

GENE4001

Advanced Studies in Genetics & Genomics

Introduction
and
Topic 1: Genomics principles

Unit Coordinator: Dr Nicole Smith
Nicole.smith@uwa.edu.au

Unit Schedule

	GENE4001 Schedule 2025 Semester 1					
	Date:	TOPIC:	Lecturer	Date, time	Venue	
Sem Week (year week)						
2 (10)	Monday 3rd March AM	NO WORKSHOP - PUBLIC HOLIDAY				
3 (11)	Monday 10th March AM	Epigenomics principles		Jessica Kretzmann	Mon 10th March, 11am-1pm	Myer: [206]
3 (11)	Monday 10th March PM	Genomics principles		Nicole Smith	Mon 10th March, 2-4pm	Laws: [G31]
4 (12)	Wednesday 19th March AM	Genome editing principles		Jessica Kretzmann	Wednesday 19th March, 9-11am	SSEH: [102]
4 (12)	Wednesday 19th March PM	Transcriptomics principles		Nicole Smith	Wednesday 19th March, 11am-1pm	SSEH: [102]
5 (13)	Wednesday 26th March AM	Genomics Applications		Nicole Smith	Wed 26th March 11am-1pm	ZOOL: [G10]
5 (13)	Wednesday 26th March PM	Epigenomics Applications		Jessica Kretzmann	Wednesday 26th March 2-4pm	ZOOL: [G10]
6 (14)	Wednesday 2nd April PM	Transcriptomics applications		Nicole Smith	Wednesday 2nd April 2-4 pm	ZOOL: [G10]
7 (15)	Wednesday 9th April PM	Genome editing applications		Jessica Kretzmann	Wednesday 9th April 2-4pm	Bayliss [G33]
8 (16)	Tuesday 15th April AM	STUDENT LECTURE PRESENTATIONS (COMPULSORY ASSESSMENT, STUDENTS WILL BE ASSIGNED TO SESSIONS AND NOTIFIED VIA LMS)			Tuesday 15th April AM 11am-1pm	Bayliss [2.15]
8 (16)	Tuesday 15th April PM				Tuesday 15th April PM 2-4pm	
8 (16)	Wednesday 16th April AM				Wednesday 16th April AM 11am-1pm	
8 (16)	Wednesday 16th April PM				Wednesday 16th April PM 2-4pm	
11 (19)	Wednesday 7th May AM	Examination			Wednesday 7th May 10am-12pm	CSSE: [207]

Academic staff

Unit coordinator

Dr Nicole Smith

Email: Nicole.smith@uwa.edu.au

Consultation: by email appointment

Research interests: Nucleic Acids
structure, function and targeting.

Technologies: Biophysical assays,
Human cell cultures, Chemical
Biology, Single Cell sequencing, 3D
bioprinting, Next Generation
Sequencing.

Dr Jessica Kretzmann

Email: jessica.kretzmann@uwa.edu.au

Location: Bayliss Building

Consultation: by email appointment

Research interests: DNA origami for gene
editing and delivery

Technologies: DNA Origami, Chemistry,
Gene Delivery and Editing.



Unit Assessments

Item	Description	Weight	Due
Lecture Outline assignment	<p>Outline in bullet points of the scope and content of your lecture</p> <p><i>Demonstrate an ability to logically and sufficiently structure the content of your lecture.</i></p>	10%	24 th March Turnitin, 11:59 pm
Lecture presentation	<p>Short lecture on an aspect of one of the topics, with a question period</p> <p><i>Demonstrate an in-depth understanding of a specific genomics application, relating to one of the modules.</i></p>	40%	15 th -16 th April, In Person
Exam (90 min)	<p>Composed of short answer questions covering all modules.</p> <p><i>Demonstrate ability to synthesize and evaluate contemporary approaches in genomic analyses, including aspects of experimental design.</i></p>	50%	7 th May

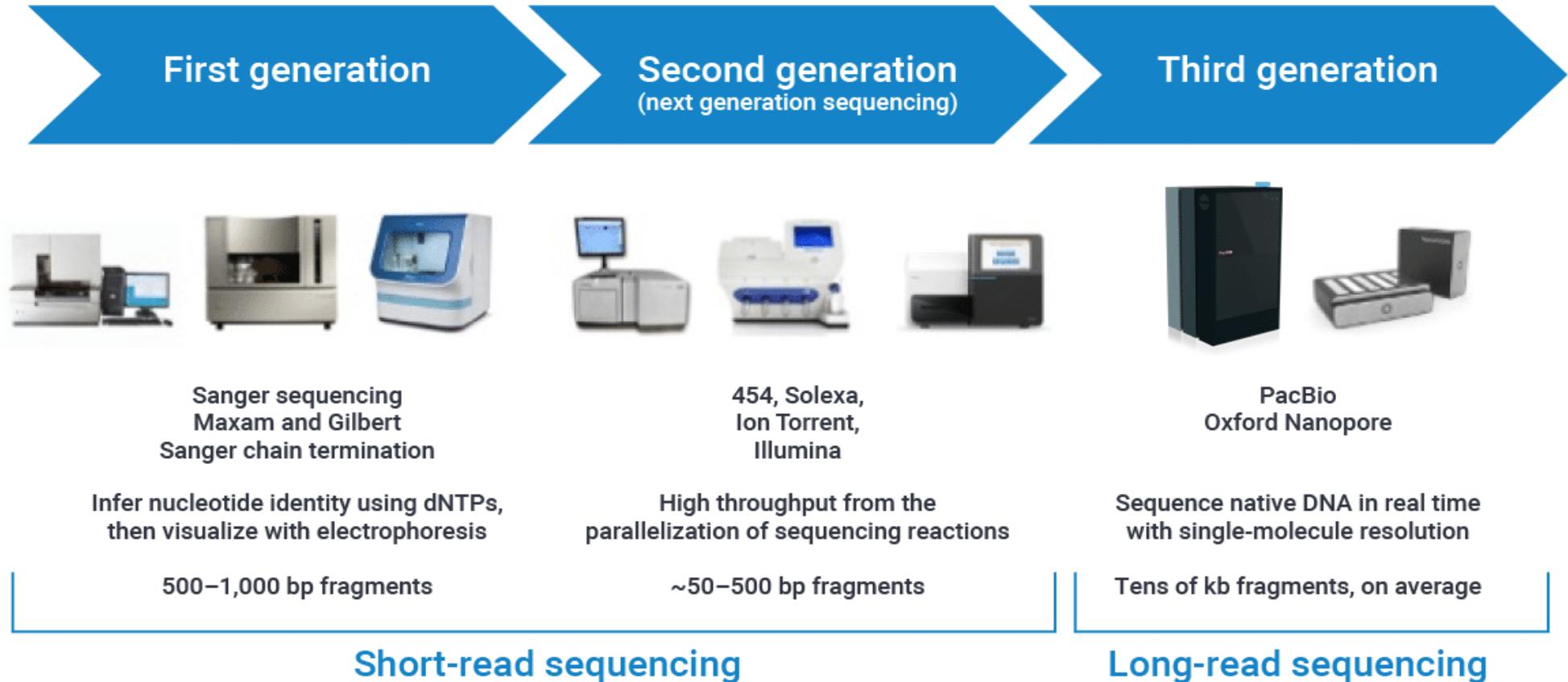
Assessment – lecture presentation

- You will be assigned one of four topics.
- You will develop a 10 minute lecture on that topic through independent reading and in consultation with an academic staff member in charge of the topic.
- Your lecture will be followed by 5 minutes of questions from the audience.
- Your lecture will highlight the research or technology that has led to the current understanding of the topic or best-practice.
- This presentation is NOT a journal club presentation (presenting a single journal article) – rather it is a synthesis of research and advancements on the topic.

Assessment – exam

- The exam will be 120 min
- You will be given one research publication for each of the four topics no later than April 7th.
- The *majority* (not all) of the exam will be based on these papers.
- You will be expected to bring your own copy of each paper (including supplementary files associated with the paper), which may have annotations. No additional papers (notebooks etc) may be brought into the exam.
- The questions will be designed to evaluate your ability to:
 - Interpret data
 - Consider the appropriateness of the approach and experimental design
 - Consider what alternative or additional approaches would be informative
 - Consider what further research approaches would be informative.
- Additional general questions will be included, based on the workshops

Sequencing Technologies



Sanger Sequencing

Manual or automated sequencing using the methods developed by Sanger, Maxam, and Gilbert is considered the "[first](#)" generation of DNA sequencing methods (1977, Cambridge University).

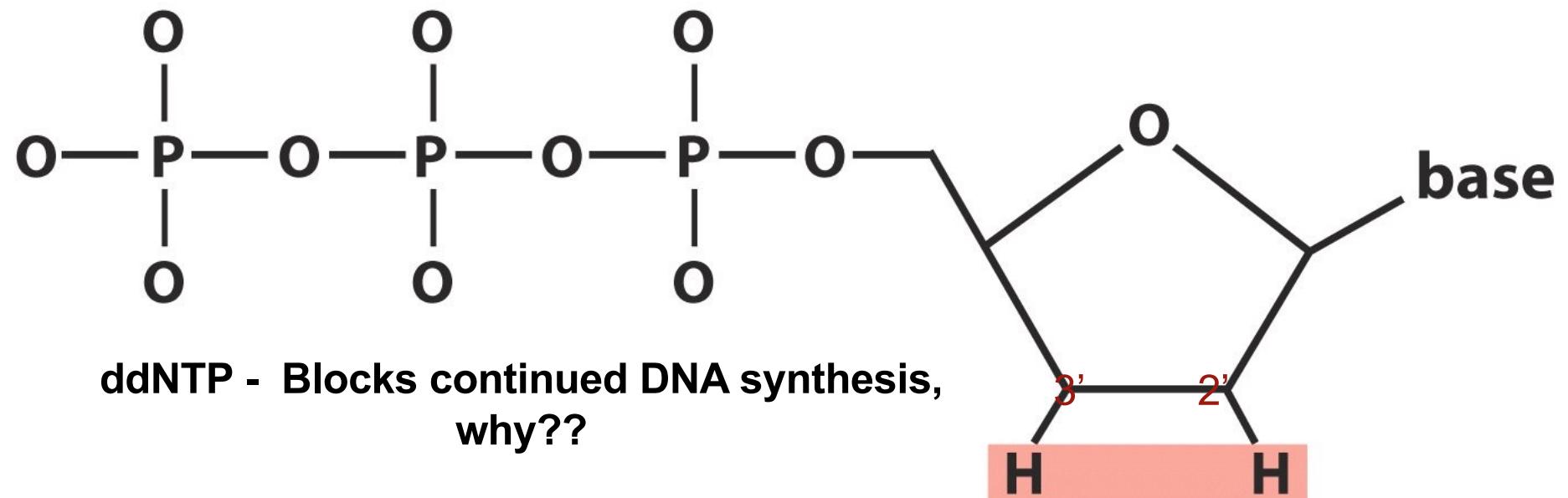
Sanger sequencing determines the sequence of large DNA fragments (up to approximately 500 to 900 bases), by collecting and aligning a series of different length products polymerized along the DNA template. The original Sanger method used radioactive markers for each nucleotide, and later adaptations have used fluorescently tagged versions.

The human genome project (1990-2003, 13 years!) ~\$2.7 Billion

Understanding DNA & RNA Chemistry is Useful

Detection of mutations in DNA by **Dideoxy or Sanger Sequencing ...**

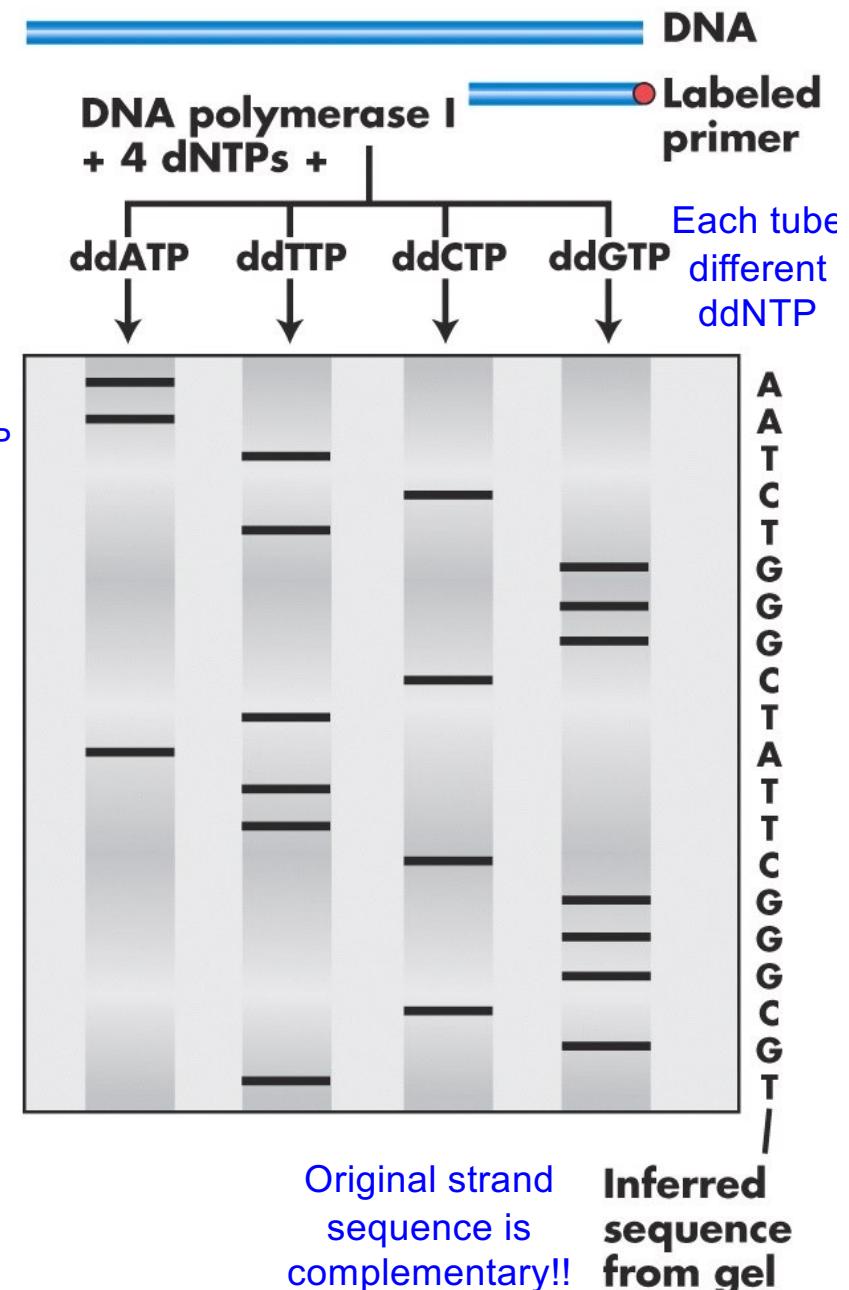
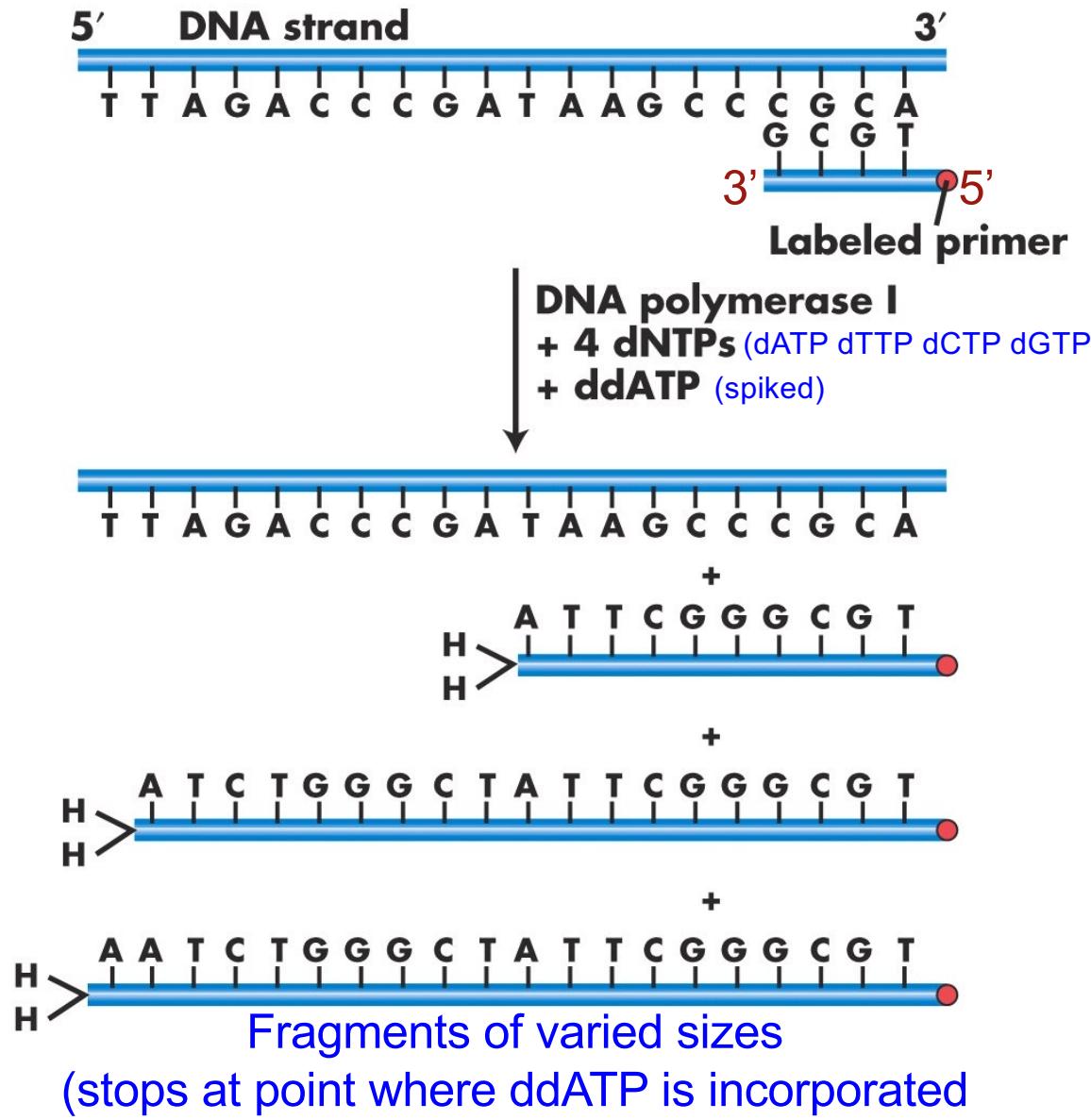
Using chemistry of DNA replication – need **primers (RNA or DNA), template (samples from patient), enzyme** (what co-factor?) & dNTPs, can synthesise DNA from any source ... & detect mutations in it.



