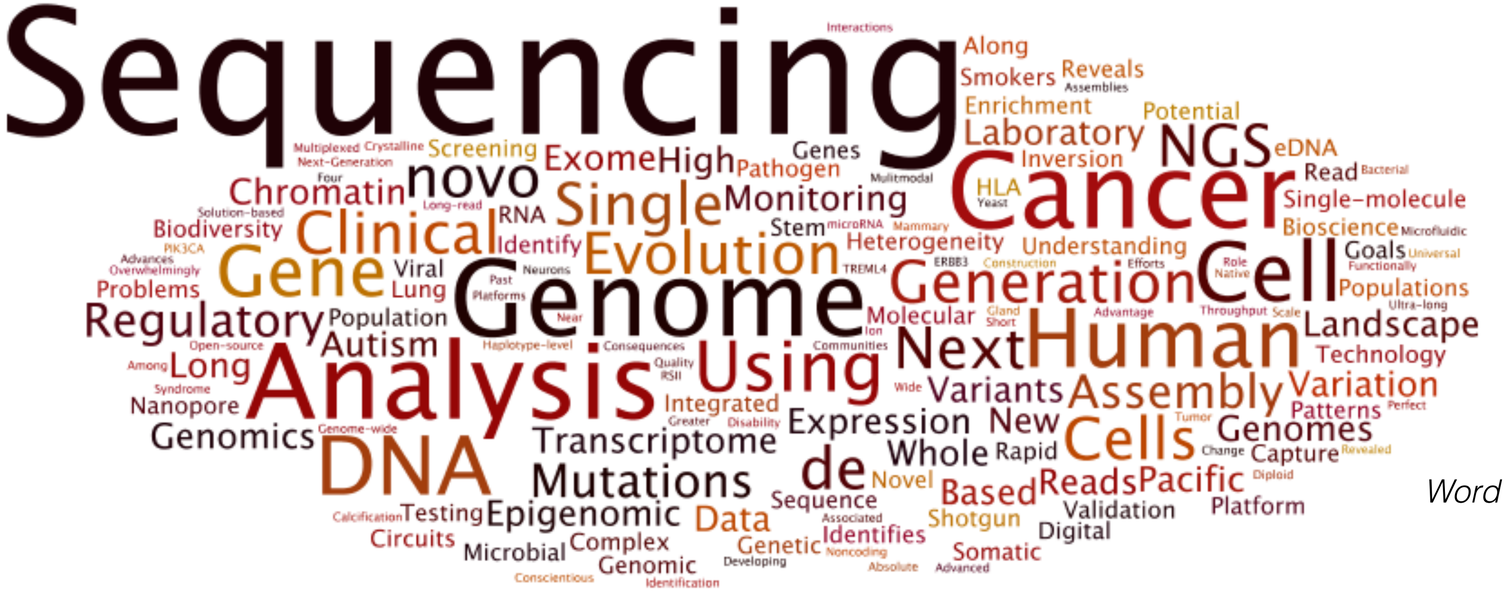
An overview of genomics and sequencing terminology and practices

Mark Stenglein

Computational Biology and
Genomics Workshop

Todos Santos Center
April 9-13, 2018

The jargon and terminology association with genomics and ‘next gen’ sequencing can be confusing and intimidating



Word cloud: Illumina

The goal of this lecture is to explain and demystify some common jargon and explain how sequencing works

There is a glossary available online that explains many of these terms

- Transcriptome
- Variant
- WGS

16S

The 16S ribosomal RNA gene is present in all bacterial and archaeal genomes. This gene is sufficiently conserved that primers that anneal to conserved regions of the gene will amplify essentially any prokaryotic 16S rRNA gene. These PCR products (amplicons) can be sequenced to provide a survey of microbial diversity in a sample.

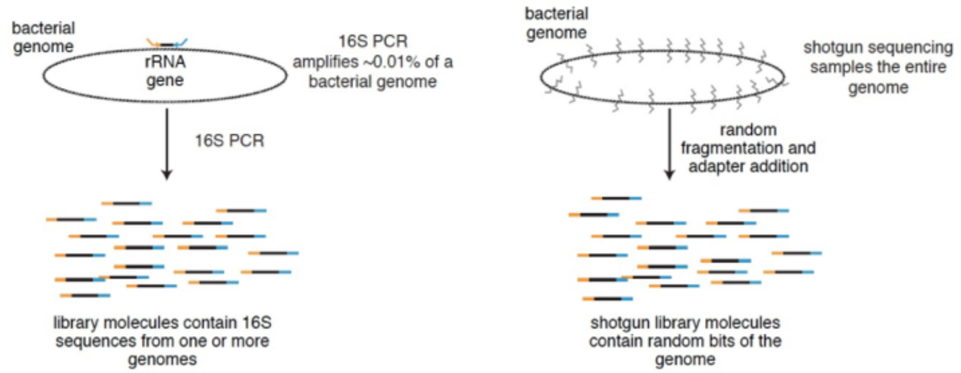


Figure: 16S vs. shotgun sequencing.

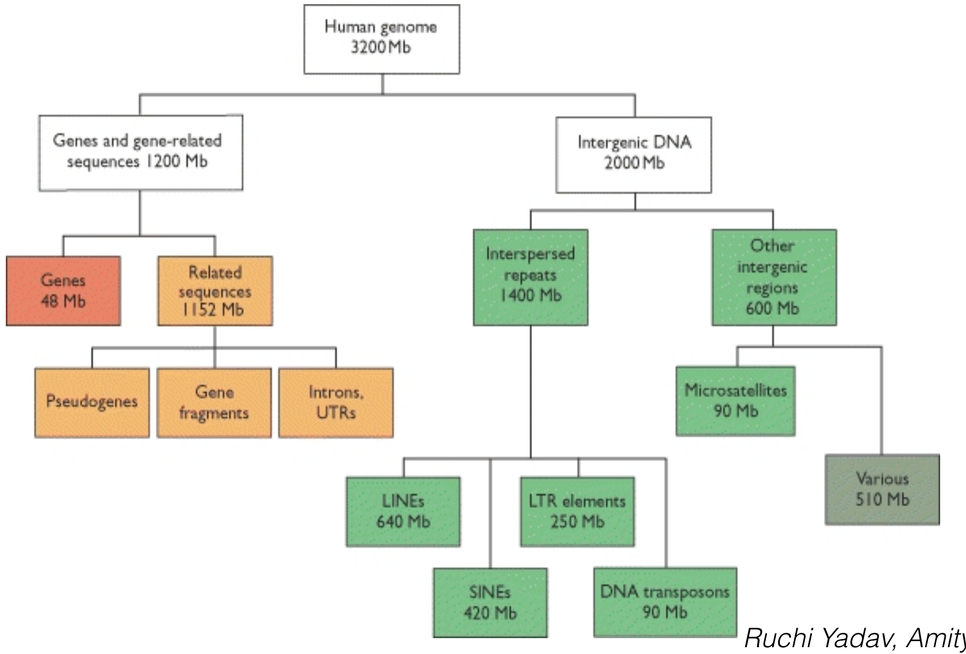
Adapter

Most NGS instruments require that dsDNA of known sequence be added to the 2 ends of library molecules that will be sequenced on the instrument. Adapters can be added in a variety of ways to starting nucleic acid molecules during library

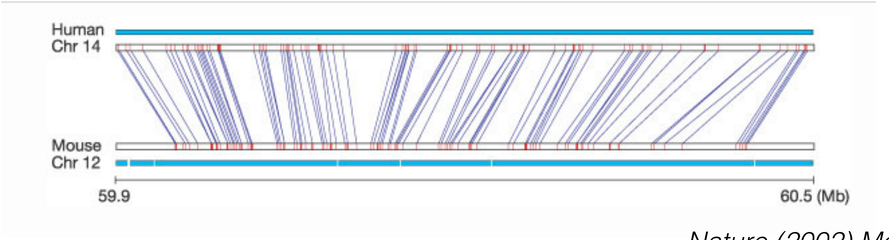
https://github.com/stenglein-lab/2018_Todos_Santos/blob/master/Genomics_and_NGS_Glossary.md

Genomics is the study of any of a number of attributes of genome or genomes

- Genome:
  - size
  - sequence
  - structure / variation
  - evolution
- Gene:
  - structure
  - expression
- Comparative genomics
- Epigenomics
- Metagenomics
- Transcriptomics
- Other -omics: Proteomics/Metabolomics

