DNA Sequencing

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DNA Sequencing

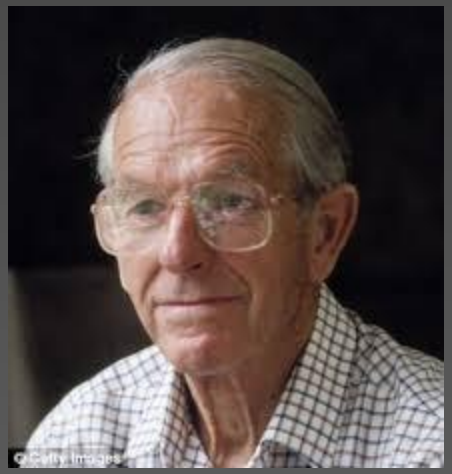
The determination of the precise sequence of nucleotides in a sample of DNA

DNA Sequencing Methods

- Sanger method (The chain termination method)
- Next generation sequencing (NGS).

Sanger sequencing

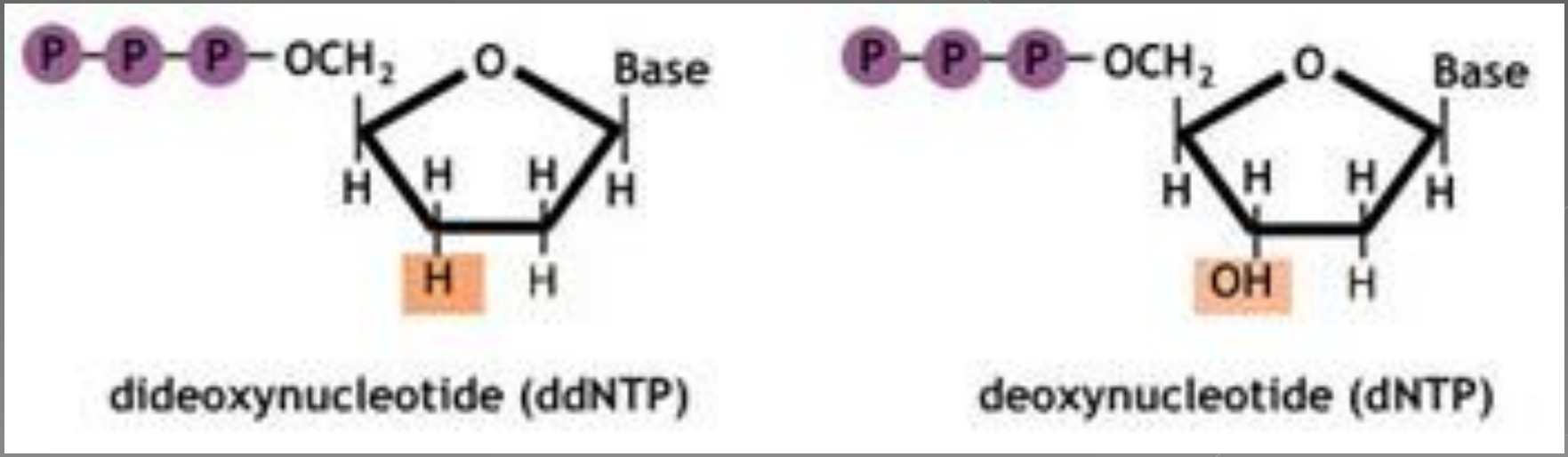
Introduction



- Sanger sequencing was named after its inventor ,***Frederick Sanger***
- who was awarded the 1980 Nobel prize in chemistry for this achievement.

Dideoxynucleotide

- synthetic nucleotides that lack the -OH at the 3′ carbon atom (ddTTP).
- When added to growing DNA strand stops the elongation .



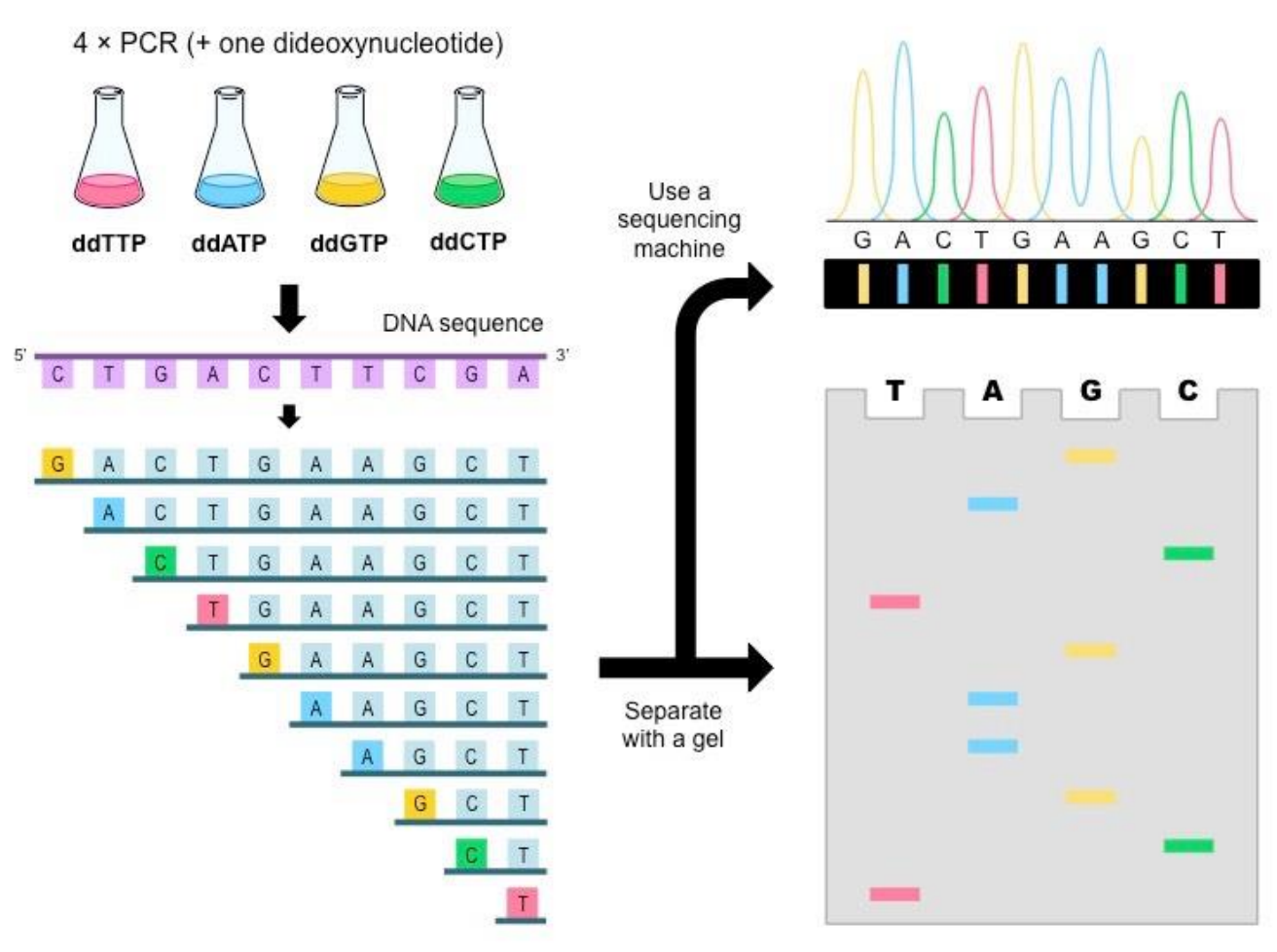
Reaction Requirements

- The template DNA to be sequenced.
- The DNA polymerase enzyme.
- A Primer.
- Deoxynucleotides (dNTPs)= (dATP, dTTP, dCTP, dGTP)
- Dideoxynucleotide (ddTTP) = (ddATP, ddTTP, ddCTP, ddGTP), each labeled with a different color of fluorescent dye.