**ddNTP - Blocks continued DNA synthesis,
why??**

2',3'-dideoxy analog of the 4 dNTPs

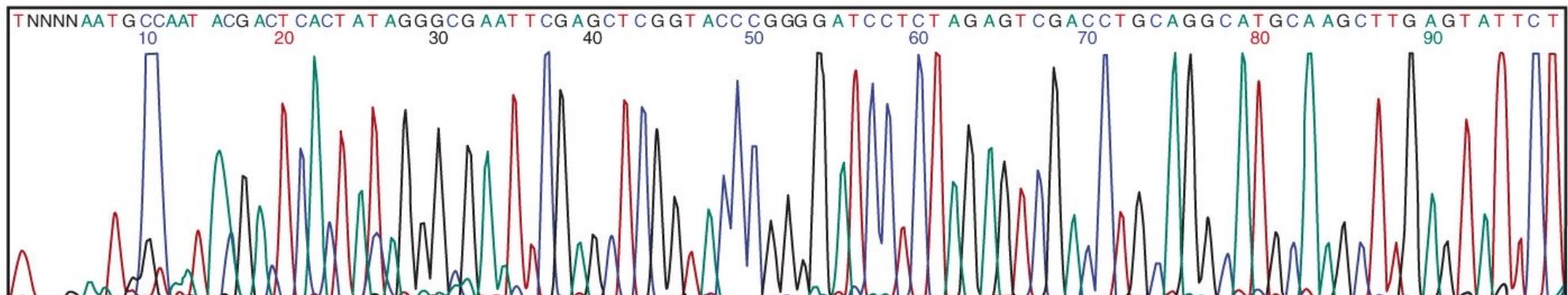
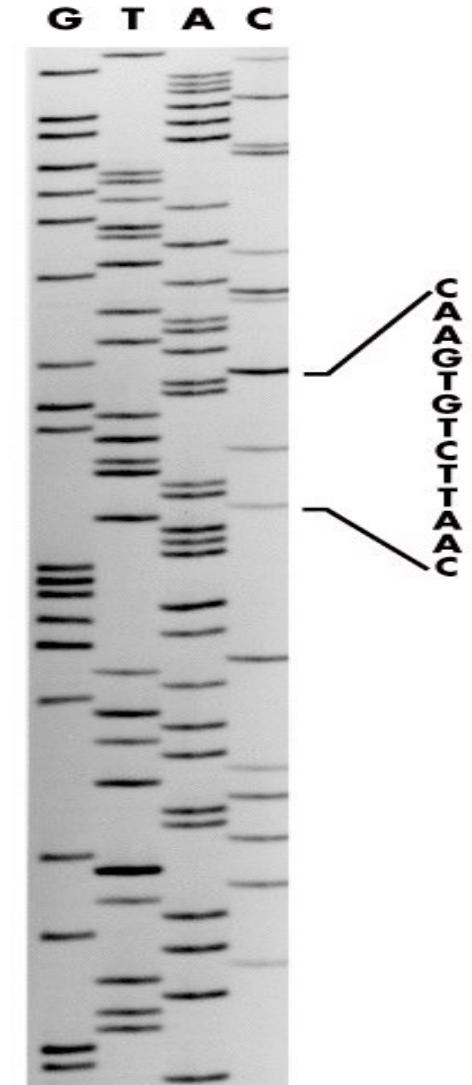
Dideoxy (Sanger) DNA Sequencing



“Label” on dideoxy nucleotides can
be a radiolabel (eg ^{32}P !) →

or a fluorescent dye, each ddNTP a
different “colour” (Same test tube!)

Automated sequencing – each coloured peak
represents a different size fragment of DNA
ending with a fluorescent base (each base is
a different colour) that was detected by the
fluorescence scanner.



Sanger sequencing principles

Innovations through:

- Capillary or fluidics vs gel-based electrophoresis
- ABI's 3730/3500 or SeqStudio
- Fluorescent vs radiolabelling.
- Multichannel detection.
- Automation, digital detection.
- But still largely linear: [low throughput e.g. 384 wells](#). Can sequence a single 600 bp PCR fragment from 384 samples or 384 PCR fragments from a single sample on 1 plate. 16 plates sequentially in 48 hours on ABI 3730 XL instrument = 6,144 sequenced reads
- **Expensive: \$6 per fragment (well), \$1 million per Gb.**



Next Generation Sequencing (NGS)

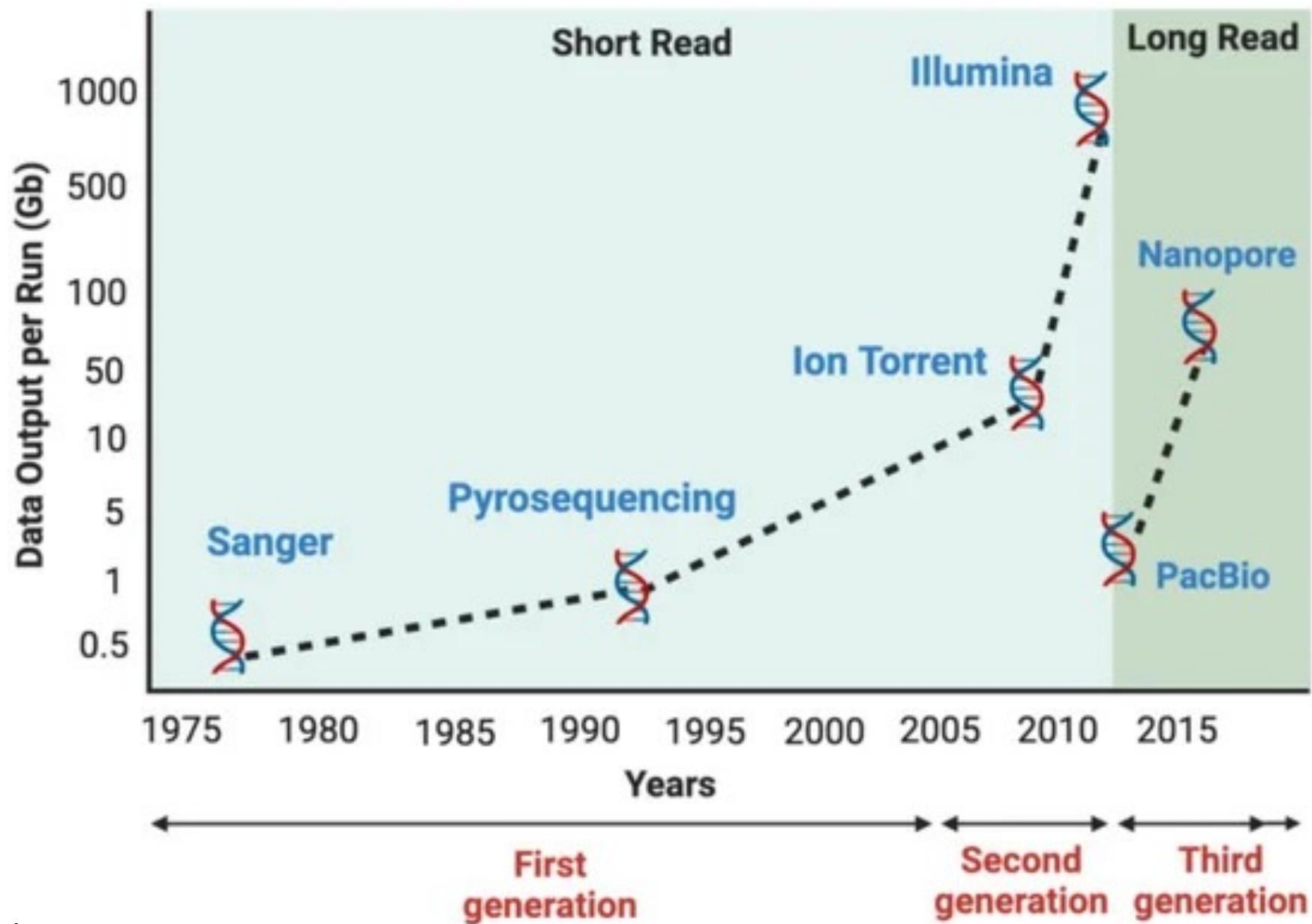
Sequencing of multiple DNA fragments, performed in parallel. This technology is also referred to by other terms (eg, short-read sequencing, high-throughput sequencing, deep sequencing, second-generation sequencing).

In contrast to Sanger sequencing, the speed of sequencing and amounts of DNA sequence data generated with NGS are exponentially greater, and are produced at significantly reduced costs. Sequencing the human genome ~\$600-\$1000

Several "platforms" (ie, sequencing instruments and associated reagents).

Across NGS platforms, there is typically a sample preparation or "library preparation" step in which the patient's DNA, which serves as the template, is purified, fragmented and amplified. Physical isolation of DNA fragments by attachment to solid surfaces or small beads. Sequence data are generated on these small fragments, and the electronic results are computationally aligned against a "reference" genome or sequence (ie, a previously sequenced genome designated as a "normal" reference).

Sequencing Technologies



Sequencing Technologies

					
Metric	iSeq100	MiniSeq	MiSeq	NextSeq500	NovaSeq6000
Run Time (hrs)	9 - 17.5 hrs	4 – 24 hrs	4 – 55 hrs	12 – 30 hrs	13- 36 hrs
Maximum output (Gb)	1.2	7.5	15	120	6000
Maximum reads per FC	4 million	25 million	25 million	400 million	10,000 million
Maximum Read length	2 X 150	2 X 150	2 X 300	2 X 150	2 X 250
Used for	Seq QC for NovaSeq, small genomes	Small Genomes, amplicon seq, 16S, plasmidseq	Small Genomes, amplicon seq, 16S, plasmidseq	All libraries	All libraries including Large whole-genome sequencing

2022 (22 human genomes @30x coverage per run)



NovaSeq X Series

Max output per flow cell
8 Tb^c

Run time (range)^d
~17–48 hr

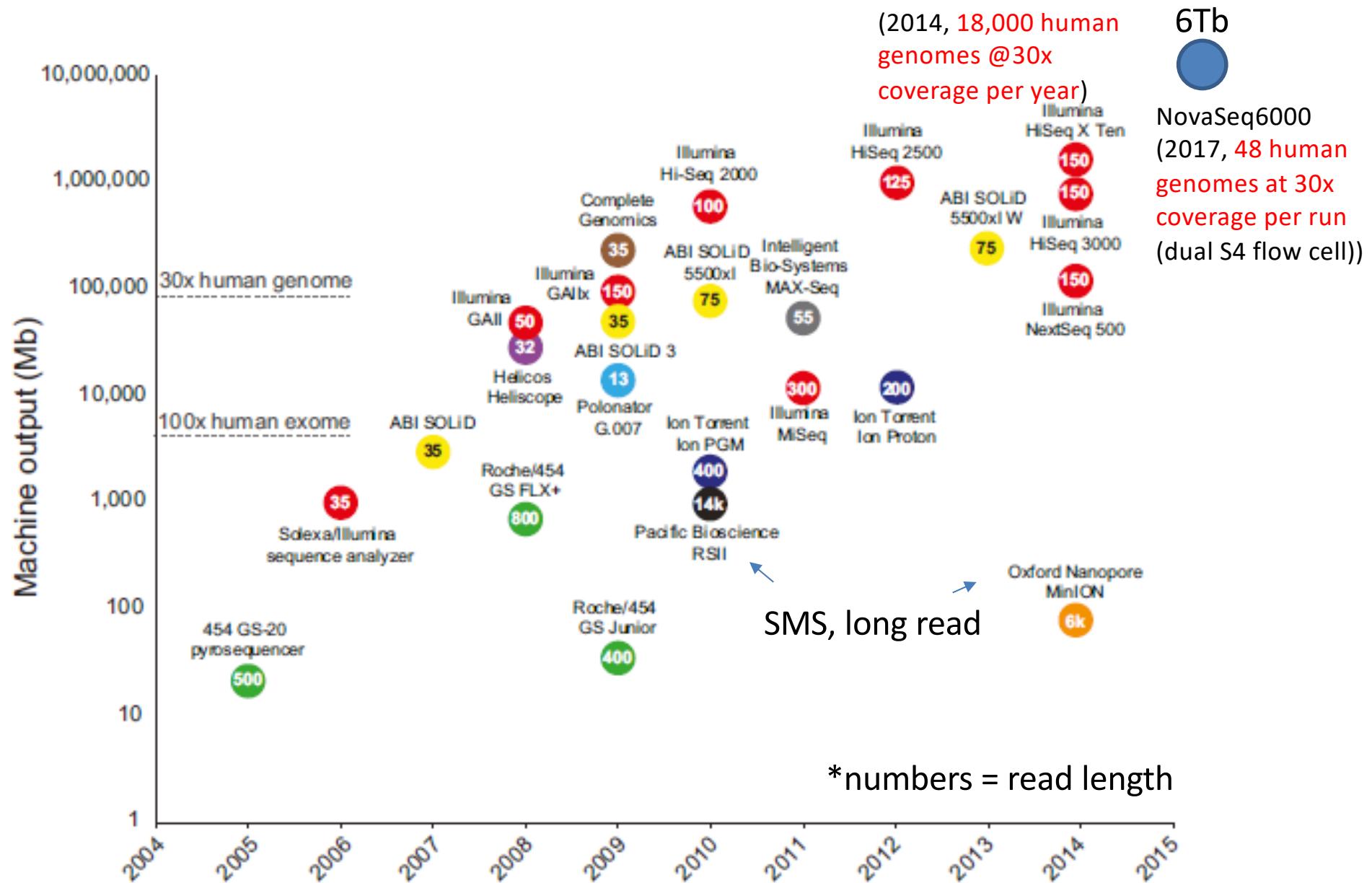
Max reads per run (single reads)
26B (single flow cell)^c
52B (dual flow cells)^{c,e}

Max read length
2 × 150 bp

All libraries including Large whole-genome sequencing

- Illumina sequencers are market leaders in this field, other sequencers are too new.

NGS technology diversity

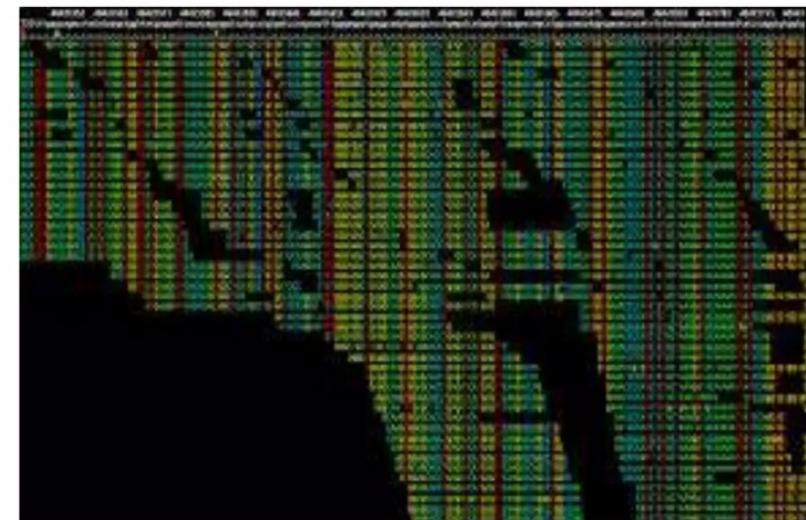
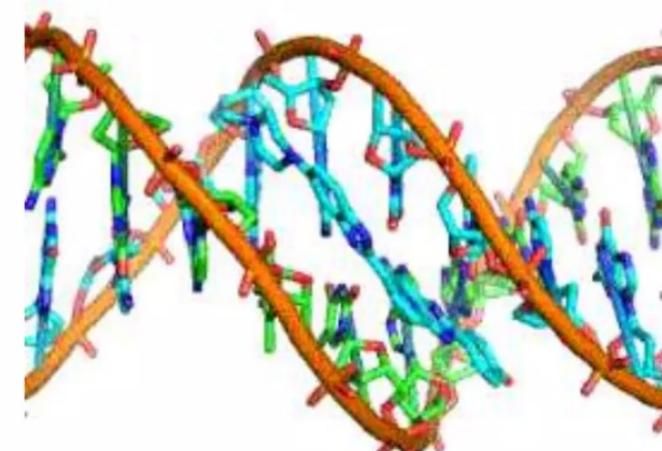


2nd Generation sequencing

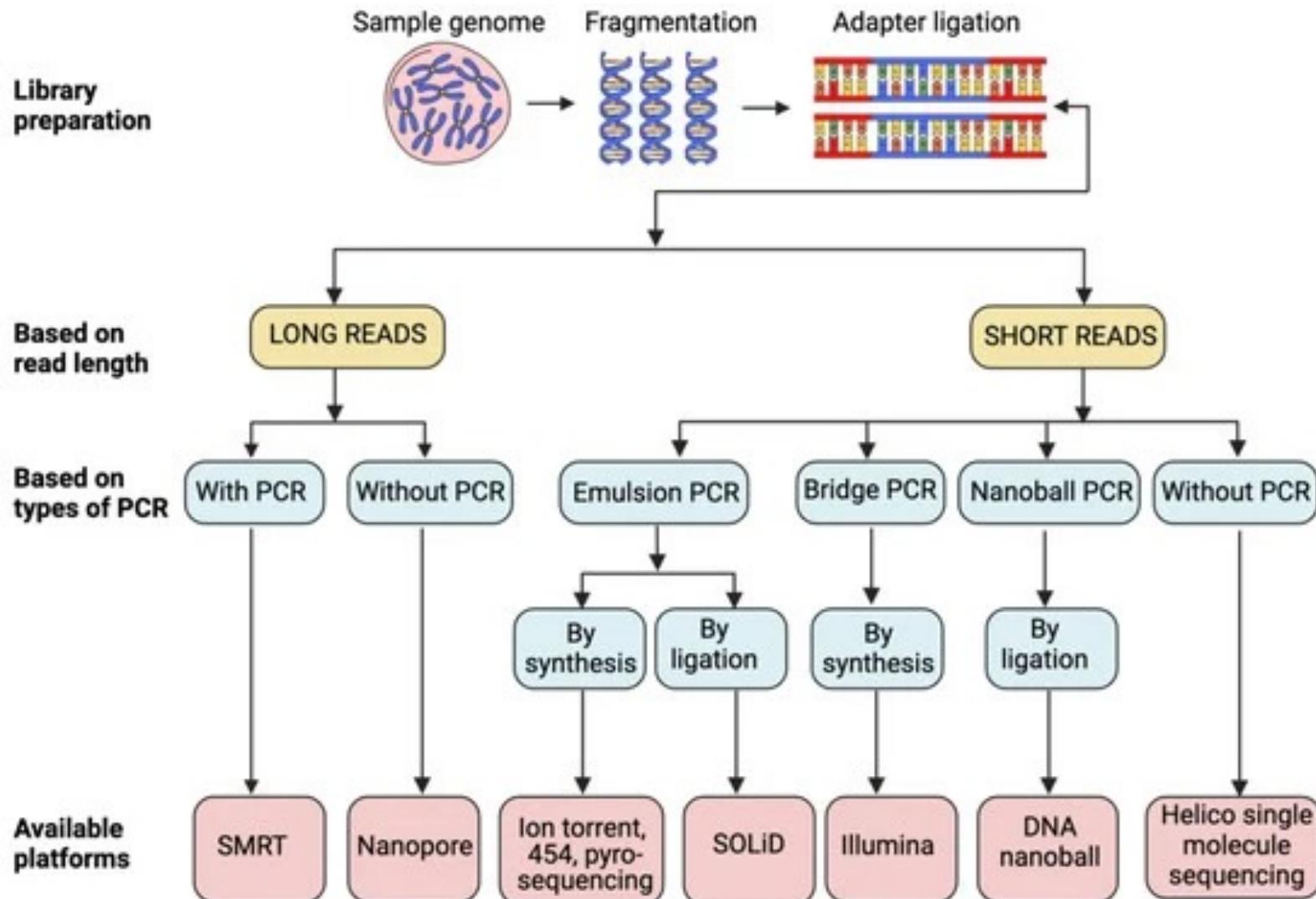
- Amplify, enabling depth.
- Short read, ~150bp.
- Sequence continuously* (nucleotides still read one at a time).
- Have real-time detection.
- Are parallel; many millions of DNA fragments from multiple samples are sequenced simultaneously
- 1 million to 25,000 million reads per run.
- Extensive sample preparation through a process called library preparation
- Cheaper, Faster and higher throughput than Sanger.
- \$2 to \$28 per Gb. The fastest sequencer can generate 50 billion reads of 300 bases each in 48 hours. This equates to **sequencing 128 human genomes in 48 hours for AUD 1,200 per sample.**

NEXT GENERATION SEQUENCING

- + High throughput DNA Sequencing Technique.
- + Employs Micro and Nanotechnologies
 - Reduce sample size.
 - Low Reagent cost
 - Less Time
- + Massive Parallel Sequencing
- + Sequence thousands of sequences at once.
- + Produce enormous amount of data .

