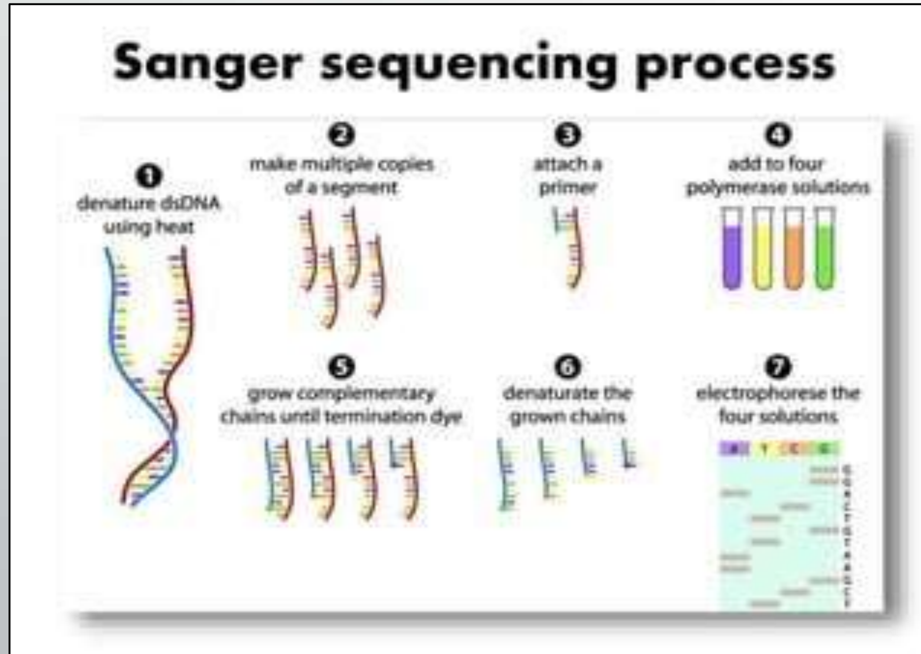
Today we will talk about:

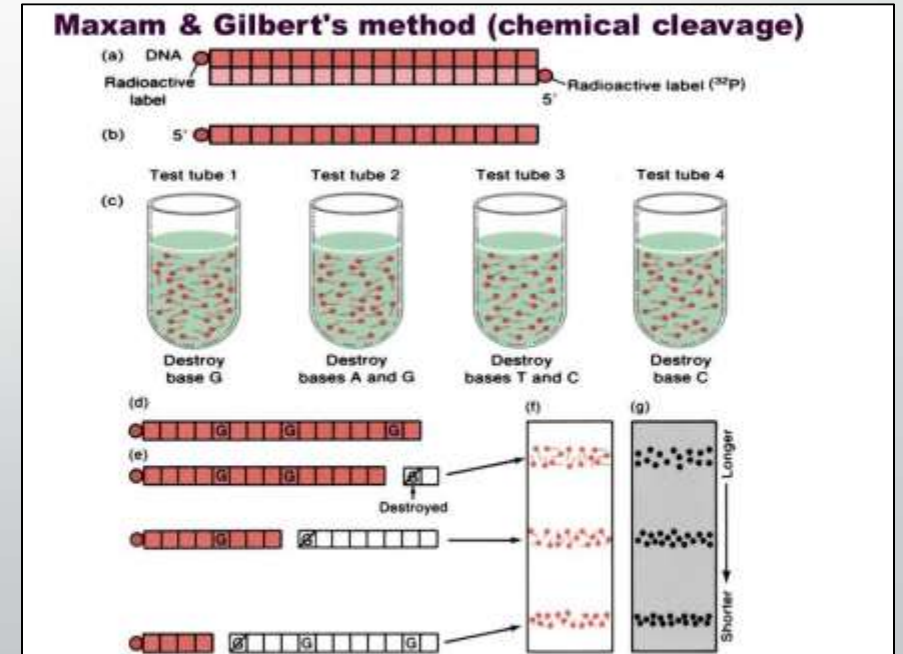
- Difference between First and Next generation sequencing .
- NGS Platforms - Roche 454, ABI SOLID, Ion torrent, Illumina .
- Advantages and disadvantages of second generation sequencing .

What is FGS?

