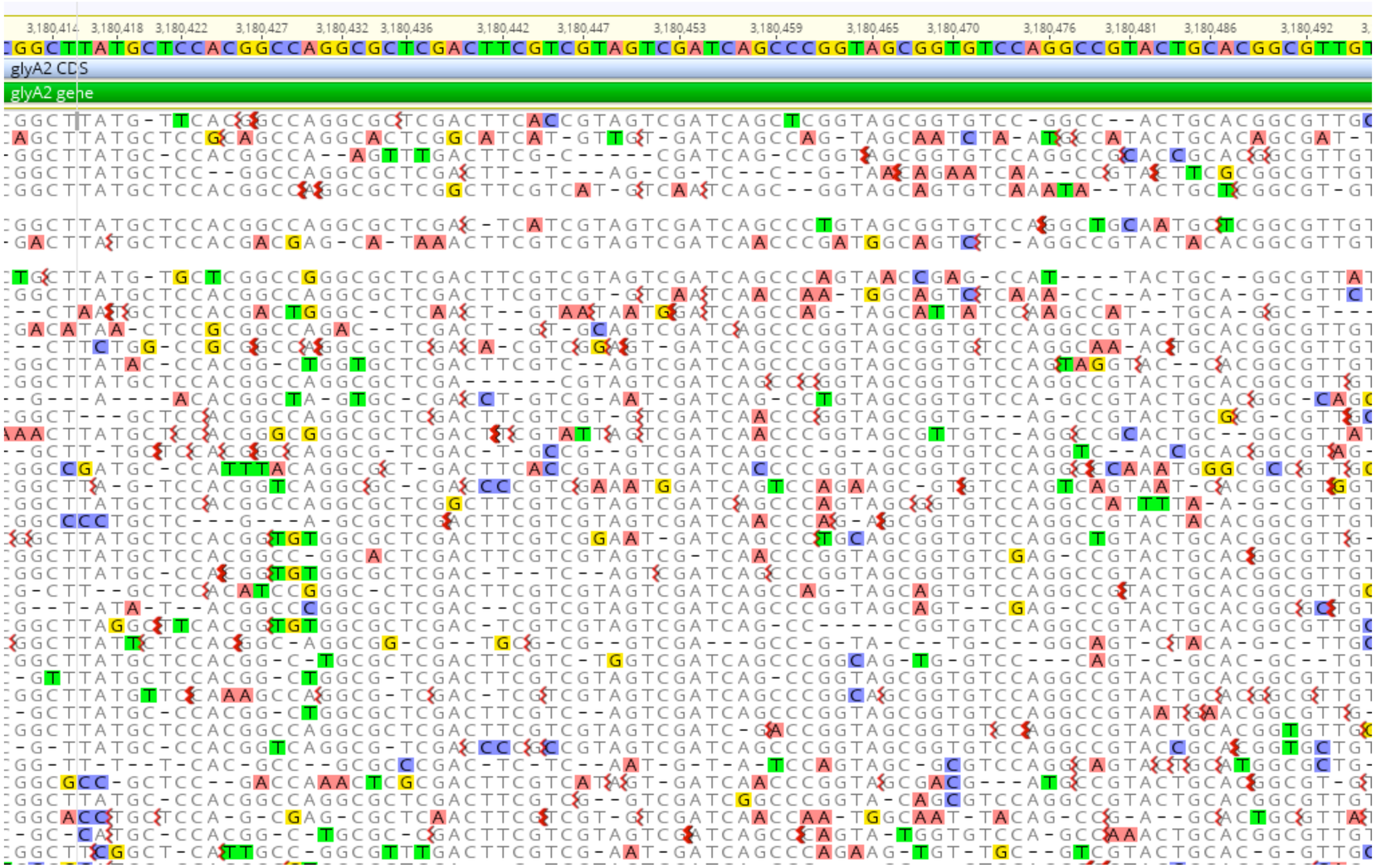
Long read sequencers sequence single molecules



Nanopore sequencing: Much longer reads, but with much higher error rates

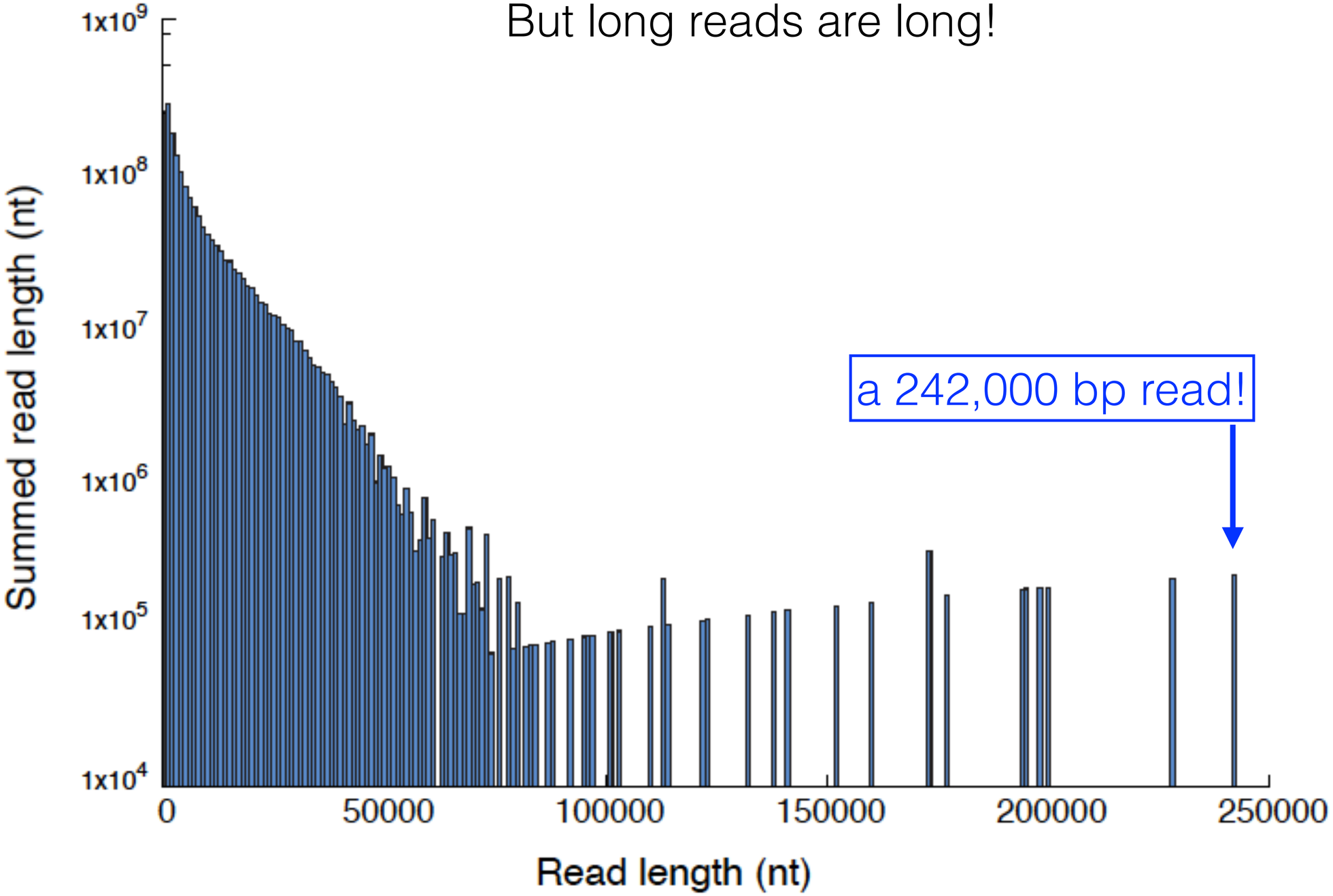
Image: Oxford Nanopore

Long reads have relatively high error rates (up to 5-10%)



Improvements to nanopore chemistry and software is pushing down error rate

But long reads are long!



