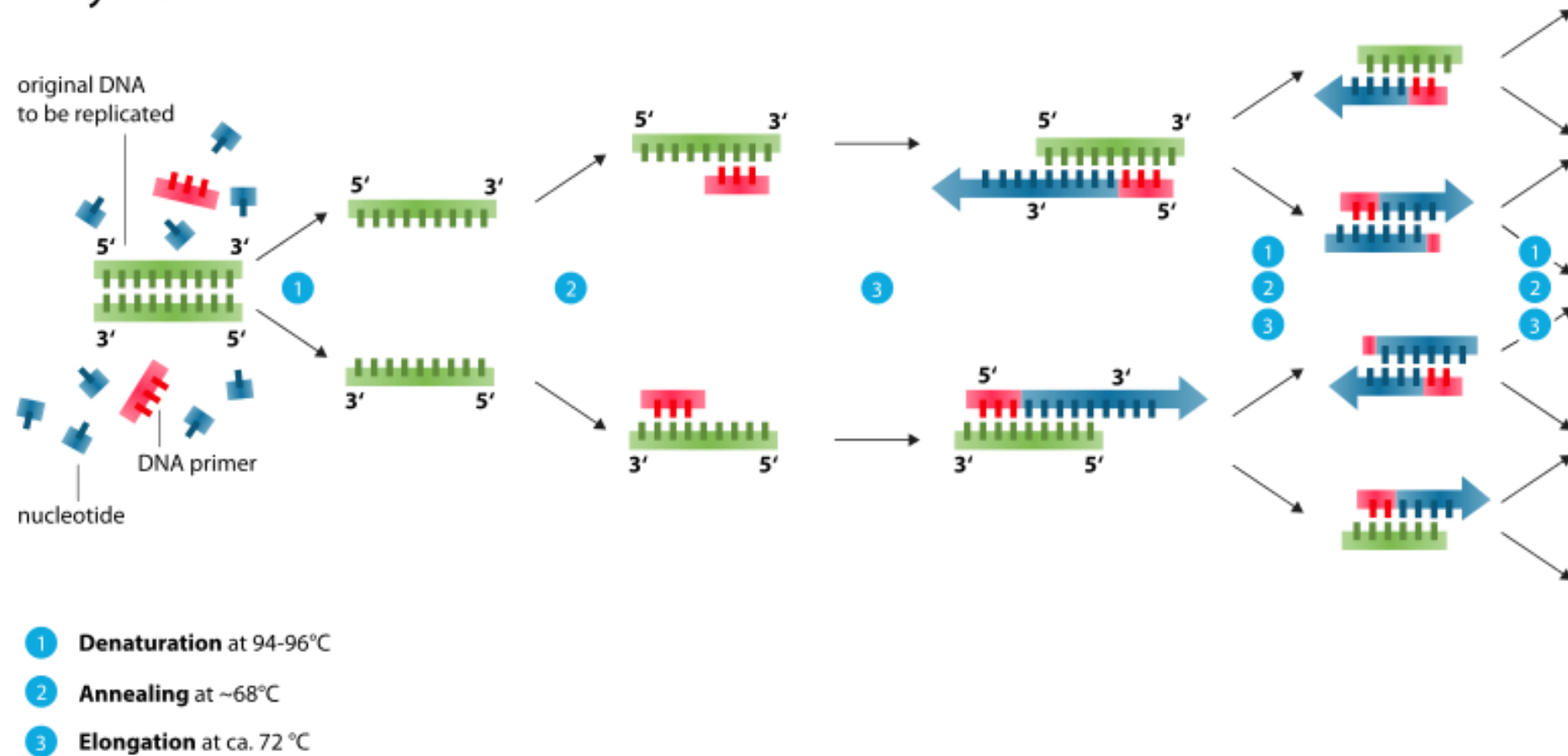