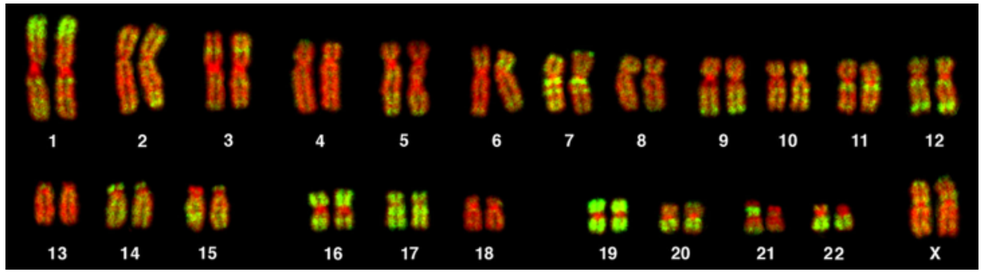


Ruchi Yadav, Amity University



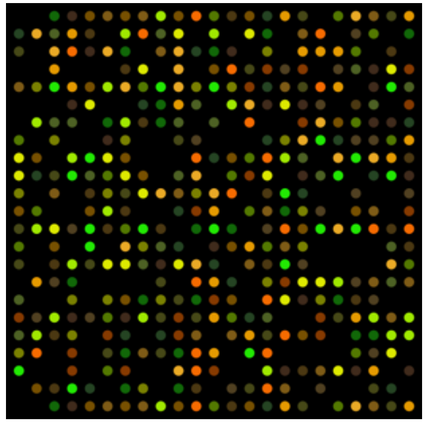
Nature (2002) Mouse Genome

Genomics isn’t the same thing as sequencing, but they’re increasingly related

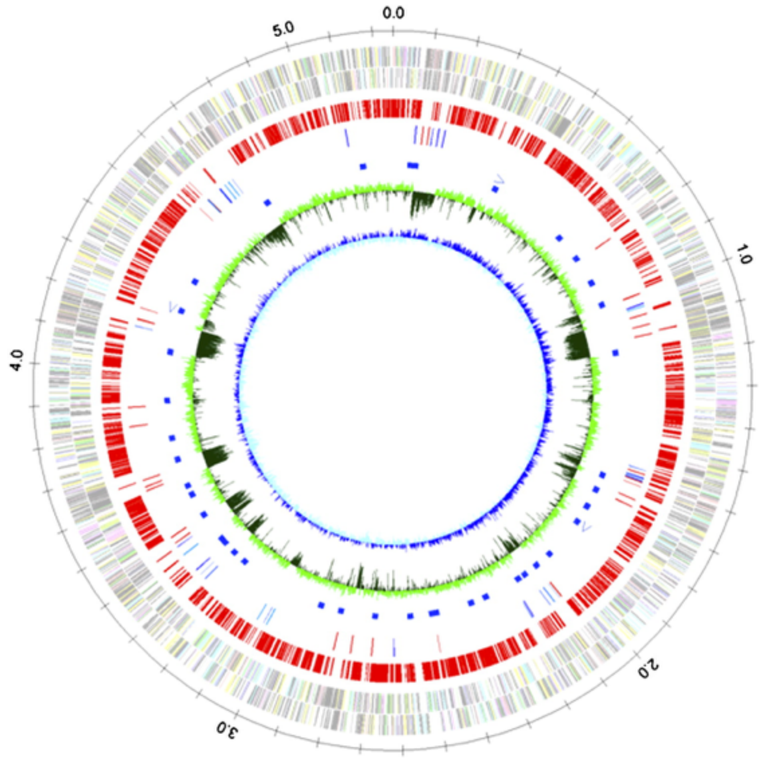


Bolzer et al (2005) PLoS Biol

Microarray

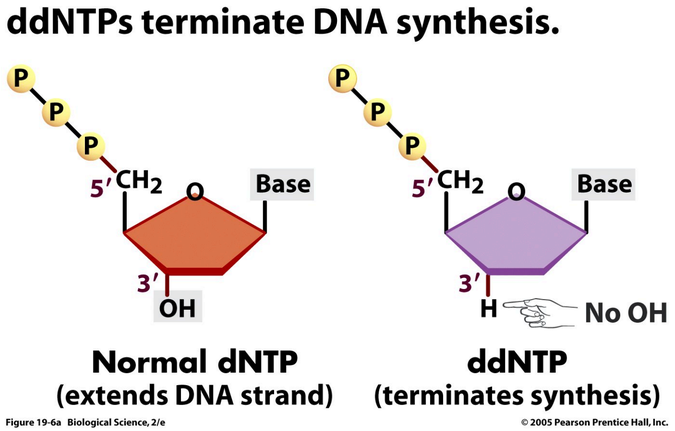


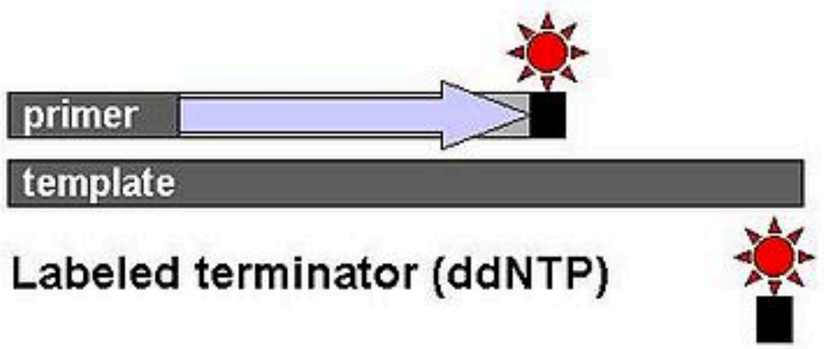
Wikimedia commons

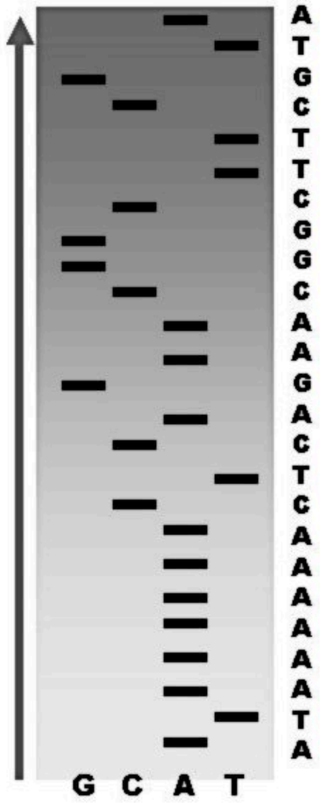


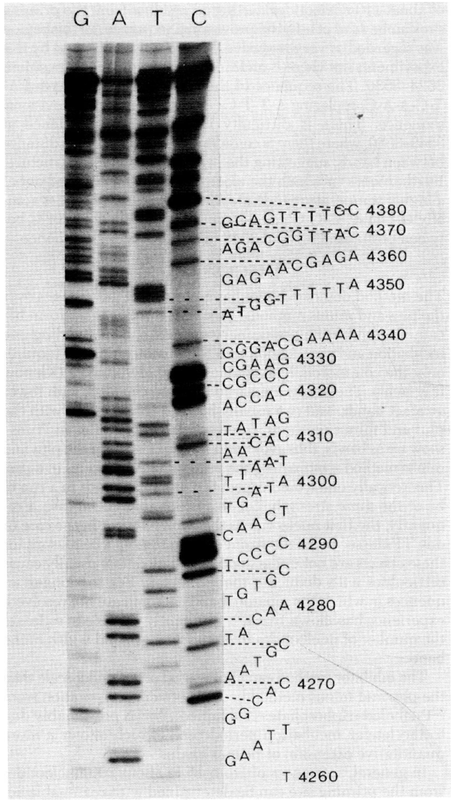
Nakazawa et al (2009) Genome Research

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









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

Improvements to Sanger sequencing and molecular methods allowed the sequencing of increasingly large genomes

1965 – First nucleic acid sequenced: Yeast trnA

1976 – First complete genome sequenced (RNA virus: bacteriophage MS2)

1977 – Maxam-Gilbert and Sanger DNA sequencing methods introduced and first complete DNA genome (Phage Φ-X174)

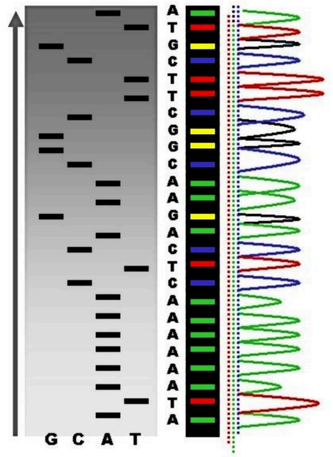
1983 – PCR introduced

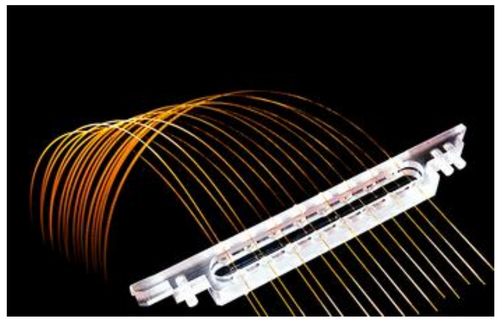
1995 – First complete cellular genome (*Haemophilus influenzae*) and eukaryotic genome (yeast) sequenced

2001 – Publication of the first sequenced human genomes

2005 – Introduction of 454 Sequencing and the NGS Revolution

2005 – present: Rapid evolution of NGS technology





Slide courtesy Dan Sloan.