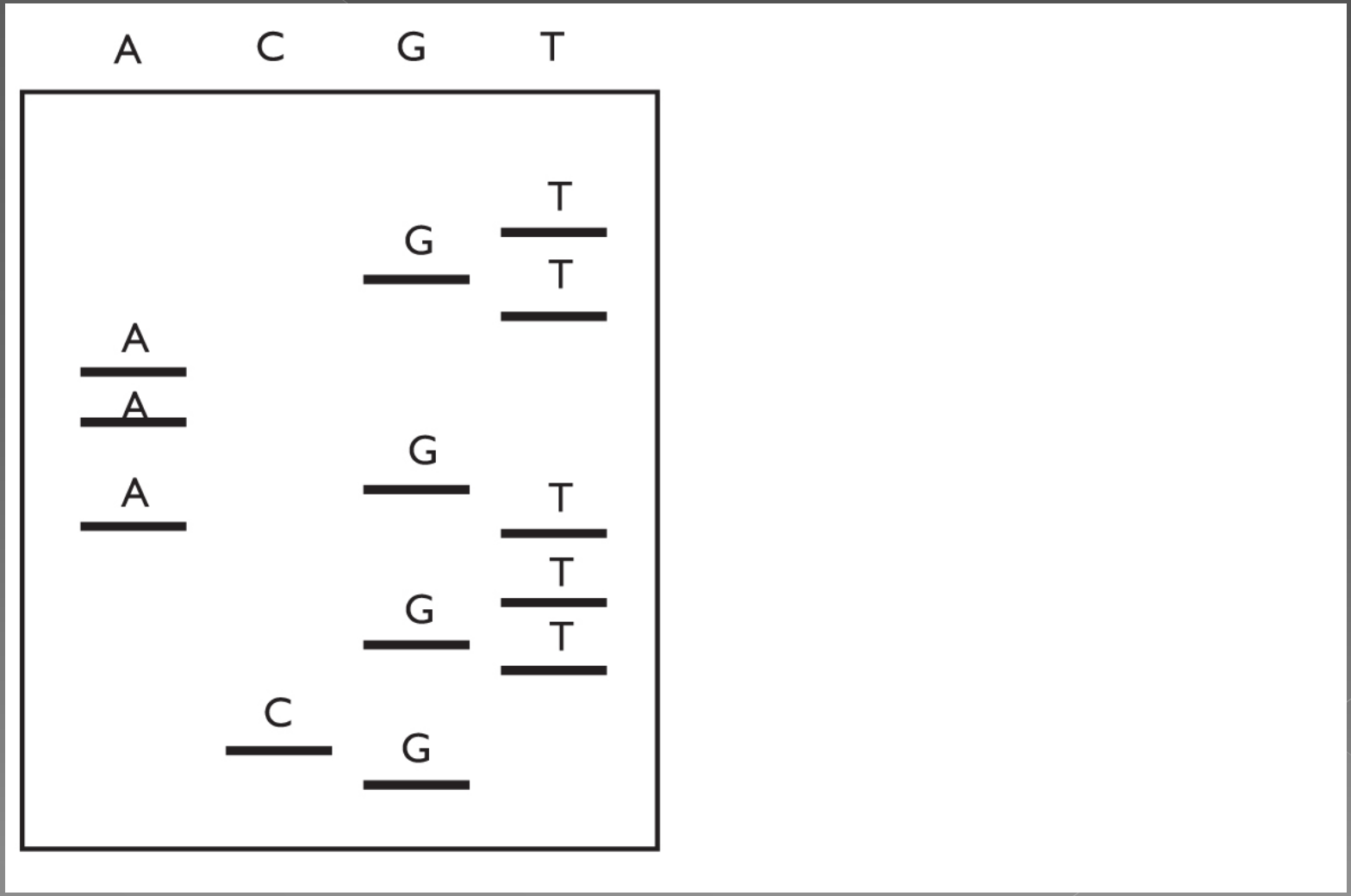
Principle

- Reaction requirements are added into 4 reaction tubes with all dNTPs but only one of ddTTP is used in each of the tubes.
- Heat Denaturing of DNA.
- Annealing with primers.
- DNA polymerase inserts normal nucleotides (dNTPs)
- During several cycles , by chance DNA polymerase adds ddTTP so stop that stand .
- At the end we have a mixture of different size DNA fragments
- Polyacrylamide gel electrophoresis then UV- light vigilization .

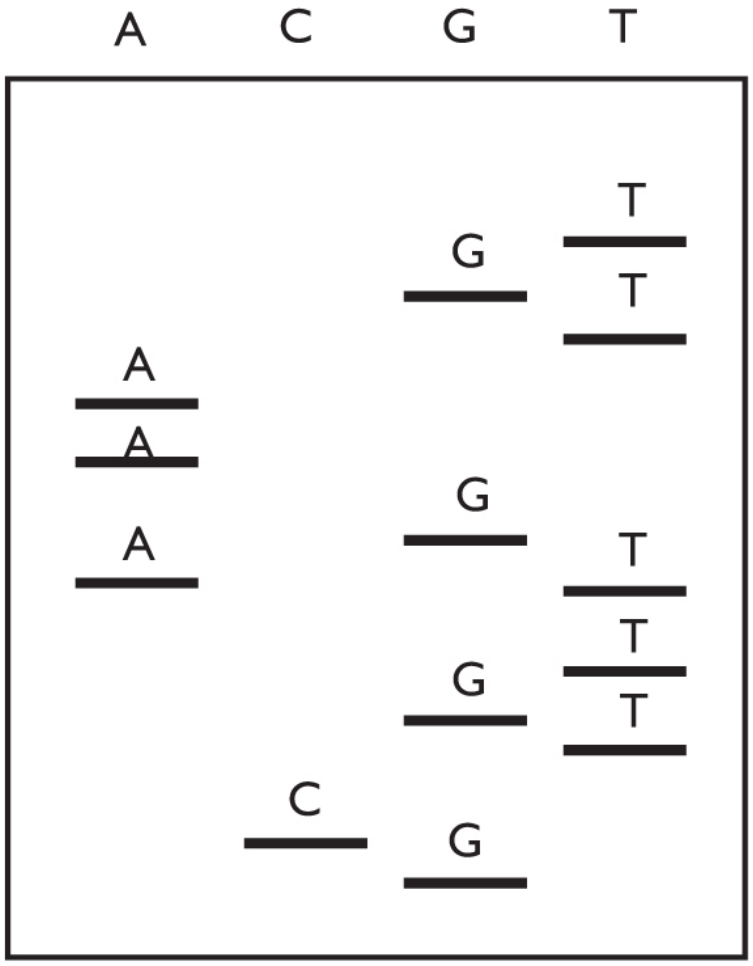
Principle


4 × PCR (+ one dideoxynucleotide)
ddTTP
ddATP
ddGTP
ddCTP
DNA sequence
5'
3'
C T G A C T T C G A
G A C T G A A G C T
A C T G A A G C T
C T G A A G C T
T G A A G C T
G A A G C T
A A G C T
A G C T
G C T
C T
T
Use a sequencing machine
Separate with a gel
G A C T G A A G C T
T A G C