First Generation Sequencing method consist of 2 methods:

- Sanger sequencing method (Chain termination)
- Maxam Gilbert method (Chemical degradation)



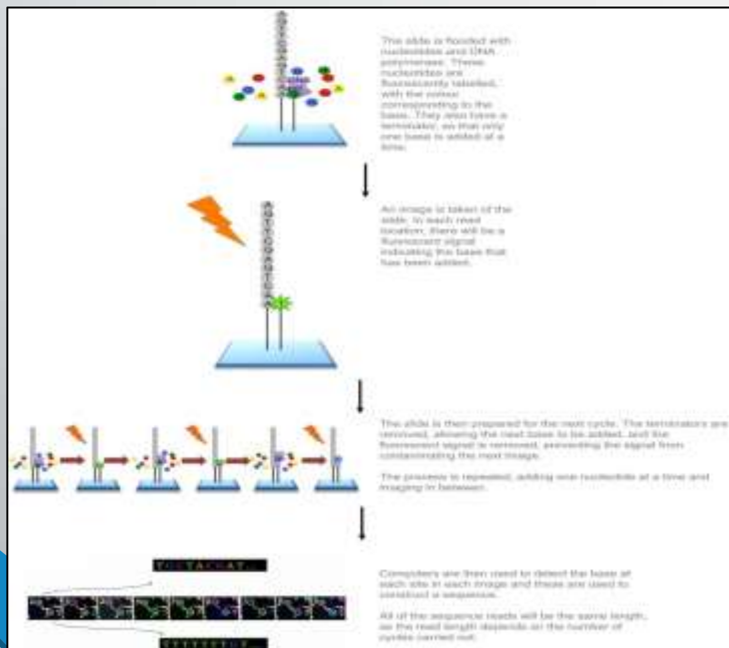


What is NGS?

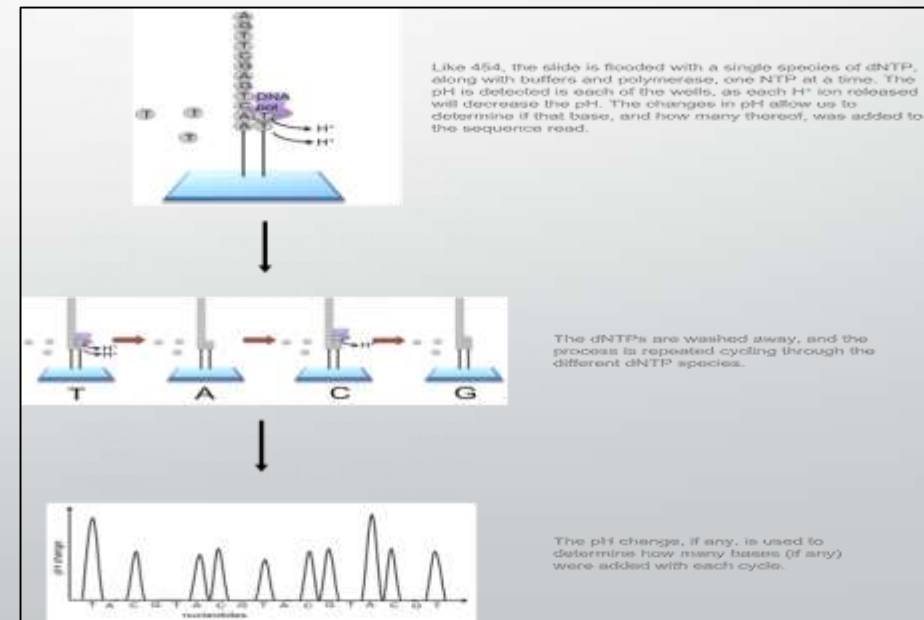
The Next-generation sequencing (NGS) method, also known as the high-throughput sequencing method, was developed in response to the constraints of the first generation sequencing.

It is based on a variety of techniques including:

Illumina (Solexa) sequencing



Ion torrent Sequencing



First Generation Sequencing Vs Next Generation Sequencing

Aspect	First Generation Sequencing	Next Generation Sequencing
Method	Sangar Sequencing	Various methods (e.g., Illumina, Ion Torrent, Pacific Biosciences)
Principle	Chain termination using dideoxynucleotides (ddNTPs)	Parallel sequencing of millions of DNA fragments
Read length	Limited (a few hundred bases)	Varies (short to long reads depending on platform)
Instrumentation	Typically requires specialized equipment	Varied, ranging from benchtop to high-throughput instruments
Error rates	Error rates	Varies depending on platform and protocol
Scalability	Limited scalability due to labor-intensive nature	Highly scalable due to parallel processing
Applications	Suitable for sequencing small genomes, targeted sequencing, and Sanger validation	Genome sequencing, transcriptomics, epigenetics, metagenomics, etc.