Justin Lee

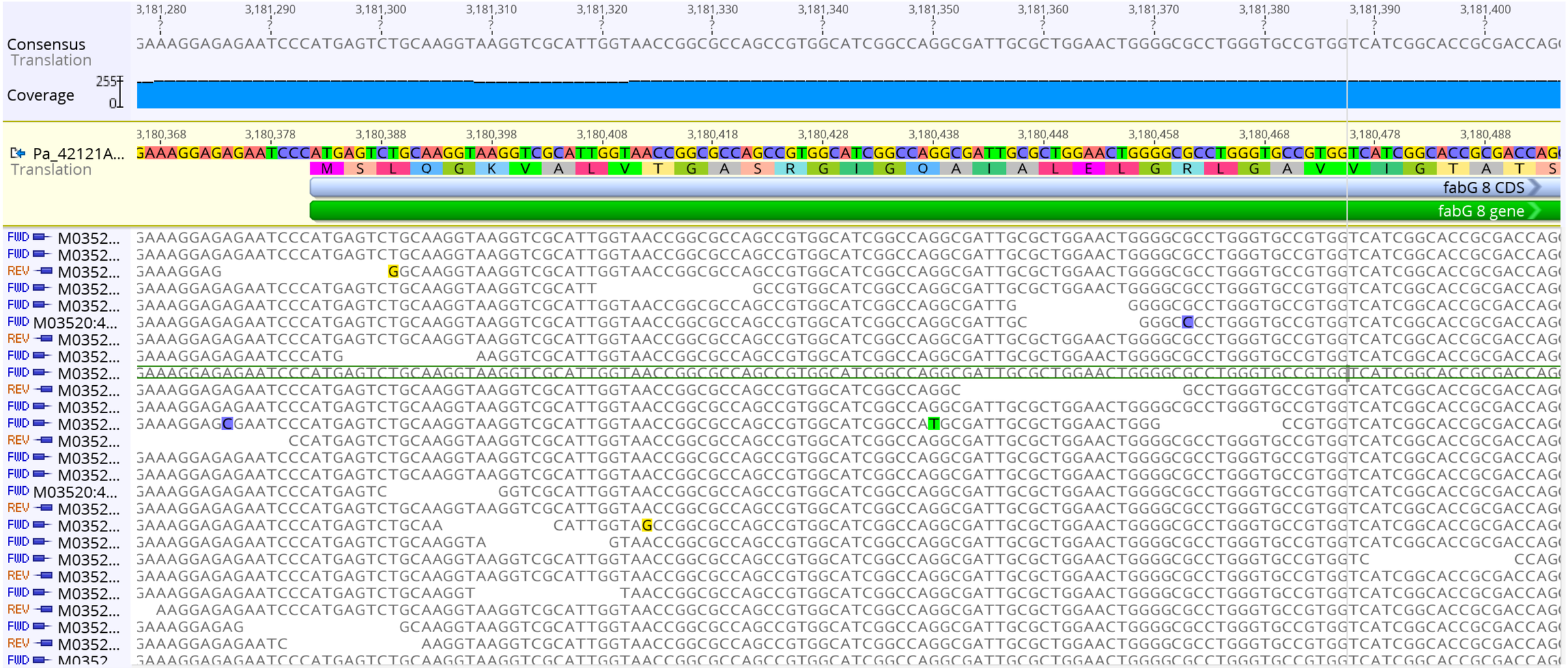
Some nanopore sequencers are portable

Oxford Nanopore
@nanopore

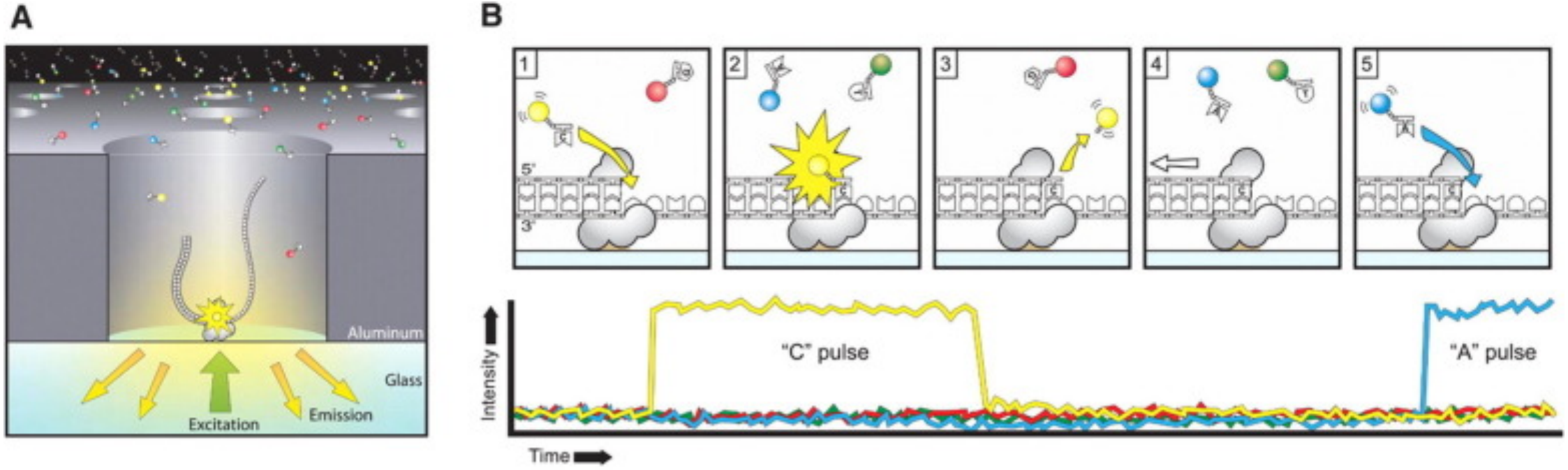
Our new field kits can be stored at ambient temperature for up to a month, the workflow is simple with no compromise on throughput. Sequencing anywhere made easier: bit.ly/2v1ilCG #ECCMID2019

2:08 AM · Apr 12, 2019 · HubSpot

Illumina reads have much lower error rates (~0.1% – 1%)

