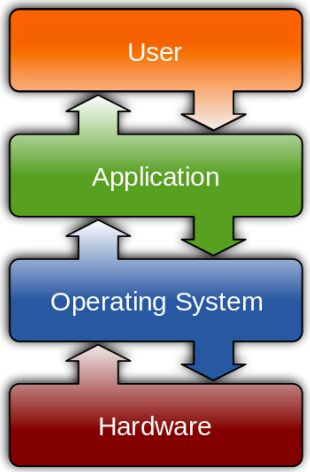
Next-gen Sequencing Technologies

Bioinformatics Applications (PLPTH813)

Sanzhen Liu

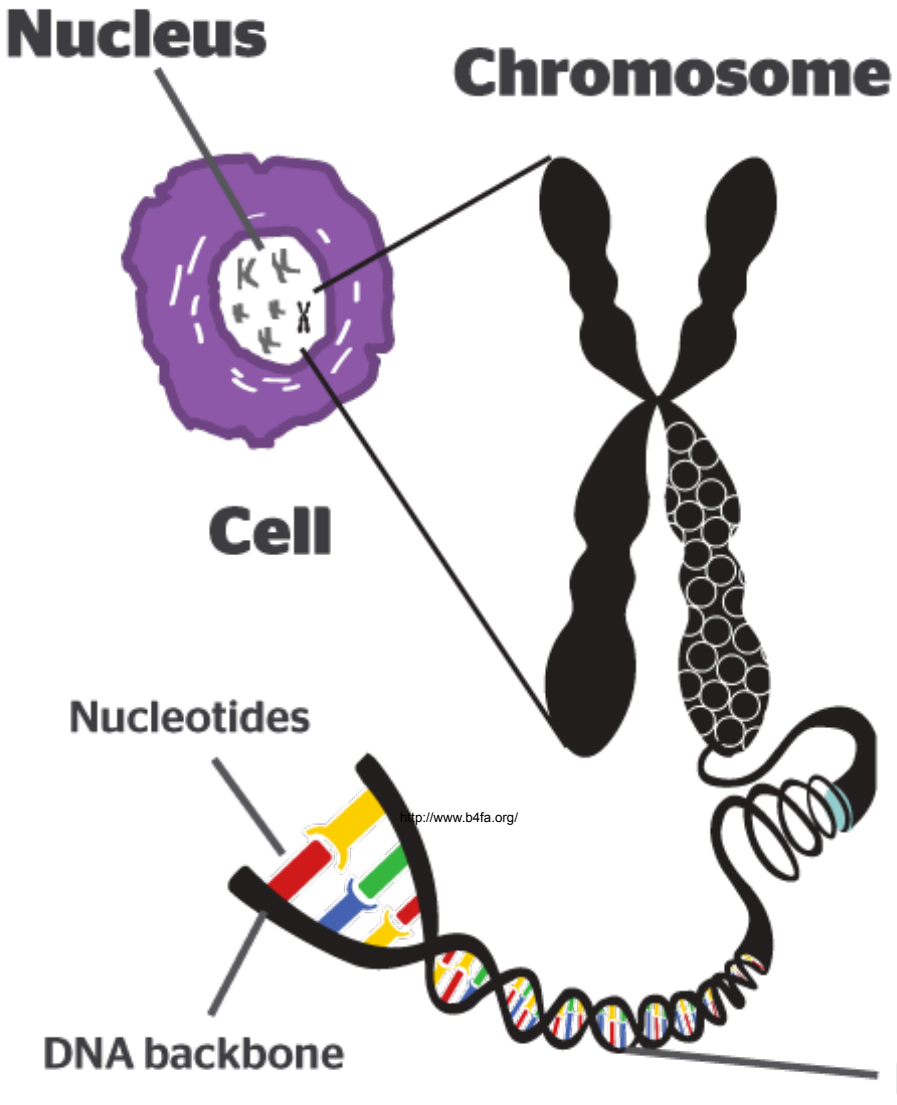
1/31/2019

Unix commands



- **cd** - change the working directory
- **mkdir** - make directories
- **pwd** - print name of current working directory
- **ls** – list directory contents
- **chmod** - change the access permissions to files and directories
- **head** - output the first part of files
- **tail** - output the last part of files
- **more** and **less** display contents of large files page by page or scroll line by line up and down
- **cat** - concatenate files
- **paste** - merge lines of files
- **wc** - print line, word, and byte counts for each file
- **grep** - print lines matching a pattern
- pipe: "|"

Genome sequencing



DNA sequence

```
   1 ccctgaggct tttcggagcg agctcctcaa atcgcatcca gattttcggg tccgagggaa
  61 ggaggaccct gcgaaagctg cgacgactat cttcccctgg ggccatggac tcggacgcca
 121 gcctggtgtc cagccgcccg tcgtcgccag agcccgatga ccttttctg ccggcccgga
 181 gtaagggcag cagcggcagc gccttcactg ggggcaccgt gtcctcgtcc accccgagtg
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 301 ctggggacaa gctaggaggc agtggcttca agtcgtcctc gtccagcacc tcgtcgtcta
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 661 cgtcggcctg cggcggcctg gcgcactccg cgcccctgcc cgccgccacc gcgcacccgg
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 841 ccgggctgcc gtcggtcggc tccatccgtc caccgcacgg cctactcaag tctccgtctg
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1021 acgtgtcggc tatgggcgcc ggcagcctgc cgcgcctcac ctccgacgcc aagtgagccg
1081 actggcgccg gcgcgttctg gcgacagggg agccaggggc cgcggggaag cgaggactgg
1141 cctgcgctgg gctcgggagc tctgtcgcga ggaggggcgc aggaccatgg actgggggtg
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1261 ggggagtgag gggatgttct ctccgggacc tgatcgagcg ctgtctggct ttaacctgag
1321 ctggtccagt agacatcgtt ttatgaaaag gtaccgctgt gtgcattcct cactagaact
```
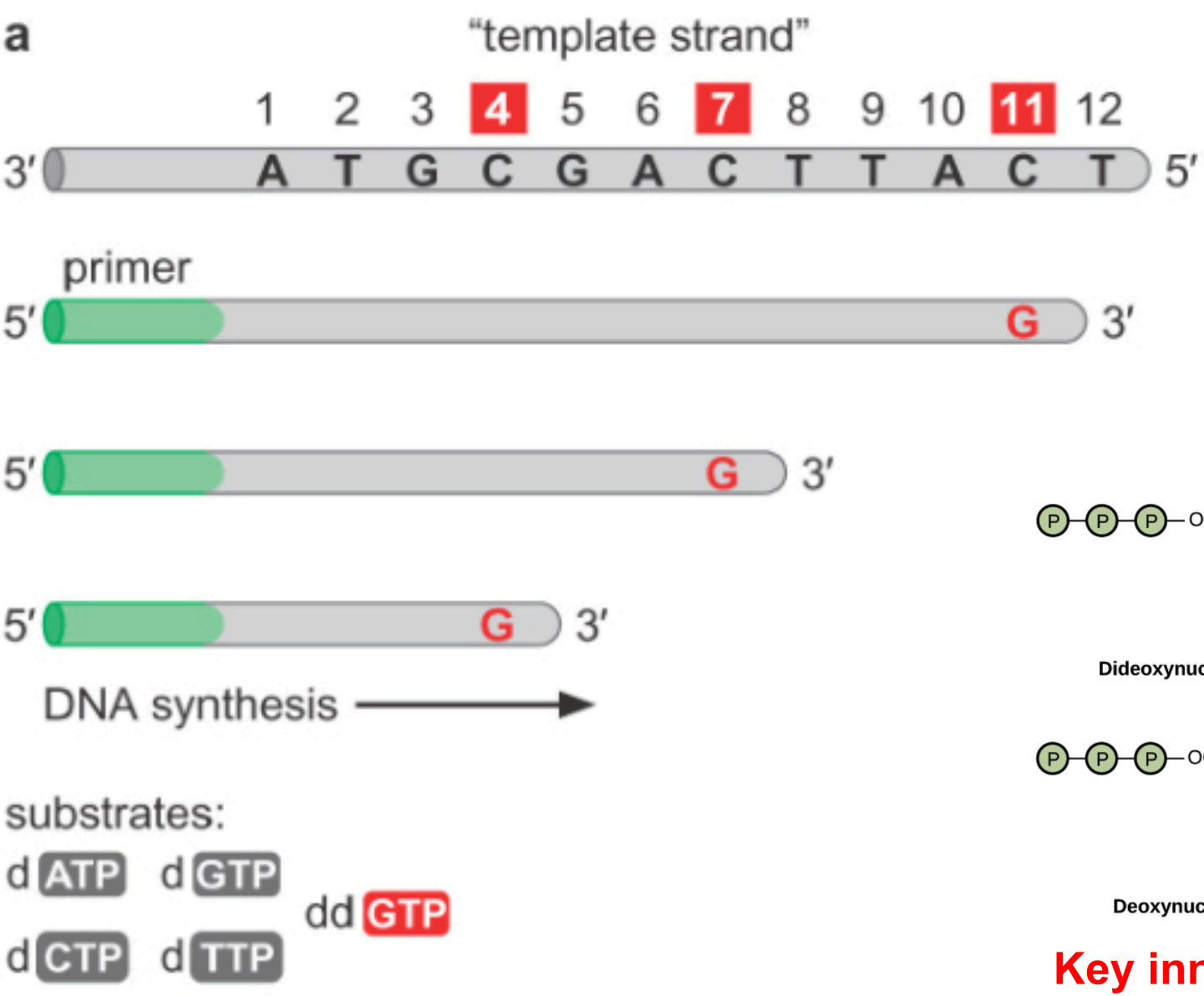
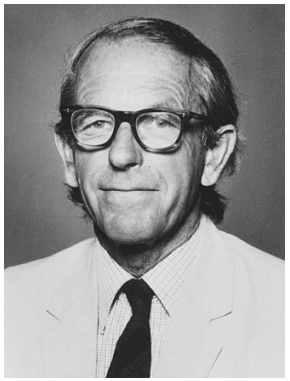
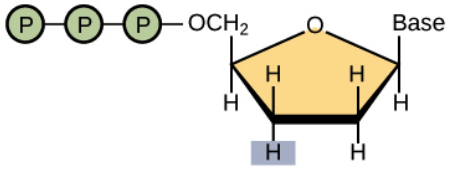