Next Generation Sequencing

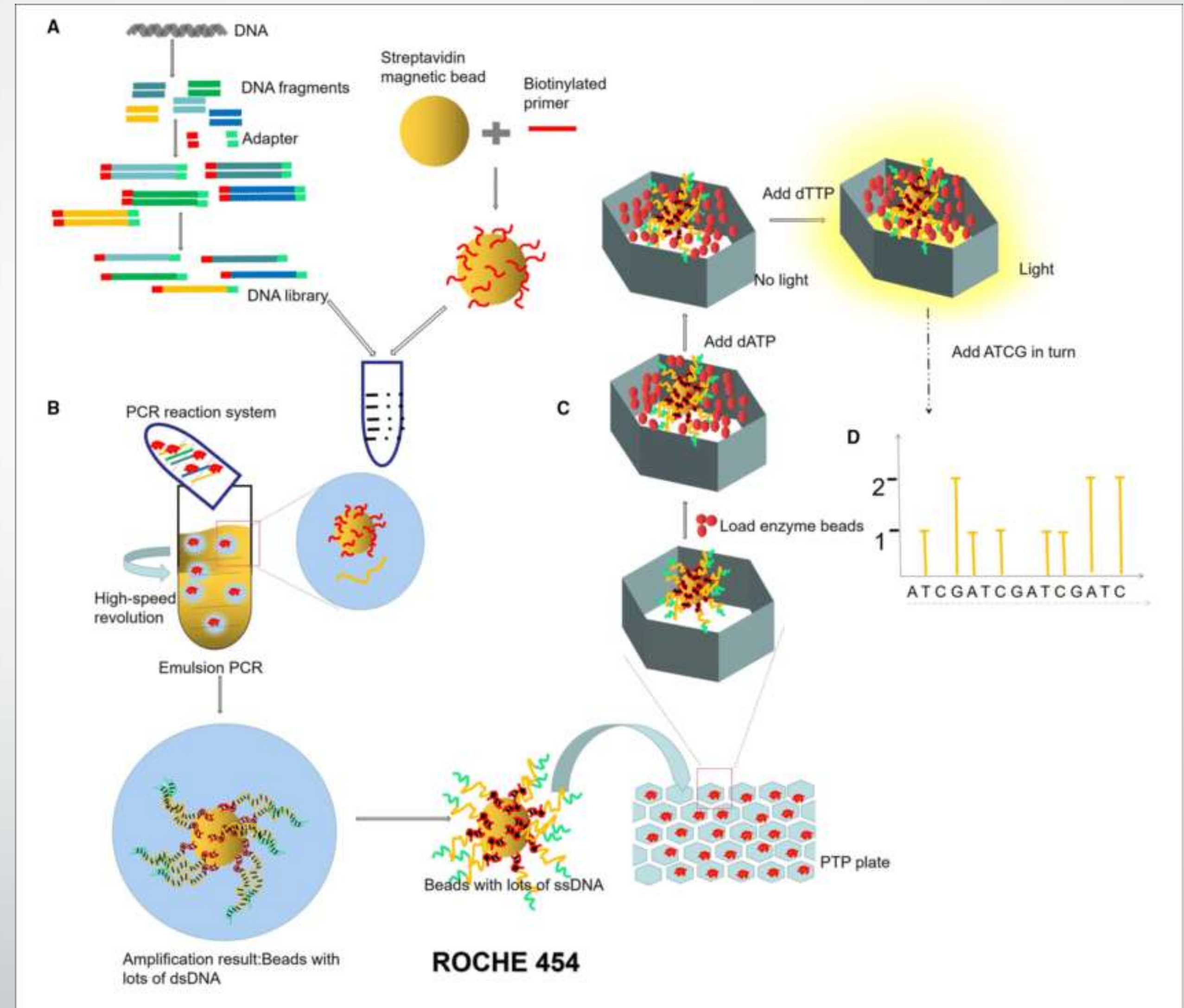
Also known as

- High throughput sequencing
- Ultra –deep sequencing
- Massively parallel sequencing

MAJOR NGS PLATFORMS

454 by Roche (Pyrosequencing)

- 454 sequencing was the first successful next generation sequencing method, developed in 2005 by Roche.
- It was much quicker than the earlier techniques used as part of the Human Genome Project, which took 13 years to sequence 3.2 billion bases.
- Comparatively, 454 sequencing could sequence up to one billion bases in a day.
- However, the platform was not without its weaknesses and it was discontinued in the mid-2010s, in favour of more accurate methods.