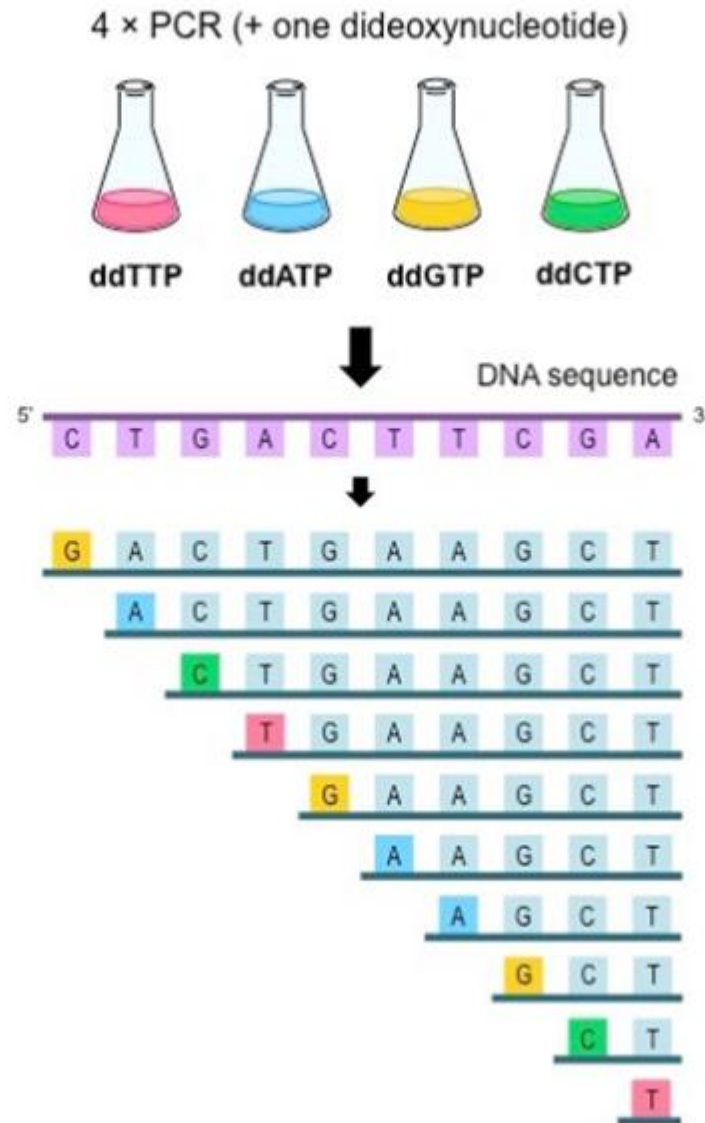