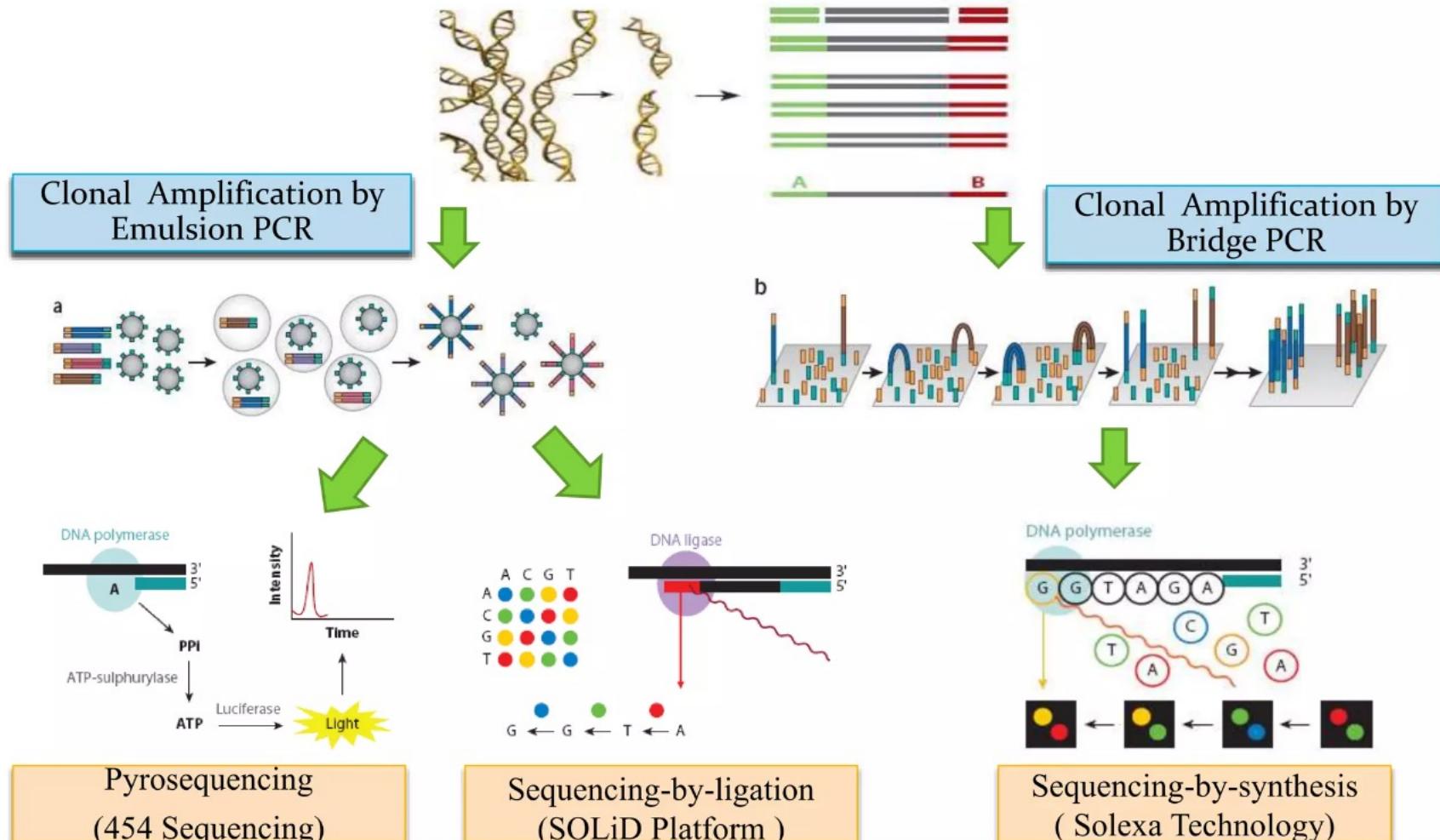


NGS Platforms and Principles



NGS WORKFLOW

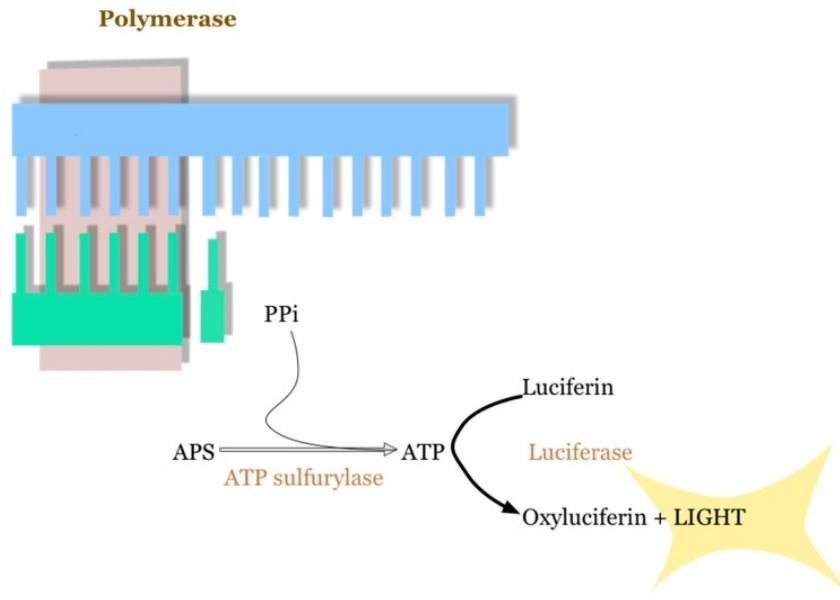
Sample Extraction , DNA fragmentation and *invitro* adapter ligation



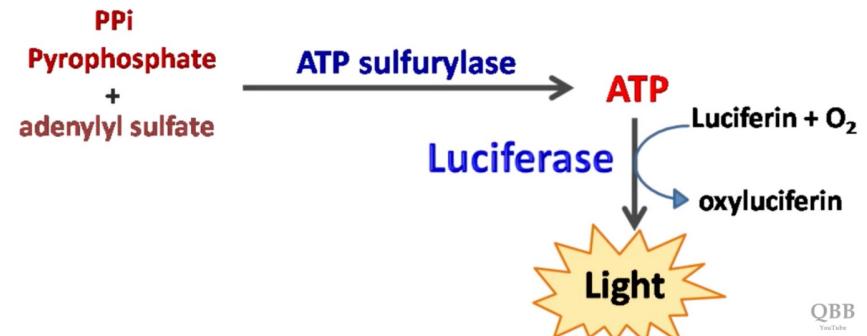
Roche 454 - obsolete

Sequencing by
Oligonucleotide Ligation and
Detection - Obsolete

2nd Gen – 454 Pyrosequencing

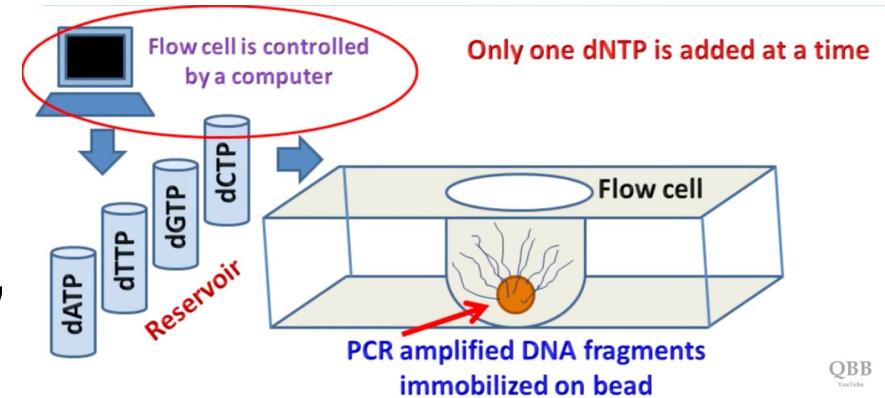


Addition of **any dNTP (A, T, G or C)** will generate PPi
How do we know which nucleotide is added ?



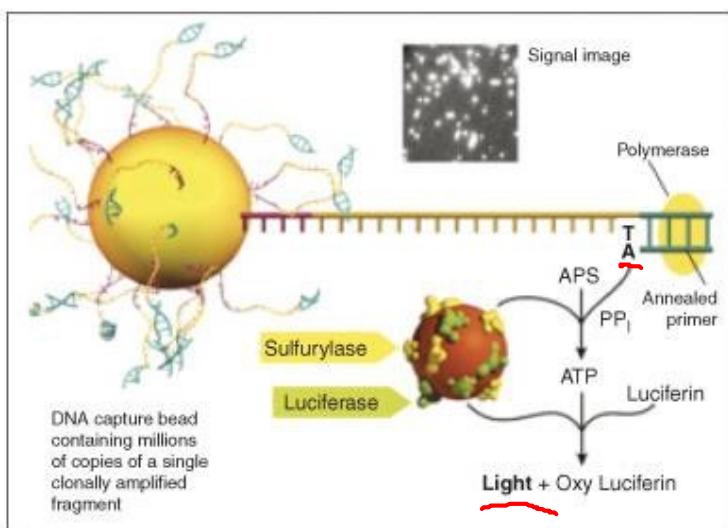
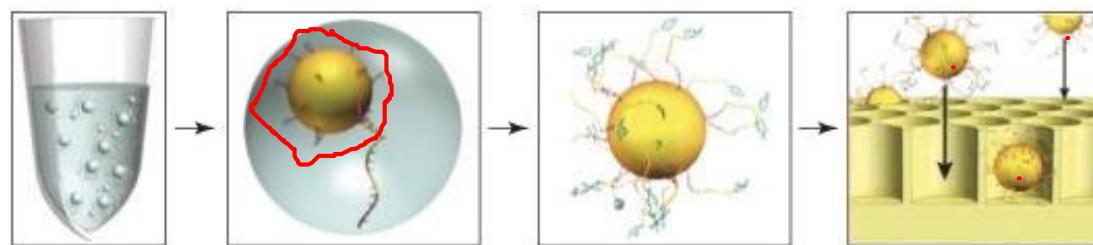
https://www.youtube.com/watch?v=wY8to-_zAEo

<https://www.youtube.com/watch?v=qKouzbp1RW>



2nd Gen – 454

- clonal amplification by emulsion PCR, beads with amplified fragments are deposited into wells of picotitre plate

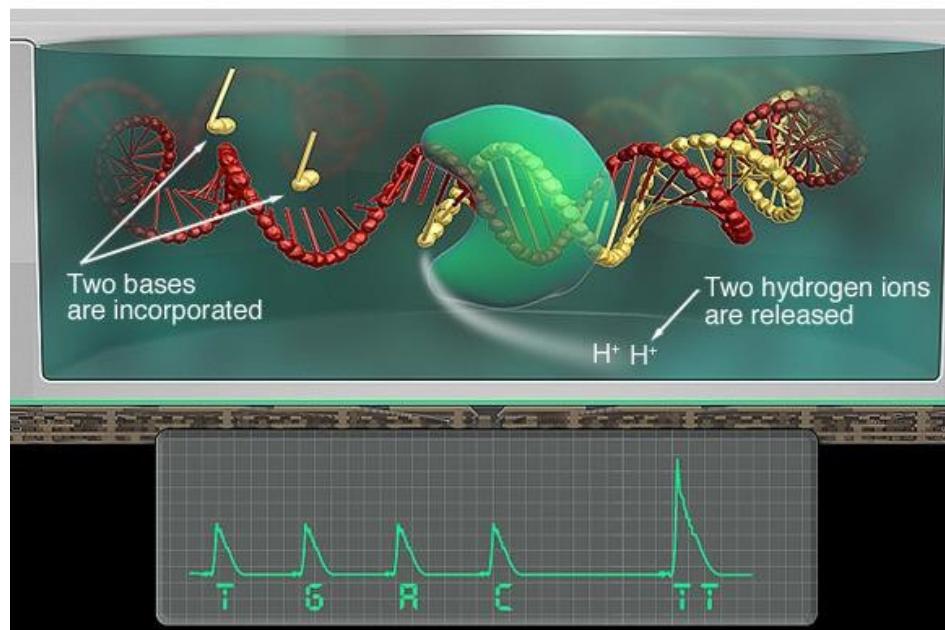


- dNTPs added sequentially then excess degraded.
- pyrosequencing – polymerisation coupled phosphate release triggers luminescent signal, optical detection, read lengths up to 1000 bp.

<https://www.youtube.com/watch?v=KzdWZ5ryBIA>

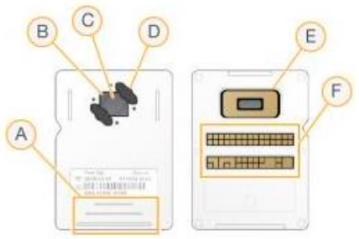
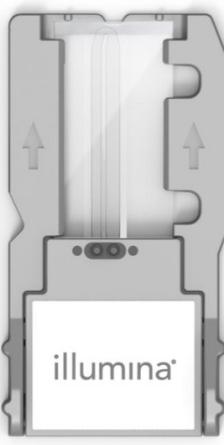
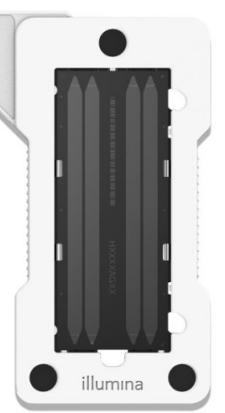
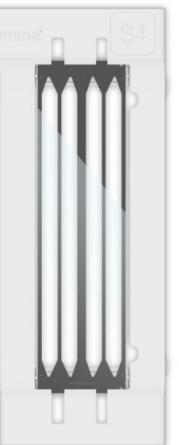
2nd Gen – Ion Torrent (ThermoFisher)

- clonal amplification by emulsion PCR
- as with 454, dNTPs added sequentially then excess degraded.
- semiconductor sequencing, microchip detects change of pH caused by hydrogen ions released during DNA polymerisation, no modified nucleotides or optics required, read lengths 400 bp



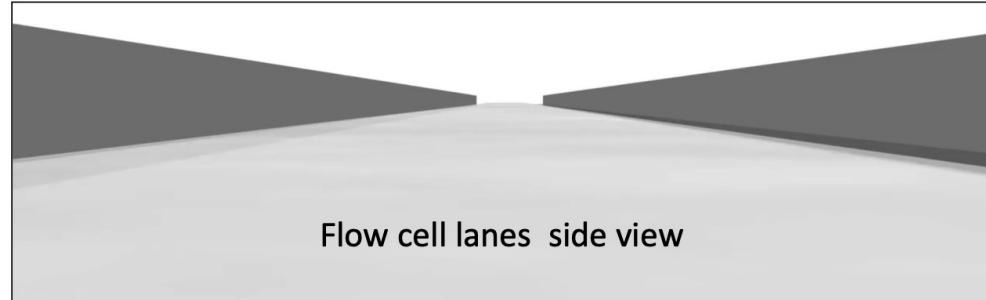
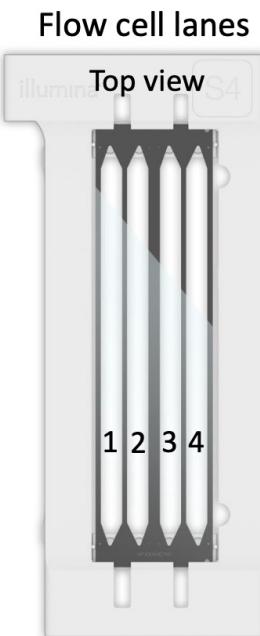
<https://www.youtube.com/watch?v=WYBzbxIfuKs>

Flow cells determine output and performance

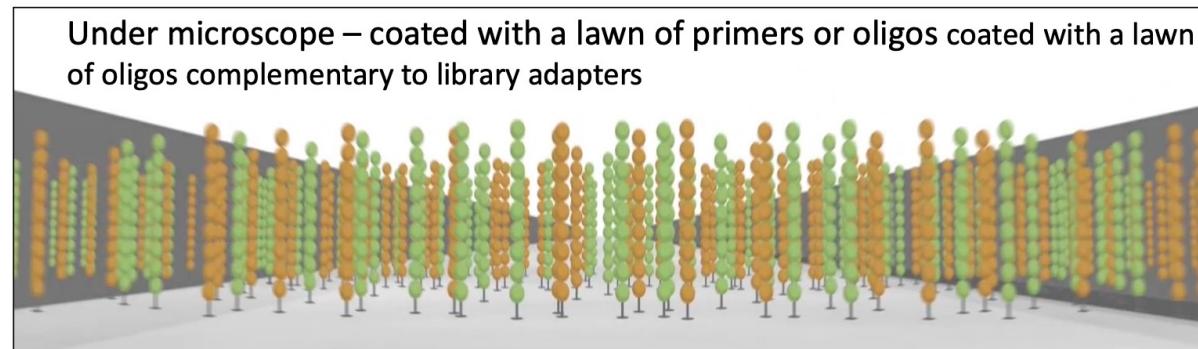
 <p>A. Grip points B. CMOS sensor (top) C. Imaging area D. Gasket (one of two) E. CMOS sensor (bottom) F. Electrical interface</p> <p>iSeq Flow cell (FC) with a complementary metal-oxide semiconductor (CMOS) chip. Light emitted is recorded by CMOS sensor. Output : 4M read pairs Format: 2 X 150 cycles</p>	 <p>MiSeq Flow cell Output: 1M, 4M, 15M, 25M read pairs. Format: 2 X 150, 2 X 250, 2 X 300, 1 X 50 cycles</p>	 <p>NextSeq 500 Flow cell Output: 150M, 400M read pairs Format: 2 X 150 cycles</p>	 <p>NovaSeq 6000 Flow cell, 2 lanes or 4 lanes. Output: 800M, 1600M, 4000M, 10,000M read pairs Format: 2 X 50, 2X 100, 2 X 150, 2 X 250 cycles</p>
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- Number of lanes - where separate sequencing reactions occur simultaneously
- Clustering capacity - lawn of oligonucleotides that facilitate amplification and clustering of DNA templates.
- Read length – depends on flow cell and sequencing chemistry
- Output capacity - total number of gigabases or terabases generated per run dependent on all the above