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

All simultaneously sequence many molecules in parallel

Short read sequencing

- Millions of reads
- Relatively short: ~50-300 nt (Illumina)
- Relative low error rates
- Illumina has virtually all of the market share

MiSeq

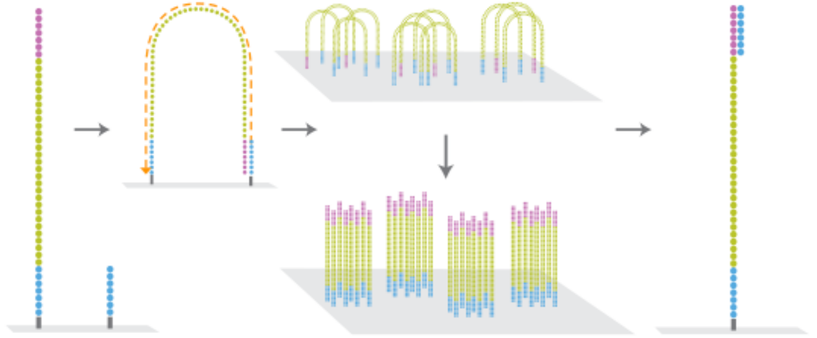
Long read sequencing

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

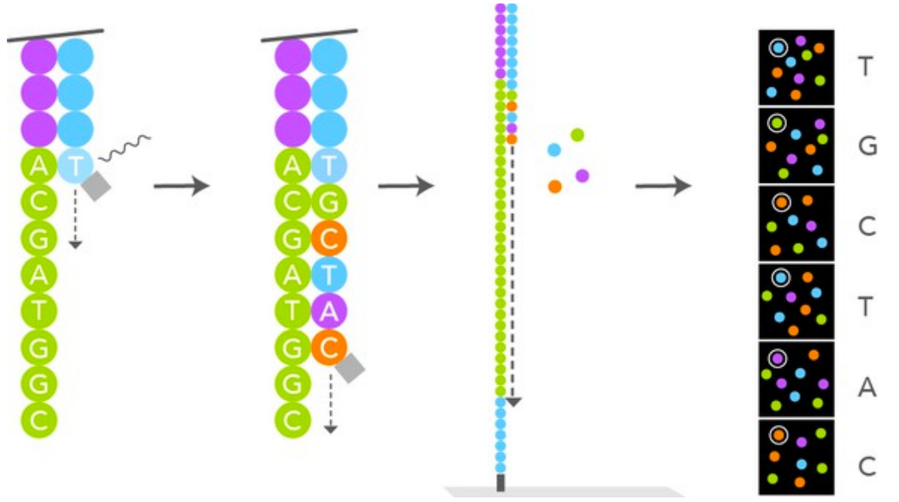
Oxford Nanopore MinION

PacBio RS-II

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

real raw Illumina sequencing data

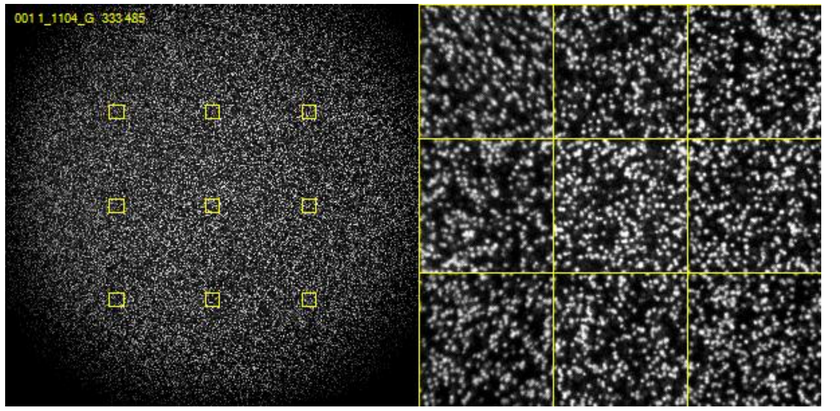
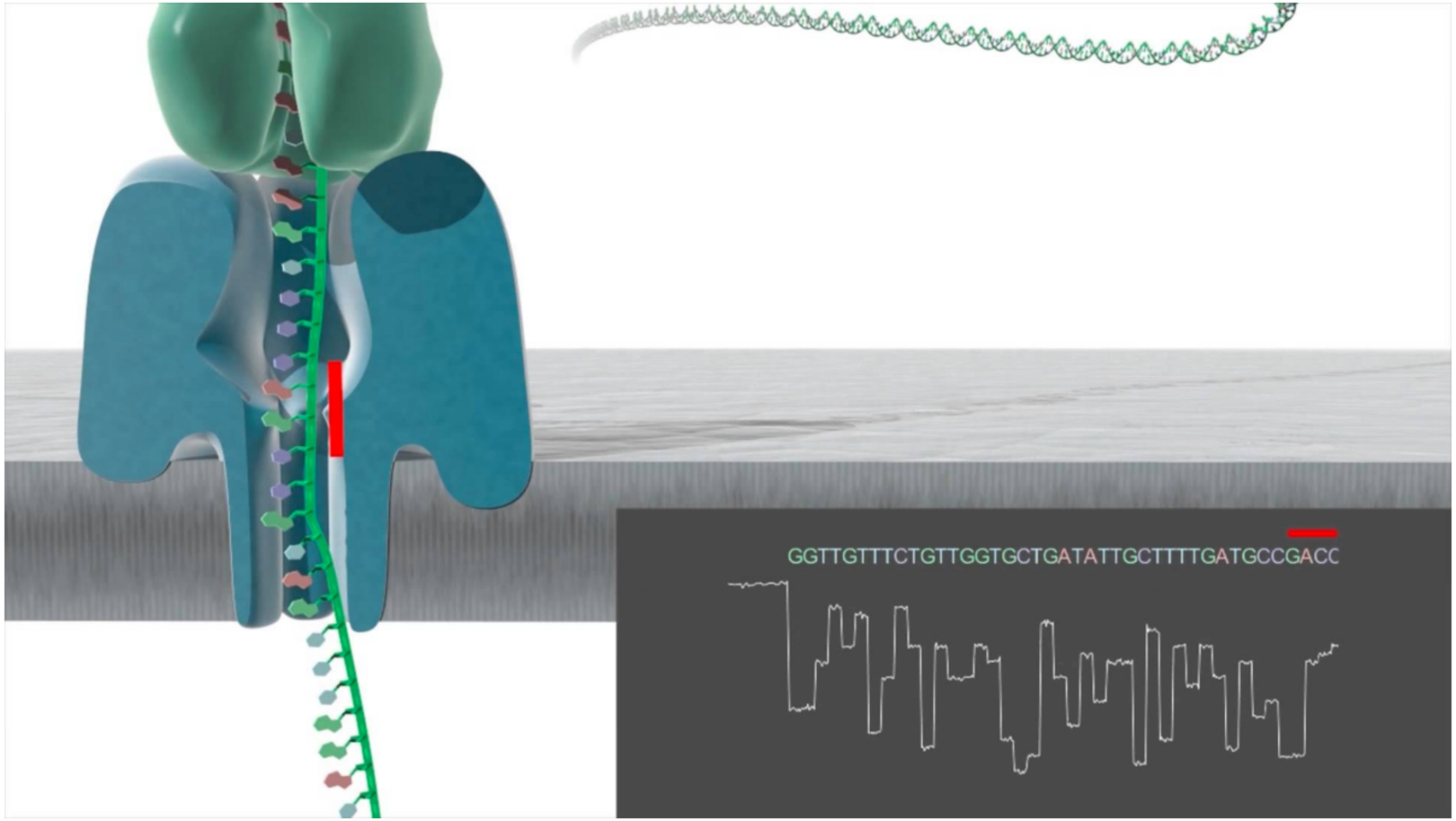