Let's work it out



Right answer

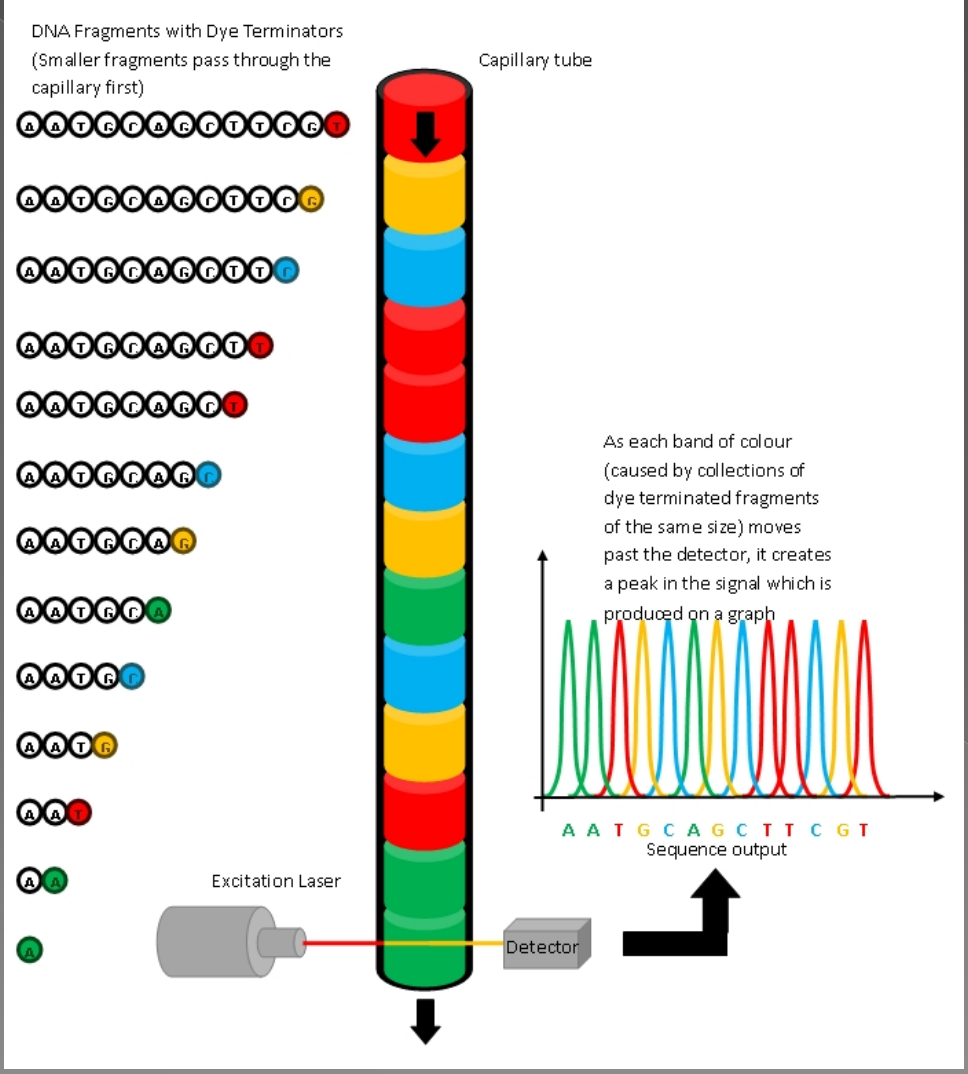


Deduced Sequence	Fragment Size
G	19
C	20
T	21
G	22
T	23
T	24
A	25
G	26
A	27
A	28
T	29
G	30
T	31

Automated sanger sequencing

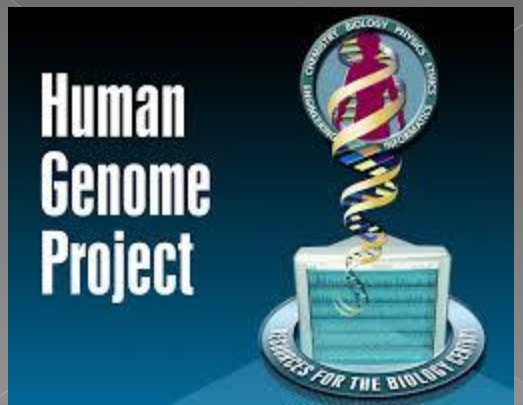
- One reaction tube containing the 4 ddTTP.
- capillary electrophoresis.
- Laser light to detect the fluorescent end of each fragment at the end of the capillary tube .
- Computerized result generation

Automated Sequencing



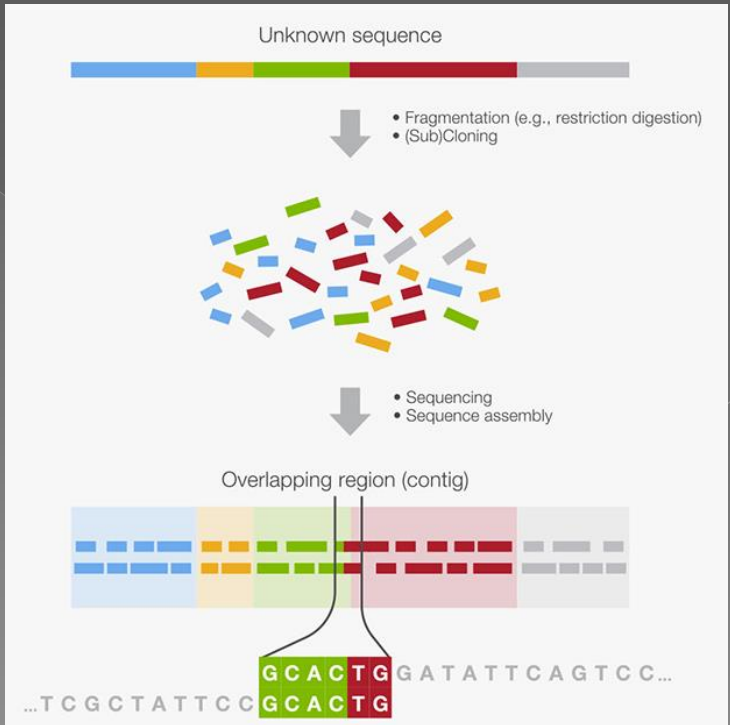
The human genome project

- was an international scientific research project with the goal of :
- determining the sequence of nucleotide base pairs that make up human DNA.
- identifying and mapping all of the genes of the human genome from both a physical and a functional standpoint.
- a 15-year-long project .



The human genome project

- Started in 1990 in US
- The first 'draft' sequence published in 2001
- The first COMPLETE sequence released in 2003, further analysis published in 2011
- shotgun sequencing .