Sanger sequencing technology - I

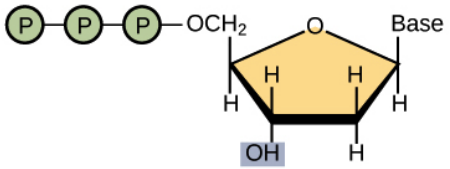




Frederick Sanger



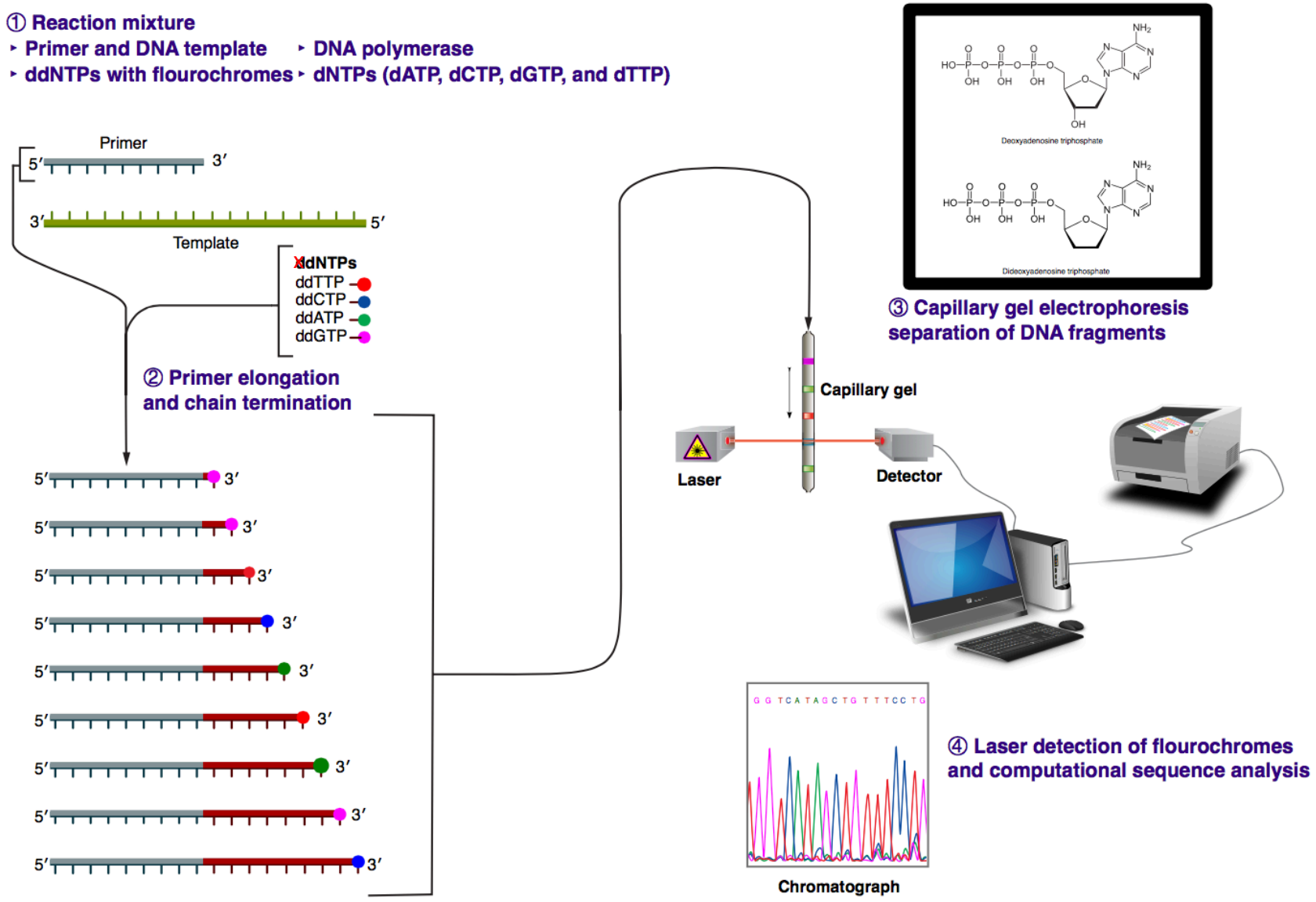
Dideoxynucleotide (ddNTP)



Deoxynucleotide (dNTP)

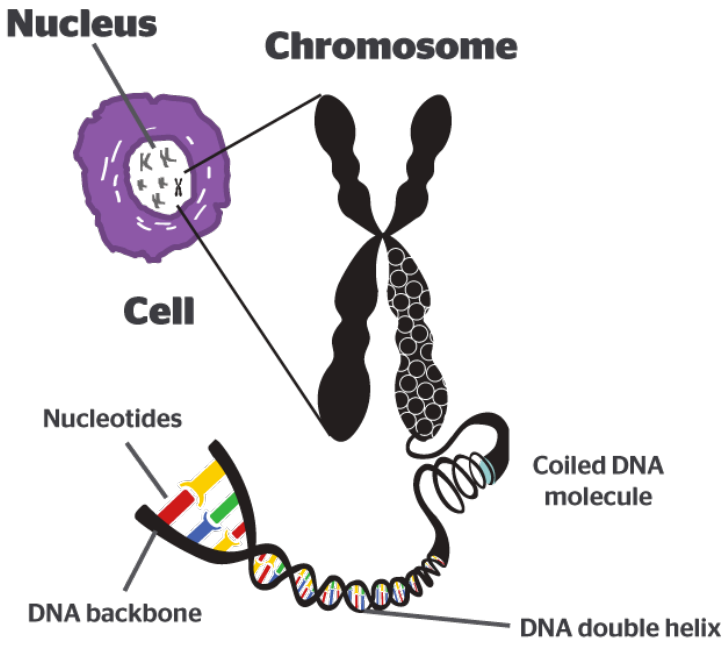
Key innovation

Sanger sequencing technology - II



wikipedia

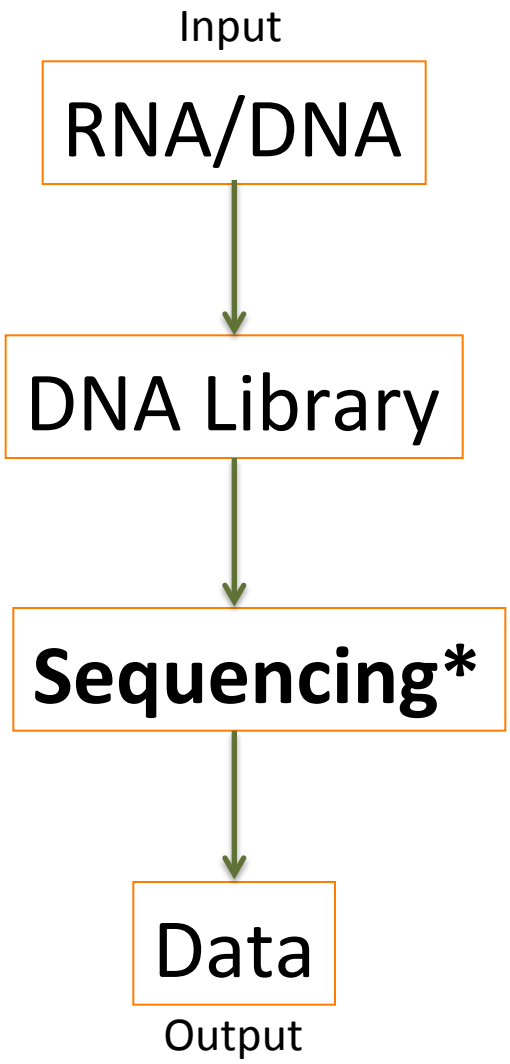
Major NGS technologies in market



sequencing sensitivity and read length

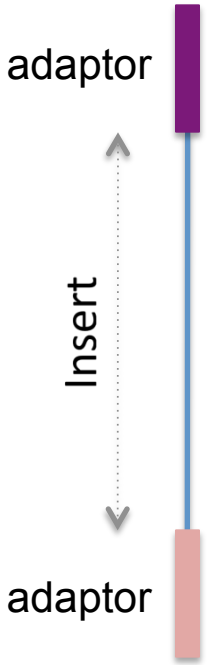
Before single molecular & "super long" sequencing technology, **fragmentation** and **amplification/cloning** of a single nucleotide molecule are needed for sequencing.

COMMON in all NGS platforms



The adaptor is required for library preparation

Hundreds to thousands of millions of fragments are sequenced ***in parallel***

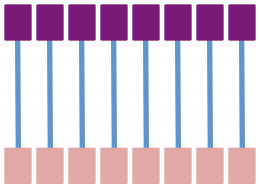


Single-molecule and ***amplification-based*** approaches

Nucleotide detector:
VERY sensitive

Directly read sequence
single-molecule

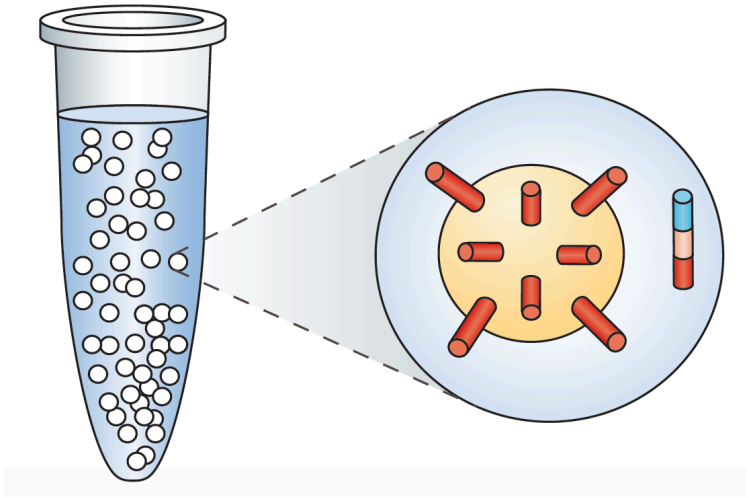
Nucleotide detector:
Not sensitive at the
single molecular level



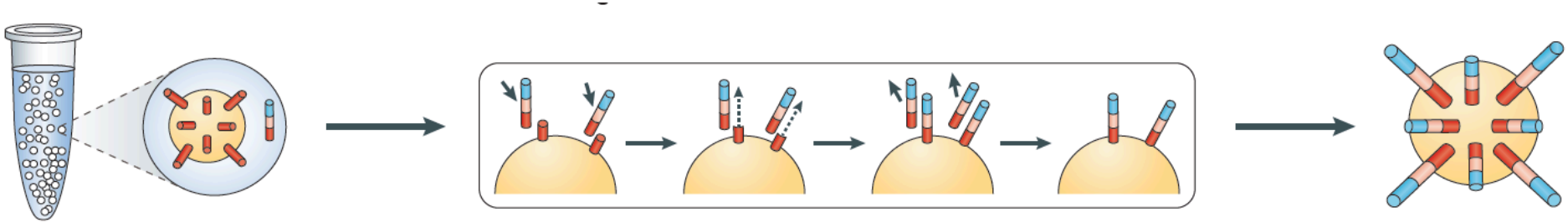
amplify and then
read sequence

"Having many thousands of identical copies of a DNA fragment ***in a defined area*** ensures that the signal can be distinguished from background noise."