Image credit: Illumina

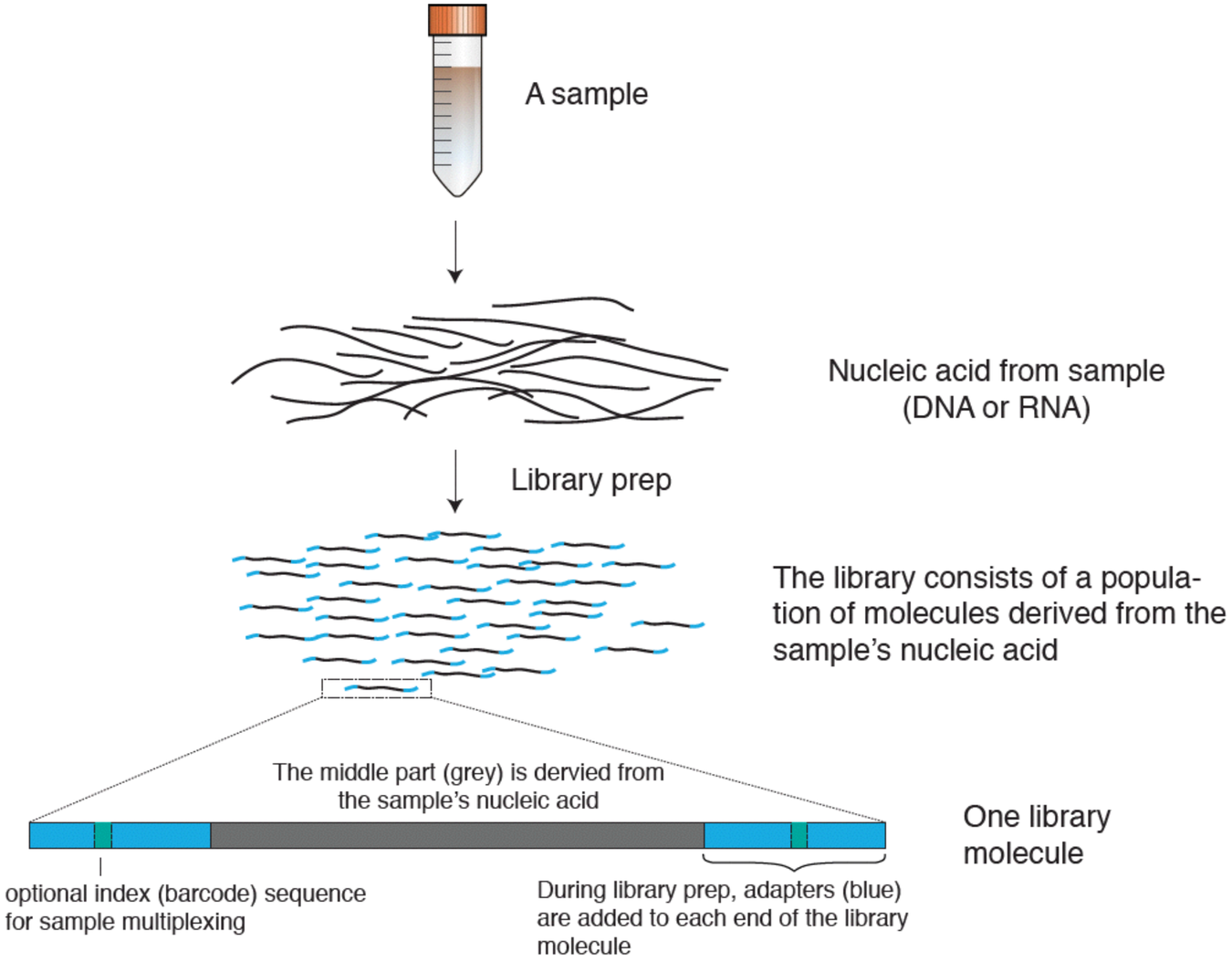
Long read sequencers sequence single molecules



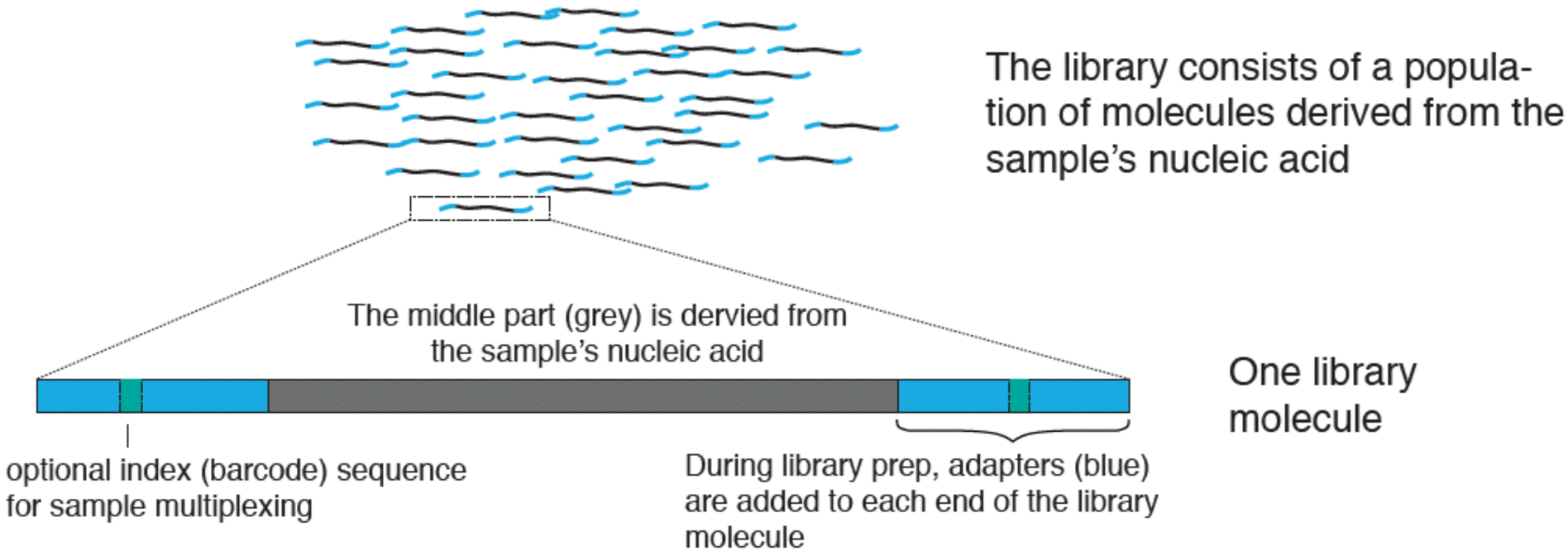
Much longer reads, but with much higher error rates

Image: Oxford Nanopore

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

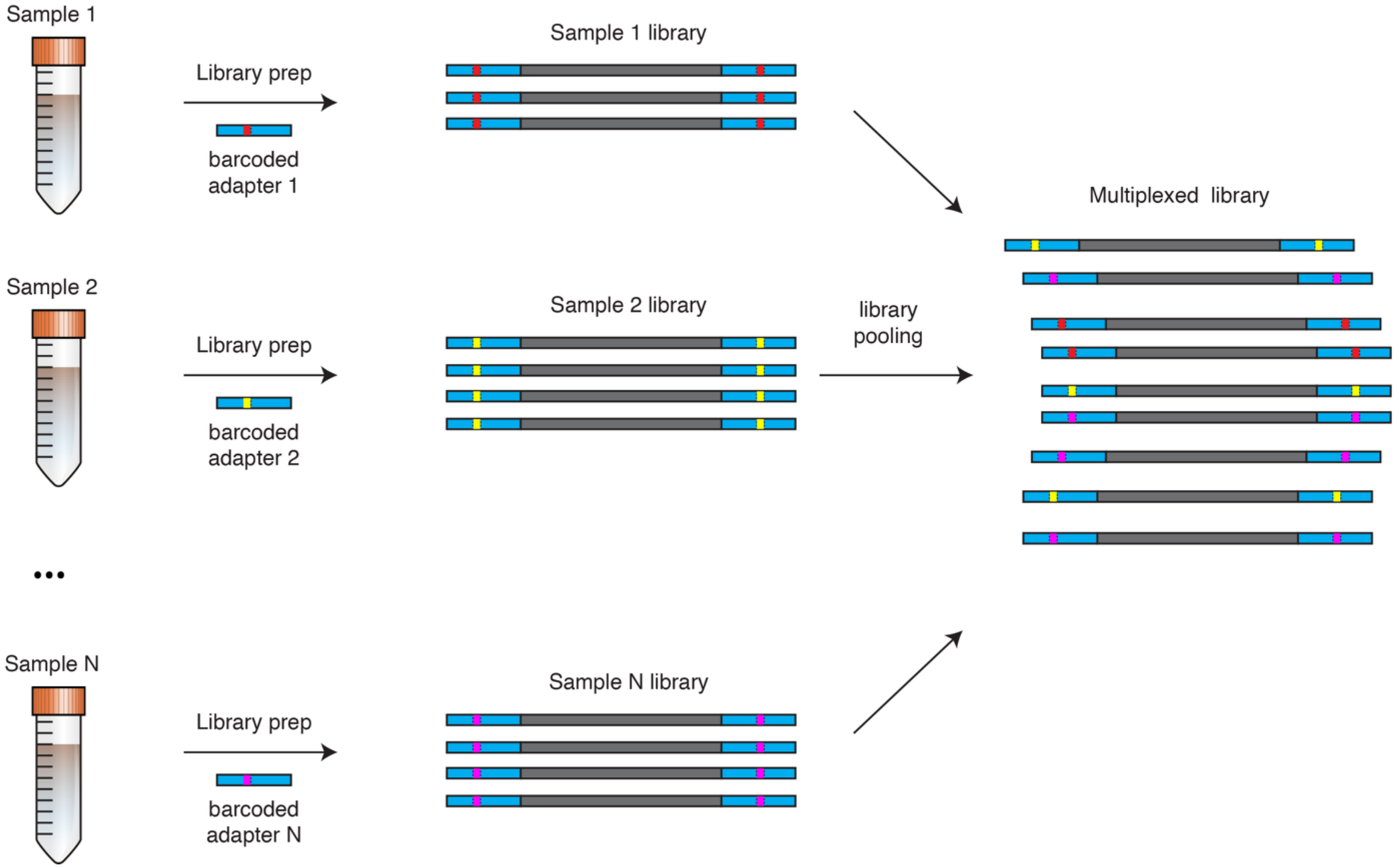


Library prep converts nucleic acids into a form suitable to be sequenced



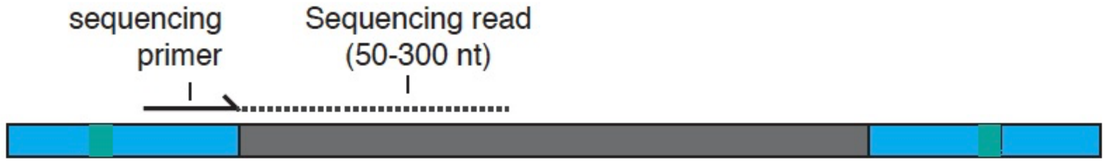
An example Illumina library molecule

Barcodes (or indexes) allow sample multiplexing



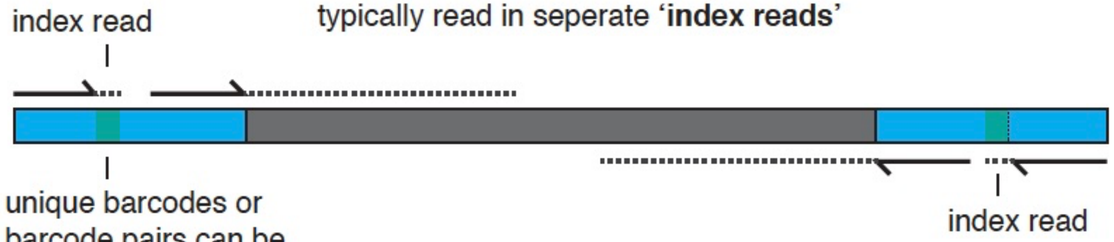
Illumina sequencing produces 1-4 reads per library molecule