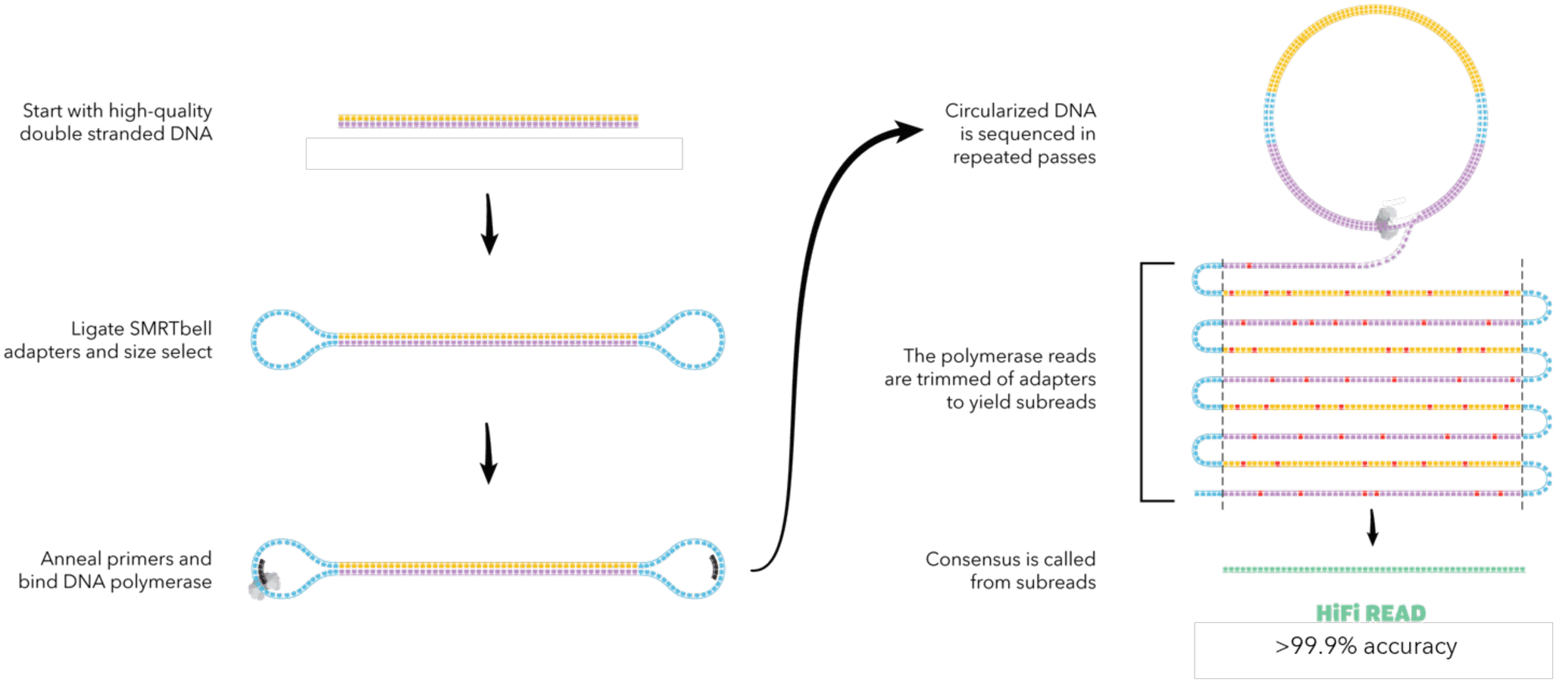


PacBio single molecule real-time (SMRT) sequencing is the other main long-read technology



Rhoads and Au, Genomics Proteomics Bioinformatics 13 (2015)

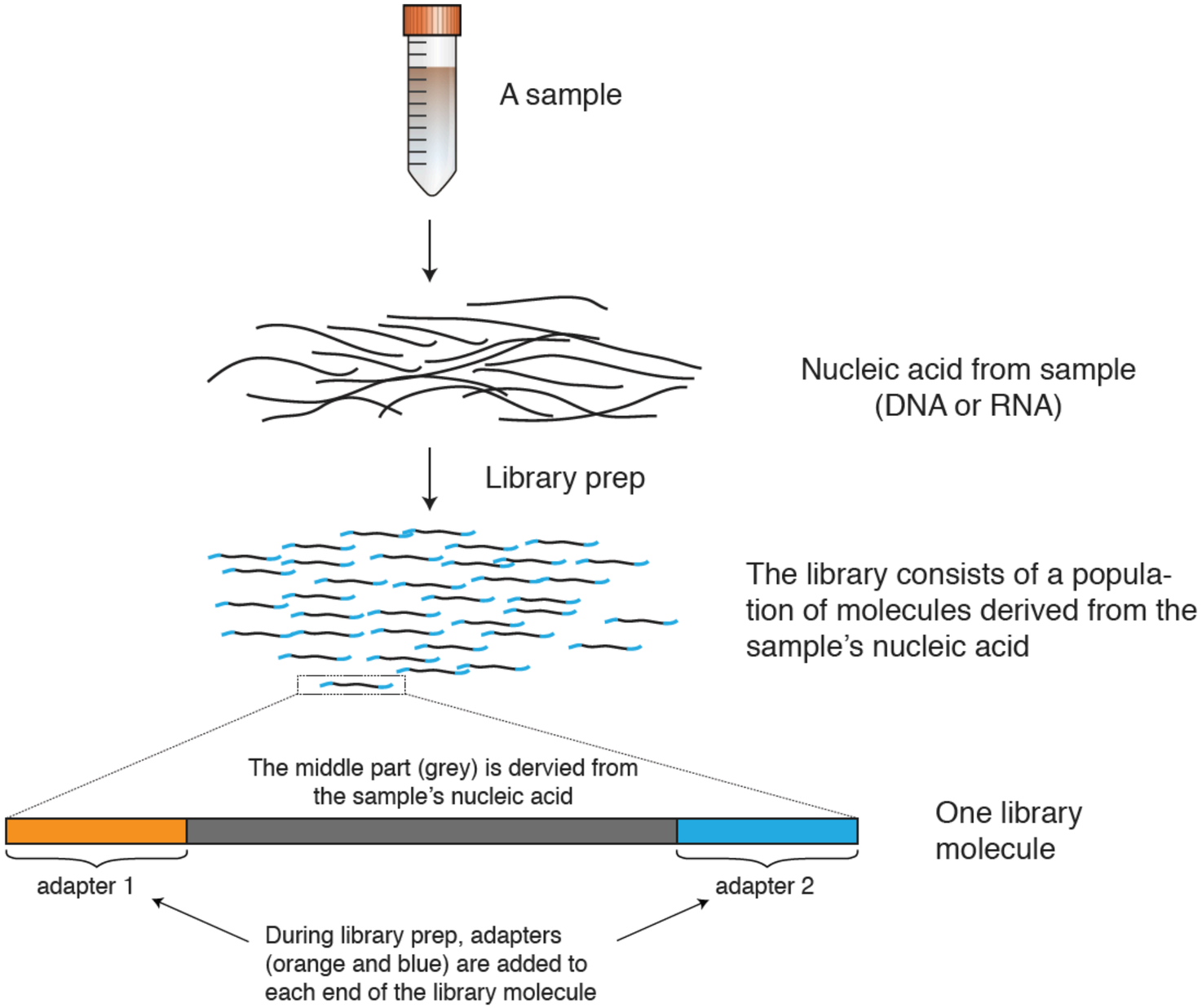
PacBio HiFi sequencing sequences the same molecule many times to reduce error rate