Illumina Flow cells – lawn of oligos

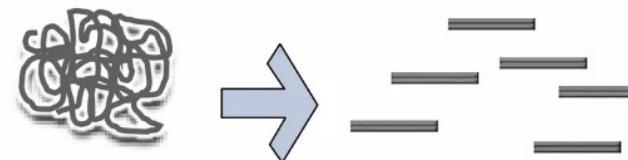


Thick glass slide
with lanes

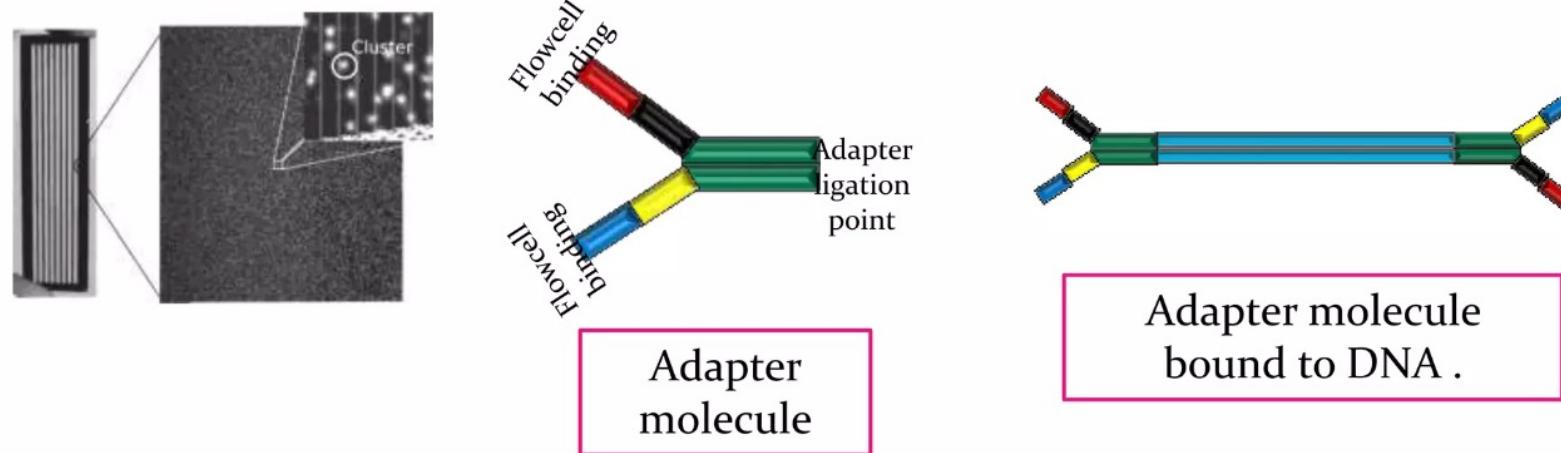


NGS WORKFLOW

1. Create DNA fragments

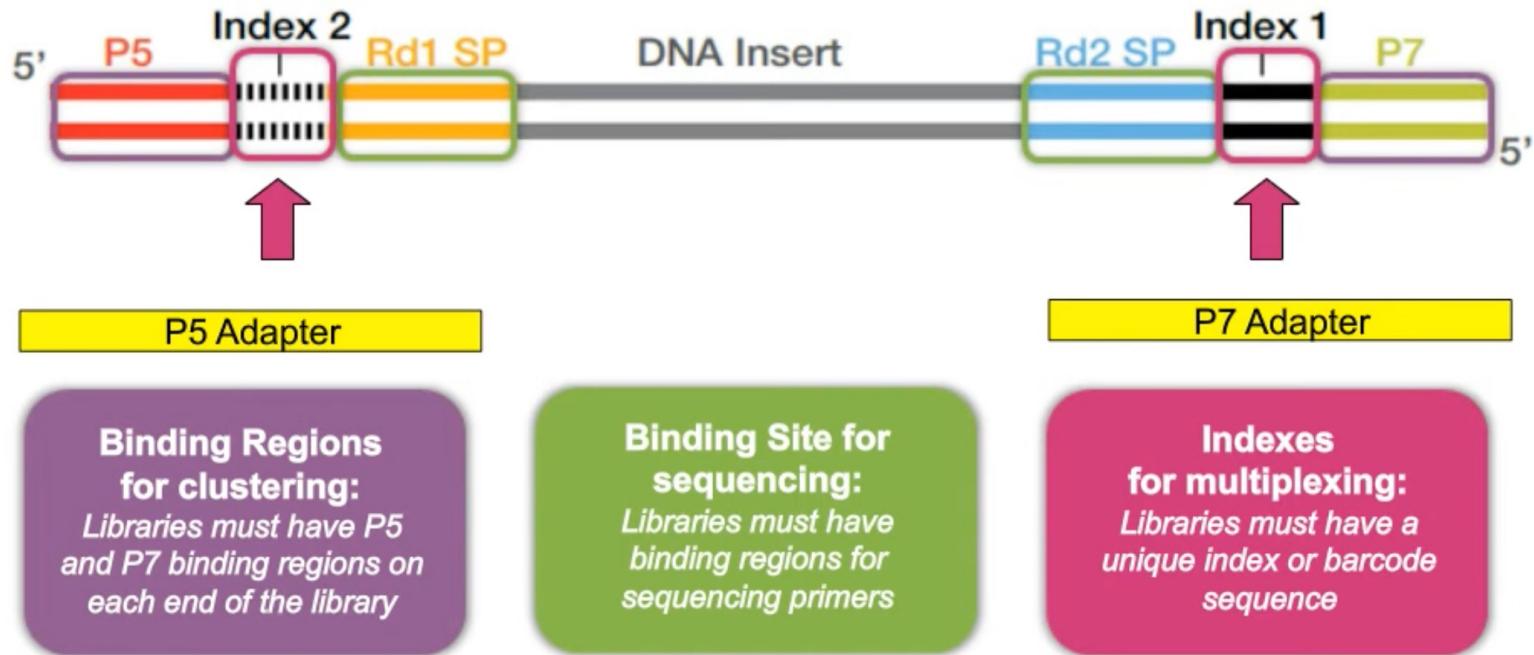


2. Add platform-specific adapter sequences to every fragment.



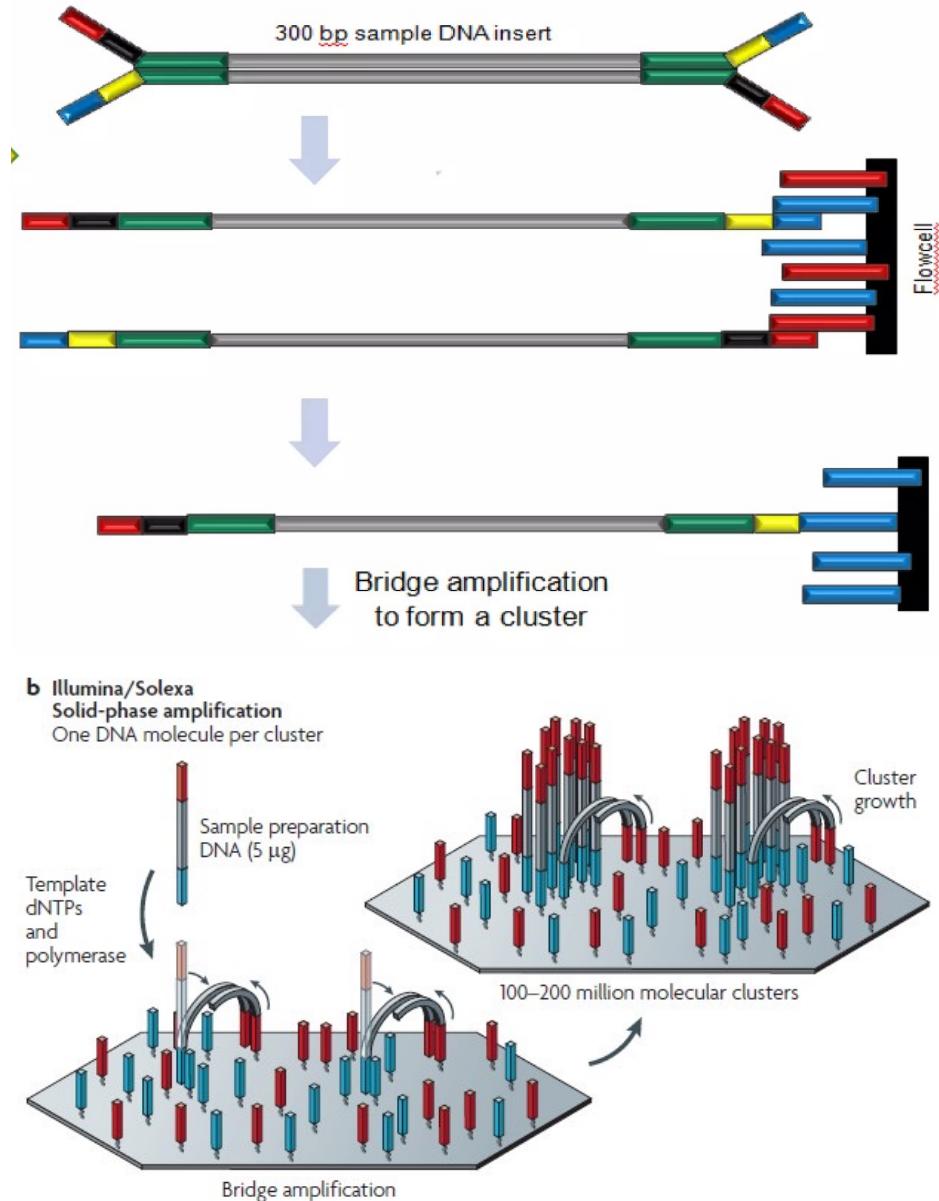
- Adapter molecules : Bind library to a flowcell or bead; Add sequence primer binding sites & Add barcodes for multiplexing.

NGS WORKFLOW

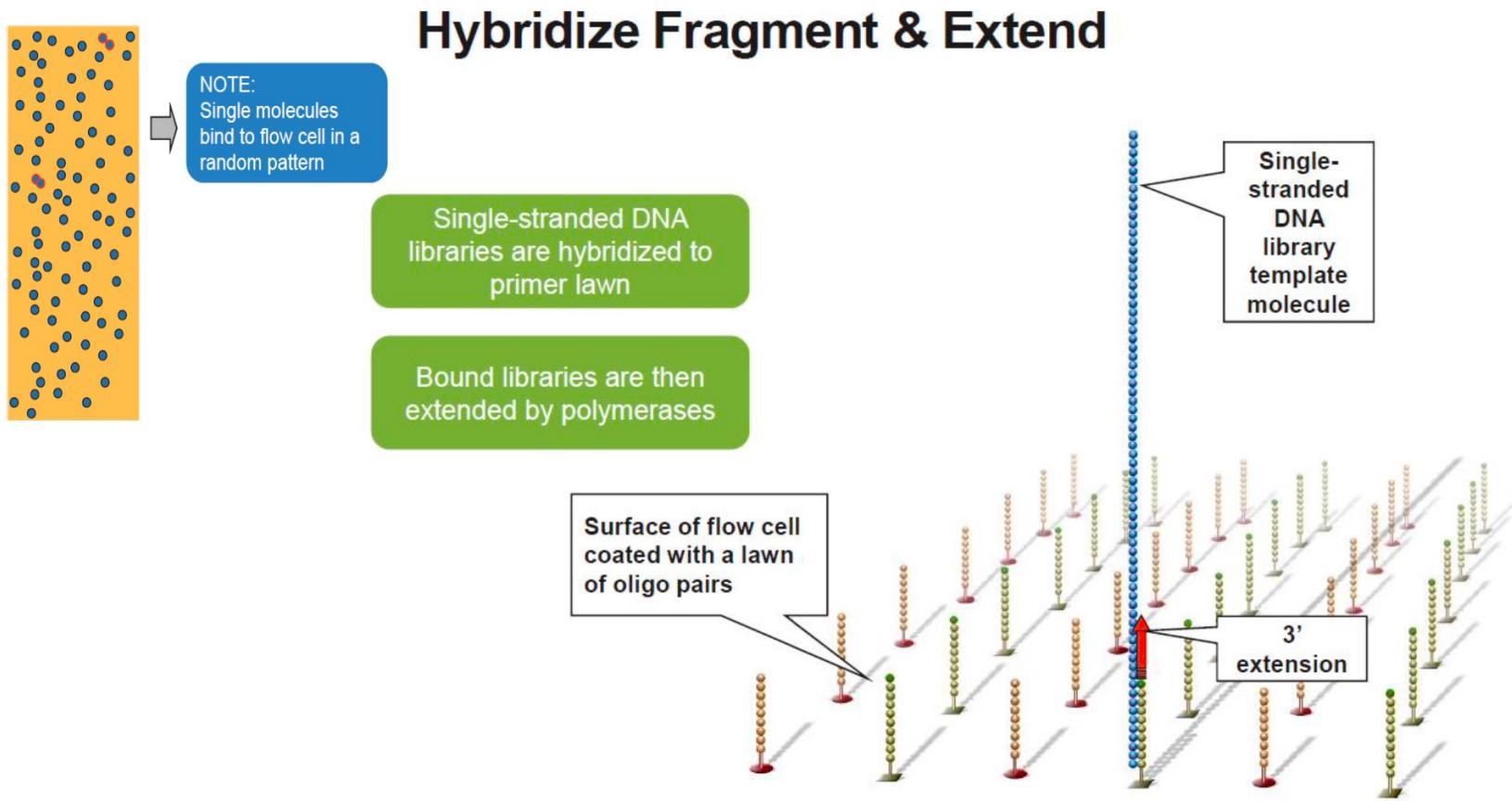


Sequencing process

- Cluster generation - a group of DNA strands positioned closely together. Each cluster represents one to two thousand amplified clonal copies of the same DNA strand in a 1–2 micron spot.
- Clustering are generated through bridge amplification
- Sequencing by synthesis – 4-channel chemistry has now progressed to 2-channel and 1-channel chemistry, thus reducing sequencing cost



Bridge amplification



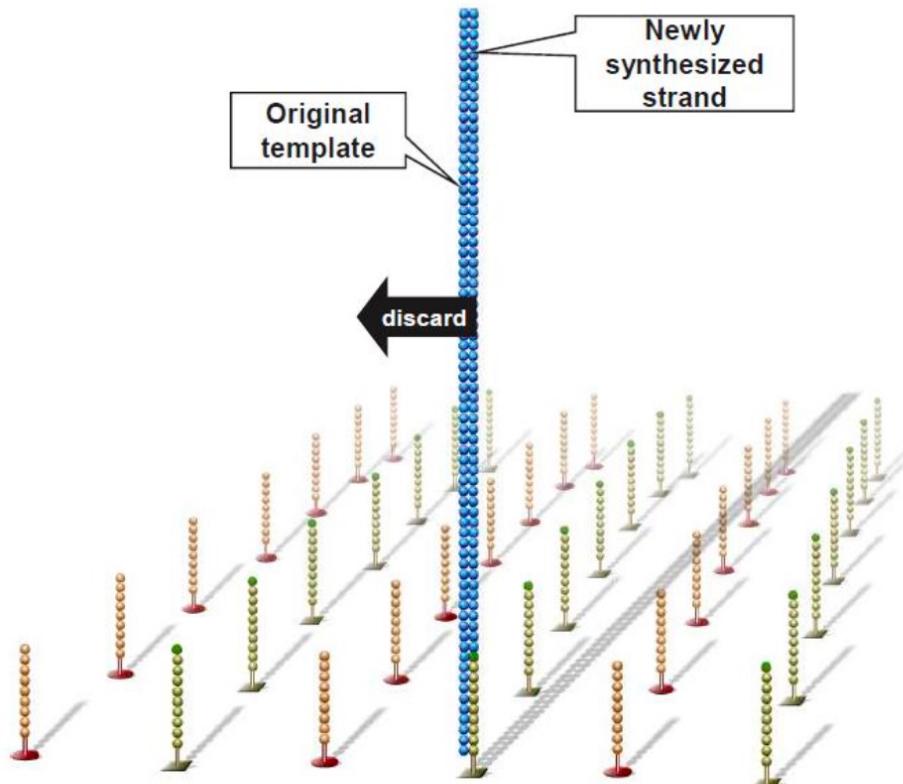
Bridge amplification

Denature Double-Stranded DNA

Double-stranded molecule
is denatured

Original template washed
away

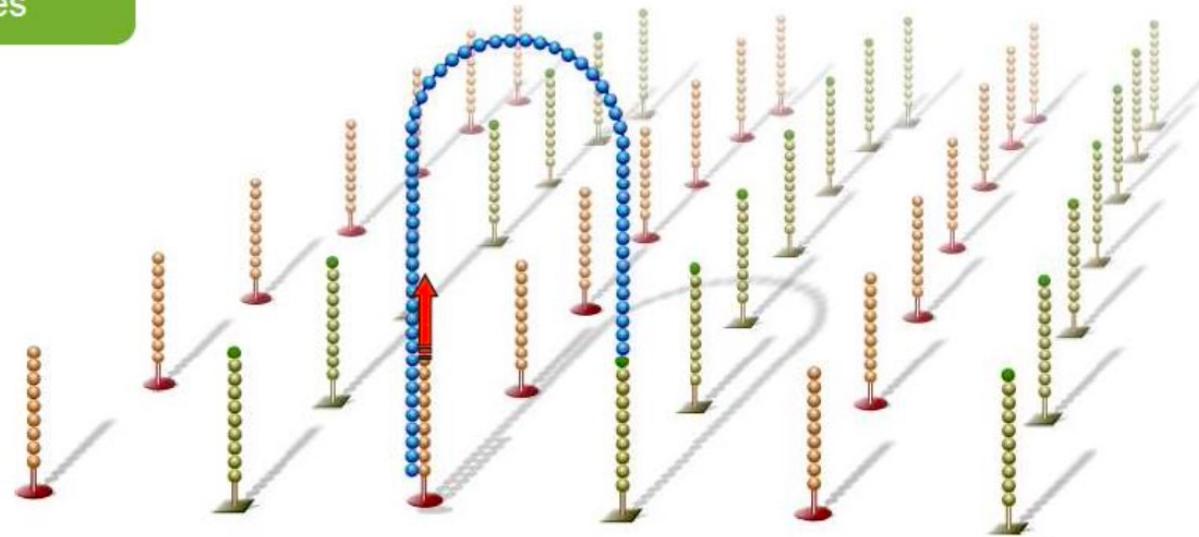
Newly synthesized strand
is covalently attached to
flow cell surface