(Thermofisher.com, 2017)

Next generation sequencing

NGS

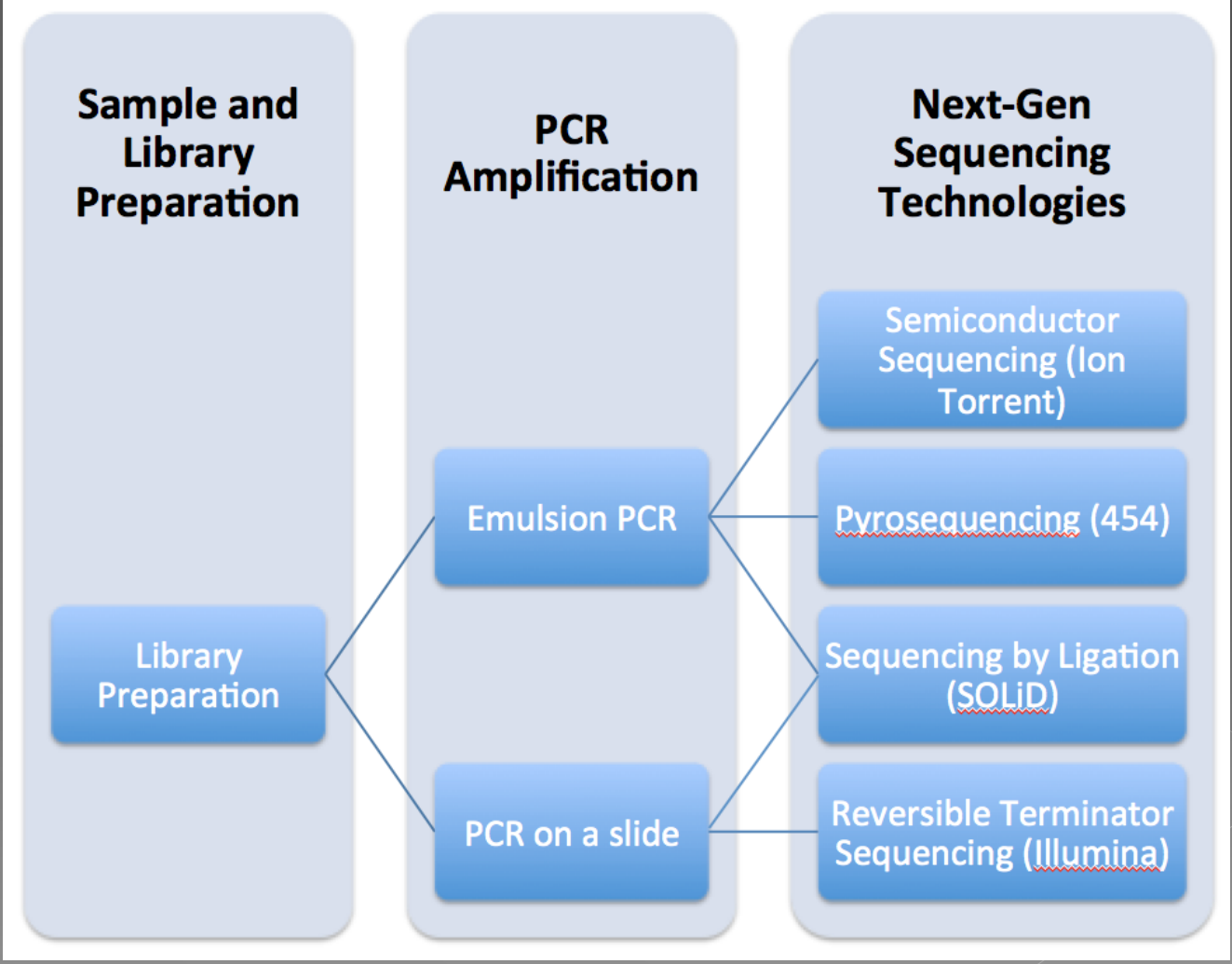
- second generation sequencing .
- massively parallel sequencing.
- Technologies:
  - ✓ IiluminaHiSeq2500
  - ✓ HiSeq2000
  - ✓ Roche 454, SOLiD

- can sequence 5 whole human genomes in 11 days (IiluminaHiSeq2500).

Steps

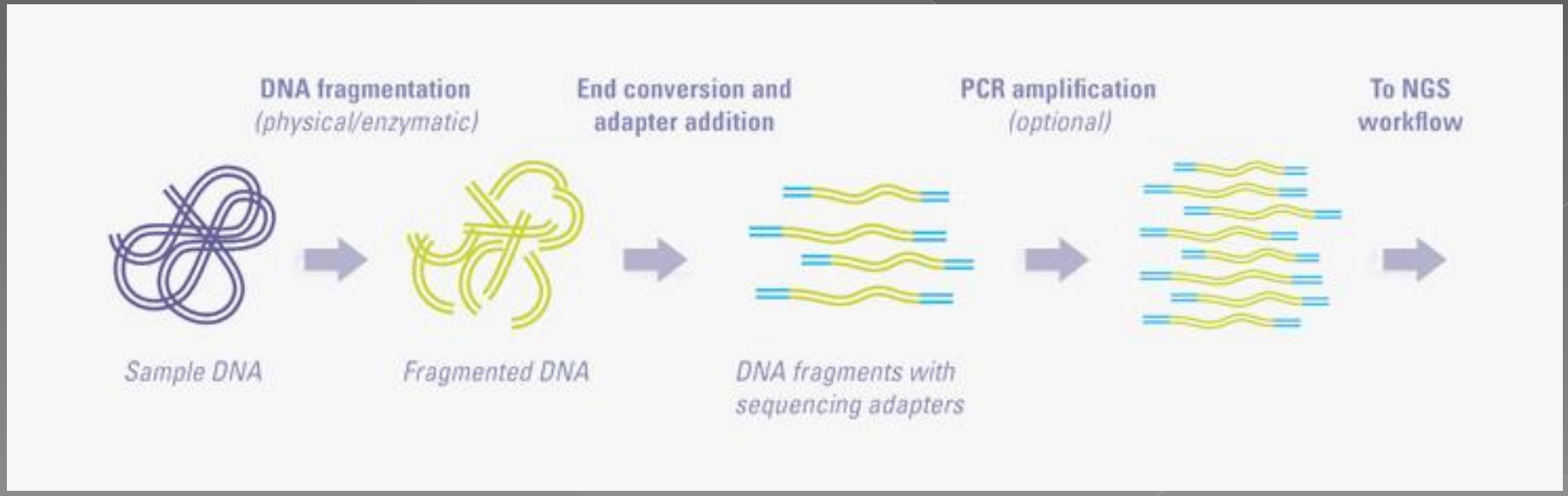
- Library preparation.
- Amplification.
- Sequencing.