Review: Polymerase chain reaction

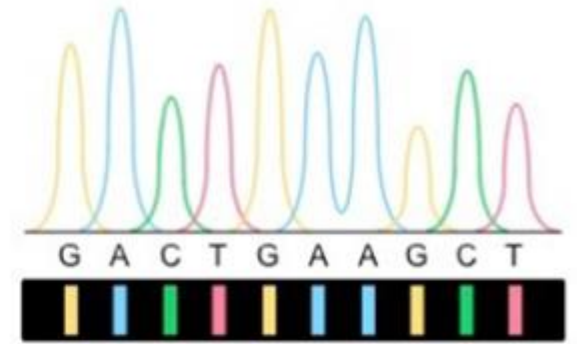
Polymerase chain reaction - PCR



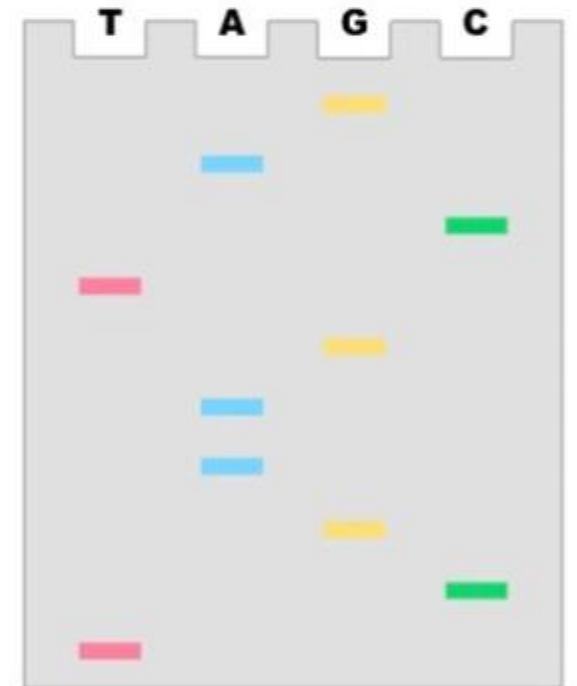