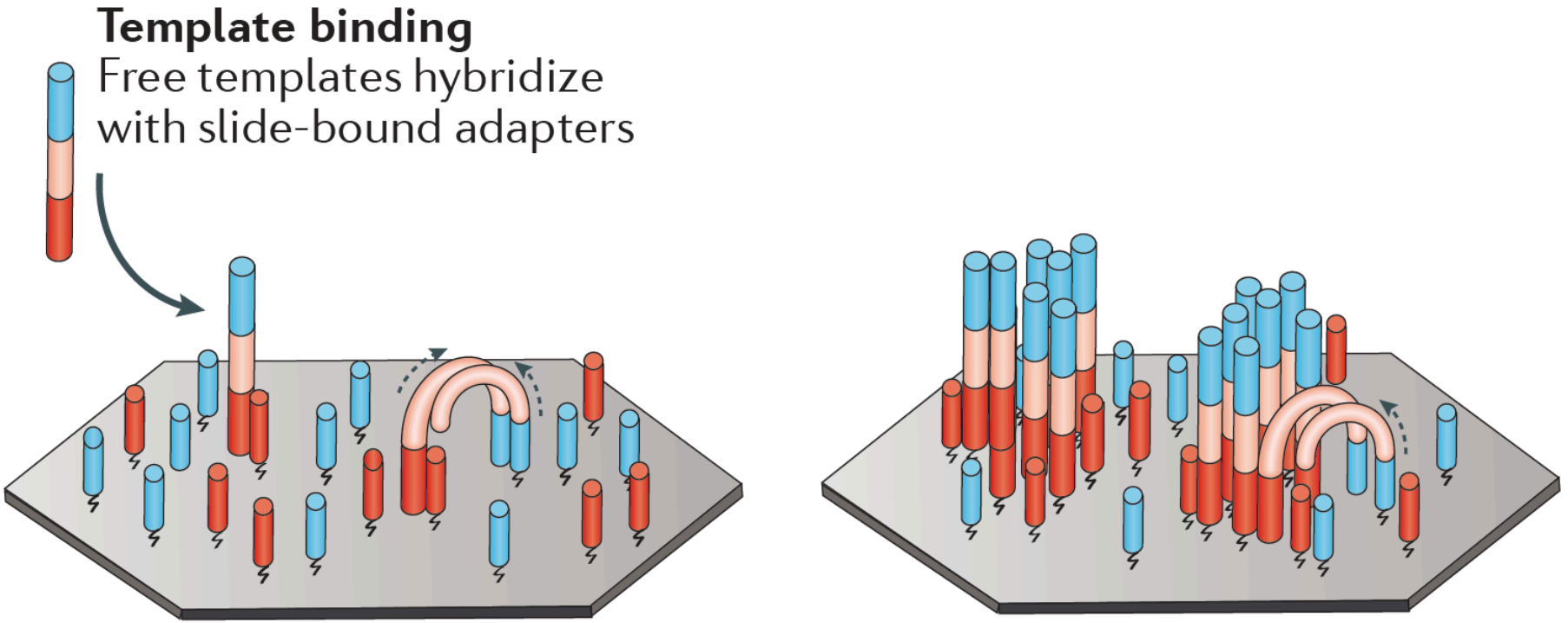
Massive independent amplifications – emulsion PCR



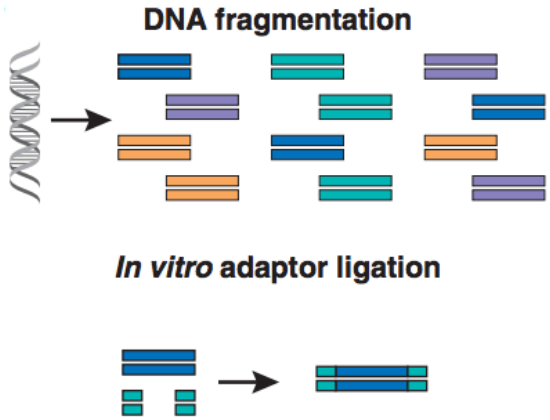
Water-in-oil emulsion PCR
(454 and Ion Torrent)



Massive independent amplifications – bridge PCR

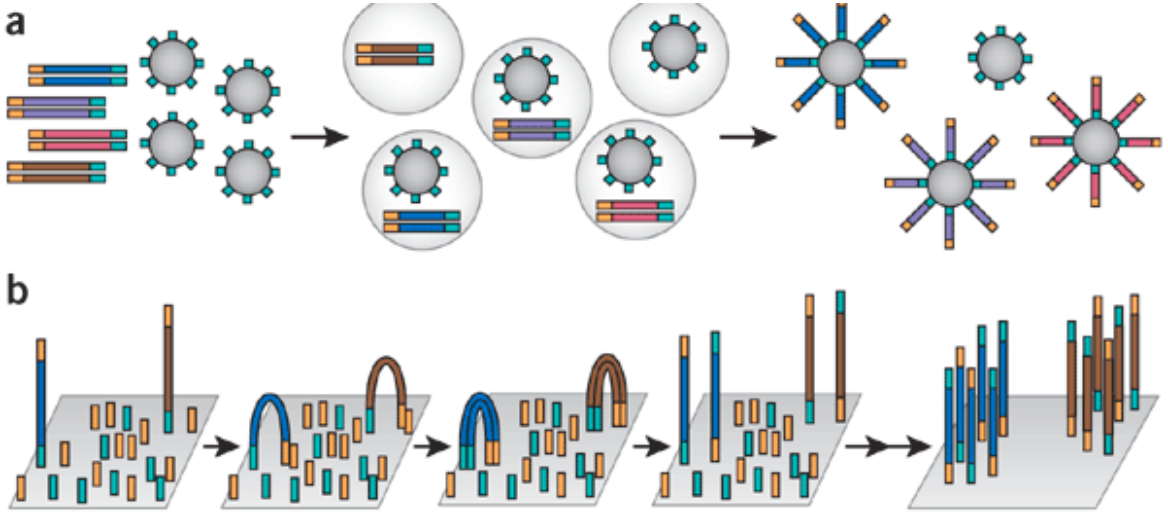


DNA amplification



Adaptor ligation

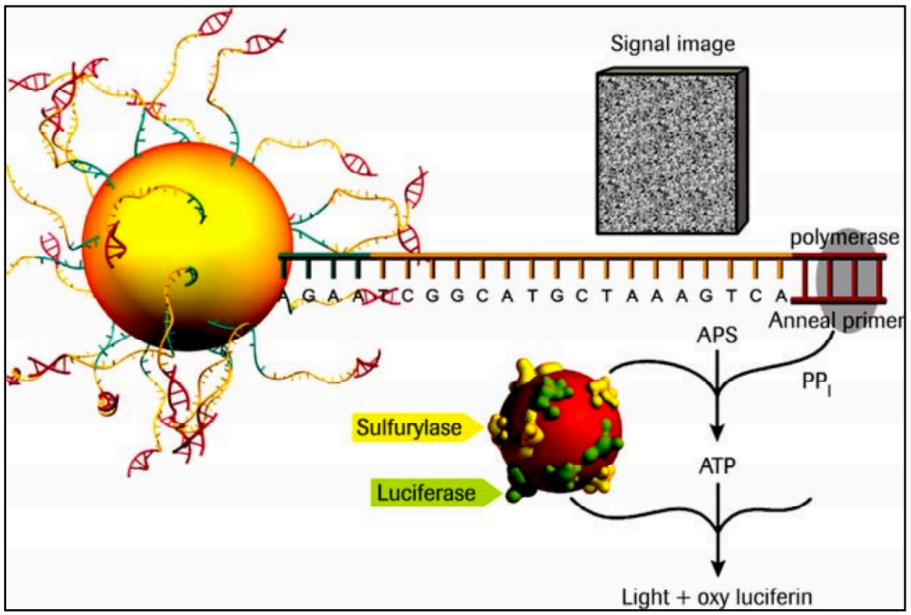
Water-in-oil emulsion PCR
(454 and Ion Torrent)



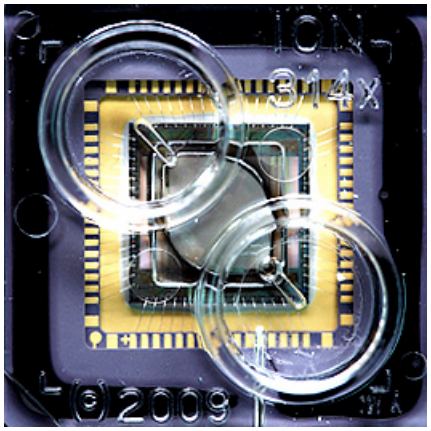
Bridge PCR on slides
(Illumina)

Nature Biotechnology, 2008, 26: 1135-45

454 and Ion Torrent signal detectors



454 technology, Nature 2005, 437: 376-380



Ion Torrent video

1. Sequencing by synthesis
2. Pyrosequencing (454)

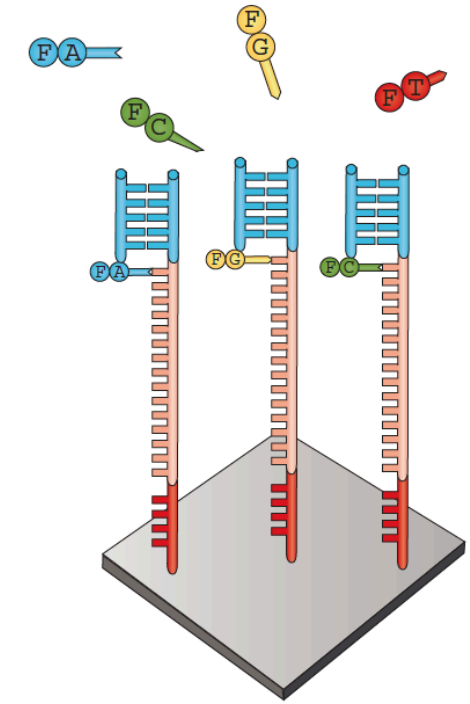
1. Ion Torrent technology is similar to 454 technology

2. The signal is H^+ rather than pyrophosphate

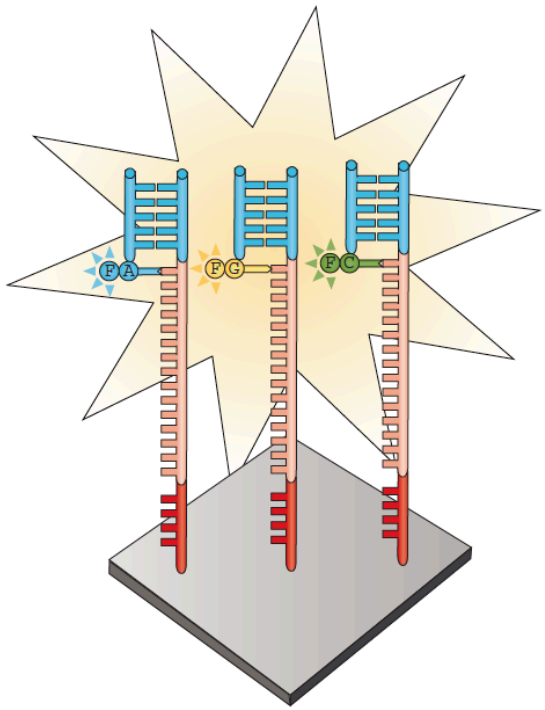
Illumina

Two key technologies:

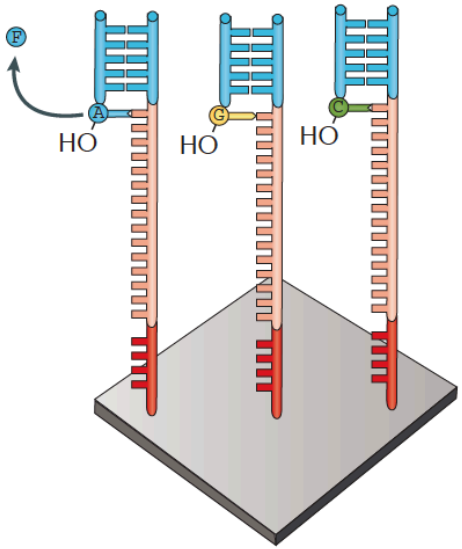
1. Bridge PCR
2. Reversible terminator chemistry



Nucleotide addition

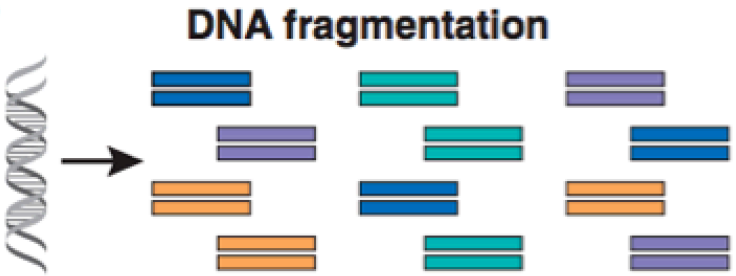


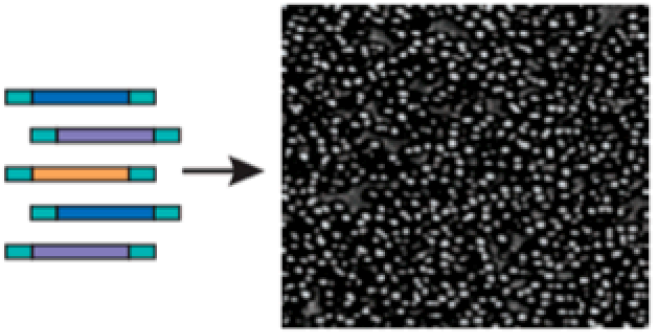
Imaging

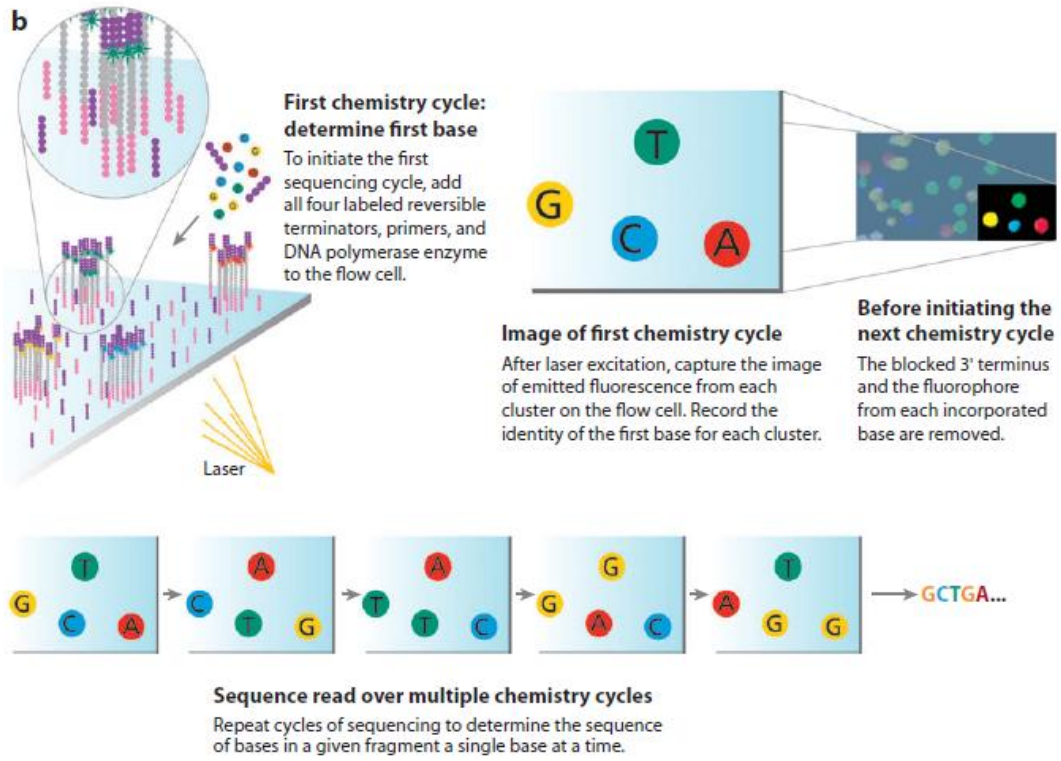


Cleavage

Illumina sequencing







Two key technologies:

1. Bridge PCR
2. Reversible terminator chemistry

Illumina Sequencers

Illumina *versus* Ion Torrent & 454

Illumina
Record signal per **nucleotide position**:

A T G C A A A A

A T G C A A A A

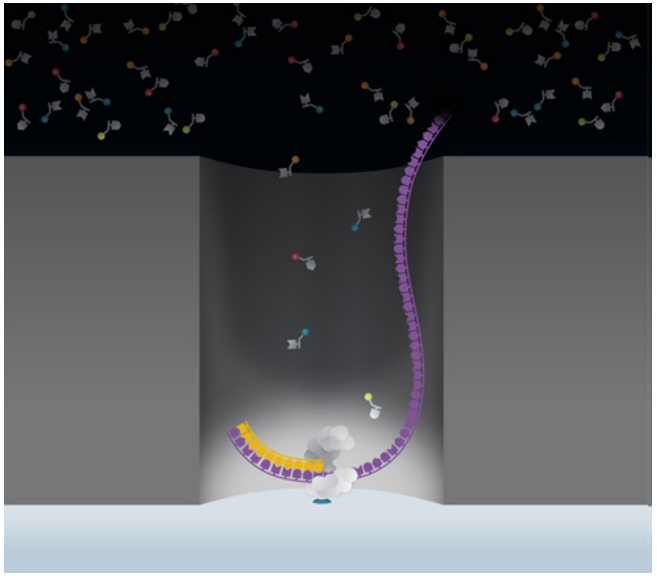
Life technology Ion Torrent & Roche 454
Record signal per **nucleotide type**:

A T G C A A A A

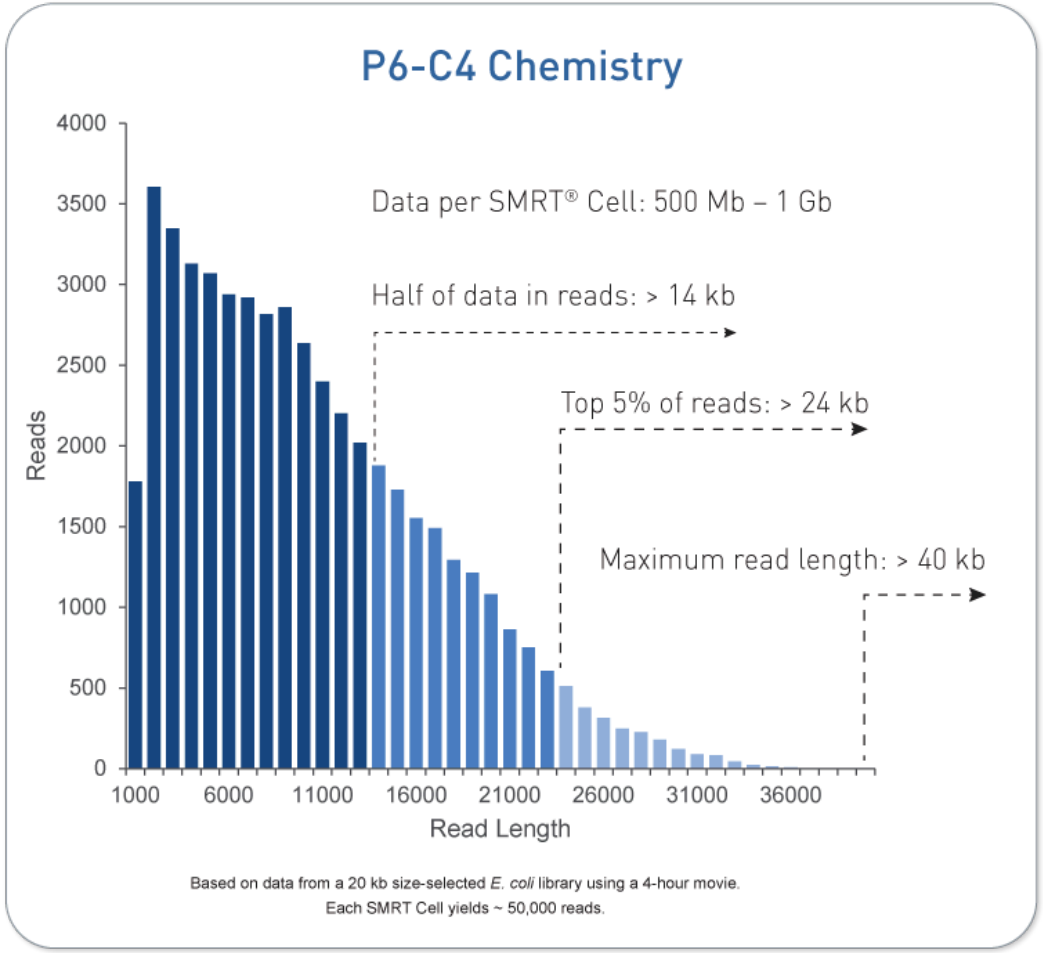
Sequencing errors at homopolymers

When the single molecular sequencing technology is ready, **amplification or cloning** is not necessary.