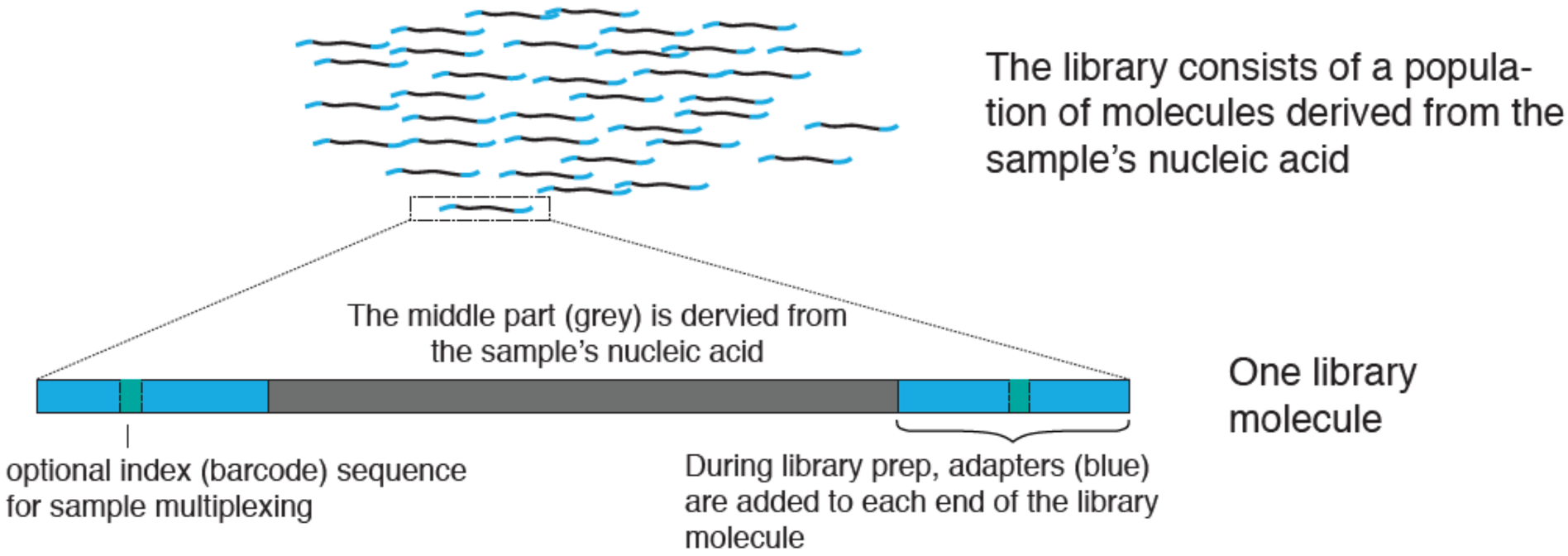
Best of both worlds: long reads with low error rate

Image: PacBio

Library prep converts nucleic acids into a form suitable to be sequenced

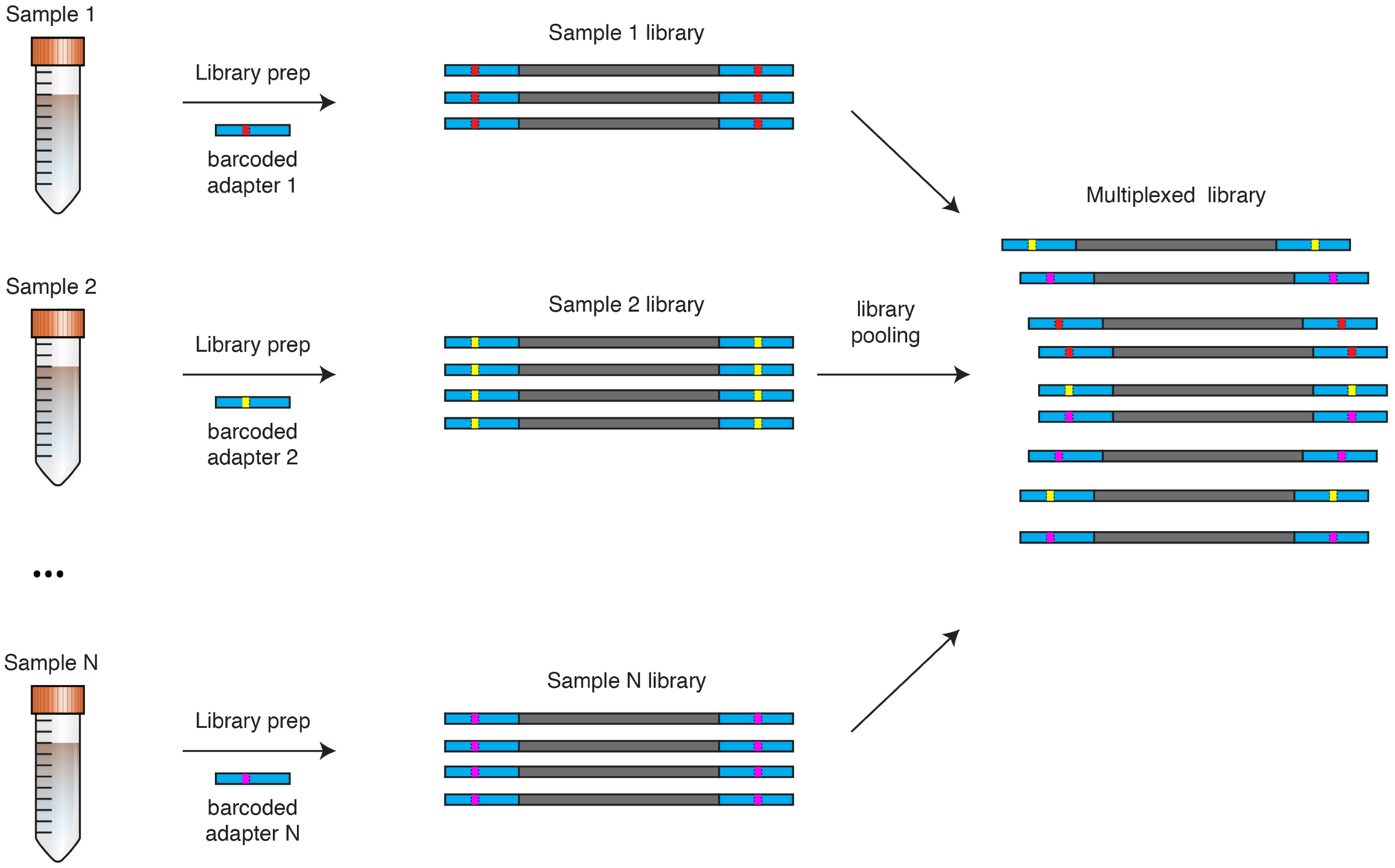


Library prep converts nucleic acids into a form suitable to be sequenced



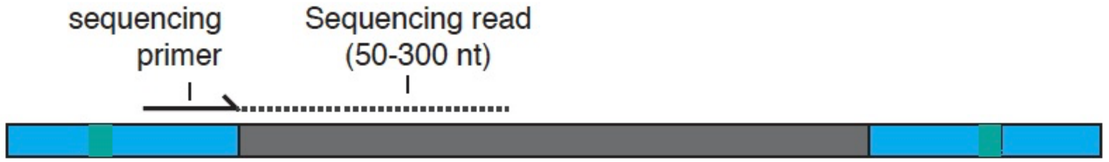
An example Illumina library molecule

Barcodes (or indexes) allow sample multiplexing



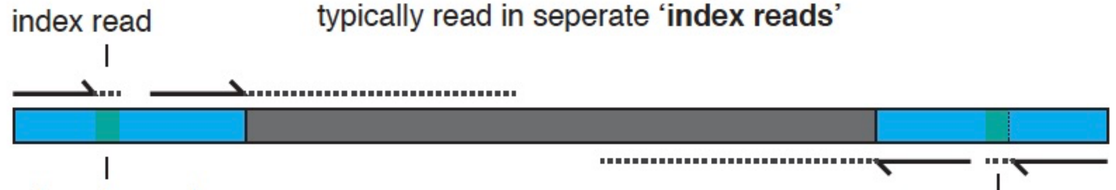
Illumina sequencing produces 1-4 reads per library molecule