1st Generation: Sanger Sequencing



Use a
sequencing
machine



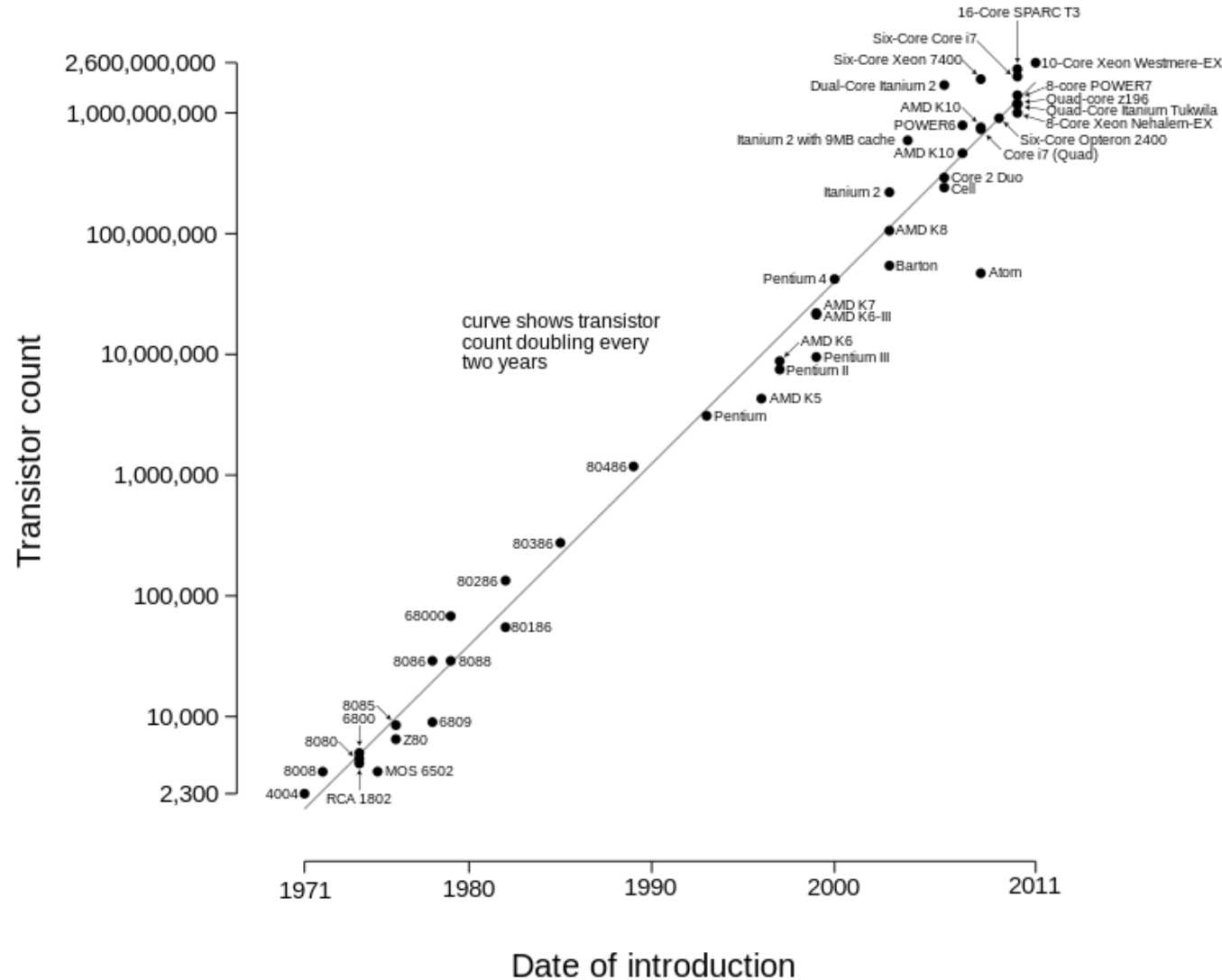
Separate
with a gel



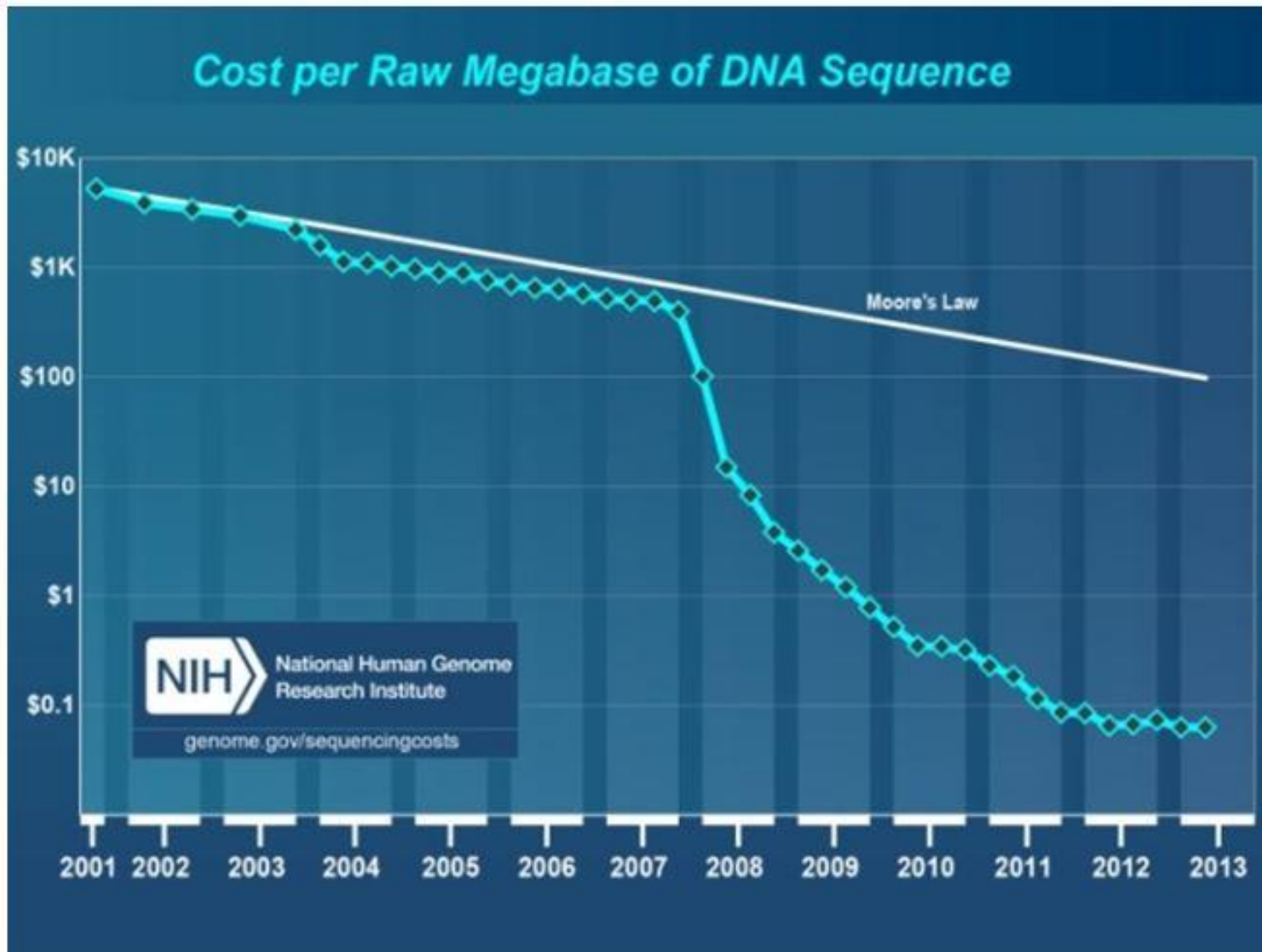