Bridge amplification

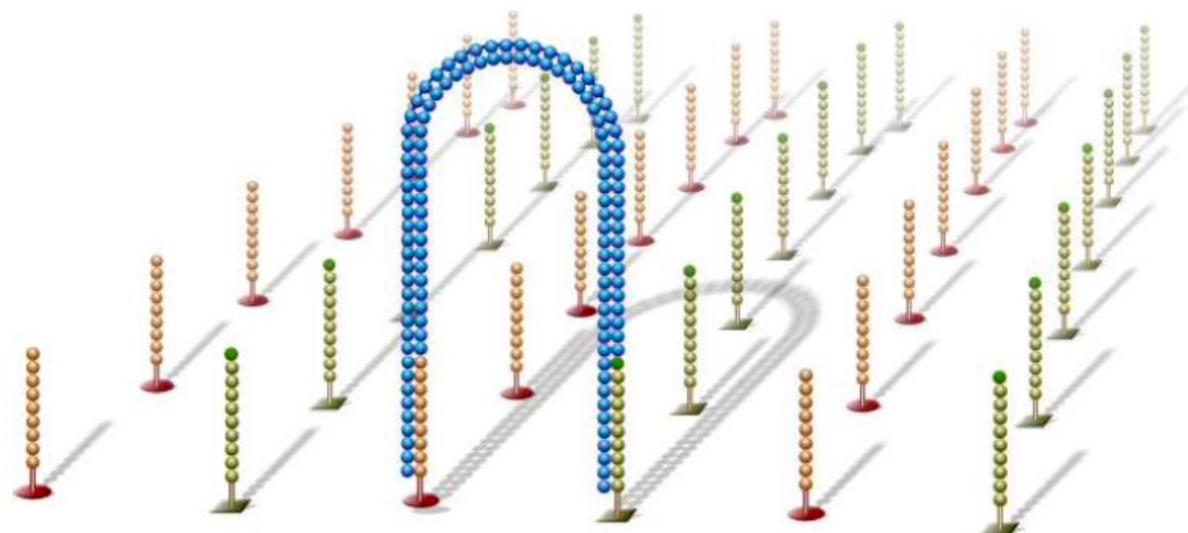
Single-stranded molecule flips over
and forms a bridge by hybridizing to
adjacent, complementary primer

Hybridized primer extends
by polymerases



Bridge amplification

Double-stranded bridge is formed

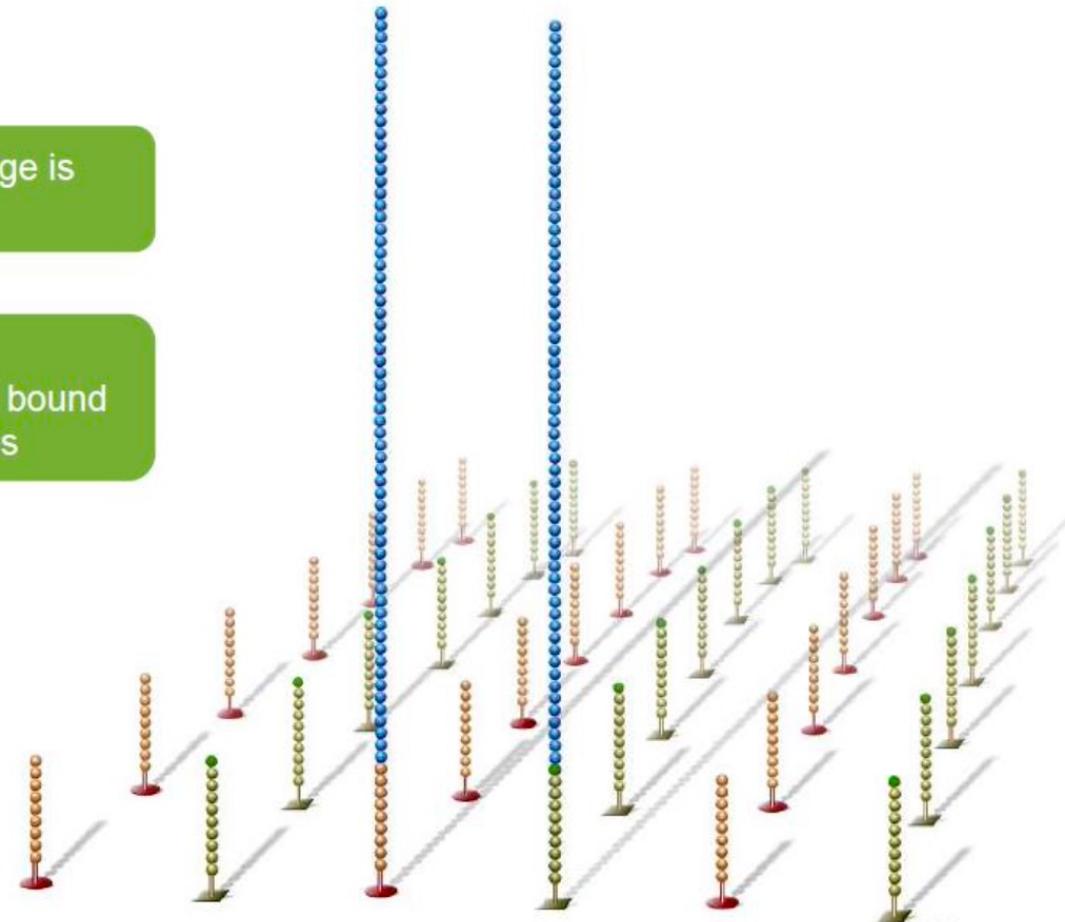


Bridge amplification

Denature Double-Stranded Bridge

Double-stranded bridge is denatured

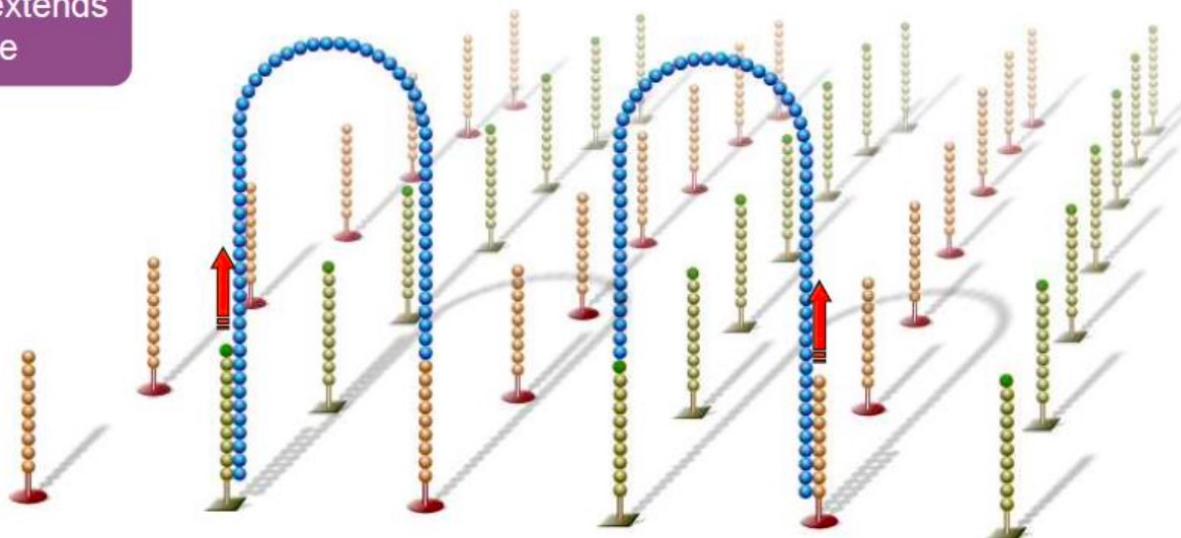
Result:
Two copies of covalently bound single-stranded templates



Bridge amplification

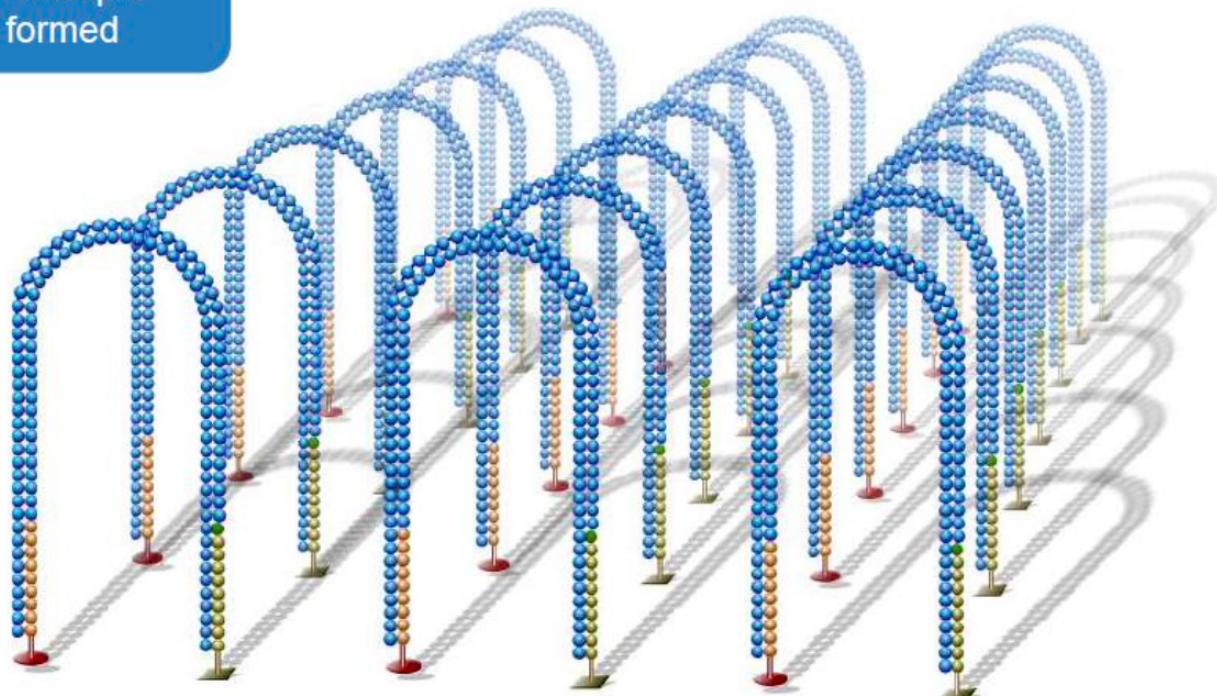
Single-stranded molecules flip over
to hybridize to adjacent primers

Hybridized primer extends
by polymerase



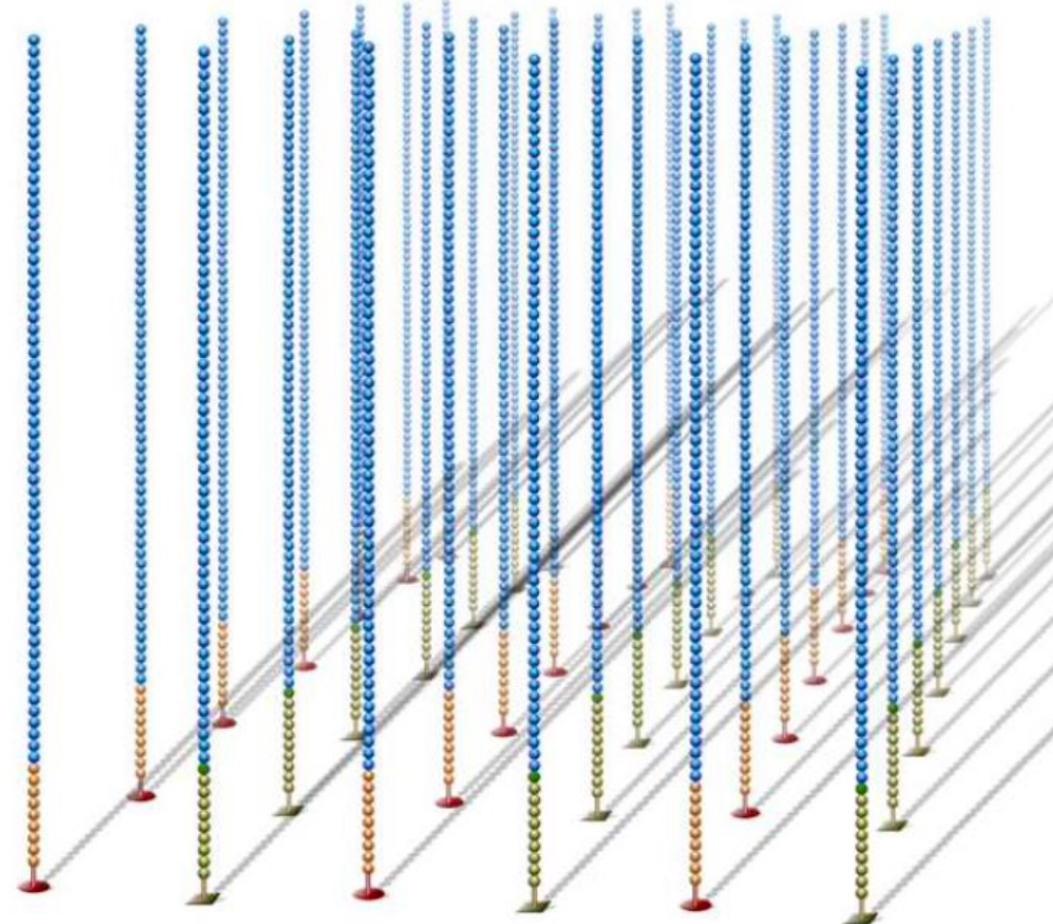
Bridge amplification

Bridge amplification cycle is repeated until multiple bridges are formed



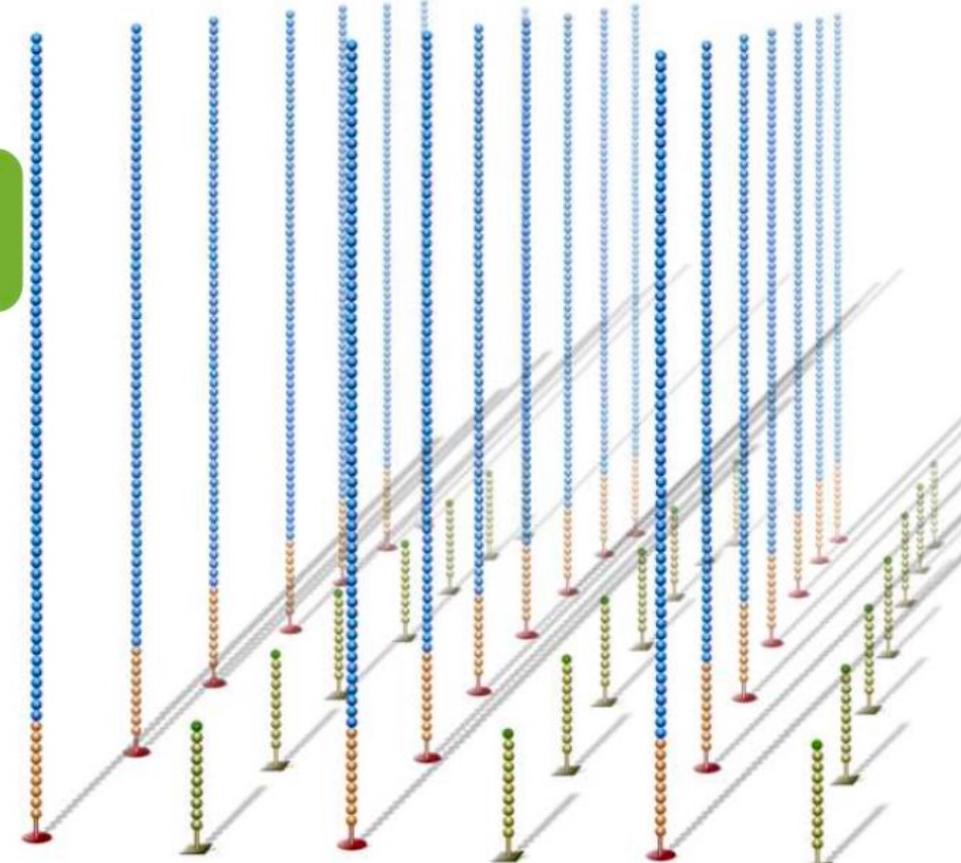
Bridge amplification

dsDNA bridges are denatured

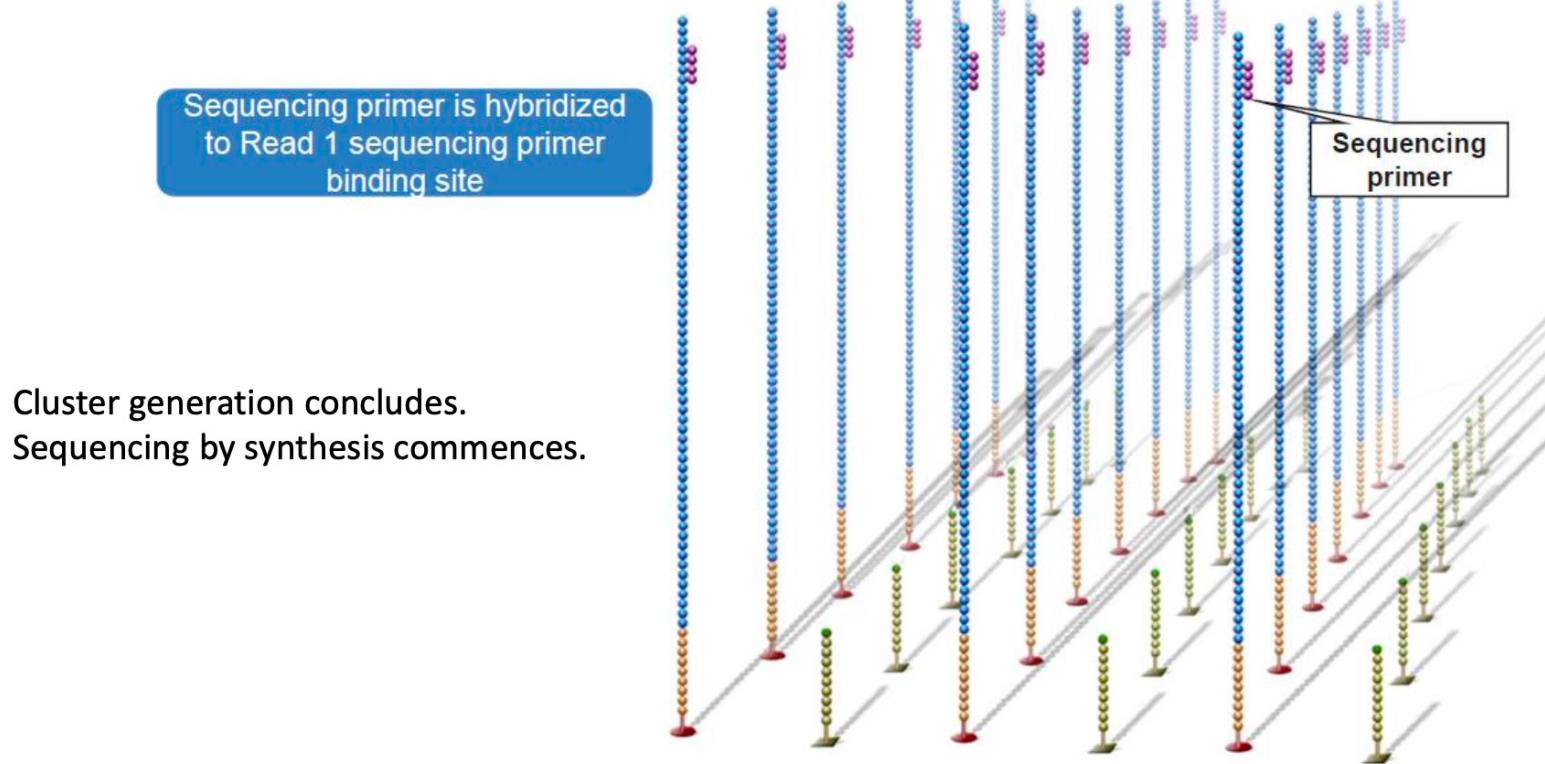


Bridge amplification

Reverse strands are cleaved and washed away, leaving a cluster with forward strands only