What were the benefits and limitations of the 454 method of DNA sequencing?

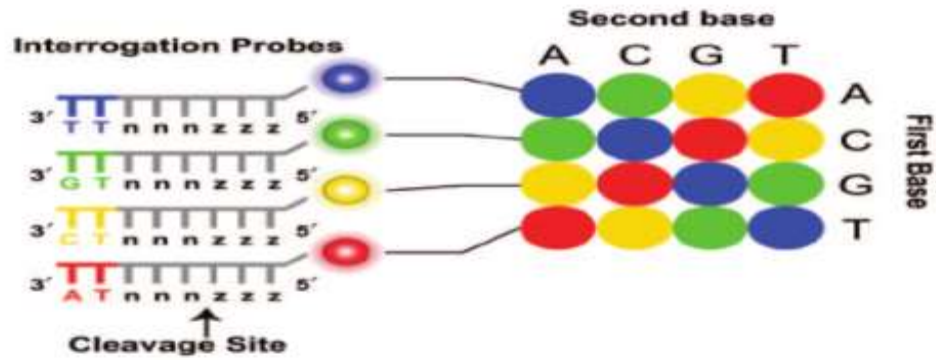
- Compared to other techniques, 454 sequencing was fast and efficient. It made it possible to sequence a large number of samples at the same time.
- However, it wasn't without its limitations, including the high cost of reagents and its relatively high error rate. For example, it had difficulty distinguishing the number of bases in a run of identical bases (such as AAAA).
- 454 sequencing was discontinued in the mid-2010s.

SOLiD (Sequencing by Oligonucleotide Ligation and Detection) by Applied Biosystems

The technology for sequencing used in ABISolid sequencing is oligonucleotide ligation and detection.

In this, a pool of all possible oligonucleotides of fixed length are labelled according to the sequenced position.

This sequencing results in the sequences of quantities and lengths comparable to illumine sequencing.


Color-Space Coding
Second base
A C G T
First Base
A
C
G
T
Interrogation Probes
3′ TTnnnzzz 5′
3′ GTnnnzzz 5′
3′ CTnnnzzz 5′
3′ ATnnnzzz 5′
Cleavage Site

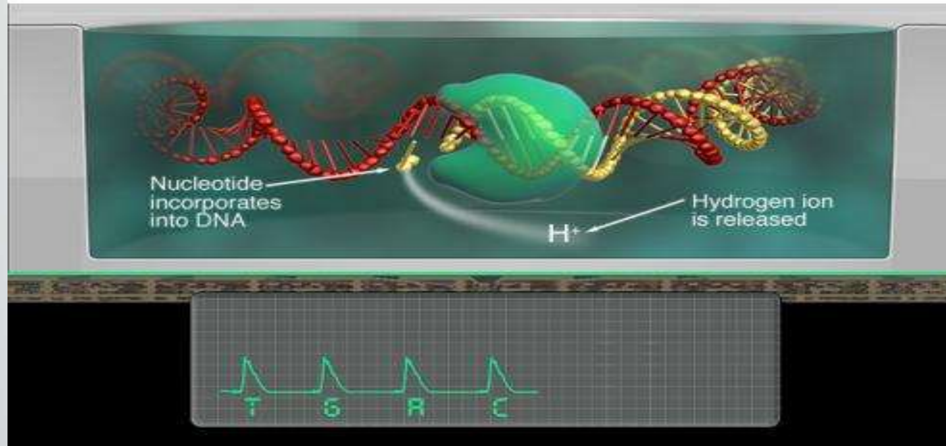
Emulsion Bead
Ligase
A
Primer n
P1 Adapter
TA
Template
B
cleavage
ATnnn
p 5′
C
AT
TTnnnzzz
AA
D
AT TT CT GT TT CA GC
TA AA GA CA AA GT CG
E
Primer n-1
AAnnnGTnnnzzz
TT CA
Second interrogation of base