Beckman Coulter CEQ 8000

Moore's Law

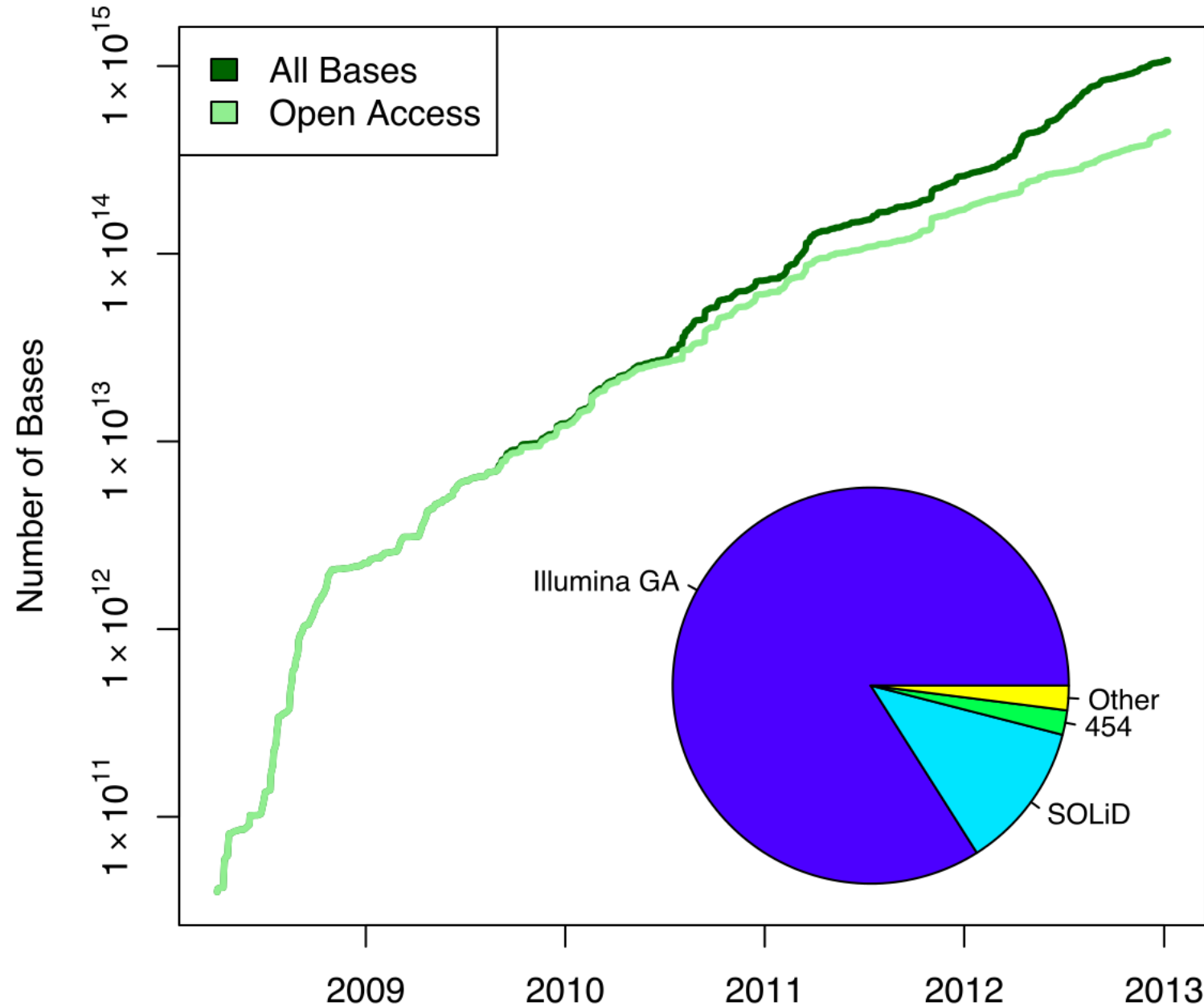
Microprocessor Transistor Counts 1971-2011 & Moore's Law