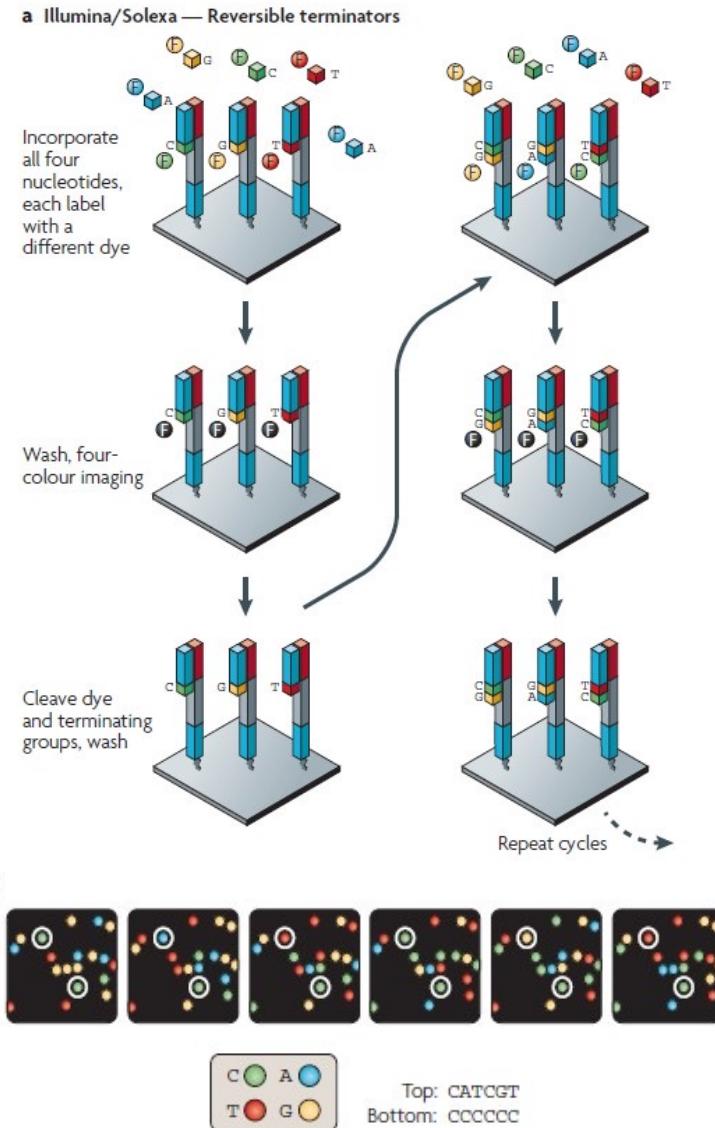
Sequencing by synthesis (SBS)



Sequencing by synthesis (SBS)

- clonal amplification onto surface of glass flowcell by bridge amplification
- reversible termination sequencing allows incorporation of one base at a time, optical detection, read length up to 300 bp
- Multichannel, all dNTPs present simultaneously.
- Each cluster appears in only 1 of 4 channels

<https://www.youtube.com/watch?v=HMyCqWhwB8E>

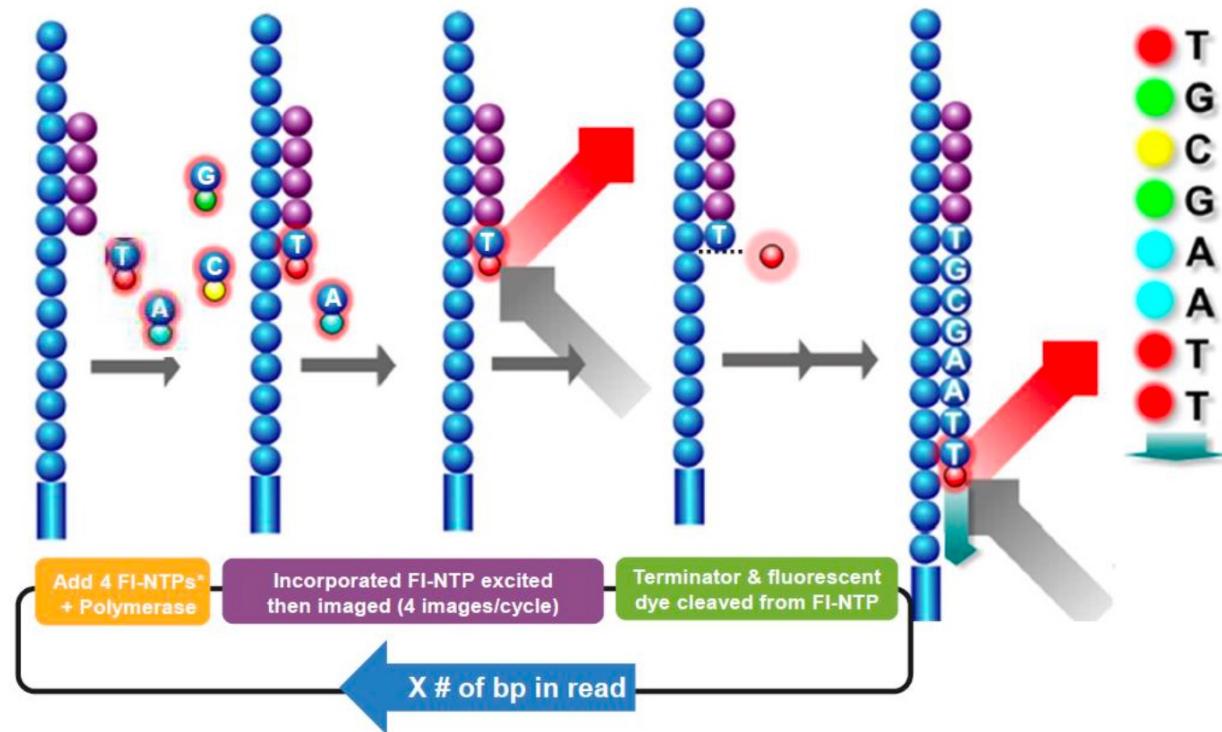


By substituting the 3'-OH group for a 3'-*o*-azidomethyl group

SBS – 4-channel chemistry

4- Colour Chemistry:

4 images are taken using 4 channels (Red, Blue, Green and Yellow) per cycle. Each cluster appears in only 1 of 4 channels.



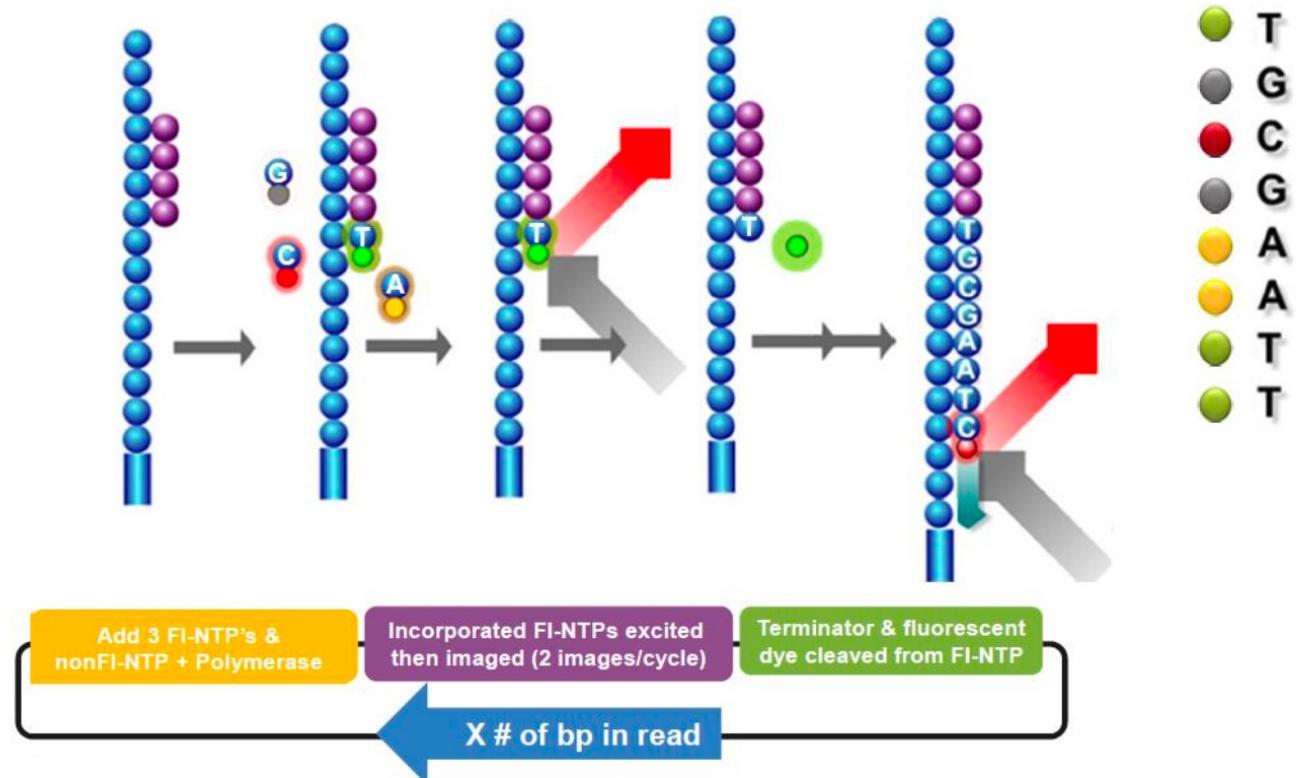
SBS – 2-channel chemistry

2 - Colour Chemistry:

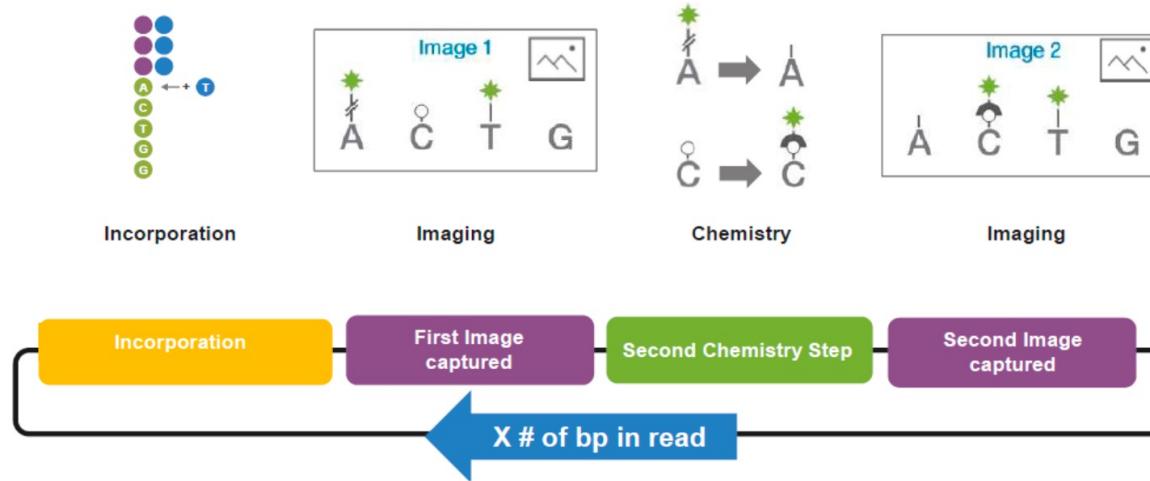
2 images are taken using 2 channels per cycle.

Clusters with T appear in only green channel,

Clusters with C appear in only red channel, Clusters with A appear in both green and red channels and Clusters with G appear in none.



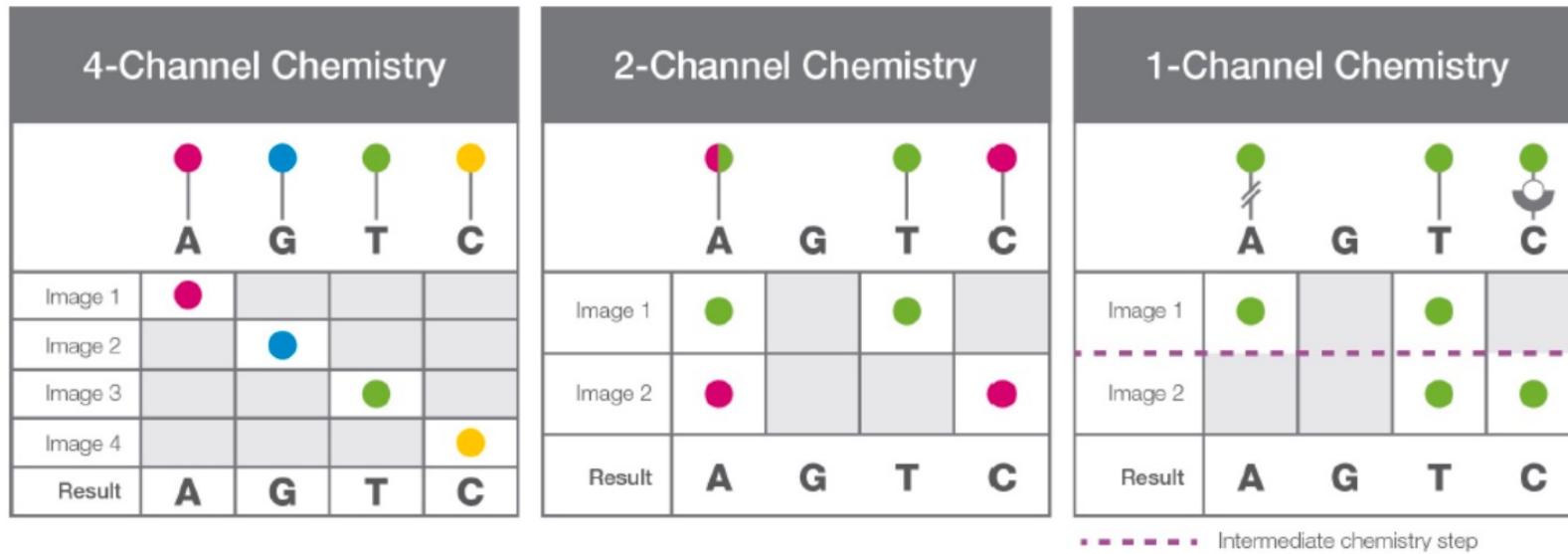
SBS – 1-channel chemistry



1 Colour chemistry:

A binary signal for each base: A (1,0), G (0,0), T (1,1) and C (0,1). This is accomplished by using a removable label on A which is cleaved before the second imaging step generating the 1,0 signal; G is not labeled giving a 0,0 signal, T is permanently labelled so generates 1,1 and C has a linker group that can bind a label and is labeled in the second image only.

SBS – Chemistry comparison



4-channel SBS

- Bases are identified using four different fluorescent dyes, one for each base and four images per sequencing cycle

2-channel SBS

- Simplified nucleotide detection by using two fluorescent dyes and two images to determine all four base calls

1-channel SBS

- Base calling uses one fluorescent dye and two images, with chemistry step in between, to determine all four base calls

2nd Gen sequencing technology errors

- Because they rely on amplification, there may be bias and any errors will accumulate (amplify).
- Pyrosequencing (454) errors predominantly INDELS, particularly in homopolymers (*e.g.* AAAAA...), as it relies on proportional signal (also applies to IonTorrent).
- Illumina use reversibly terminating dNTPs, similar to Sanger. dNTPs and polymerase are modified/ artificial, so errors typically by base substitution.

Third-generation sequencing

Third-generation sequencing (also known as long-read sequencing) uses parallel sequencing similar to NGS.

Simultaneous sequencing of millions of single molecules at moderate throughput ~6 million reads of length 30 Kb.

Uses **single DNA molecules** rather than amplified DNA as a template. Thus, third-generation sequencing potentially eliminates errors in DNA sequence introduced in the laboratory during the DNA amplification process.

Under development and generally are not clinically available. Approaching the accuracy of short-read technology which is considered the clinical NGS gold standard..

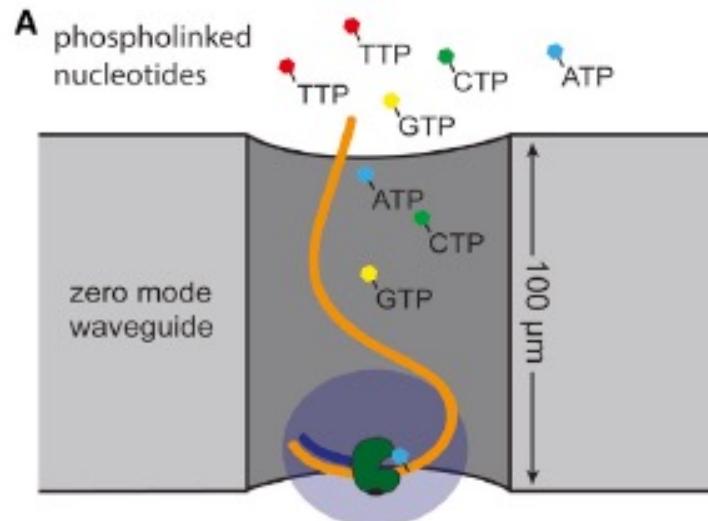
Third-generation sequencing

Third-generation sequencing allows for sequencing some of the most challenging areas of the genome (challenging due to the high percentage of guanine and cytosine bases, referred to as "GC content") and for providing better analysis of structural variation in the genome .