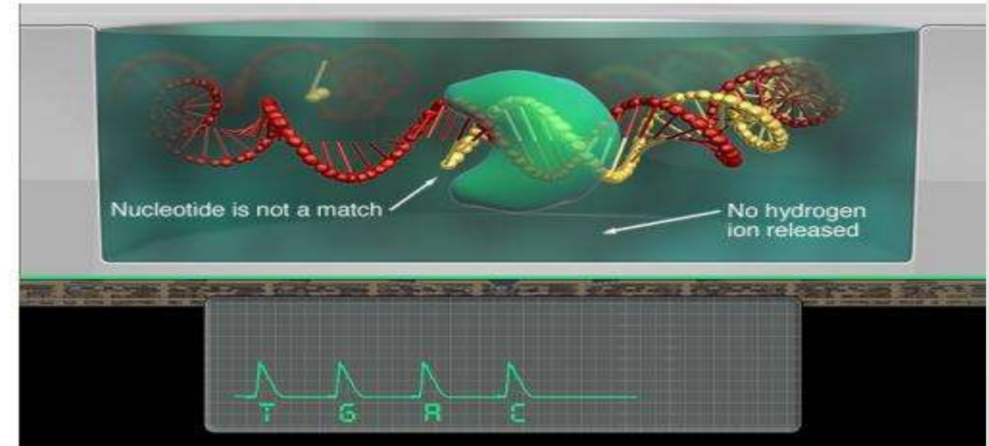
Ion Torrent Sequencing

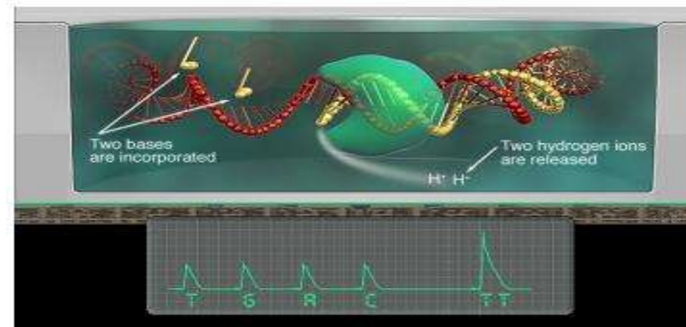
- Life Technologies commercialized the Ion Torrent semiconductor sequencing technology in 2010.
- It is similar to 454 pyrosequencing technology but it does not use fluorescent labelled nucleotides like other second-generation technologies.
- It is based on the detection of the hydrogen ion released during the sequencing process.
- Specifically, Ion Torrent uses a chip that contains a set of micro wells and each has a bead with several identical fragments.

Ion Torrent Sequencing

Flow one nucleotide through at a time
If nucleotide incorporates, then a current pulse is measured
If no match, then no pulse occurs