In **single end sequencing**, a library molecule is sequenced from one end

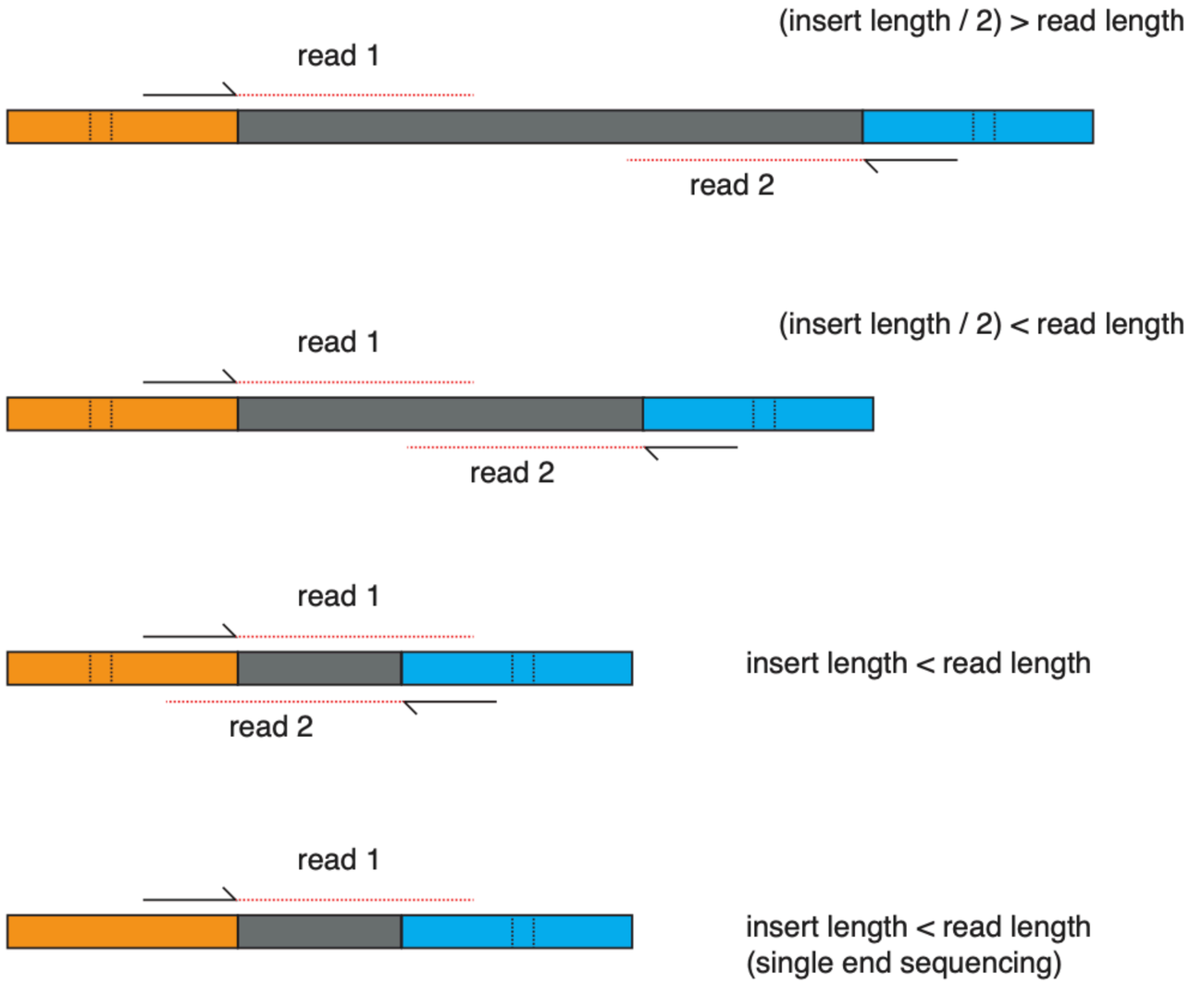


In **paired end sequencing**, a library molecule is sequenced from both end

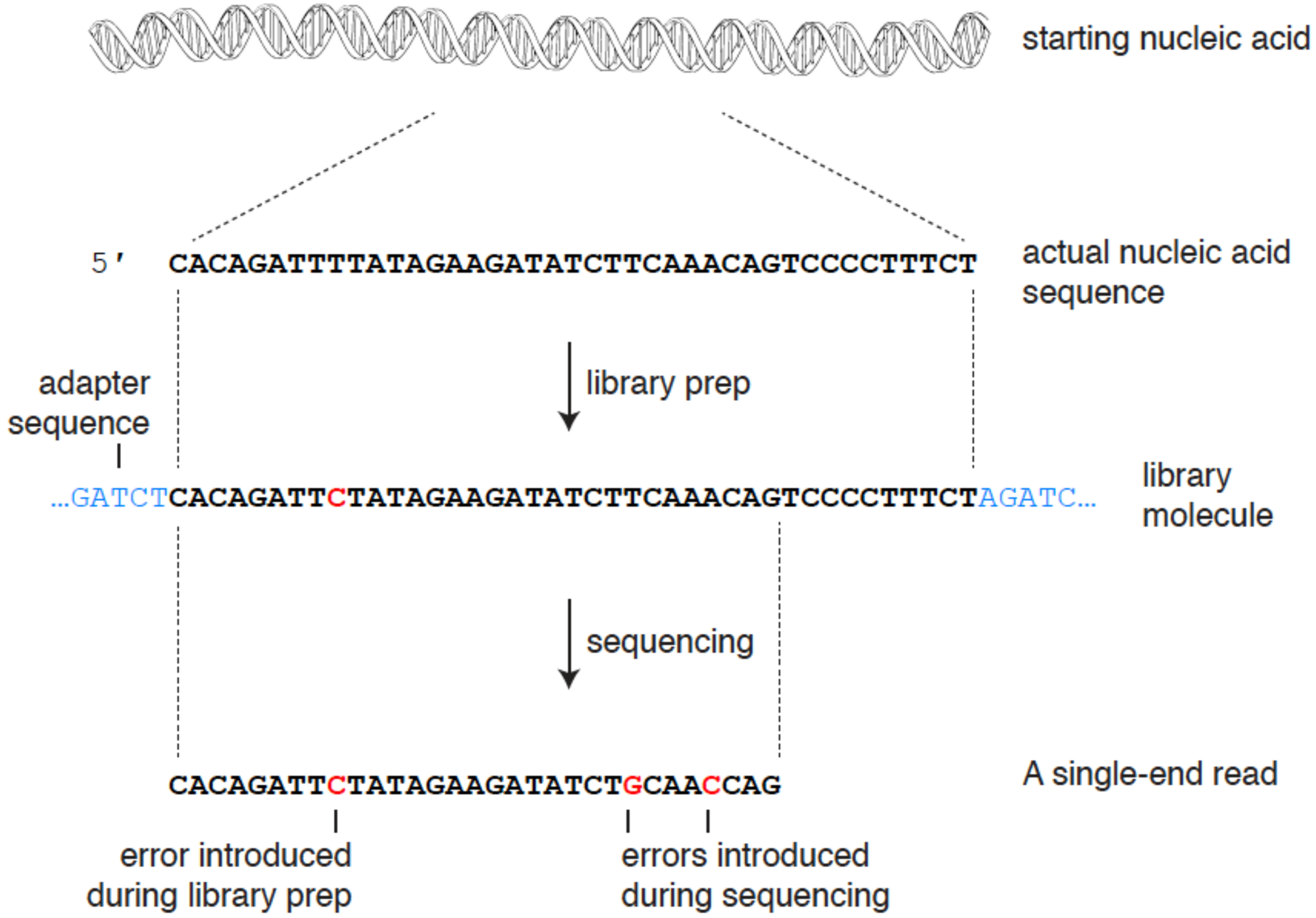
The library molecule's barcodes (indexes) are typically read in seperate '**index reads**'