PacBio – Single Molecule Real Time (SMRT)



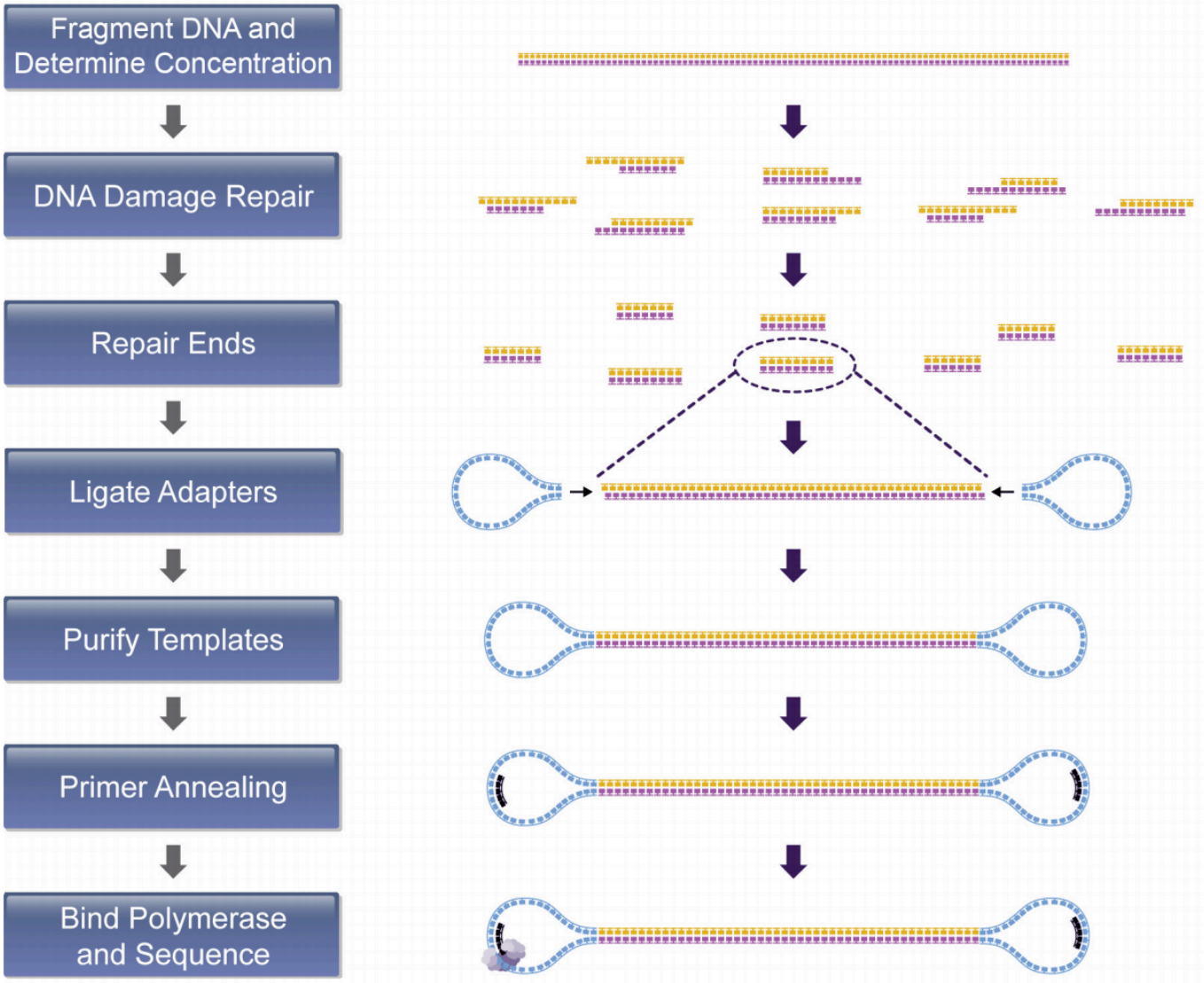
[PacBio tech video]



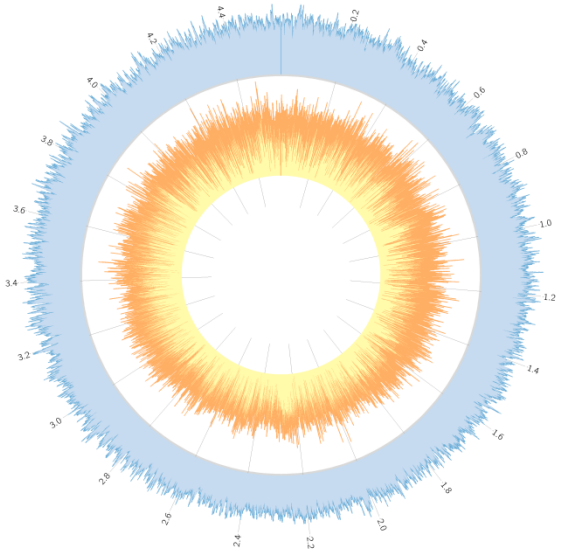
- Single molecule sequencing
- no amplifications required
- up to 70+ kbp sequencing
- Moderate sequencing throughput
- high sequencing error rate (~10%, random, no-context-specific errors)

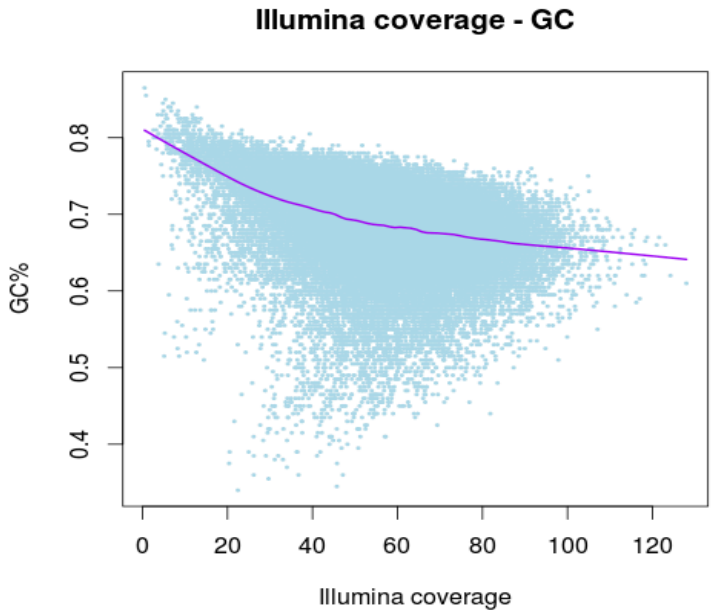
pacbio.com

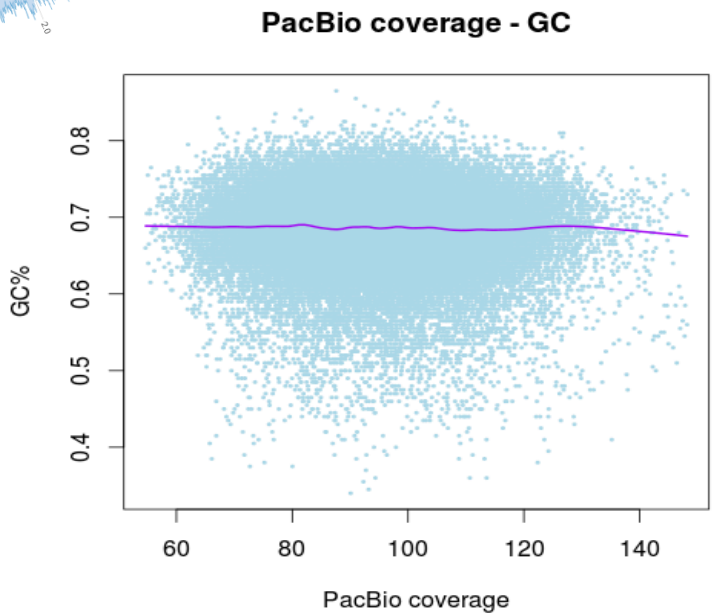
PacBio library prep workflow



Less GC-related biases

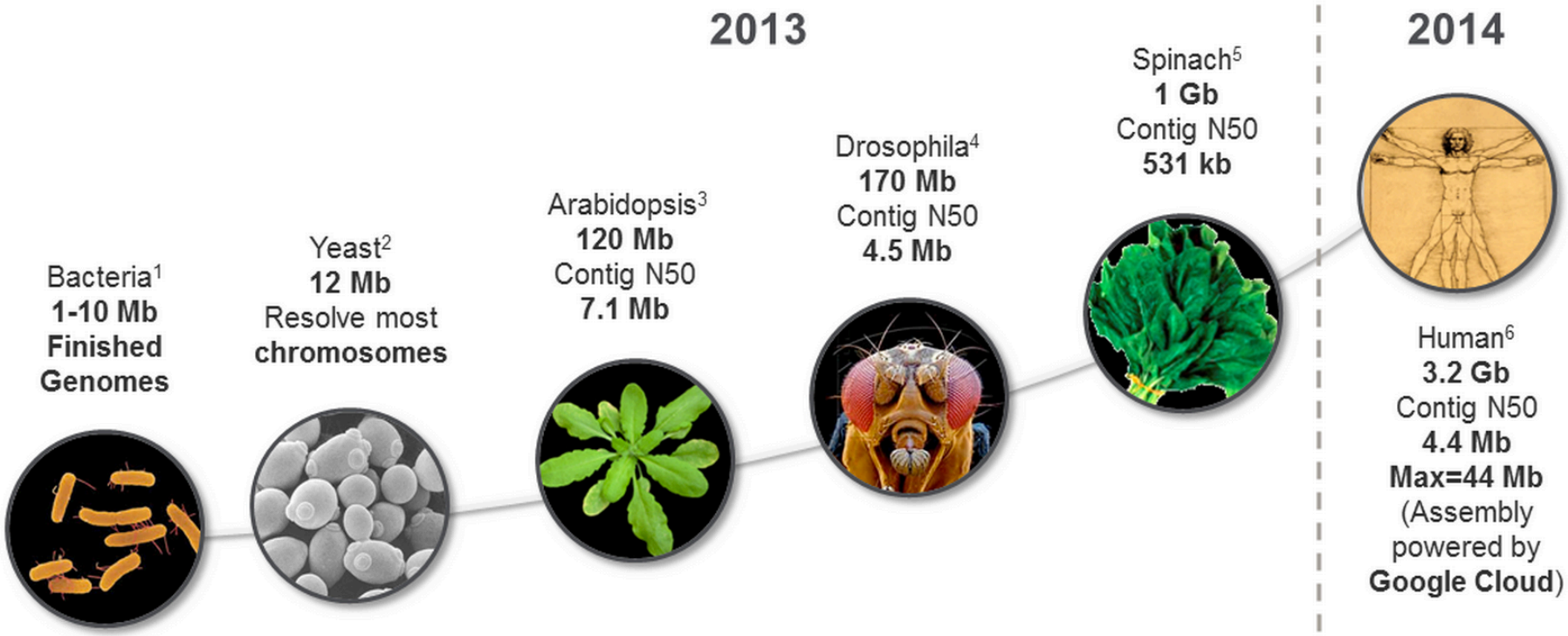






Peng *et al*., BMC Genomics, 2016, 17:21

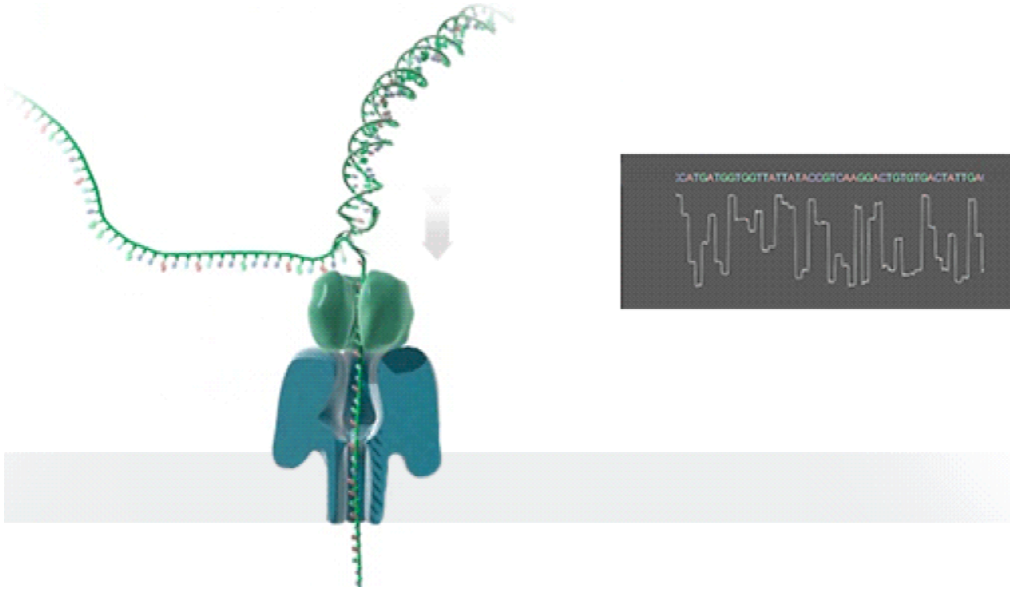
PacBio for genome assembly



PacBio has solved *de novo* assemblies of most bacterial genomes and it will solve assemblies of small “simple” genomes (e.g., <500 Mbp) with increasing read length and improved sequencing quality.