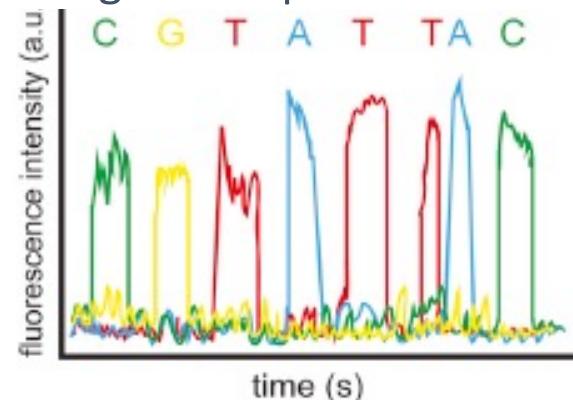
Permits de-novo assembly, true whole genome sequencing with no gaps.

3rd Gen sequencing (single-molecule)

Pacific Bioscience

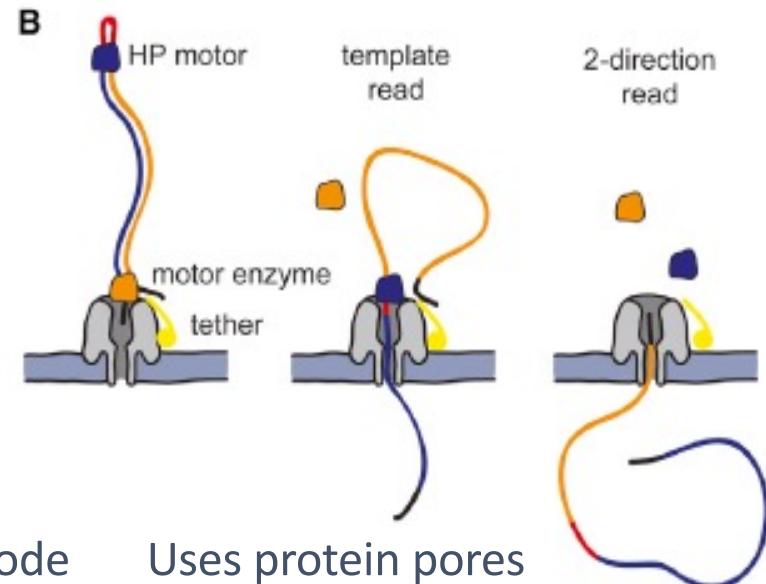


8 million or 25 million tiny wells called Zero Mode Waveguides. 1 polymerase bound library per well.
Fluorescent signal is captured in a movie

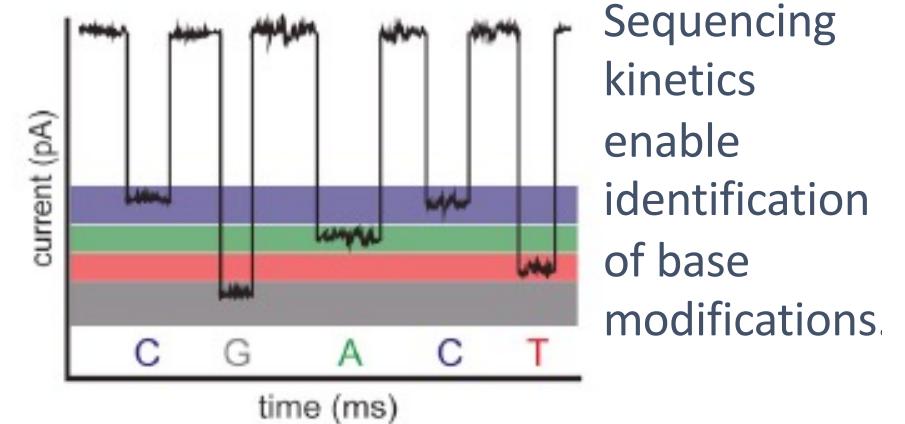


<https://youtu.be/NHCJ8PtYCFc>

Oxford Nanopore



Uses protein pores



Sequencing kinetics enable identification of base modifications.

<https://youtu.be/RcP85JHLmnl>

NGS principles

Next-generation shotgun sequencing approaches require sequencing every base in a sample several times for two reasons:

- You need multiple observations per base to come to a reliable base call.
- Reads are not distributed evenly over an entire genome, simply because the reads will sample the genome in a random and independent manner.

NGS principles

Two important terms are ‘coverage’ and ‘depth’:

- Depth: “The average number of times that a particular nucleotide is represented in a collection of random raw sequences.” This is what you will read as *e.g.* 30x read depth/depth of coverage

Purpose: Higher sequencing depth increases the confidence in calling a variant at a specific location. It is particularly important when looking for rare variants or when sequencing heterogeneous samples (like tumor tissues).

Measurement: Expressed as an average, *e.g.*, "100x depth." This means that, on average, each nucleotide in the sequenced region was read 100 times.

NGS principles

Two important terms are ‘coverage’ and ‘depth’:

- Coverage: “the breadth of a target genome that is sequenced; the percentage of target bases that are sequenced a given number of times.”
 - Purpose: Ensures that the entirety (or as much as possible) of the target region, whether it's the whole genome, exome, or a specific panel, has been sequenced.
 - Measurement: Typically expressed as a percentage. For example, "95% coverage" means that 95% of the intended region has been sequenced at least once.

NGS principles

The Lander/Waterman equation is a method for computing depth.

The general equation is:

$$D = LN / G$$

D stands for depth

G is the haploid genome length

L is the read length

N is the number of reads (~280 mill for HiSeq4000)

So, if we take one lane of single read human sequence with v3 chemistry, we get

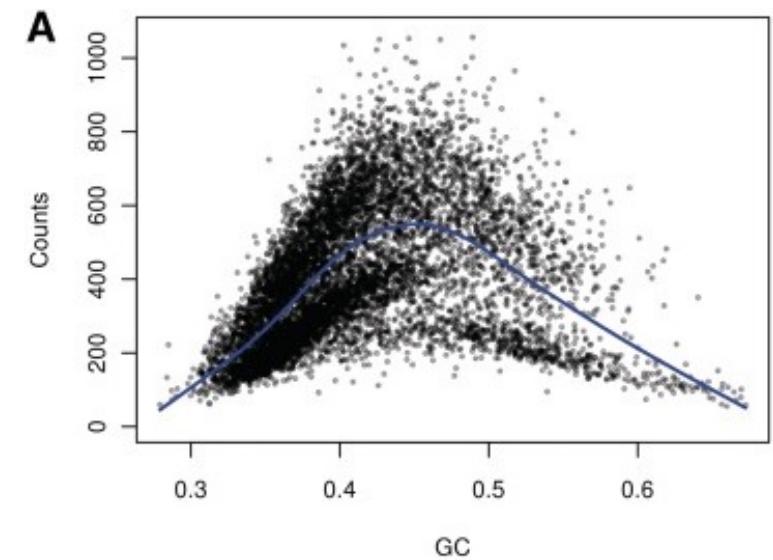
$$D = (100 \text{ bp}) * (280 \times 10^6) / (3.2 \times 10^9 \text{ bp}) = 8.75$$

This tells us that each base in the genome will be sequenced between 8 and 9 times on average.

NGS principles

- Why is PCR an issue for 2nd Gen sequencing strategies?
- GC bias describes the dependence between fragment count (depth) and GC content found.
 - $G \equiv C$, whereas $A = T$, although H-bonding doesn't fully explain the bias.
 - Bias can be introduced at each step, not just PCR.
 - Several innovations, unique library preps, data analysis methods, or amplification-free library preps developed to target GC-bias.

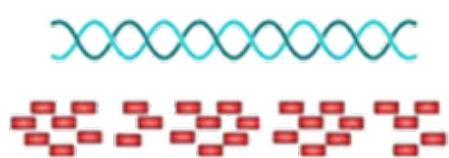
<https://doi.org/10.1186/gb-2011-12-2-r18>



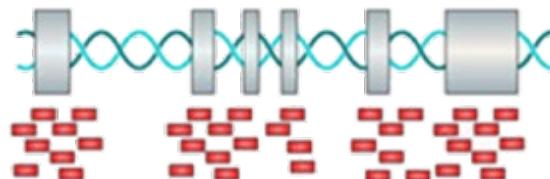
<http://dx.doi.org/10.1093/nar/gks001>

Genomic Comparisons

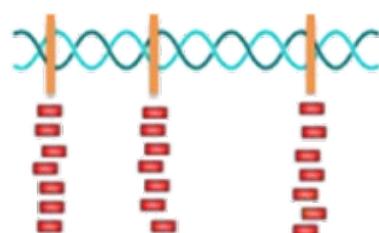
Whole genome sequencing



Whole exome sequencing



Targeted sequencing

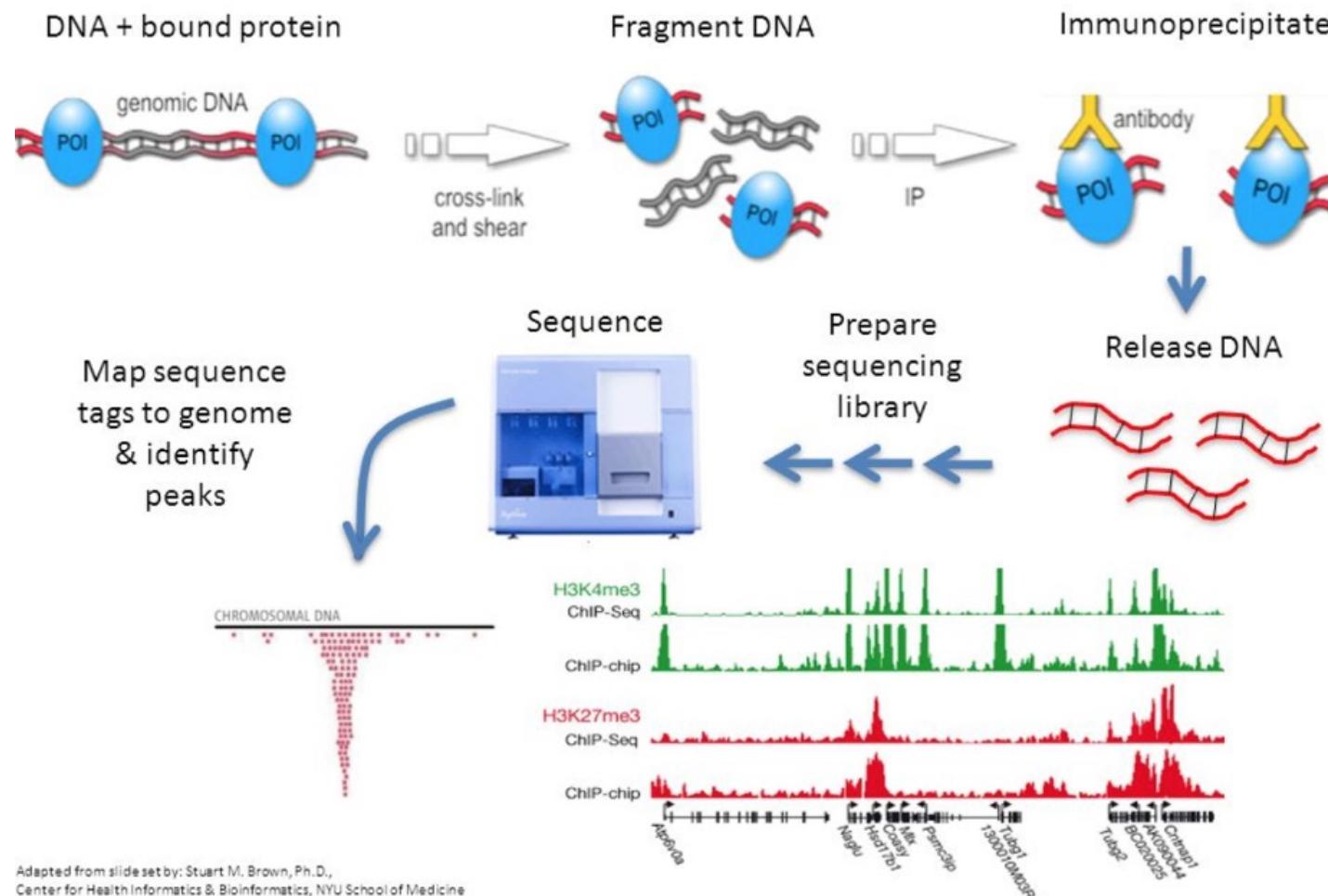


- Sequencing region : whole genome
- Sequencing Depth: >30X
- Covers everything – can identify all kinds of variants including SNPs, INDELs and SV.

- Sequencing region: whole exome
- Sequencing Depth : >50X ~ 100X
- Identify all kinds of variants including SNPs, INDELs and SV in coding region.
- Cost effective

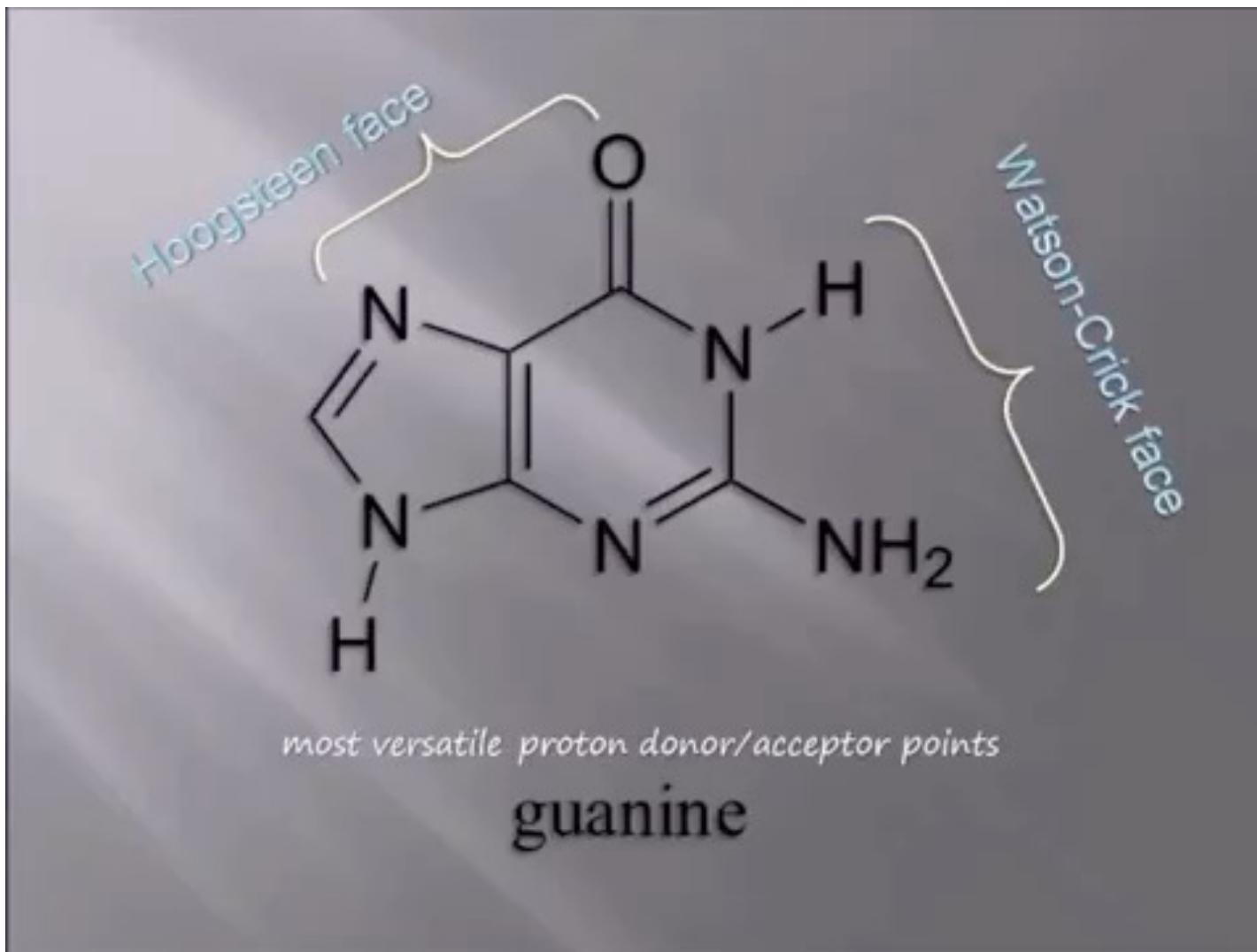
- Sequencing region: specific regions (could be customized)
- Sequencing Depth : >500X
- Identify all kinds of variants including SNPs, INDELs in specific regions
- Most Cost effective

Chromatin Immunoprecipitation (ChIP-seq)