Whether or not paired reads overlap and whether or not a read extends into the opposite adapter is a function of insert size and read length



Reads are measurements of the real sequence that often contain errors and are usually shorter than the starting nucleic acid

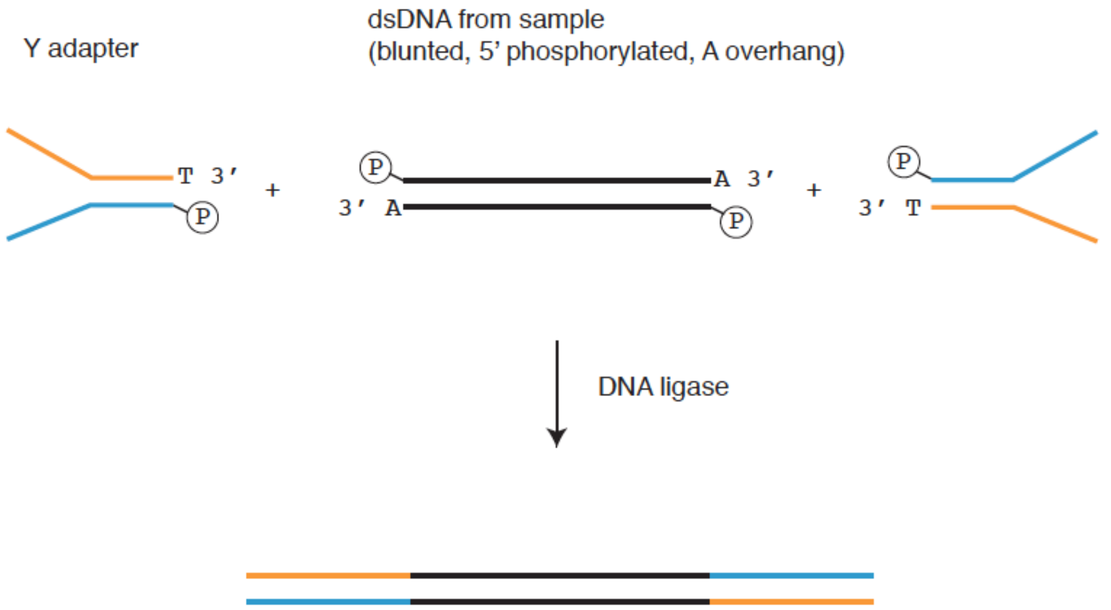


There are many good ways to make sequencing libraries

Common Illumina library prep steps (not always included and not always in this order)

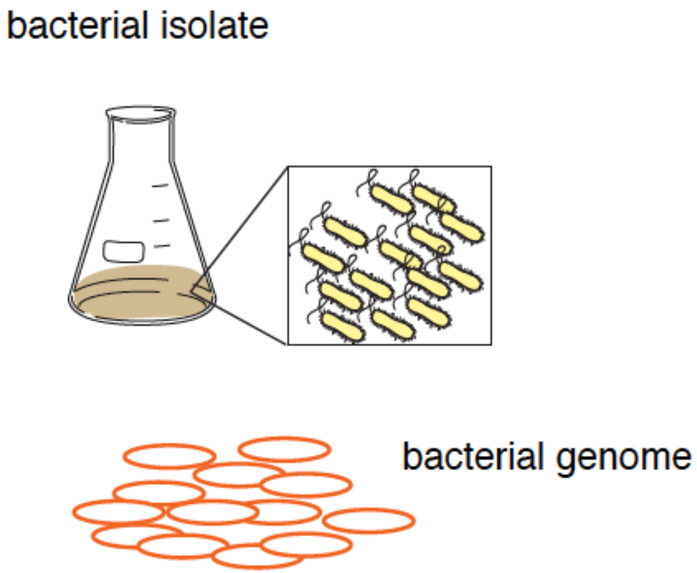
- Nucleic acid isolation
- Enrichment (of nucleic acid subtypes you want) or subtraction (of those you don't want)
- Nucleic acid fragmentation
- Conversion of RNA into dsDNA (for RNA sequencing)
- Addition of adapters to ends of library molecules, possibly with barcodes for multiplexing
- Library amplification
- Pooling of multiplexed samples
- Library QC / quantification

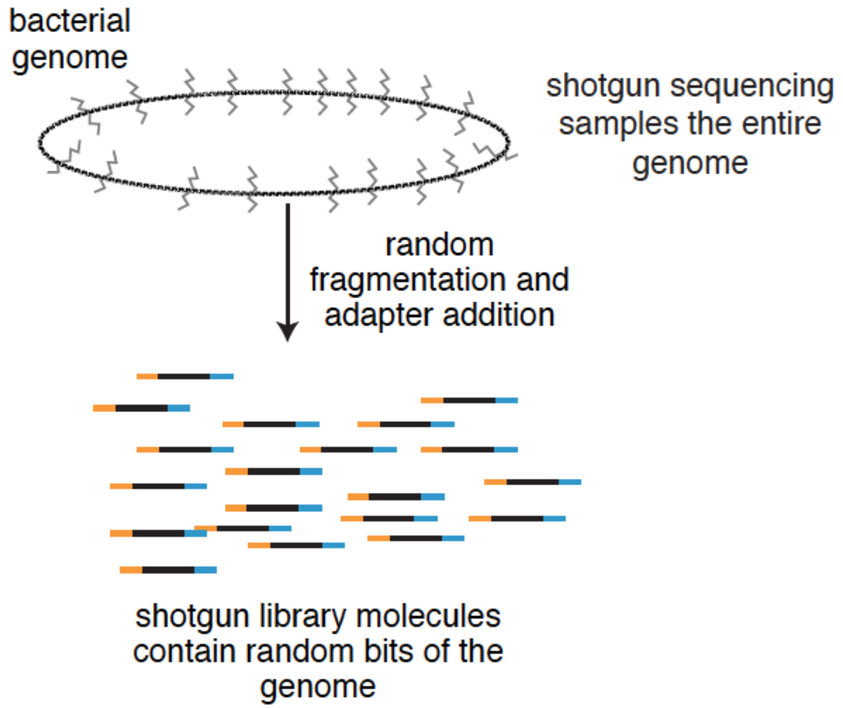
Adapters can be added to sample-derived dsDNA by ligation



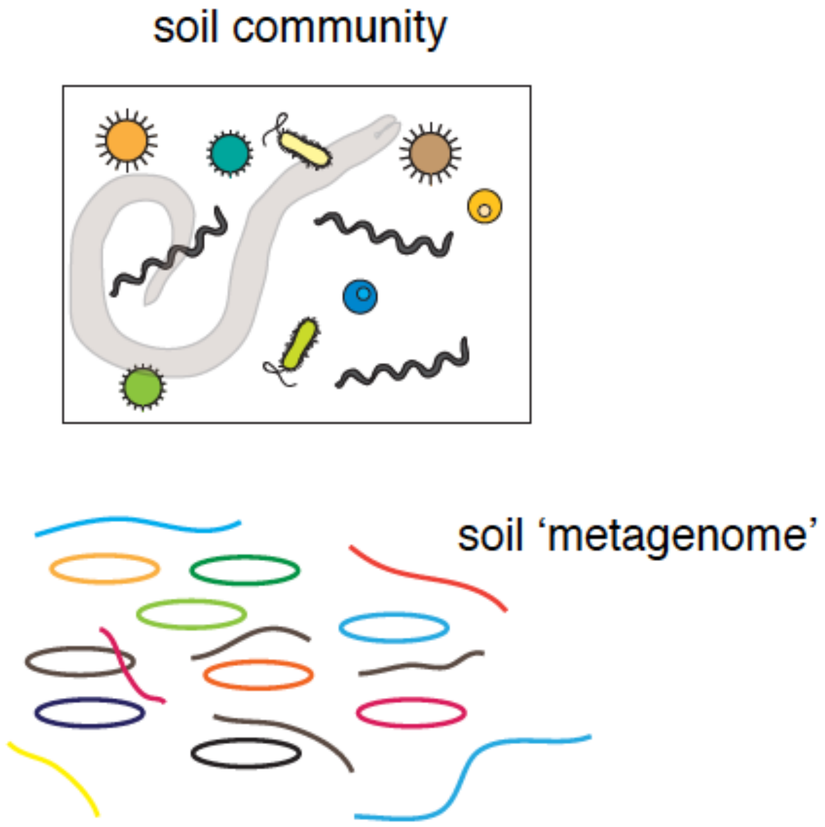
How you make a library determines what type of sequencing you're doing