Moore's Law vs. Sequencing Technology



Sequencing Read Archive (NCBI)



Blue and green =
'Next Generation Sequencing'

2nd Gen: Sequencing Platforms



MiSeq®



NextSeq® 500



HiSeq® 2500



HiSeq® 3000

Next Generation Sequencing
platforms from trusted names



Ion Torrent™

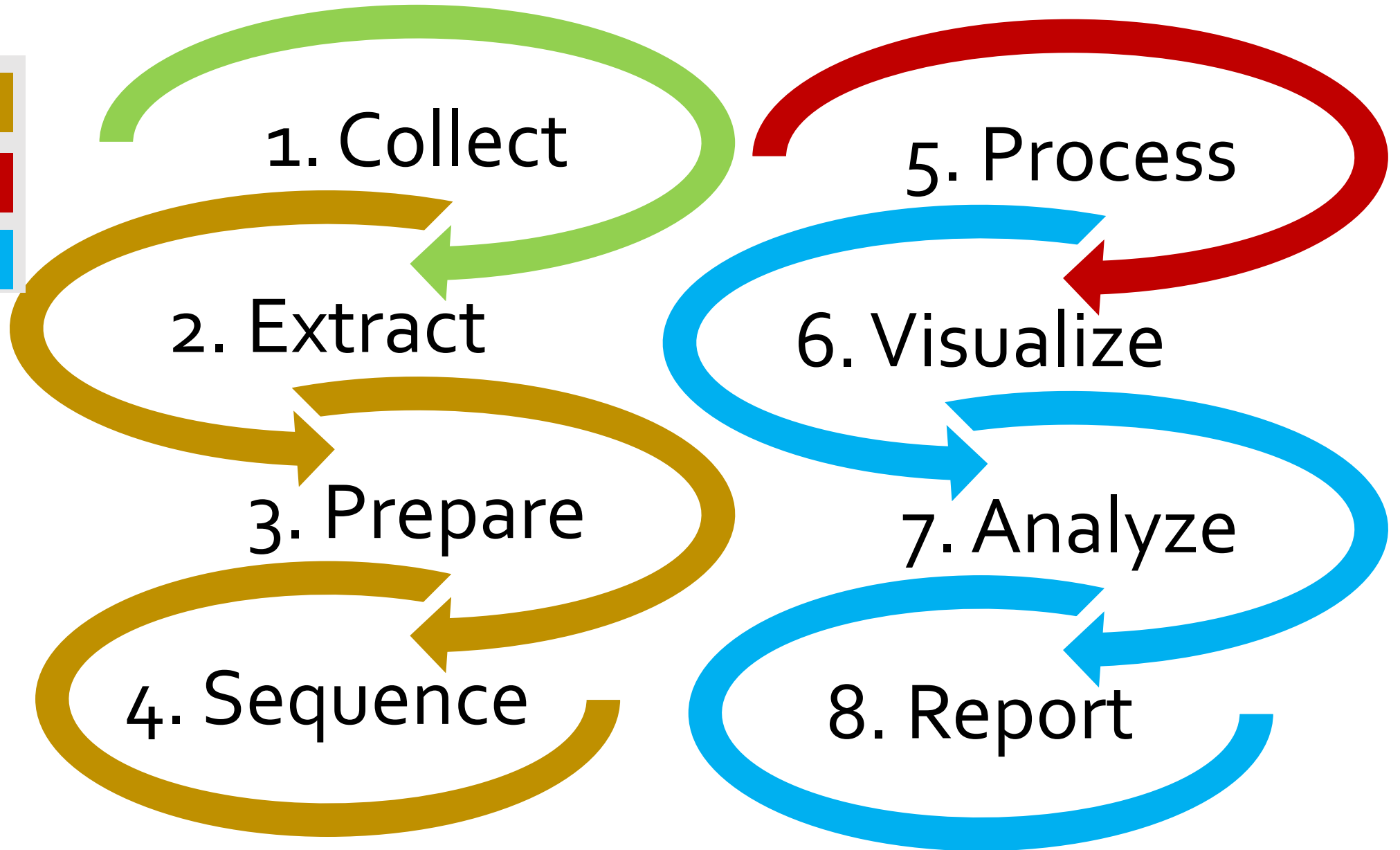
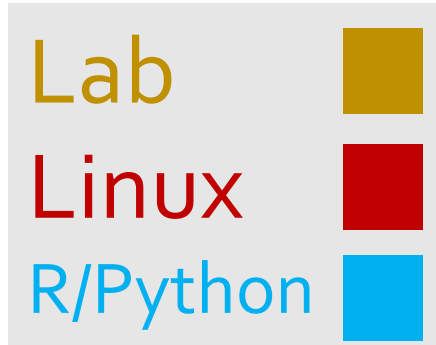