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

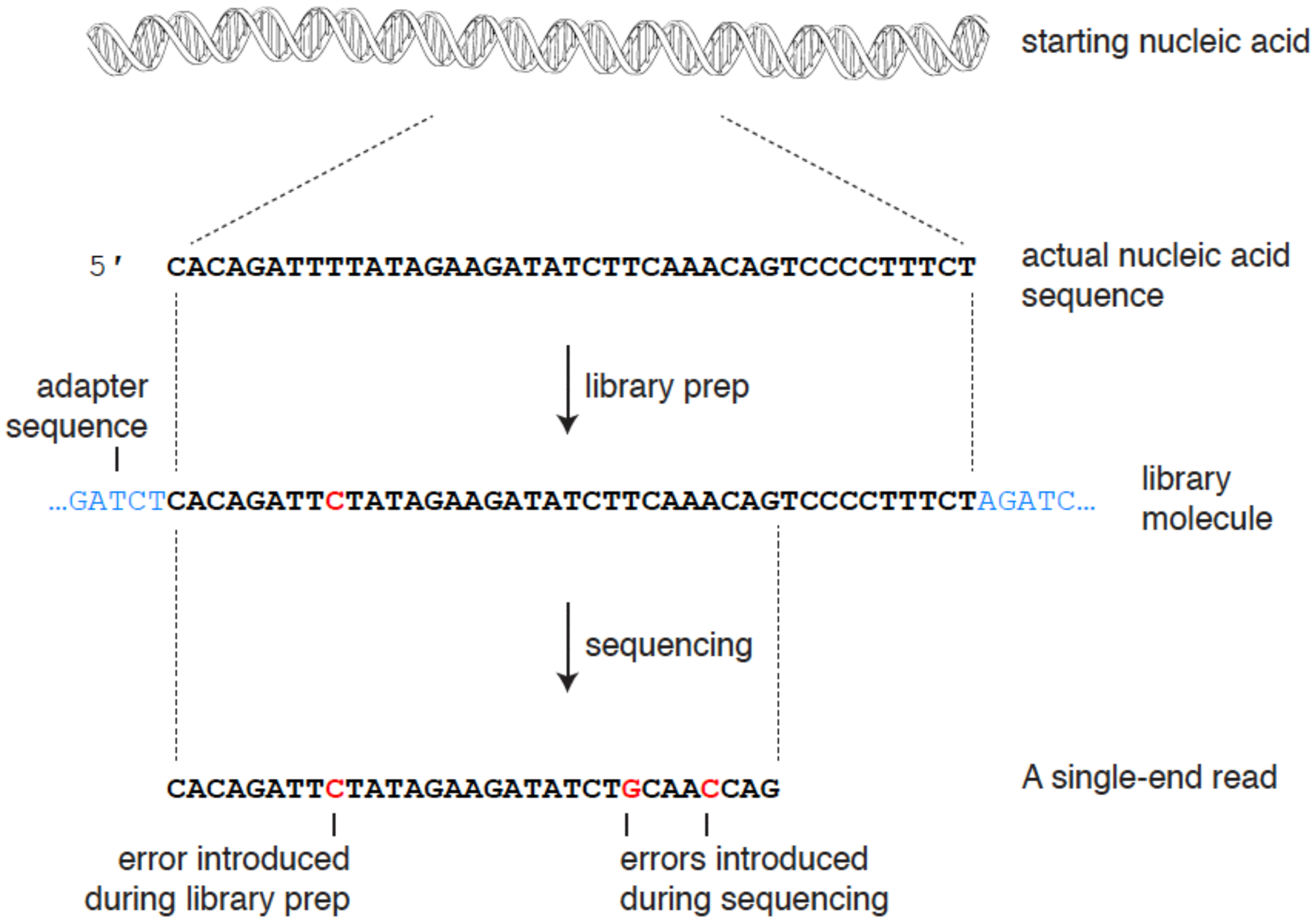


In **paired end sequencing**, a library molecule is sequenced from both end

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



Reads are sub-sequences of the starting nucleic acid that often contain errors

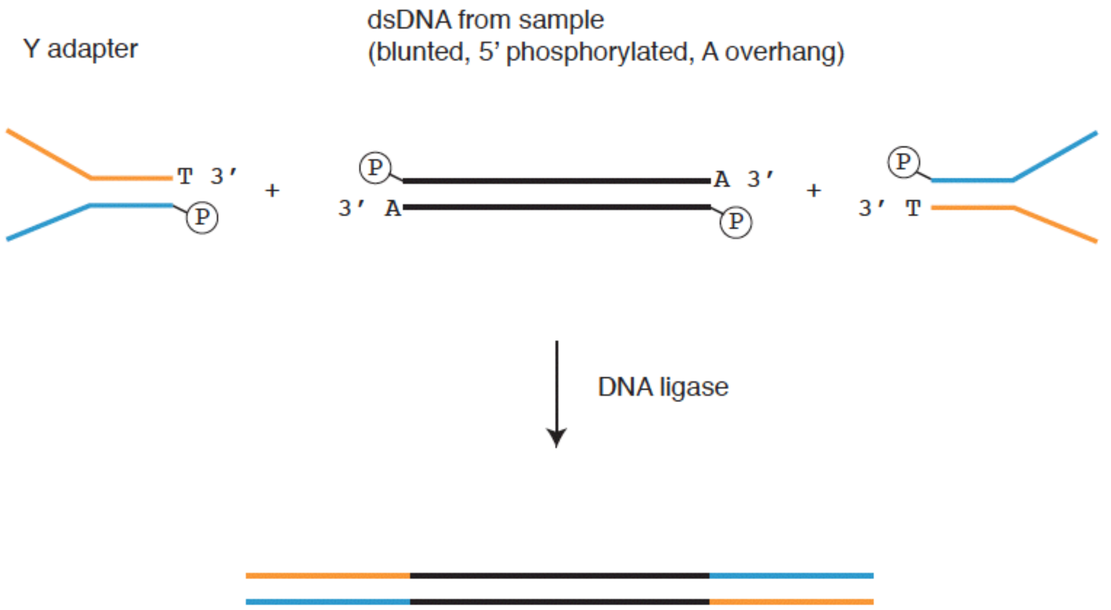


There are many good ways to make sequencing libraries

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

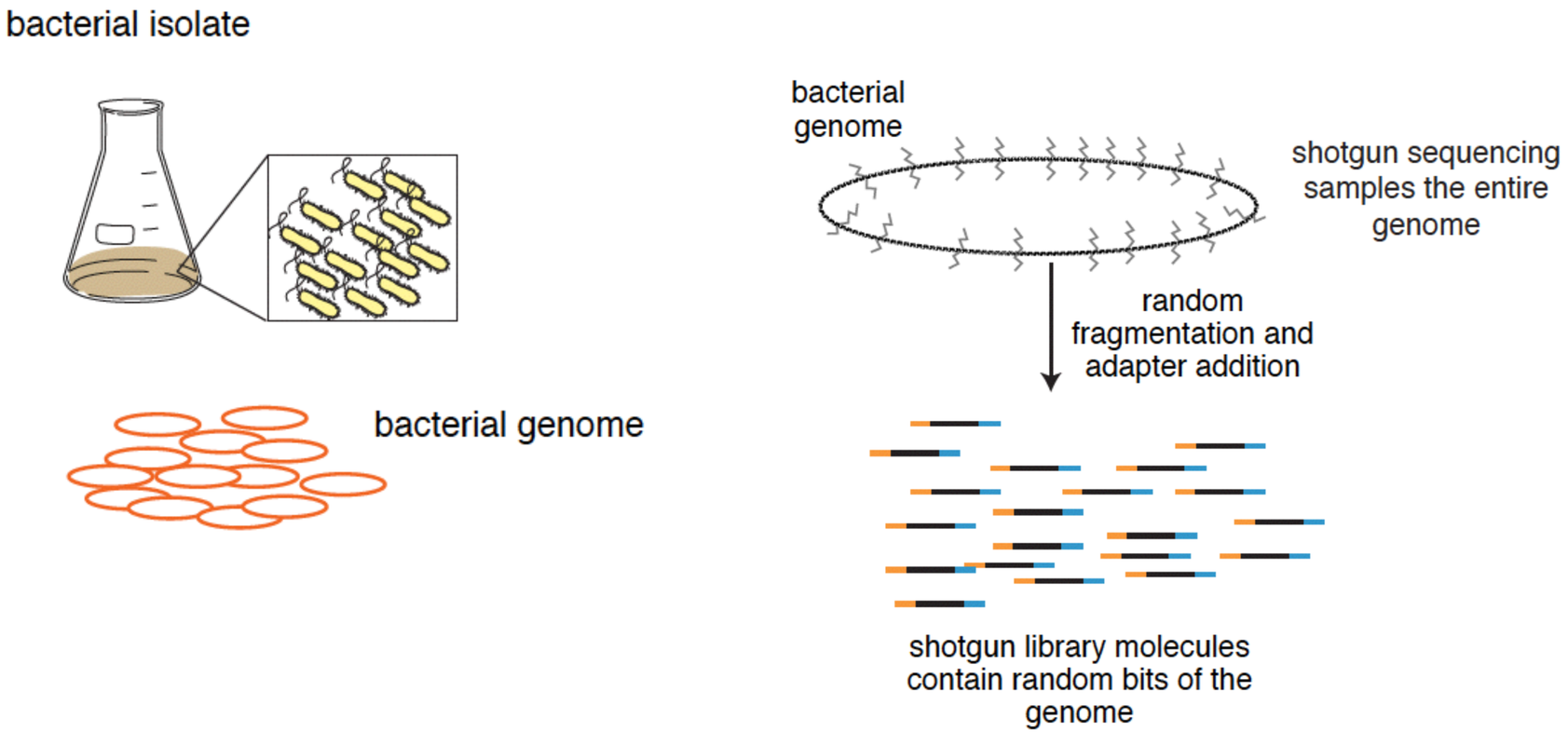
- Nucleic acid isolation
- Enrichment (of nucleic acid subtypes you want) or subtraction (of those you don't want)
- Nucleic acid fragmentation
- Conversion of RNA into dsDNA (for RNA sequencing)
- Addition of adapters to ends of library molecules, possibly with barcodes for multiplexing