New Platform – **Oxford Nanopore**

A promising technology



As each nucleobase passes through the pore the current is affected and this change allows sequence to be read out.

- Single molecular sequencing
- No amplifications
- **Long reads (typically 10-200kb)**
- **Error rate is high (~15%)**

Nanopore devices

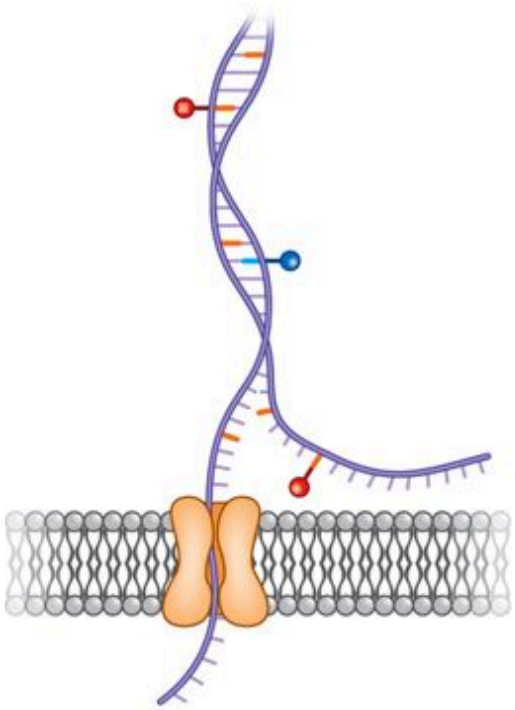
MinION
1. USB disposable sequencer
2. ~10Gb in about two days

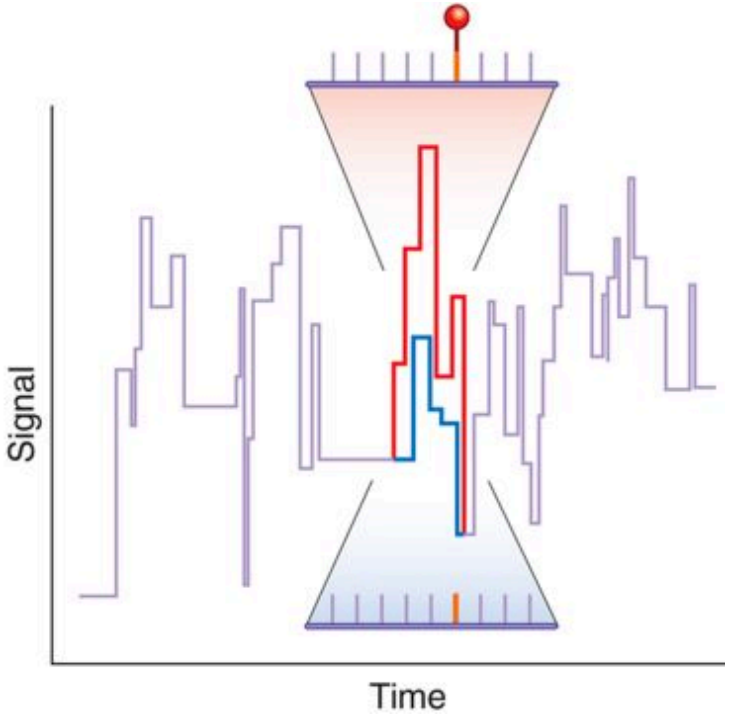
PromethION
1. High-throughput
2. lower cost (~$1000 per human genome)

portable device:
MinION Mk1C

Applications of Nanopore sequencing

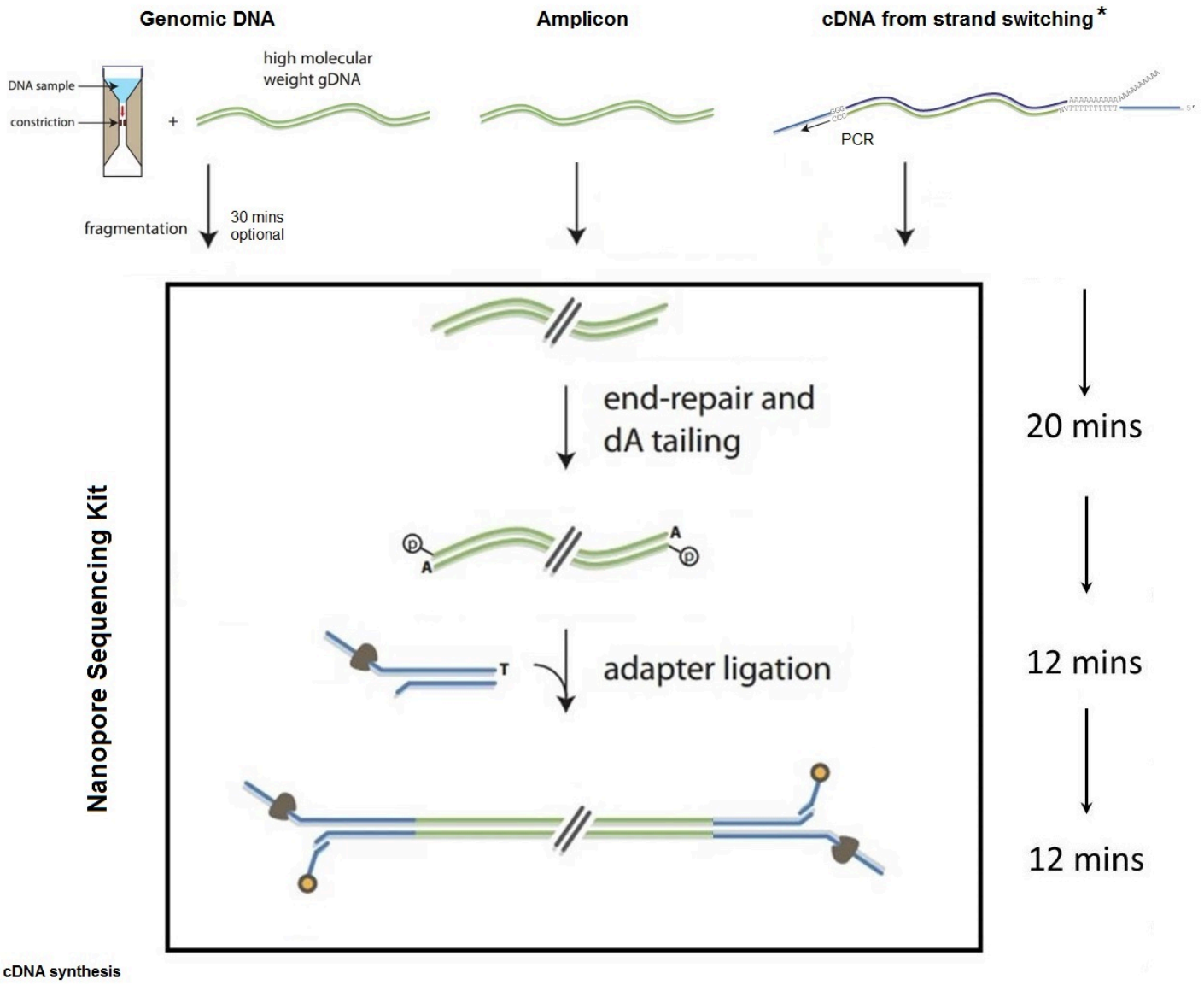
1. Genomic DNA sequencing
2. RNA sequencing (direct RNA or cDNA)
3. DNA methylation