Work flow


Sample and Library Preparation
PCR Amplification
Next-Gen Sequencing Technologies
Library Preparation
Emulsion PCR
PCR on a slide
Semiconductor Sequencing (Ion Torrent)
Pyrosequencing (454)
Sequencing by Ligation (SOLiD)
Reversible Terminator Sequencing (Illumina)

Library preparation

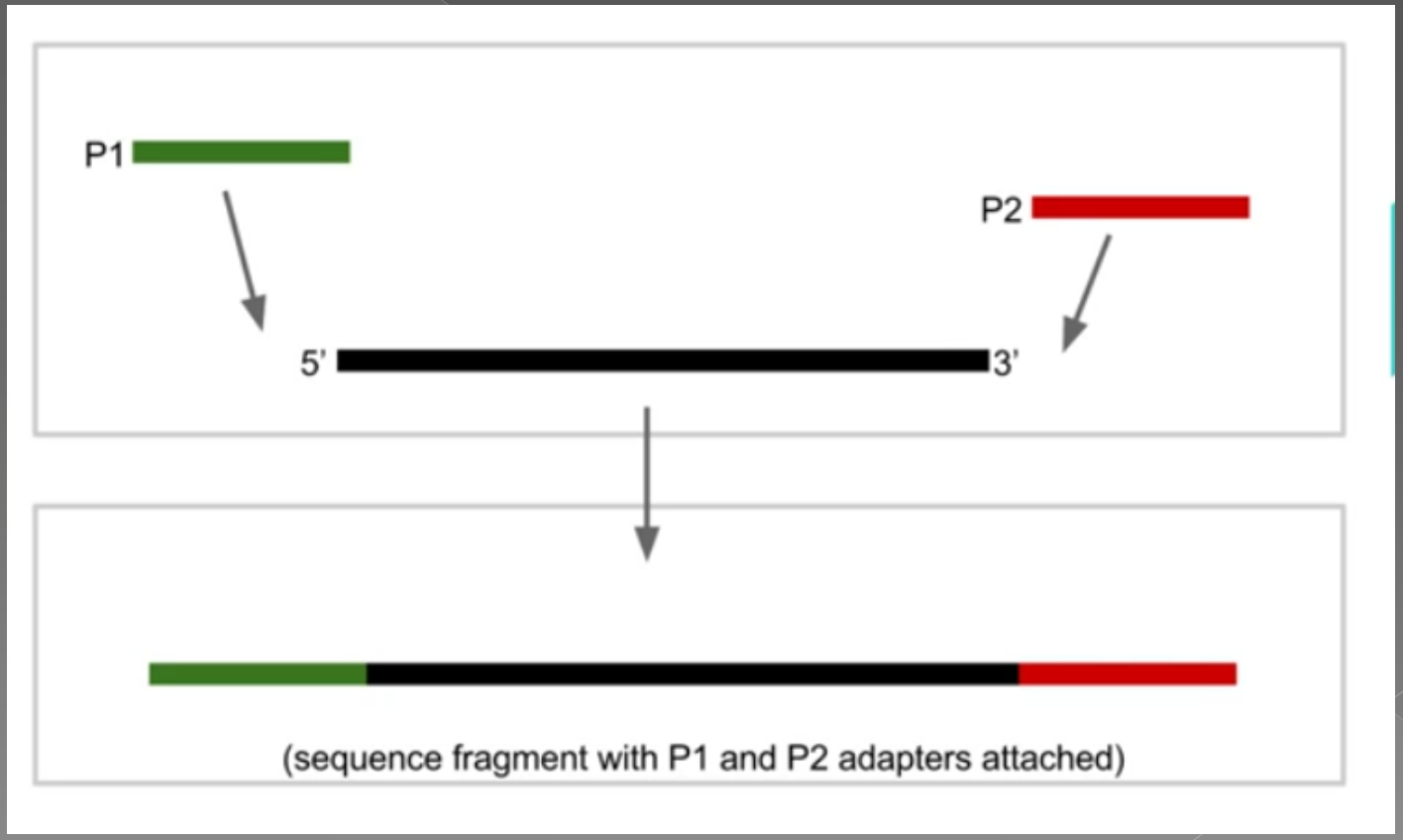
- random fragmentation of DNA to create libraries .
- followed by ligation with specific linkers (adapters).



DNA Fragmentation

NEBULIZATION	SONICATION	DIGESTION
Compressed nitrogen is used to force DNA through a small hole, creating mechanically sheared fragments	Ultrasonic waves used to create gas bubbles in sample, and shear DNA by resonance vibration	Restriction enzymes used to cleave DNA, reaction kits with enzymes commercially available

Ligation of adaptors



Amplification

- Emulsion PCR.
- Bridge PCR.

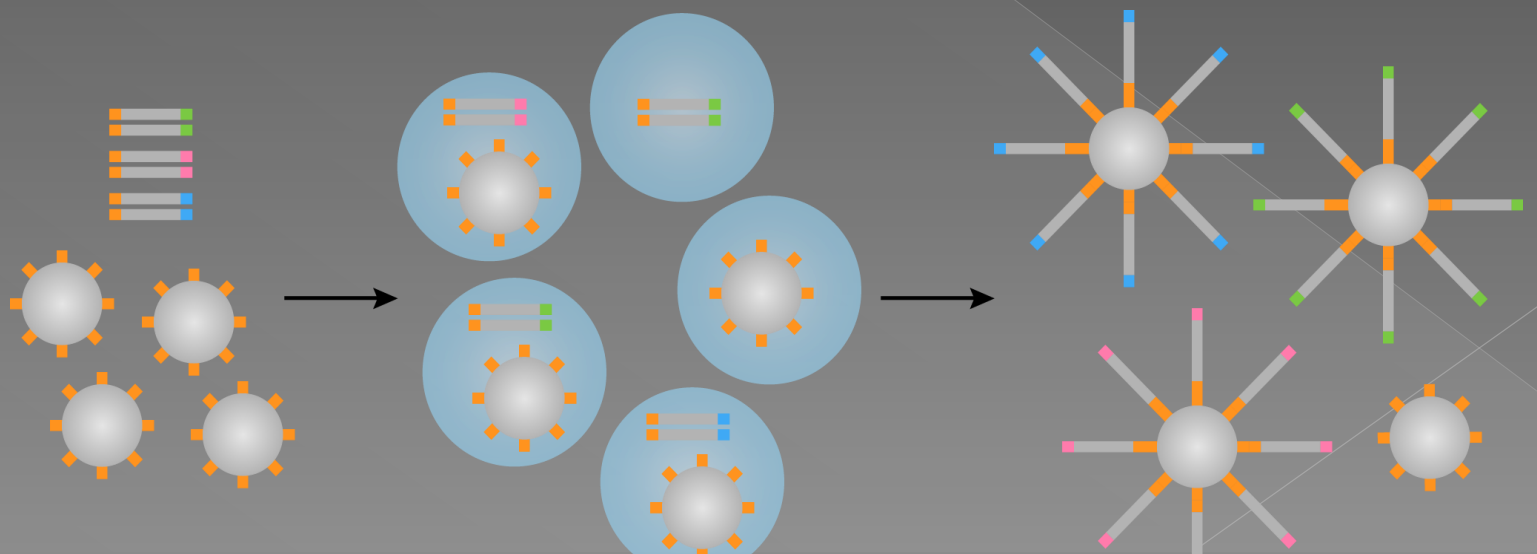
Emulsion PCR

- library DNA = fr… …d to adaptors
- Beads : co… complem… …e hybridiz…
- PCR m…
- Emulsi…