- Library amplification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

Adapters can be added to sample-derived dsDNA by ligation



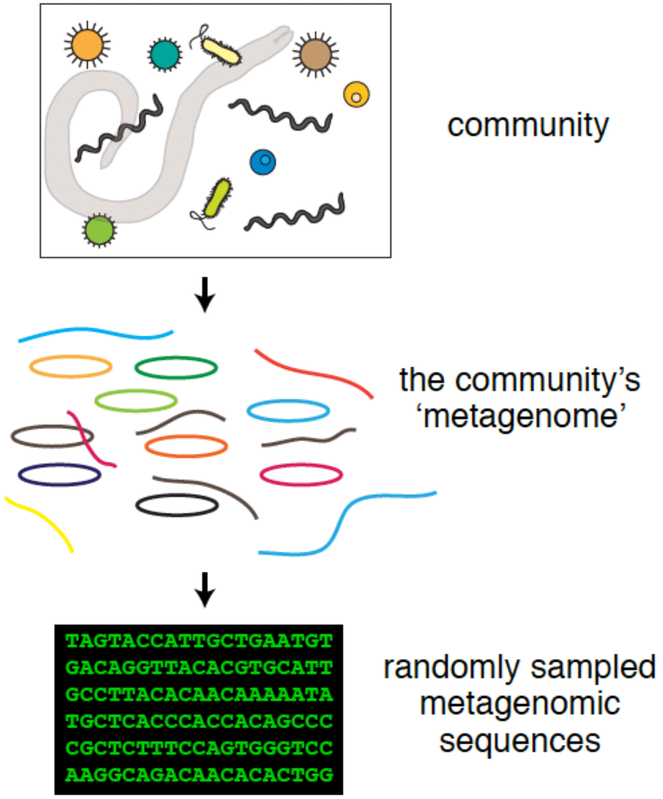
How you make a library determines what type of sequencing you're doing

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



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

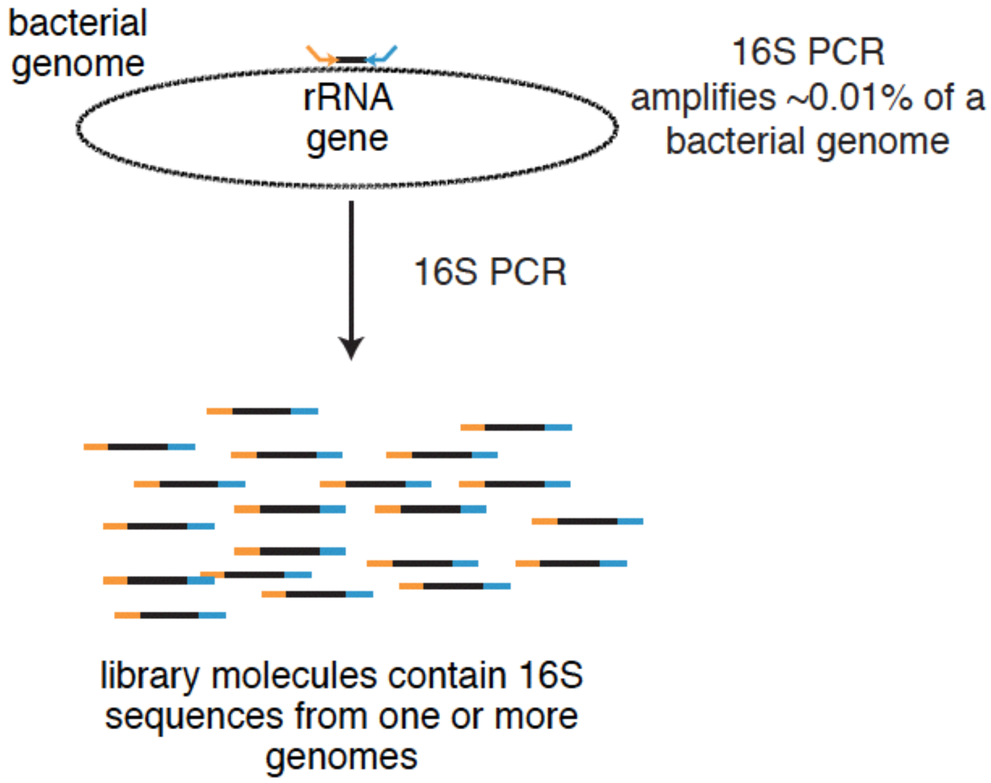
For instance, if you make a 'shotgun library' from a community DNA sample,
you're doing **metagenomics**



The library preparation methods are identical
to those used for whole genome sequencing.
You're just starting with a different kind of DNA sample.

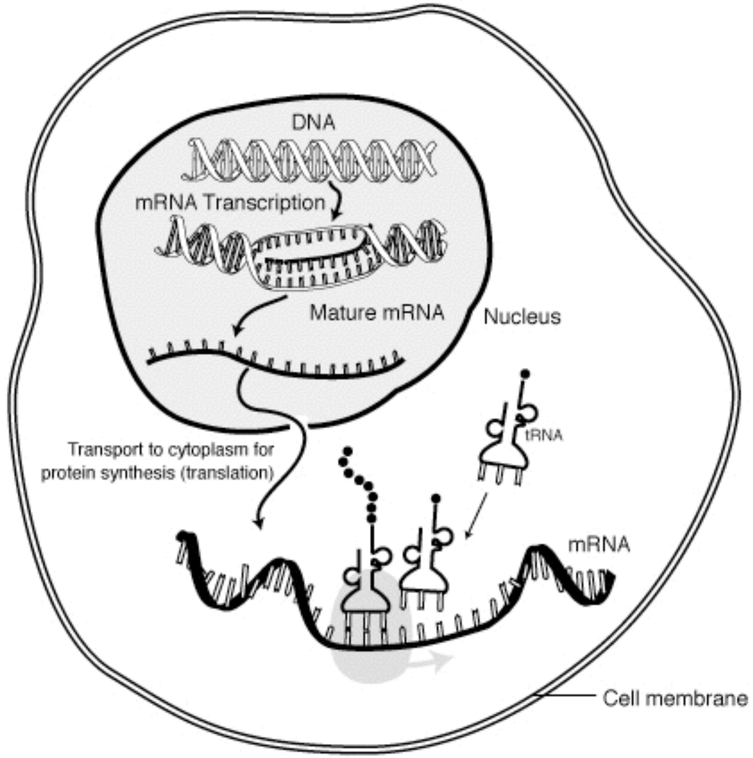
Microbiome sequencing often means **16S rRNA** sequencing

16S sequencing is one type of
'amplicon sequencing'