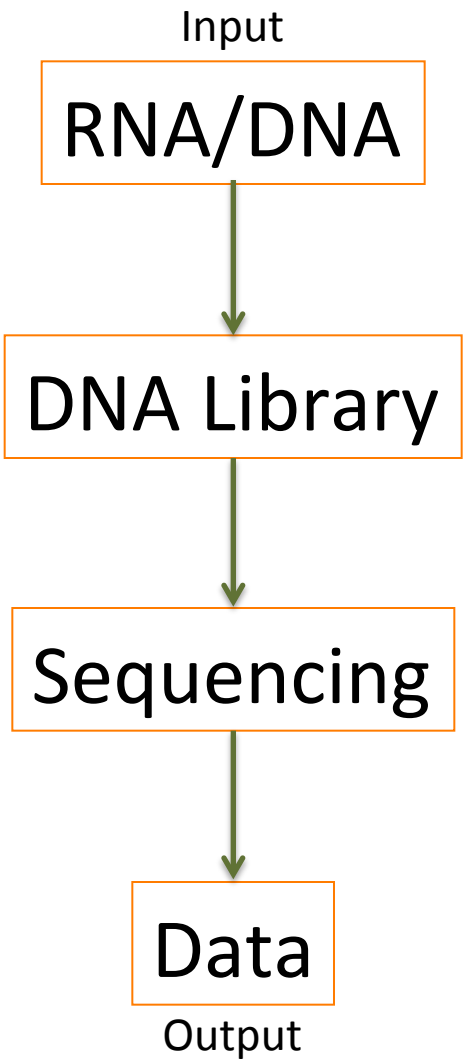
Nature Methods, 2017, 14:347–348

Nanopore library preparation



nanopore.com

COMMON in all NGS platforms

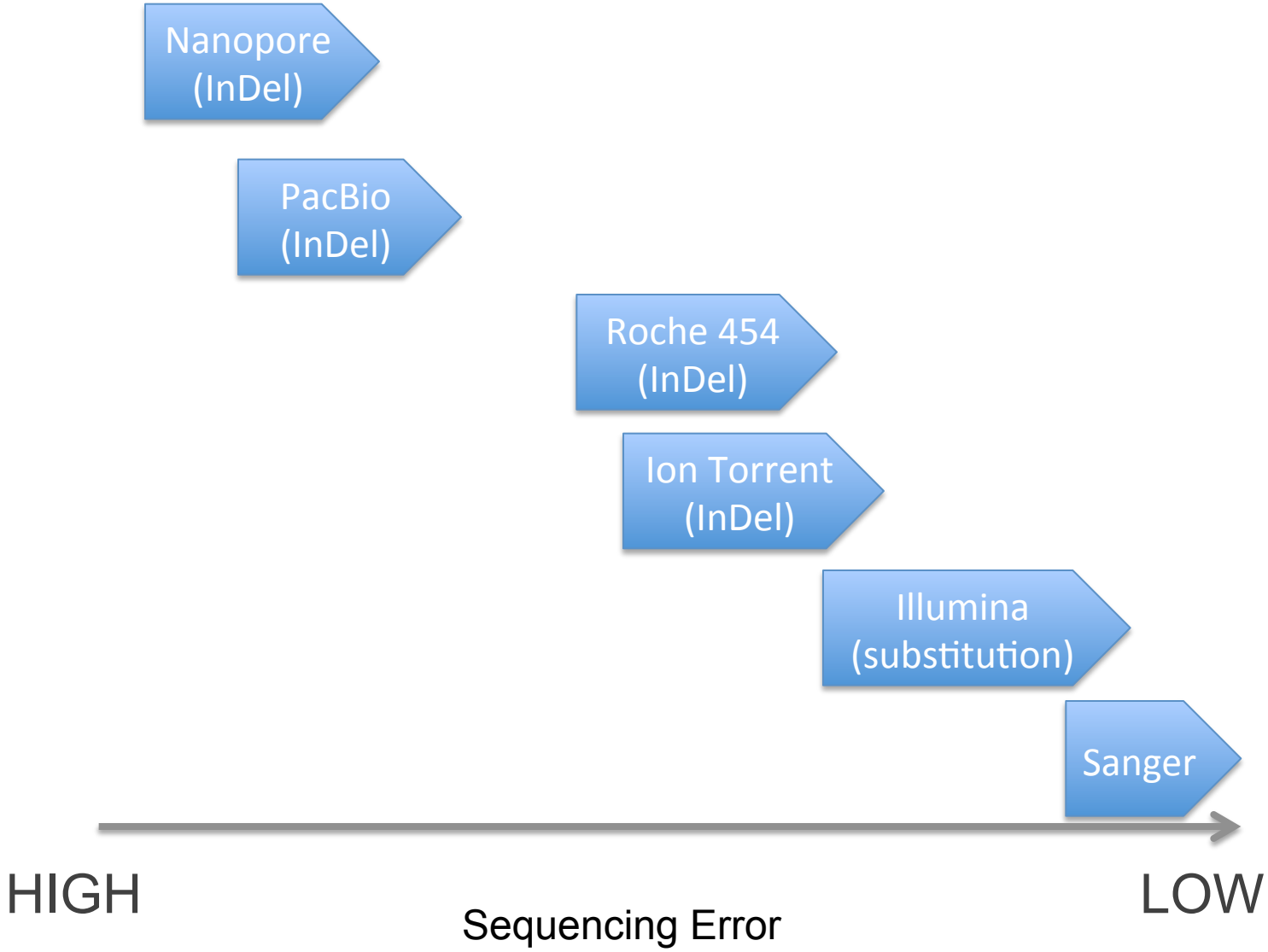


Adaptor is required for library preparation

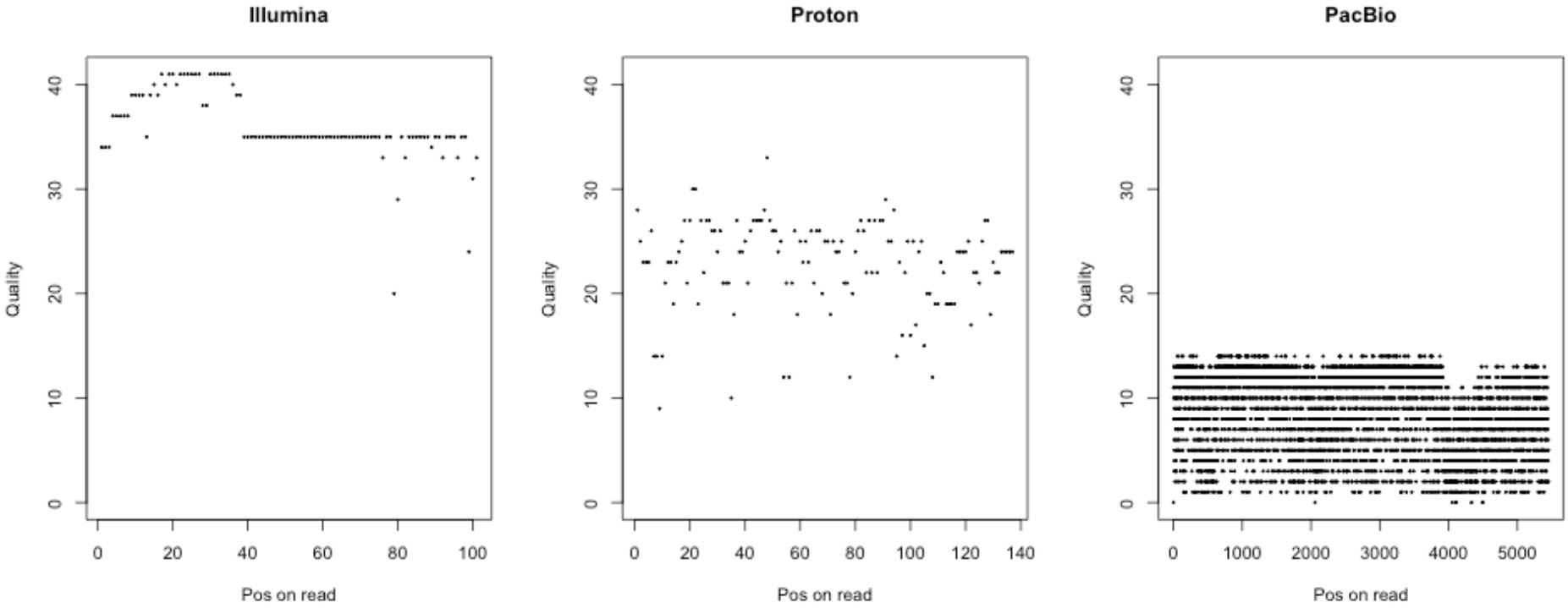
Thousands to hundreds of millions of fragments are sequenced in parallel

Standard data (FASTQ) are output

Sequencing error rates



Typical reads in different platforms



Read length
Read quality

Applications of NGS

1. Whole-genome sequencing/re-sequencing / target-region sequencing (Assembly, Variant discovery)
2. Genome-reduction sequencing (GBS, RAD-Seq)
3. RNA-Seq: differential expression, alternative splicing and variant discovery
4. Small RNA-Seq
5. ChIP-Seq: Elucidate DNA-protein interaction
6. Metagenomics
7. Others

Case study

1. *De novo* assembly of a strain of *E.coli*

2. Human whole genome sequencing for SNP discovery

Which platform(s)?

Sequencing depth?

Sequence platforms

Illumina (MiSeq, NextSeq, HiSeq)

very high throughput, up to 2x300 bp, and high accuracy (<1%)

Proton (Ion Torrent)

high throughput, up to 300-500 bp, but high errors at homopolymer regions

PacBio

Moderate sequencing throughput, very long (>70kb+), but high errors (10%)

Nanopore

Moderate sequencing throughput, very long (> 1 Mb), but high errors (10-20%)

@anne_churchland (twitter)

Experimental design

- Goal
- Platform
- Read length
- Rate and type of sequence errors
- Sequencing depth
- Replication
- Control
- Budget

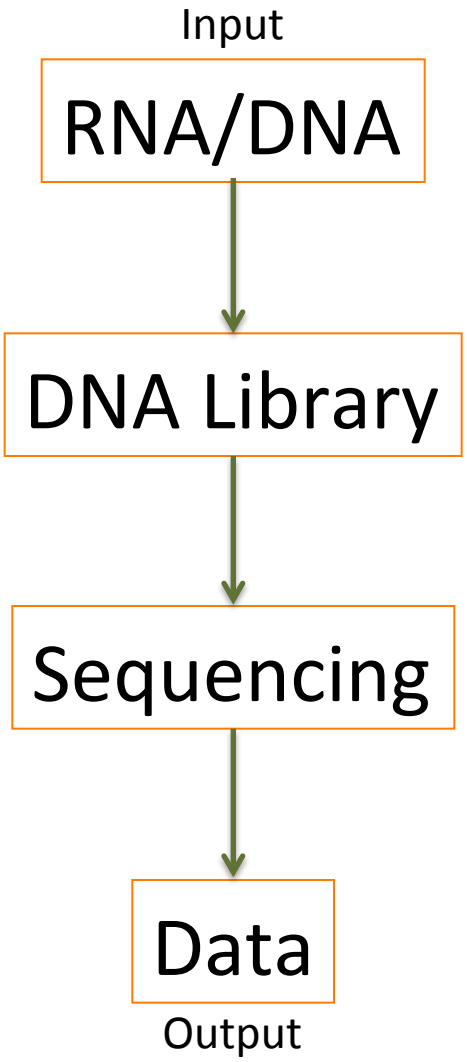
Platform	Templates	Signal	Read length	Run time	reads per run	Error type	Error rate
Illumina Miseq	PCR or PCR-free	fluorescent	up to 2x300	1-2 days	Up to 10 Gb	substitutions	~0.1-1%
Illumina Hiseq	PCR or PCR-free	fluorescent	up to 2x250	days	Hundreds of Gb	substitutions	~0.1-1%
Ion Torrent	PCR	H+	300-500	2 hours	10 Gb?	InDel	>1%
PacBio	Amplification not required	fluorescent	Average >5,000	30min	500 Mb – 1 Gb	InDel	~15%
Nanopore	Amplification not required	Electronic flow change	>1,000	hours	? Mb per MinION	Del?	~10-20%