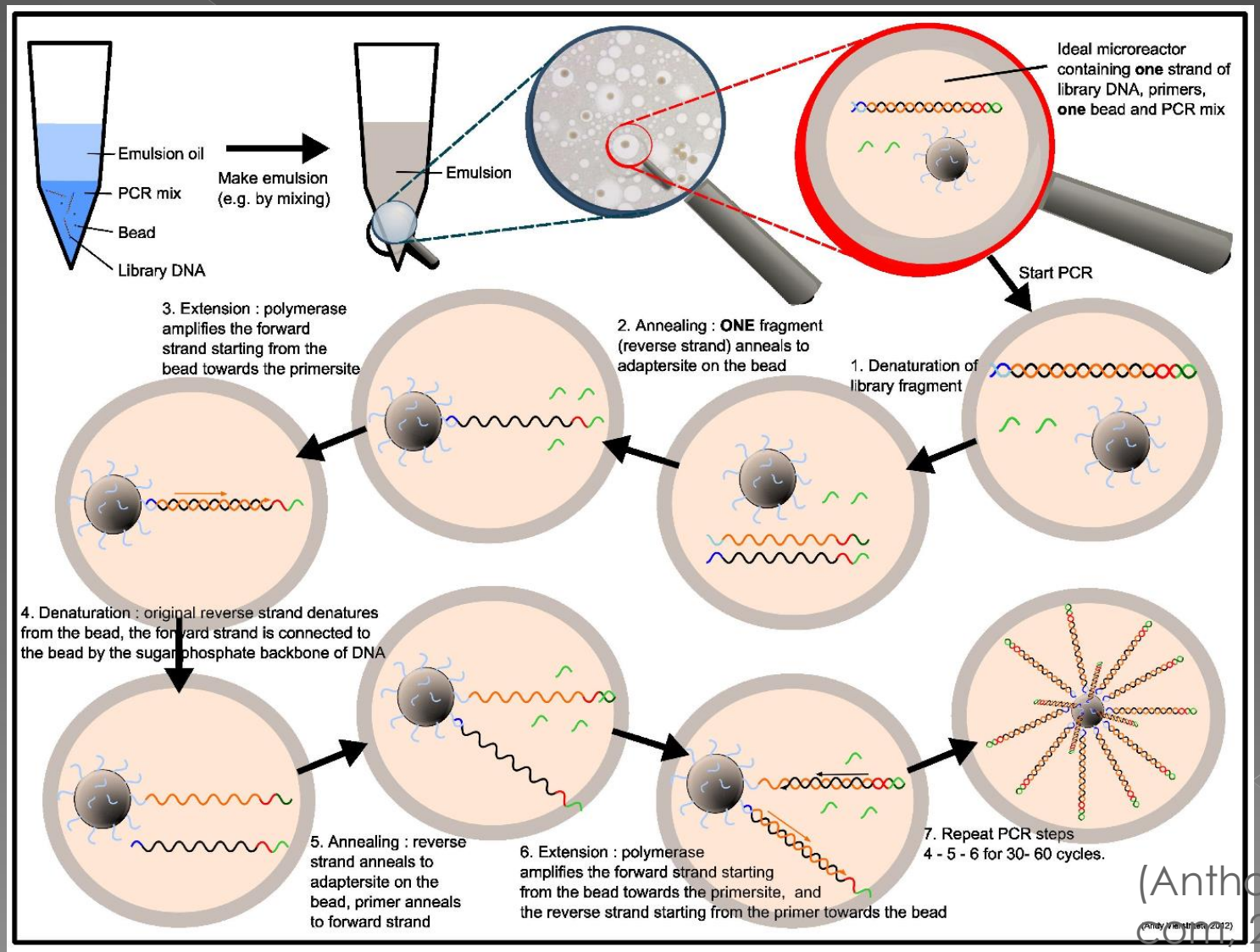
Mix all ingredients to get **Microreactors**

Microreactor

- Micro reactor is a drop of water within oil.
- micro well should contain 1bead + 1 strand of DNA. (15% of wells)
- PCR reaction is done within the drop of water to achieve polony formation.



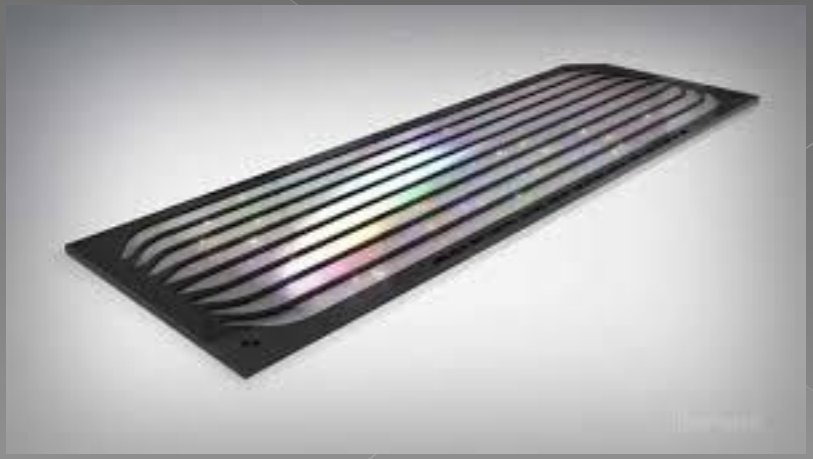
Microreactor reaction



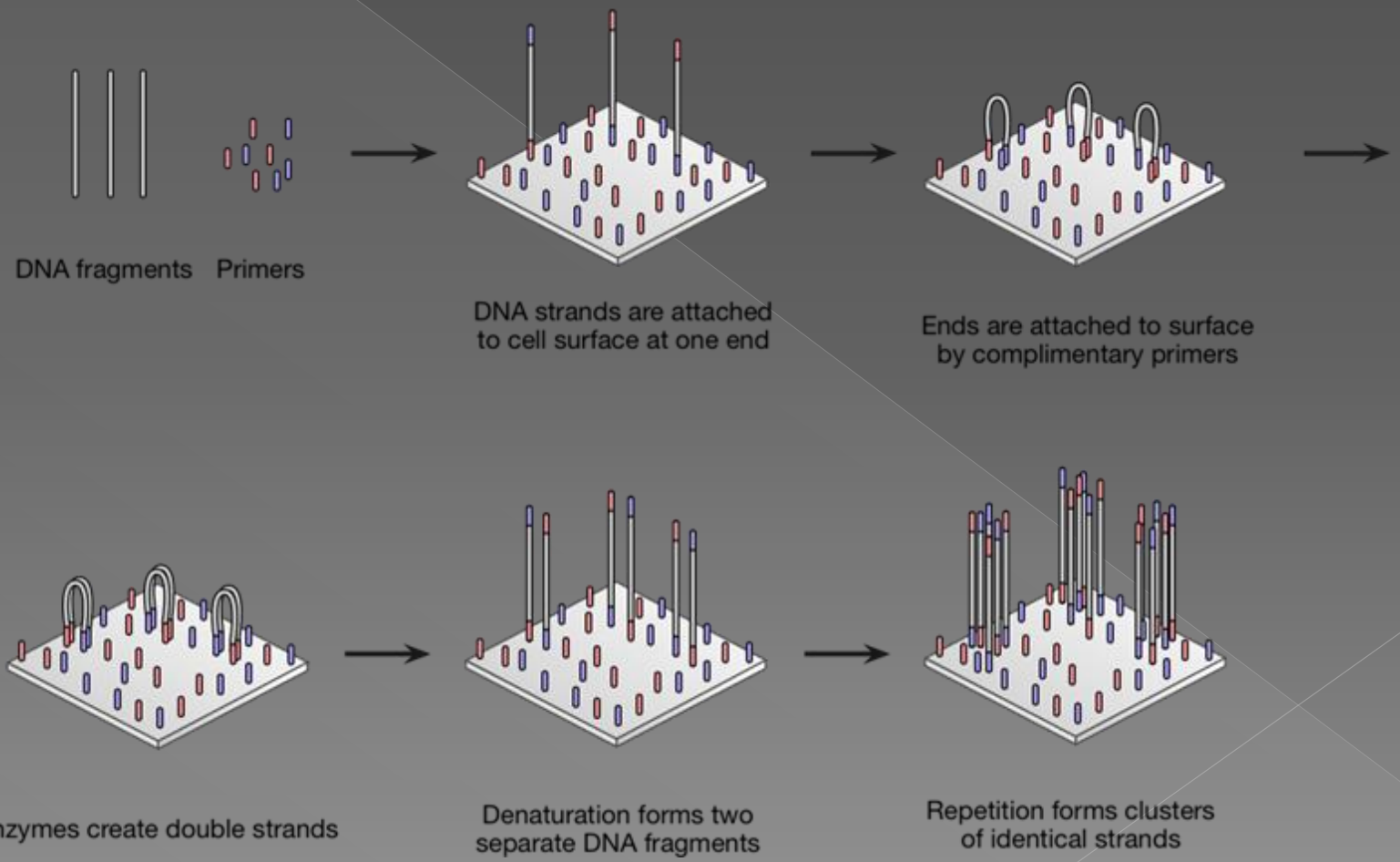
(Anthonybaldor.com, 2017)