PacBio RS II System



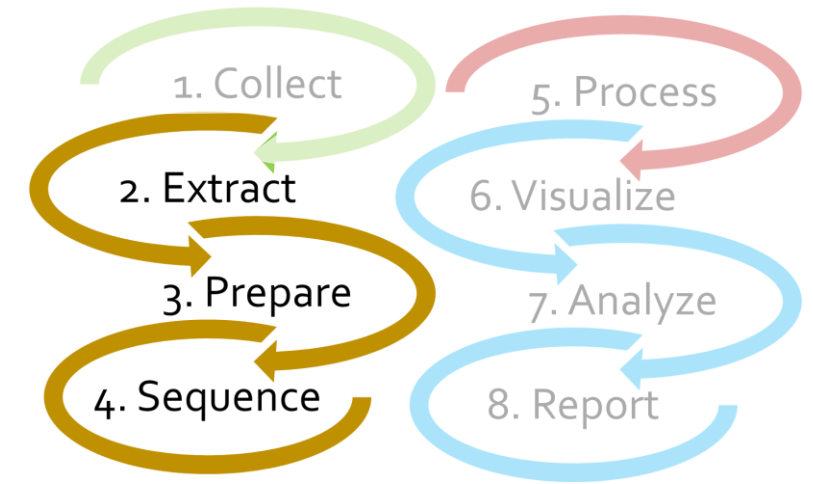
HiSeq® 4000

Next-Generation Sequencing: Typical Workflow



Sequencing Library Preparation

1. Extract & purify DNA*
2. Fragment to target size (75-750 bp)
3. Strand isolation
4. Clonal Amplification
5. Nucleotide detection



2. Fragment Sizes (longer list on website)

Platform	Instrument	Mreads	Length (bp)	Gbp	Type
Illumina	NovaSeq 6000 S4	10000	300	3000	SR & PE
Illumina	NextSeq 500 High-Output	400	300	120	SR & PE
Illumina	HiSeq X	375	300	112.5	PE
Illumina	HiSeq High-Output v4	250	250	62.5	SR & PE
Illumina	MiSeq v3	25	600	15	SR & PE
Illumina	MiniSeq High-Output	25	300	7.5	SR & PE
Ion	Proton I	60	200	12	SR
Ion	PGM 318	4	400	1.6	SR
Ion	PGM 316	2	400	0.8	SR
Ion	PGM 314	0.4	400	0.16	SR
PacBio	PacBio Sequel	0.37	20000	7.4	SR
PacBio	PacBio RS II (P6)	0.055	15000	0.825	SR
Roche 454	GS FLX+ / FLX	0.7	700	0.49	SR
SOLiD	5500xl W	267	100	26.7	SR & PE

Sequence isolation (and cloning)

(a)



One bead per
microreactor
(454 and
IonTorrent)

(b)



Beads bound
to solid surface
(SOLiD)
(Ion Torrent)

(c)