For instance, if you make a 'shotgun library' from a single organism, you're doing whole genome sequencing (WGS)





Metagenomic sequencing involves sequencing of genomes from more than one organism



Could make a 16S or a shotgun library from these genomes

Sequencing of RNAs from a complex sample like this is metatranscriptomics

“Nothing in Biology Makes Sense Except in the Light of Evolution”

- Theodosius Dobzhansky

Image credit: Steve McCluskey CC BY-SA 3.0 Link

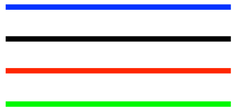
Biological sequences really only make sense when you compare them to each other

Sequence alignment is at the heart of a lot of bioinformatics and sequence analysis

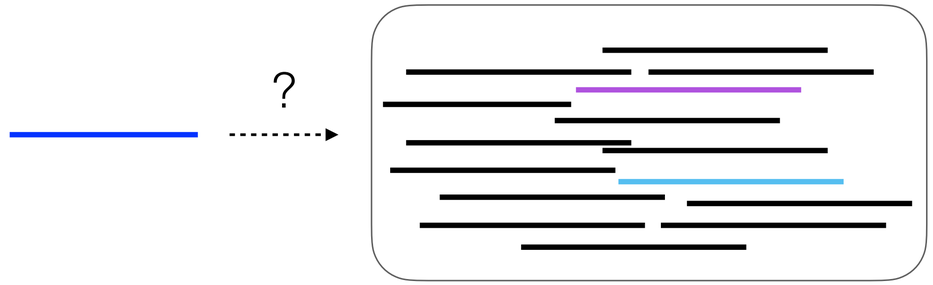
Major categories of sequence alignment

Pairwise alignment

Multiple sequence alignment

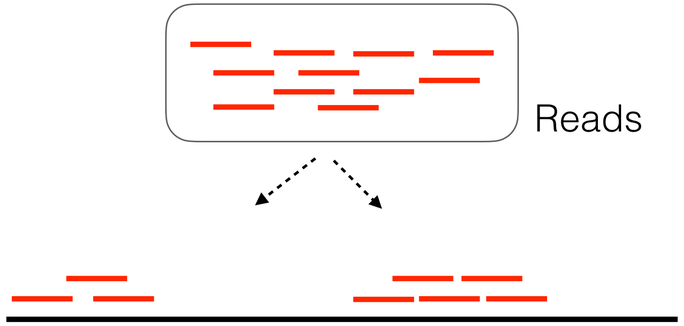


Alignment-based database searches



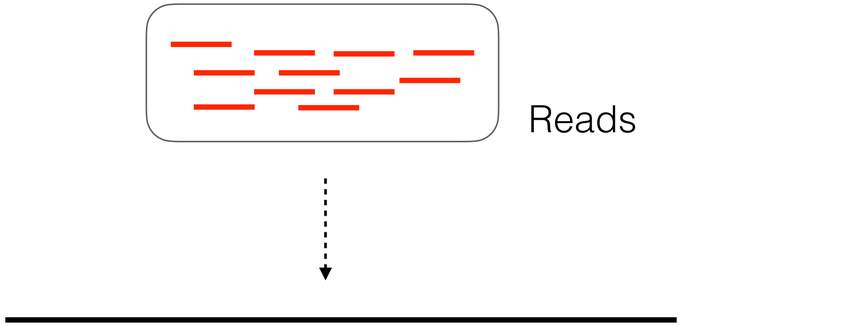
Database

Alignment to reference (mapping)