Bridge PCR

- Flow cell : coated with primers that are complementary to the primers attached to the DNA library fragments.
- Solid-phase amplification (PCR)
- 1,000 identical copies/ 1μm² of each strand
- Cluster Generation



Flow cell


DNA fragments
Primers
DNA strands are attached to cell surface at one end
Ends are attached to surface by complimentary primers
Enzymes create double strands
Denaturation forms two separate DNA fragments
Repetition forms clusters of identical strands

Sequencing

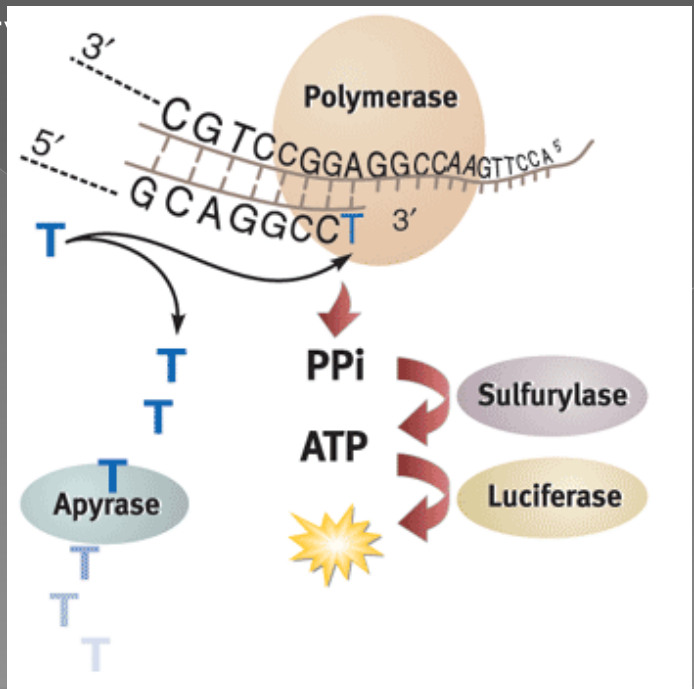
- 454 Pyrosequencing
- Ion torrent semiconductor sequencing
- Sequencing by ligation (SOLiD)
- Reversible terminator sequencing (Illumina)

Sequencing-by-Synthesis

- a new DNA strand, complementary to the target strand, is synthesized one base at a time.
- A sequencing done by detecting the nucleotide that incorporated by a DNA polymerase.
- A solution of the same nucleotide is used in each run.
- If nucleotides are labeled using different color of florescent dye they added as mix instead of separating them.

Pyrosequencing

- sequencing by synthesis
- Chemiluminescence (detection of light)
- pyrophosphate (ppi) is released when nucleotide is attached to its complementary sister.
- if more than one of the same nucleotide is added, the change in light/signal intensit
correspondingly larger.

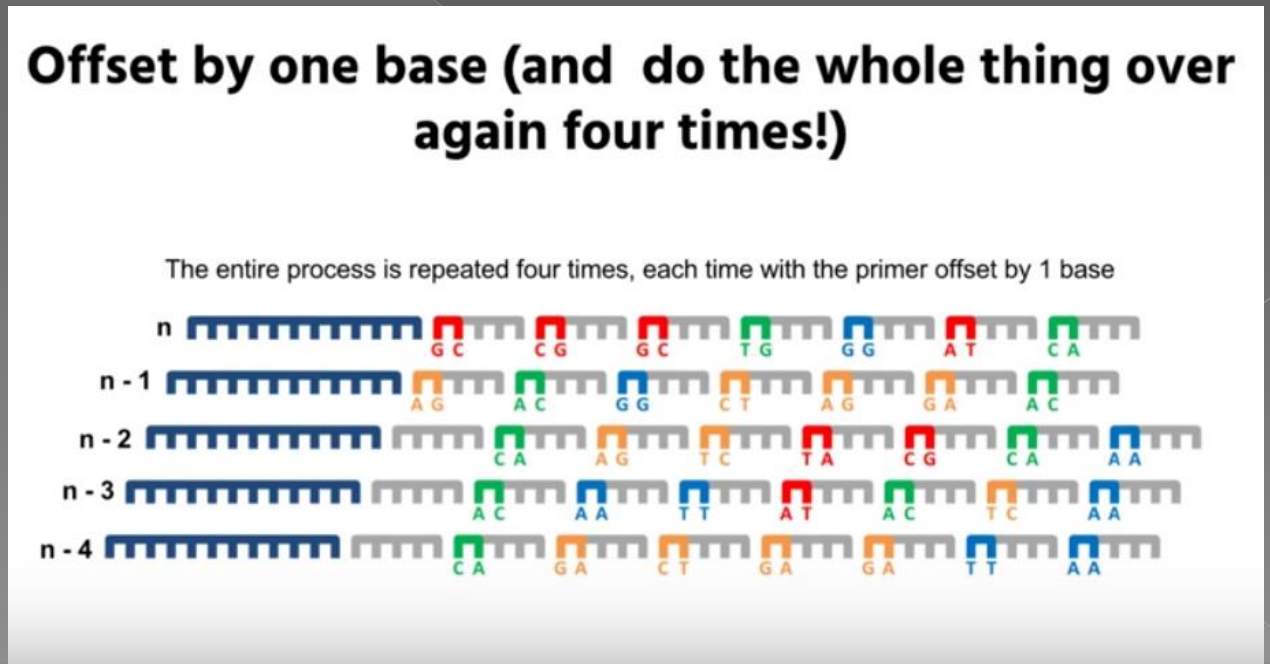


semiconductor sequencing

- sequencing by synthesis.
- hydrogen ions produced during DNA polymerization.
- A semiconductor chip detects the change in Ph.
- if more than one of the same nucleotide is added, the change in pH/signal intensity is correspondingly larger.