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

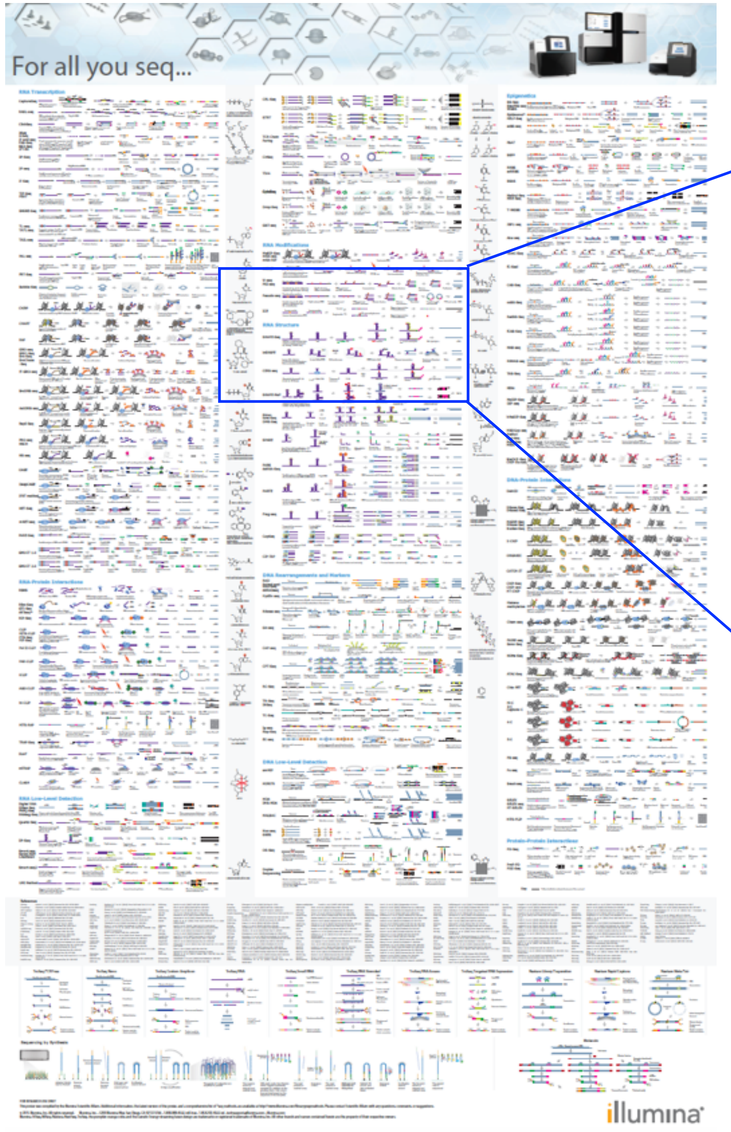
If you make a library from mRNA, that is **RNA-Seq** (transcriptome sequencing)

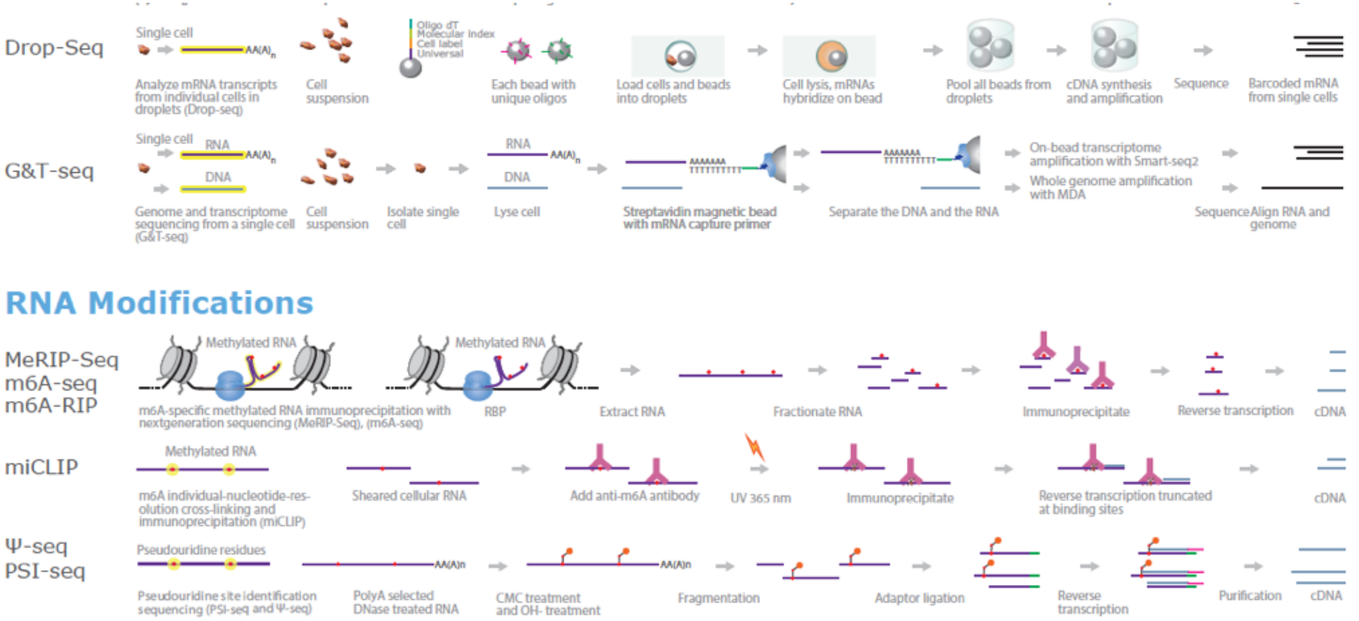


The abundance of reads from a particular mRNA is proportional to that mRNA's abundance in the cell

Image: Wikipedia

There are **5 billion** ways to make libraries and to do sequencing (all have names that end in -seq)