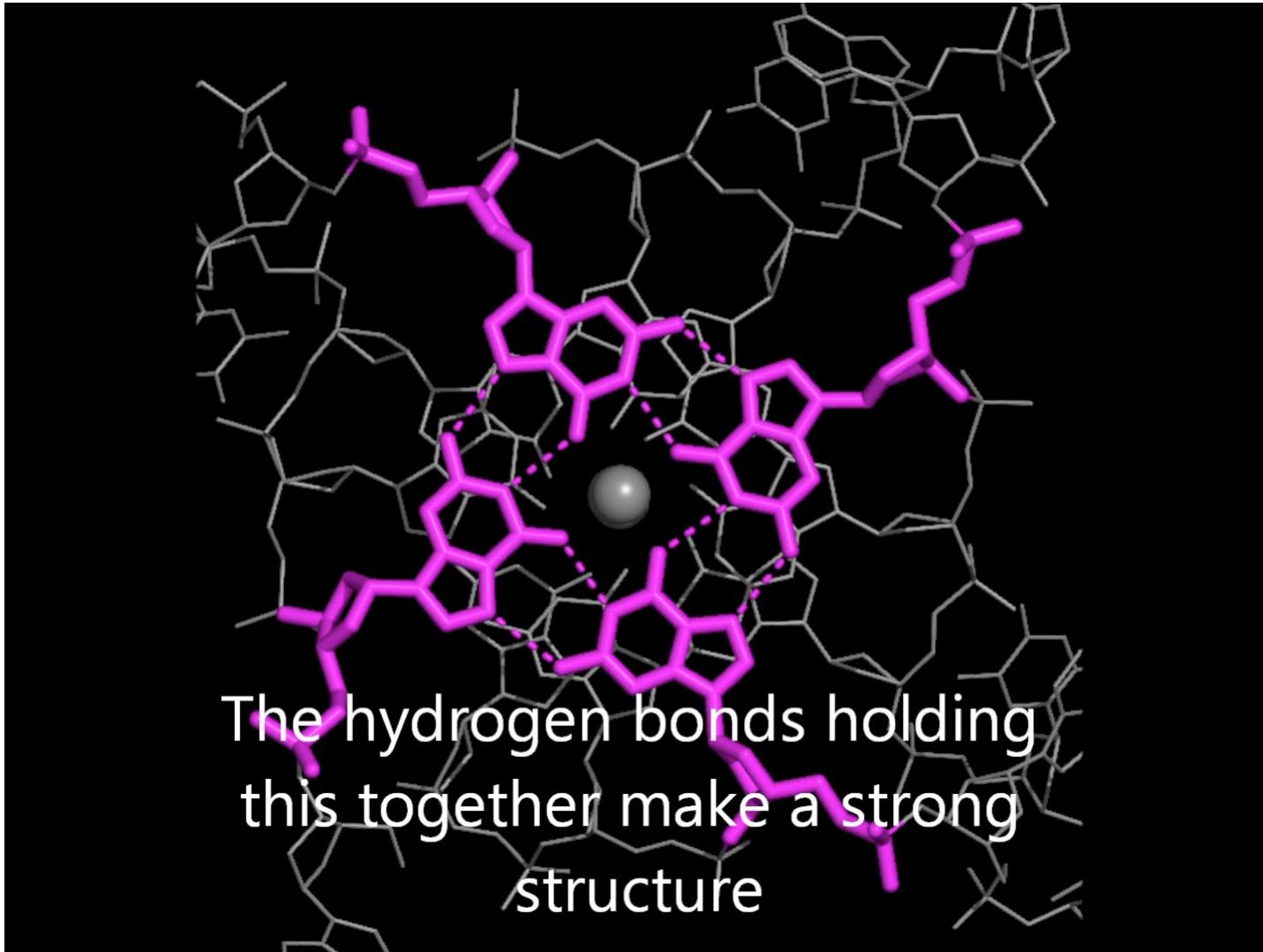


- Commonly used to identify protein binding sites in DNA
- It can also be used to identify specific DNA structures

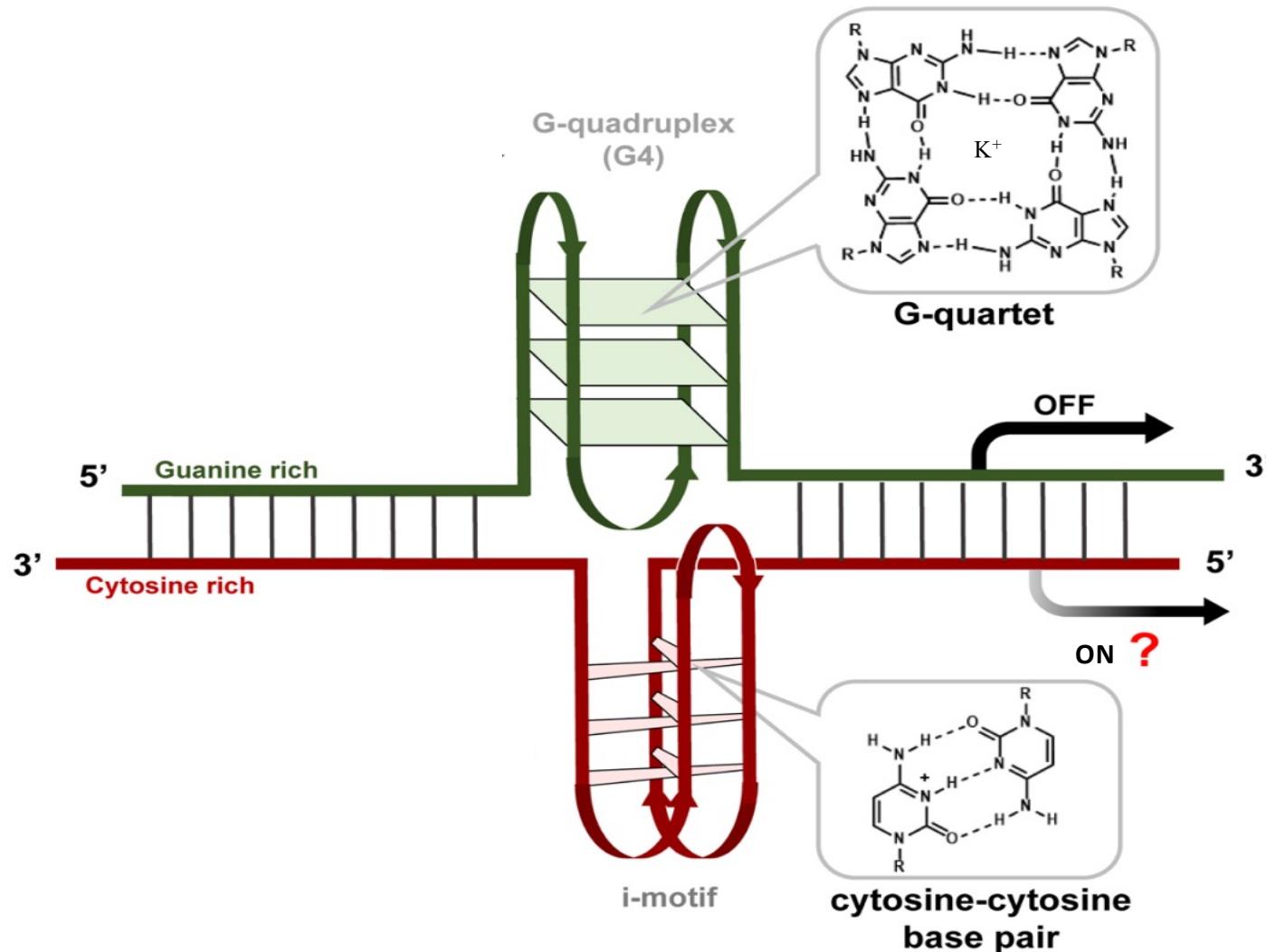
G-Quadruplex (G4-DNA): An alternative DNA structure



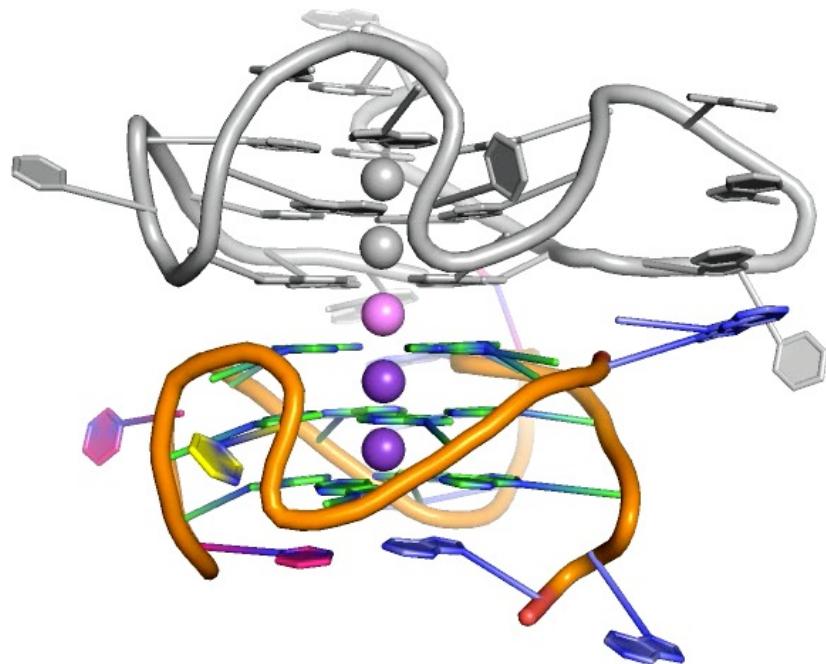
Human telomeric G4-DNA



Non-canonical DNA/RNA Structures



KRAS promoter G4 crystal structure



Head-to-head dimer

Potassium ion at the interface

Poly-A stacking

3' Capped by T10•A22

G4s in the human genome

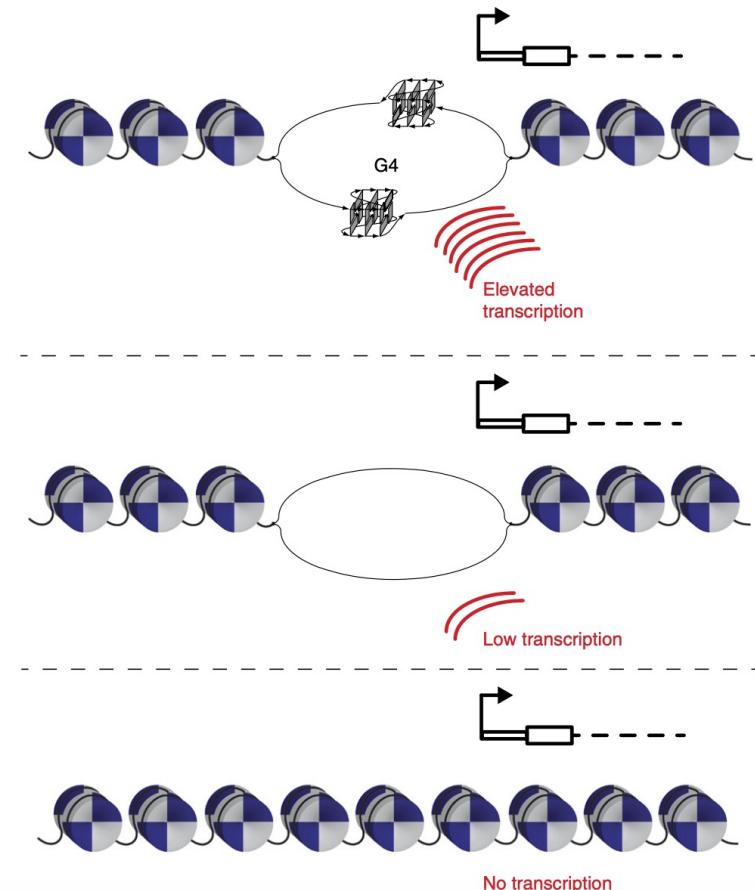
In the human genome, ~700,000 G4s (IP from naked genomic DNA)

Enriched in gene regulatory regions such as promoters region (~1 kb upstream of TSS), 5' and 3' UTRs

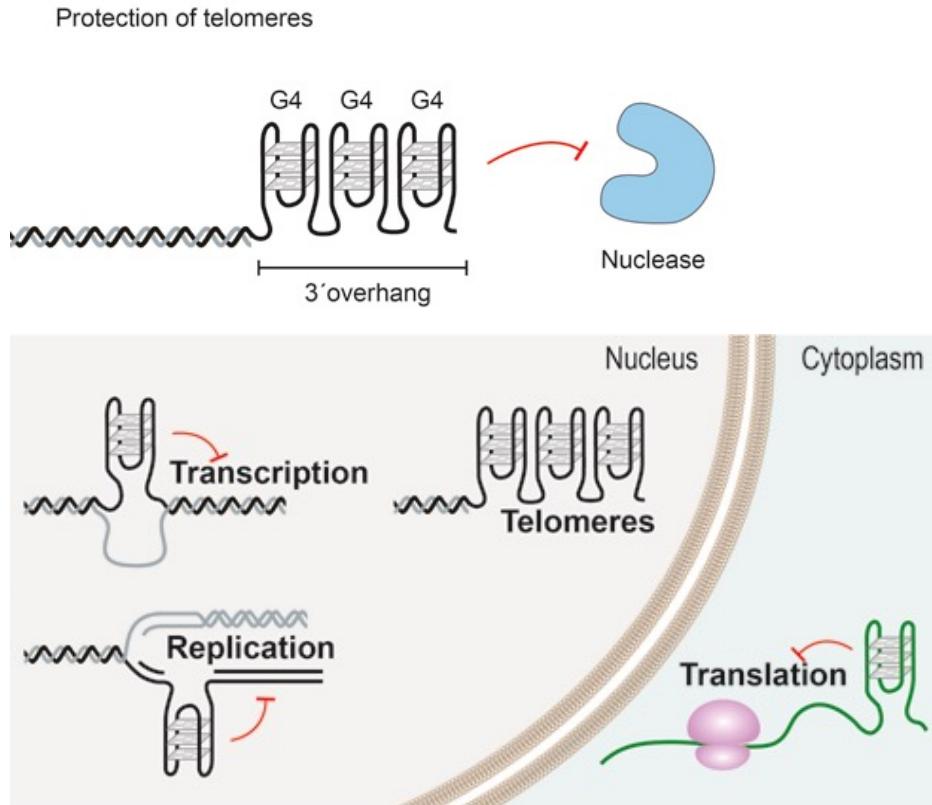
10,000 G4s found in regions of open chromatin (G4 ChIP-seq)

Oncogenes were found to be highly transcribed when a G4 coincides in a region of open chromatin (promoter)

G4s can also act as silencer elements



G4s implications in biology

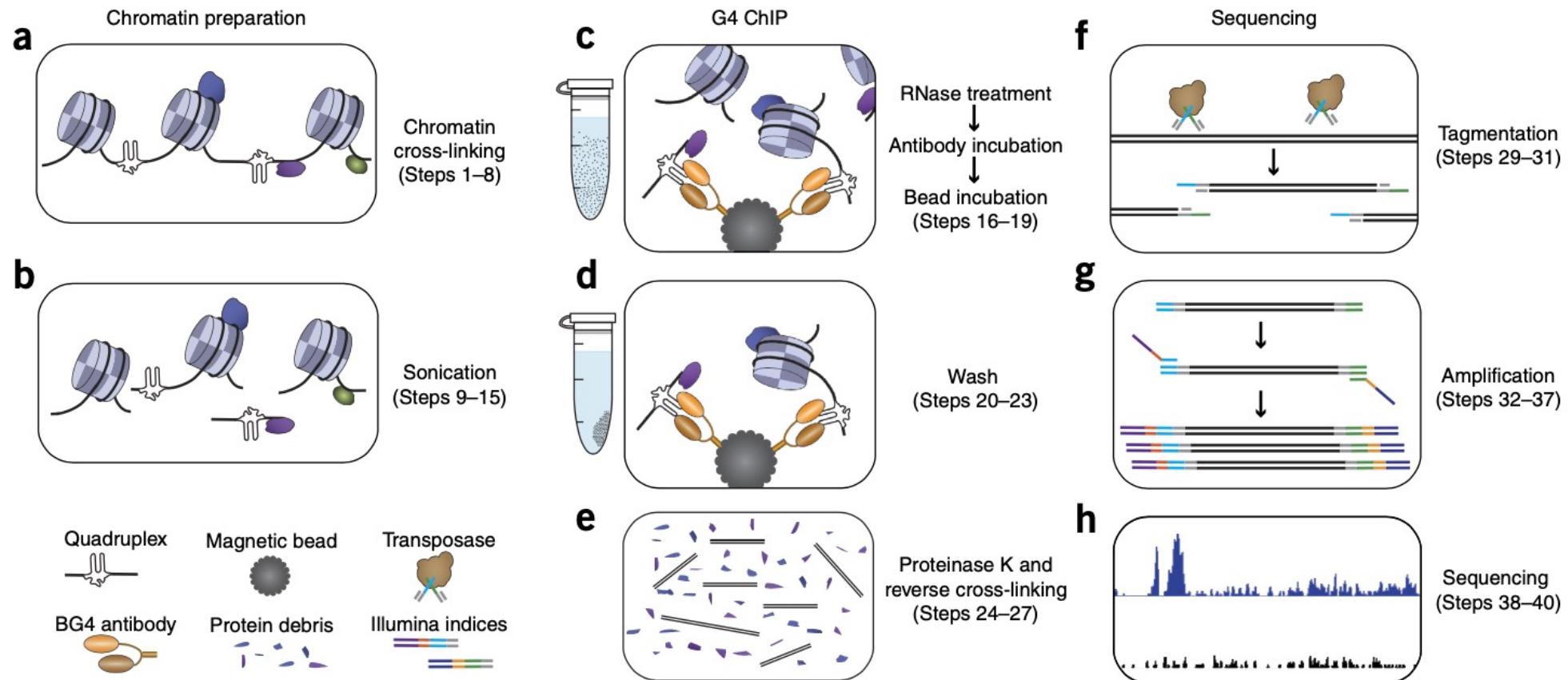


G4s have been shown to form in the 3' overhang at the end of telomeres
 $(TTAGGG)_n$

The formation of G4s can regulate telomere lengthening

Formation of G4s can also stall cellular machinery involved in transcription, regulation and translation

G4 ChIP-seq – Mapped G4s in the human Genome



Unit Schedule

	GENE4001 Schedule 2025 Semester 1					
	Date:	TOPIC:	Lecturer	Date, time	Venue	
Sem Week (year week)						
2 (10)	Monday 3rd March AM	NO WORKSHOP - PUBLIC HOLIDAY				
3 (11)	Monday 10th March AM	Epigenomics principles		Jessica Kretzmann	Mon 10th March, 11am-1pm	Myer: [206]
3 (11)	Monday 10th March PM	Genomics principles		Nicole Smith	Mon 10th March, 2-4pm	Laws: [G31]
4 (12)	Wednesday 19th March AM	Genome editing principles		Jessica Kretzmann	Wednesday 19th March, 9-11am	SSEH: [102]
4 (12)	Wednesday 19th March PM	Transcriptomics principles		Nicole Smith	Wednesday 19th March, 11am-1pm	SSEH: [102]
5 (13)	Wednesday 26th March AM	Genomics Applications		Nicole Smith	Wed 26th March 11am-1pm	ZOOL: [G10]
5 (13)	Wednesday 26th March PM	Epigenomics Applications		Jessica Kretzmann	Wednesday 26th March 2-4pm	ZOOL: [G10]
6 (14)	Wednesday 2nd April PM	Transcriptomics applications		Nicole Smith	Wednesday 2nd April 2-4 pm	ZOOL: [G10]
7 (15)	Wednesday 9th April PM	Genome editing applications		Jessica Kretzmann	Wednesday 9th April 2-4pm	Bayliss [G33]
8 (16)	Tuesday 15th April AM	STUDENT LECTURE PRESENTATIONS (COMPULSORY ASSESSMENT, STUDENTS WILL BE ASSIGNED TO SESSIONS AND NOTIFIED VIA LMS)			Tuesday 15th April AM 11am-1pm	Bayliss [2.15]
8 (16)	Tuesday 15th April PM				Tuesday 15th April PM 2-4pm	
8 (16)	Wednesday 16th April AM				Wednesday 16th April AM 11am-1pm	
8 (16)	Wednesday 16th April PM				Wednesday 16th April PM 2-4pm	
11 (19)	Wednesday 7th May AM	Examination			Wednesday 7th May 10am-12pm	CSSE: [207]