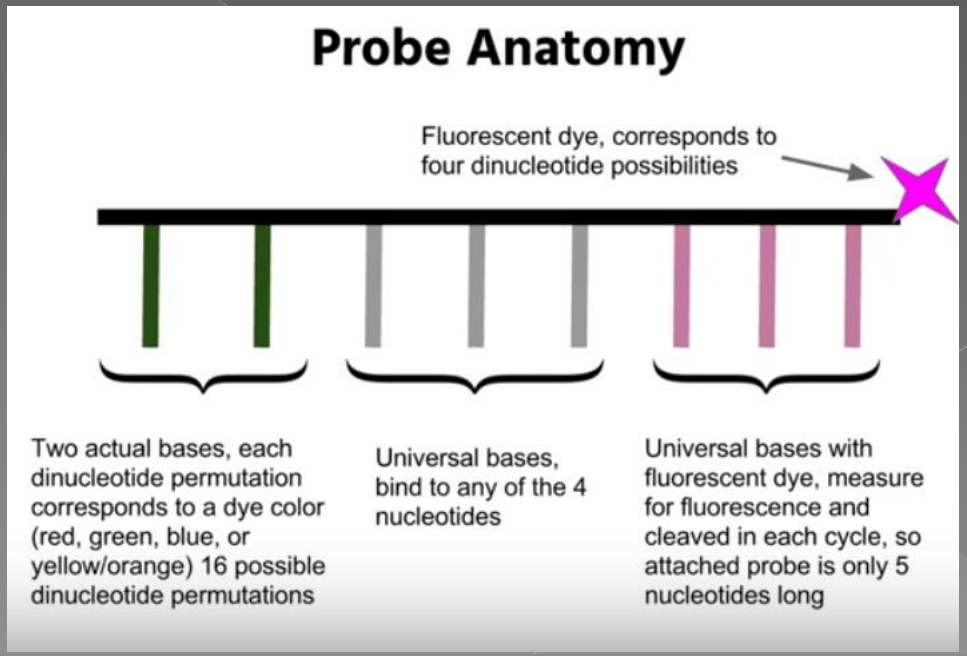
Sequencing by ligation (SOLiD)

- uses DNA ligase
- ligate double-stranded DNA strands.
- a primer of length(N, N−1, N−2, N−3……etc)
- library of 8-mer probes



library of 8-mer probes

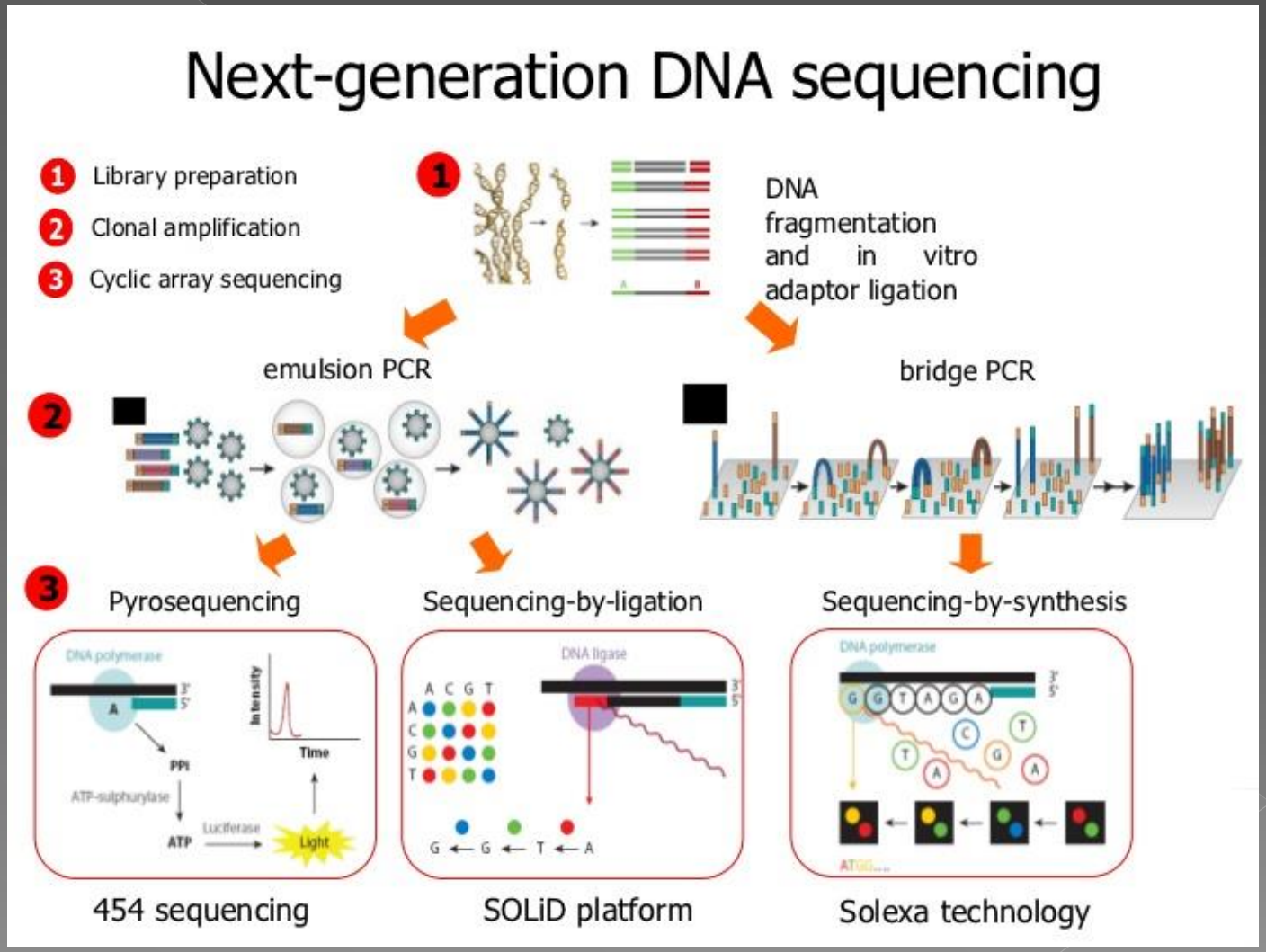
- 8 nuclides
- The first 2 chanced so 16 possibilities
- 4 fluorescent dye specific for the first nucleotide.
- Bases 1 and 2 are complementary to ssDNA
- 3-5 are degraded .
- The rest detached.



Reversible terminator sequencing (Illumina)

- sequencing by synthesis.
- Resembles Sanger method.
- But use reversible terminator instead of ddTTP .
- reversible terminator are nucleotide attached to fluorescent dyes.
- https://www.youtube.com/watch?v=womKfikWlxM&app=desktop

To sum up


Next-generation DNA sequencing
1 Library preparation
2 Clonal amplification
3 Cyclic array sequencing
1
DNA fragmentation and in vitro adaptor ligation
emulsion PCR
bridge PCR
2
3
Pyrosequencing
Sequencing-by-ligation
Sequencing-by-synthesis
DNA polymerase
Intensity
Time
PPi
ATP-sulphurylase
ATP
Luciferase
Light
DNA ligase
A C G T
454 sequencing
SOLiD platform
Solexa technology

References

- Anthonybaldor.com. (2017). Sample Preparation and DNA Amplification | Anthony Edward Baldor. [online] Available at: http://www.anthonybaldor.com/sample-preparation-and-dna-amplification/ [Accessed 7 Sep. 2017].
- hermofisher.com. (2017). *Common Cloning Applications and Strategies | Thermo Fisher Scientific*. [online] Available at: https://www.thermofisher.com/sa/en/home/life-science/cloning/cloning-learning-center/invitrogen-school-of-molecular-biology/molecular-cloning/cloning/common-applications-strategies.html [Accessed 8 Sep. 2017].