Existing Reference sequence

Assembly

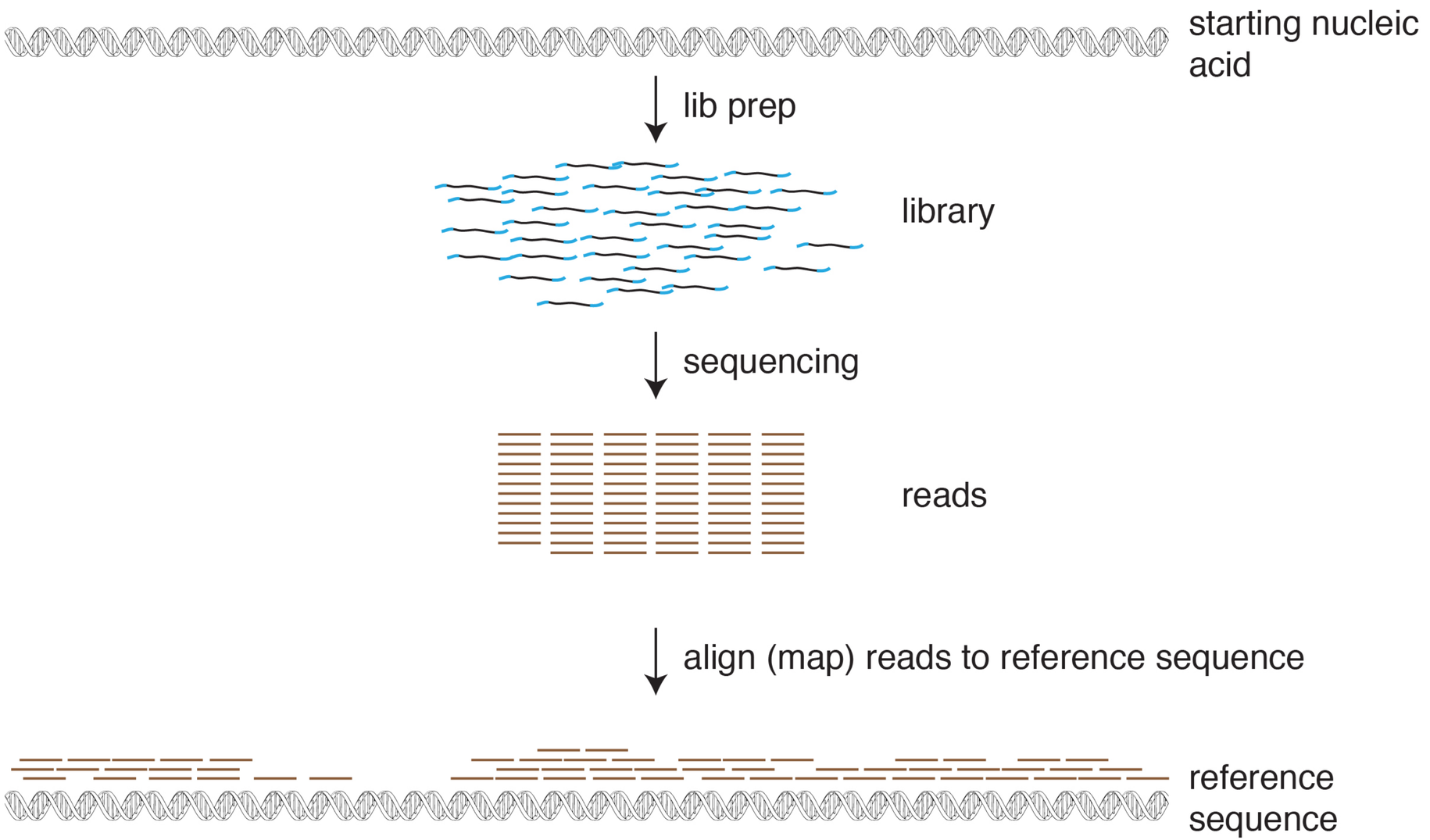


New Reference sequence

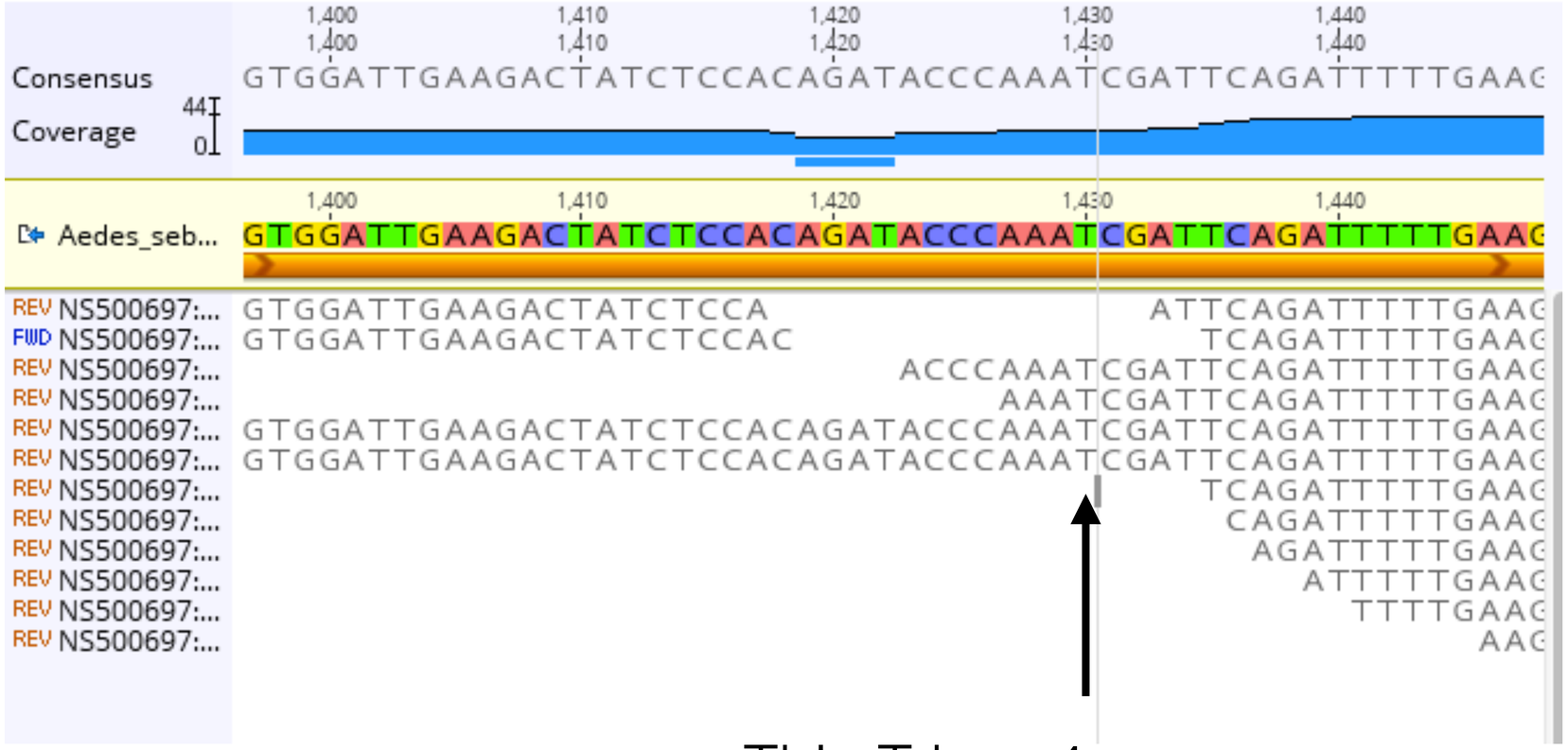
Major categories of sequence alignment

Alignment type	Purpose	Commonly-used software
Pairwise alignment	Identify the similarities or differences between two sequences	Needle (global alignment) Water (local alignment)
Multiple sequence alignment	Identify the similarities or differences between >2 sequences. Input to tree building.	MAFFT
Alignment-based search	Find the most closely related sequence in a database of sequences	BLAST
Mapping (alignment to reference)	Determine the most likely location in a reference sequence from which a shorter sequence (a read) derives	BWA Bowtie2
Assembly	Create a new reference sequence using overlapping reads	SPAdes

Mapping is the process by which sequencing reads are aligned to the region of a genome from which they derive.



Coverage is the number of individual aligned reads that support a particular nucleotide in a reference sequence

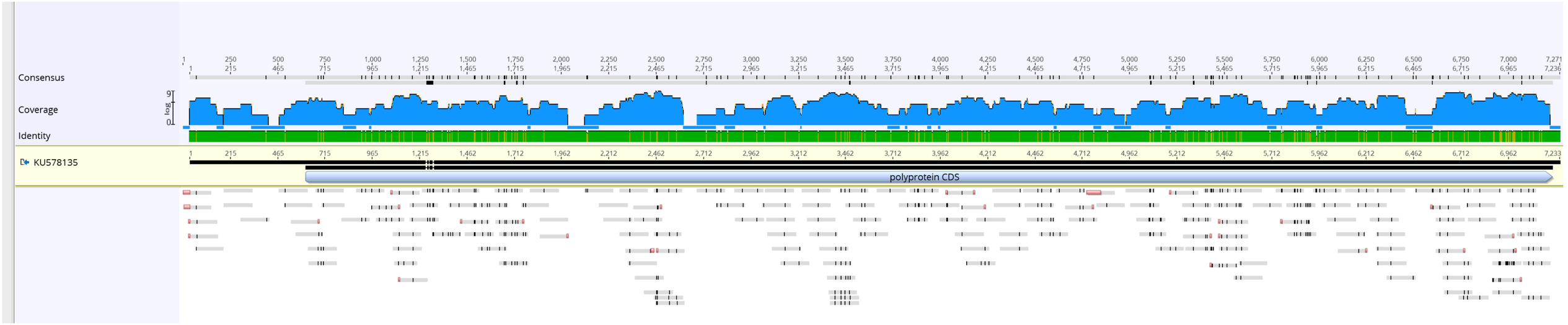


This T has 4x coverage

coverage is often referred to as ‘depth’ or ‘depth of coverage’

Coverage is also used to describe the fraction of a genome with >0x read coverage

reads from human oral swab RNA aligned to a coxsackie virus genome



96% genome coverage (96% of bases have >0x coverage)
3.4x average coverage depth (range 0-9x)

hand foot and mouth disease

(Mayo clinic)