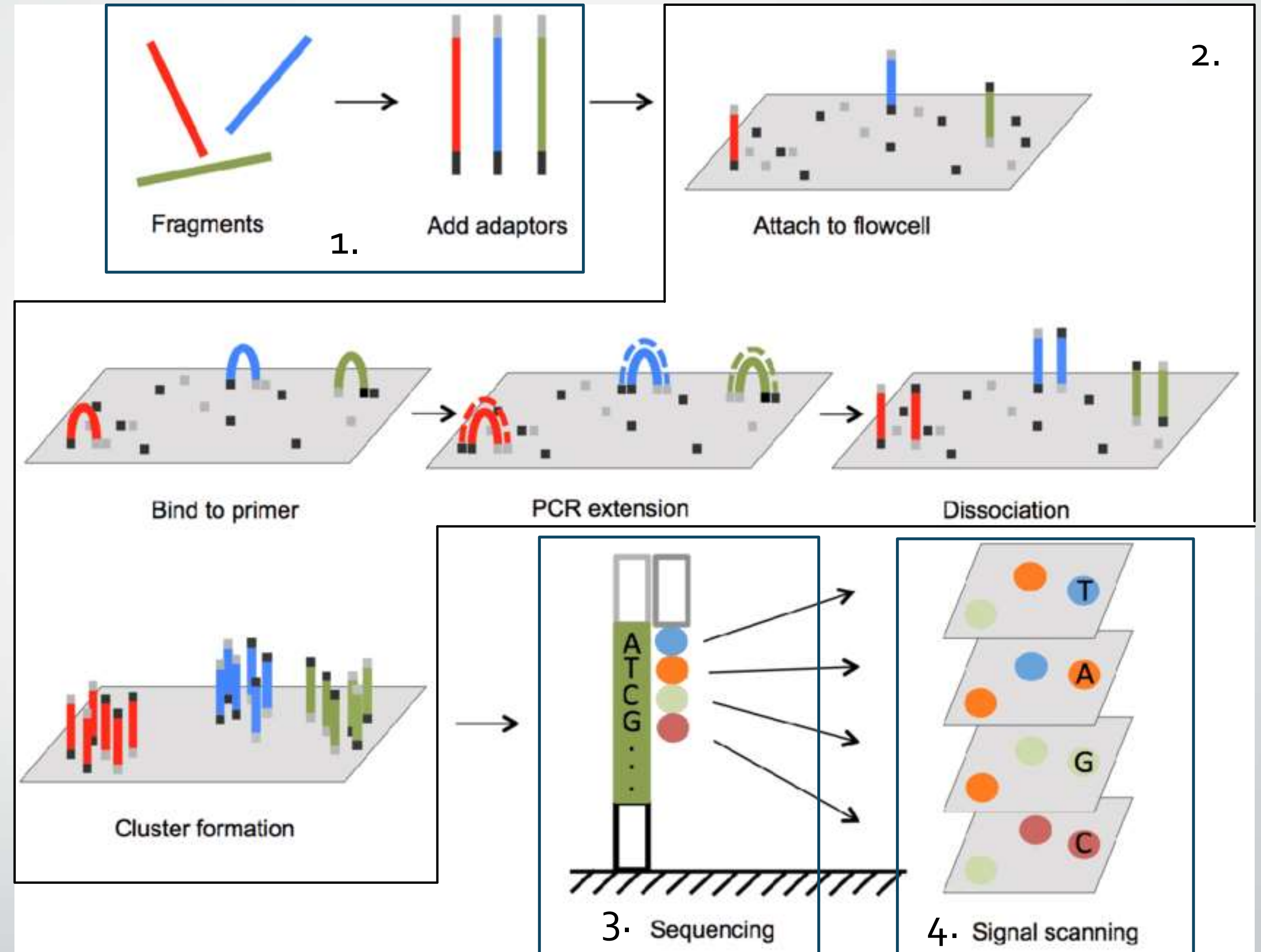
Repeated base pairs give a pulse of 2x greater magnitude