Illumina platforms and terminologies

Illumina video

1. Library preparation
2. Single-ends and paired ends
3. Reads
4. Instruments

COMMON in all NGS platforms



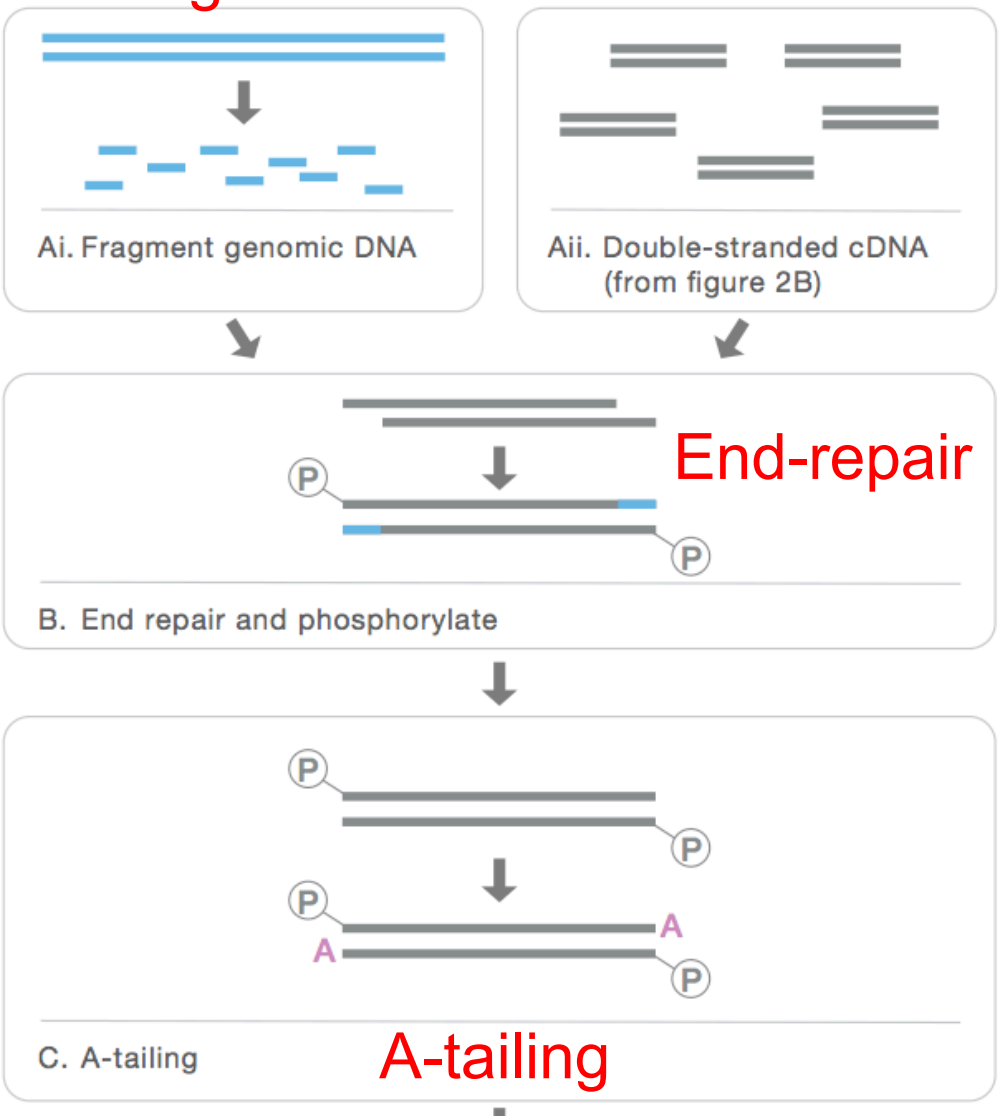
Adaptor is required for library preparation

Thousands to hundreds of millions of fragments are sequenced in parallel

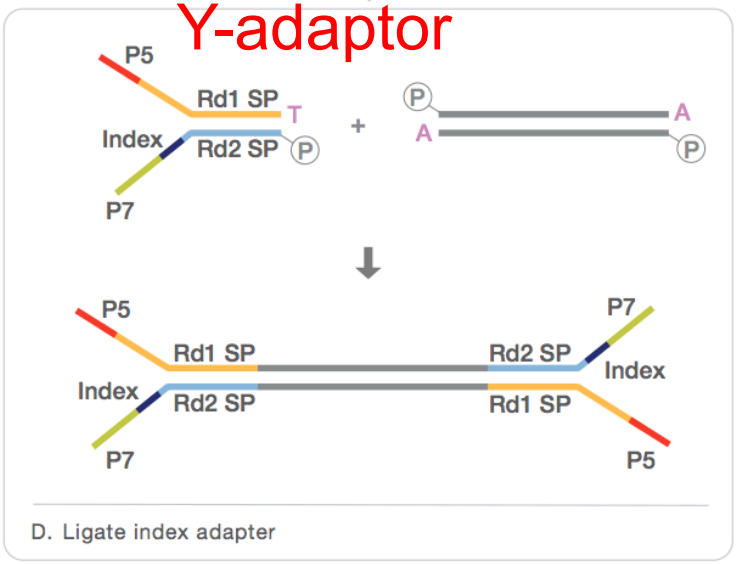
Standard data (FASTQ) are output

Library preparation – Y-adaptor method

a. Fragmentation

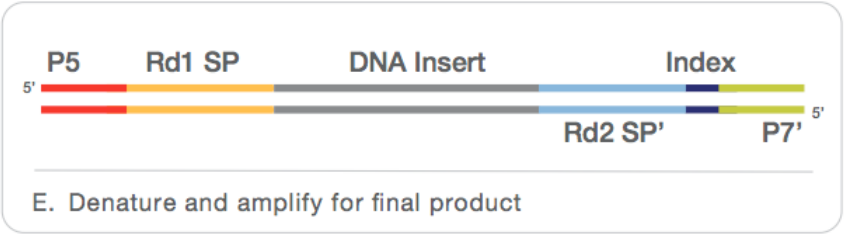


b.



PCR or PCR-free

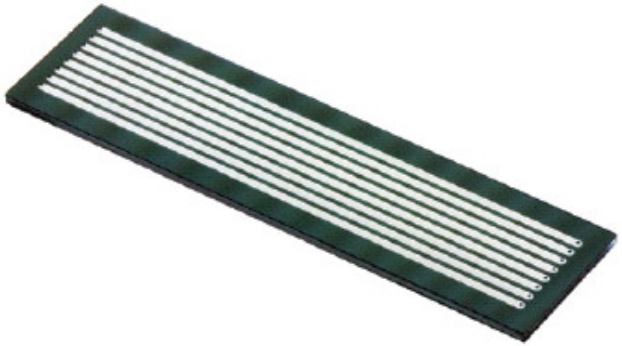
Final product



From TruSeq Manual

Multiplexing (DNA barcode/Index)

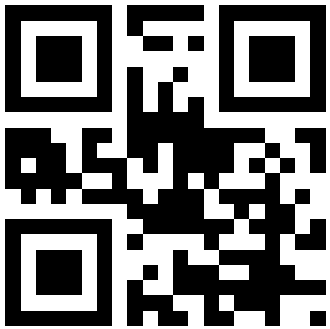
flowcell
lane



- per lane's data are more than needed in many cases

- Multiplexing: To put multiple samples in a lane via using **DNA barcodes** to distinguish samples

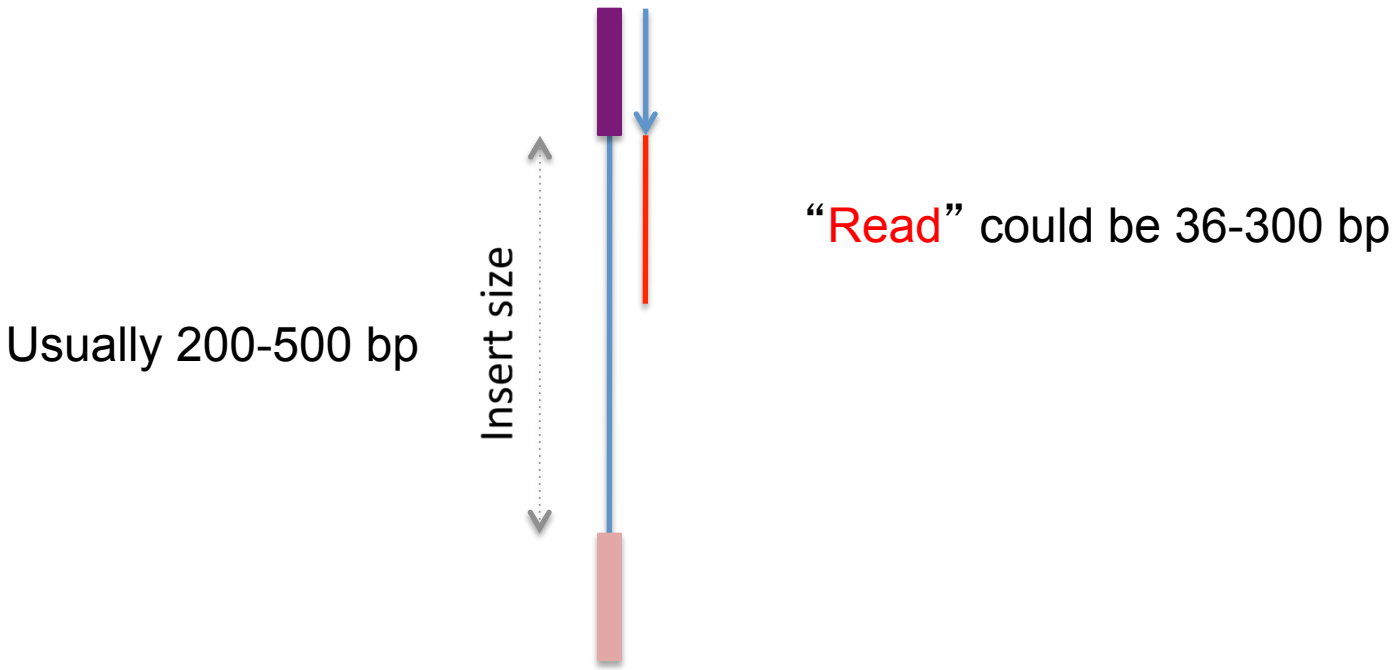
Barcode / Index



```
                AGTGCAxxxxxxxxxxxxx
sample 1        AGTGCAxxxxxxxxxxxxx
                AGTGCAxxxxxxxxxxxxx

                CATGTCxxxxxxxxxxxxx
sample 2        CATGTCxxxxxxxxxxxxx
                CATGTCxxxxxxxxxxxxx
```

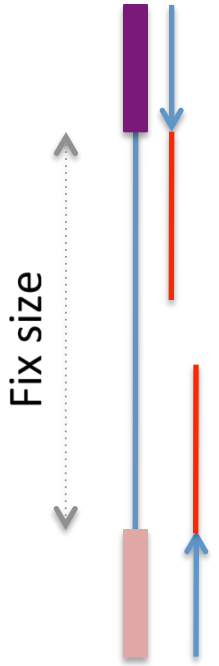
Single-end sequencing

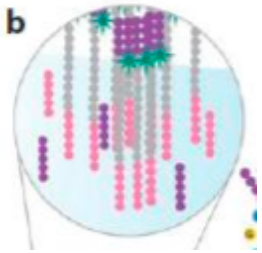
A single read is generated for each template/cluster



Paired-end sequencing

Two reads are generated for each template cluster;
the 1st is from one end with one primer;
the 2nd is for the other end with the other primer.





Summary

1. NGS platforms
2. Pro and con of each platform
3. Approaches for library preparation
4. Applications of various NGS tech