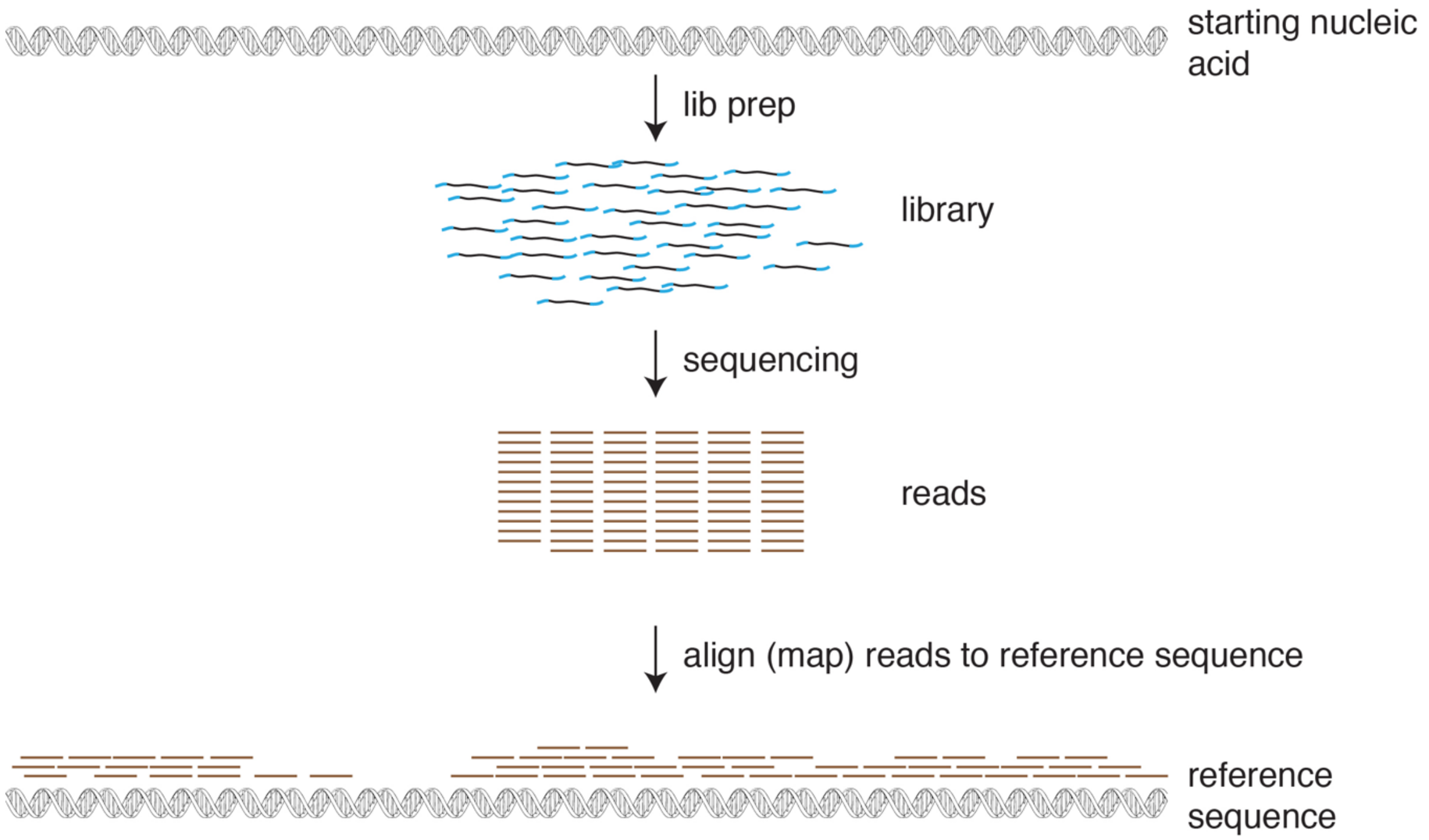




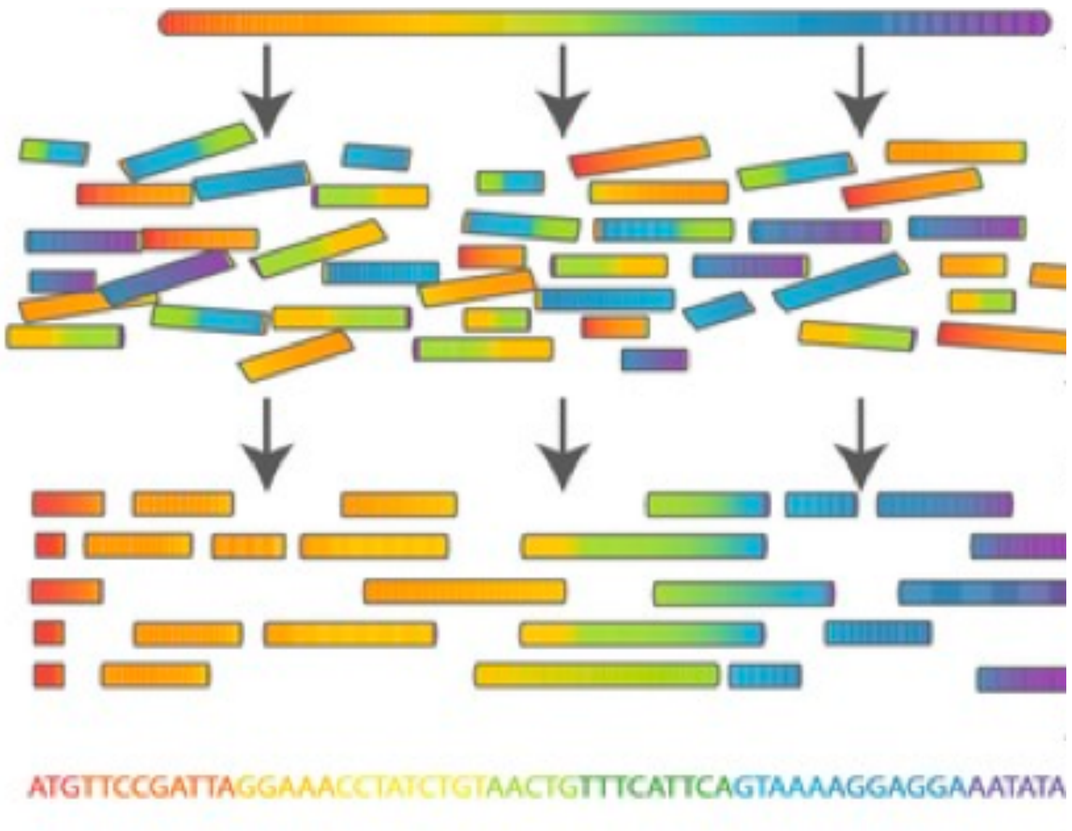
They're all variations on a few themes. Don't let it overwhelm you. Most sequencing is of a few simple types, and it's better to focus on the Biology and experimental design.

https://www.illumina.com/content/dam/illumina-marketing/documents/applications/ngs-library-prep/ForAllYouSeqMethods.pdf

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



(De novo) **assembly** is the process of trying to reconstruct a genome sequence from reads



not yet sequenced genome

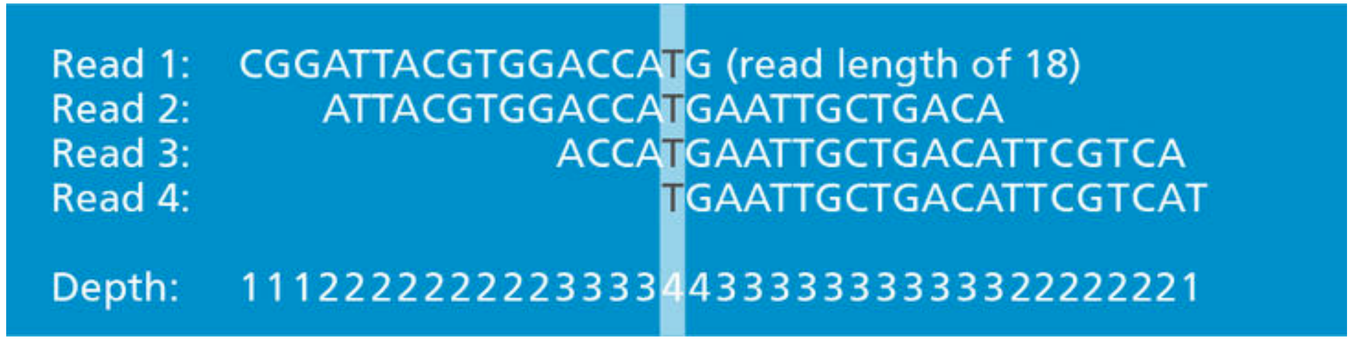
reads

contigs

reconstructed (assembled) genome

Image: Commins, J., Toft, C., Fares, M. A (2009)

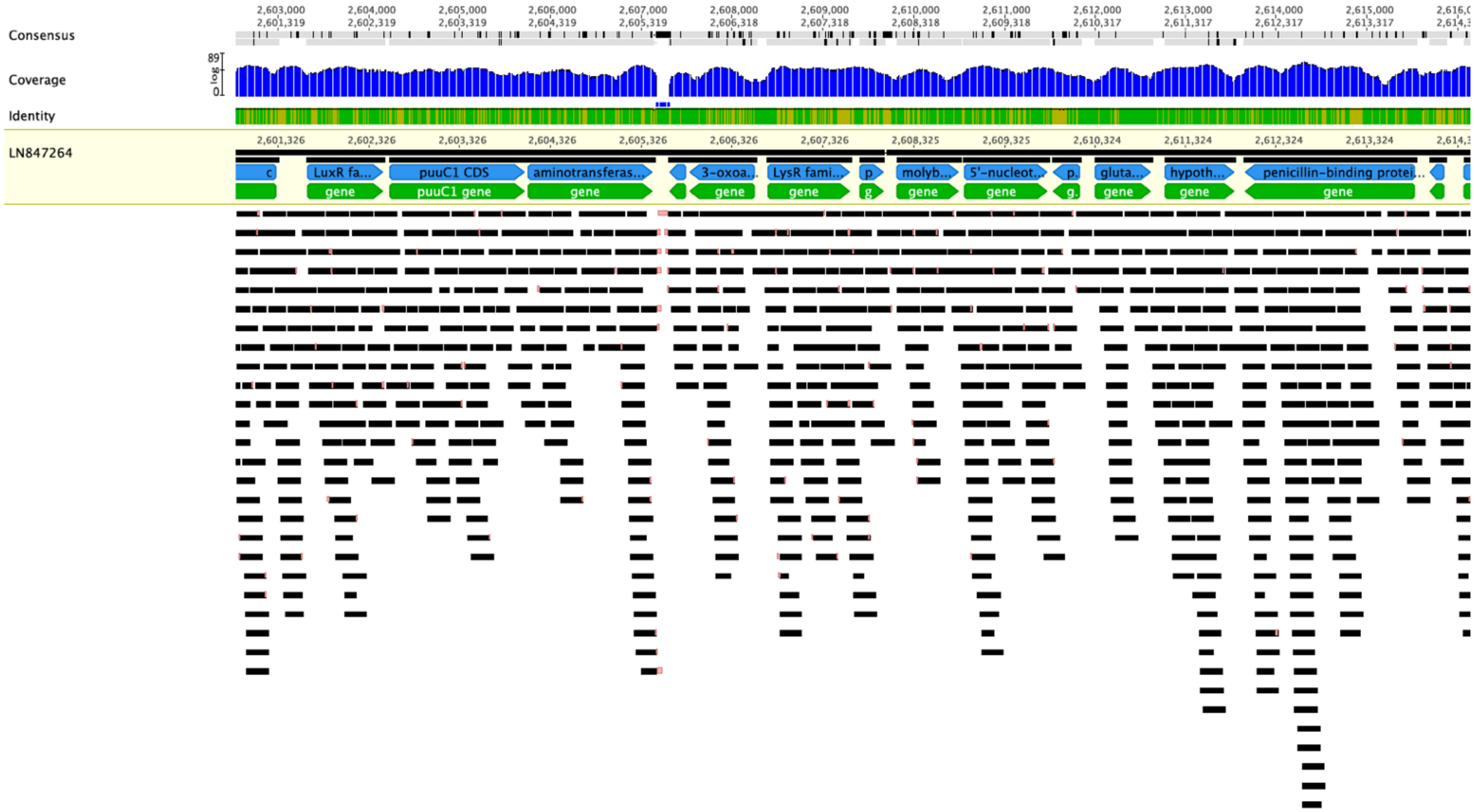
Coverage is the number of individual reads that support a particular nucleotide in an assembled (reconstructed) sequence or that align to a particular nucleotide in a reference sequence



coverage is often referred to as 'depth' or 'depth of coverage'

image credit: wikimedia.org

Coverage from WGS of a bacterial isolate



Questions?

Is there genomics or sequencing jargon about which you’re not certain?