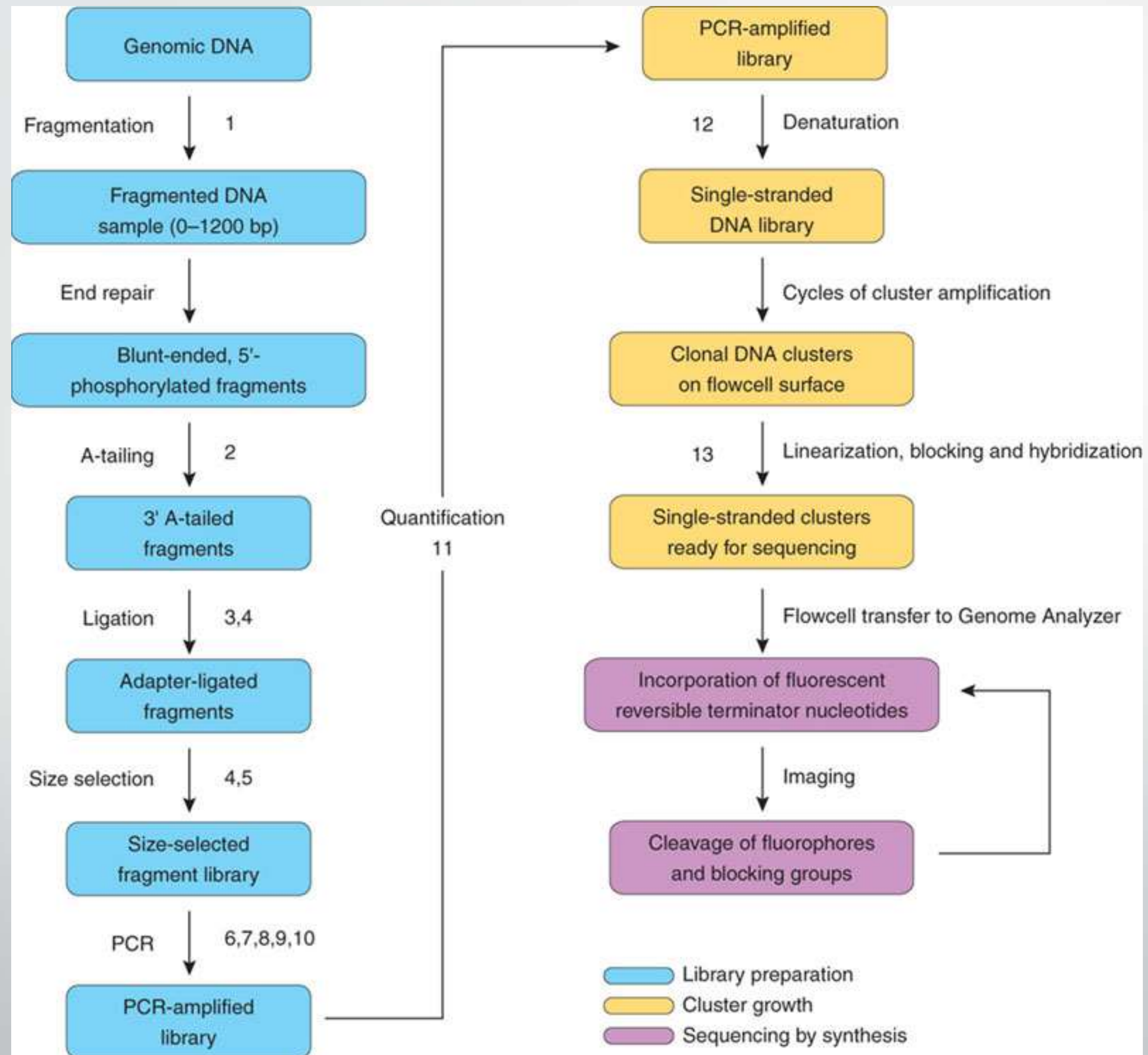
Illumina Next – Generation Sequencing

- Principle of Illumina sequencing: **Sequencing by Synthesis (SBS)**

- Workflow: Key steps involved –

1. **Library preparation =** (DNA fragmentation + ligation with adapters) → Clonally amplified DNA molecules
2. **Cluster generation =** Hybridization of fragments + Bridge amplification + cluster at each sequenced position
3. **Sequencing cycles =** Sequencing buffer introduction + Polymerase Extension + Cleavage and Washing
4. **Data analysis =** Imaging and Base calling




Genomic DNA
Fragmentation
1
Fragmented DNA sample (0–1200 bp)
End repair
Blunt-ended, 5'-phosphorylated fragments
A-tailing
2
3' A-tailed fragments
Ligation
3,4
Adapter-ligated fragments
Size selection
4,5
Size-selected fragment library
PCR
6,7,8,9,10
PCR-amplified library
Quantification
11
PCR-amplified library
12
Denaturation
Single-stranded DNA library
Cycles of cluster amplification
Clonal DNA clusters on flowcell surface
13
Linearization, blocking and hybridization
Single-stranded clusters ready for sequencing
Flowcell transfer to Genome Analyzer
Incorporation of fluorescent reversible terminator nucleotides
Imaging
Cleavage of fluorophores and blocking groups
Library preparation
Cluster growth
Sequencing by synthesis

Applications of Illumina Next – Generation Sequencing

1. Genome – wide analysis: Whole – Genome Sequencing(WGS) + Targeted Resequencing
2. Epigenetics: study DNA methylation patterns + other epigenetic modifications that influence gene expression without altering the DNA sequence itself.
3. Metagenomics: Characterizing microbial communities

Advantages of Next – Generation Sequencing (NGS)

1. High – thoroughput → allows for parallel sequencing of millions of DNA fragments simultaneously
2. Cost – effectiveness → reduced cost per base pair as compared to traditional methods
3. Speed → elimination of time – consuming processes such as gel electrophoresis and radioactive labelling
4. High data resolution → provides detailed information about genetic variations and modifications
5. High accuracy + low error rates

Disadvantages of Next – Generation Sequencing (NGS)

1. Data analysis and Interpretation → require sophisticated bioinformatics tools and expertise
2. Computational cost → expensive for individual labs and smaller institutions
3. Storage requirements → storing massive amount of data requires significant storage capacity

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