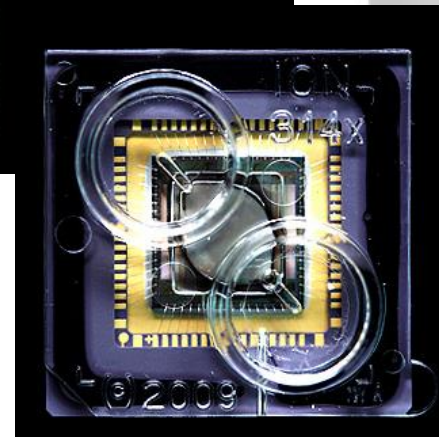
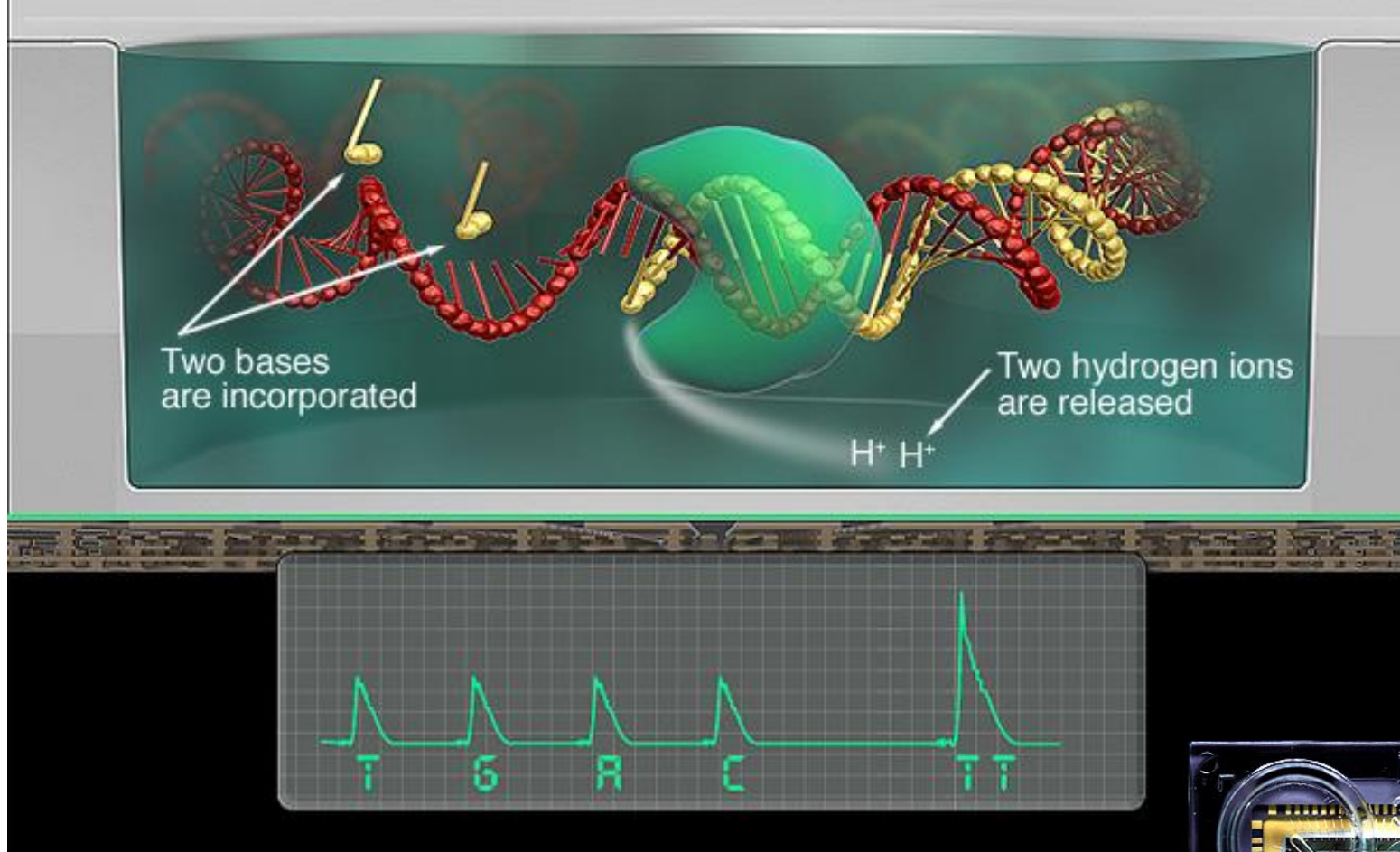
Amplified islands
on glass plate
(Illumina)

(e)

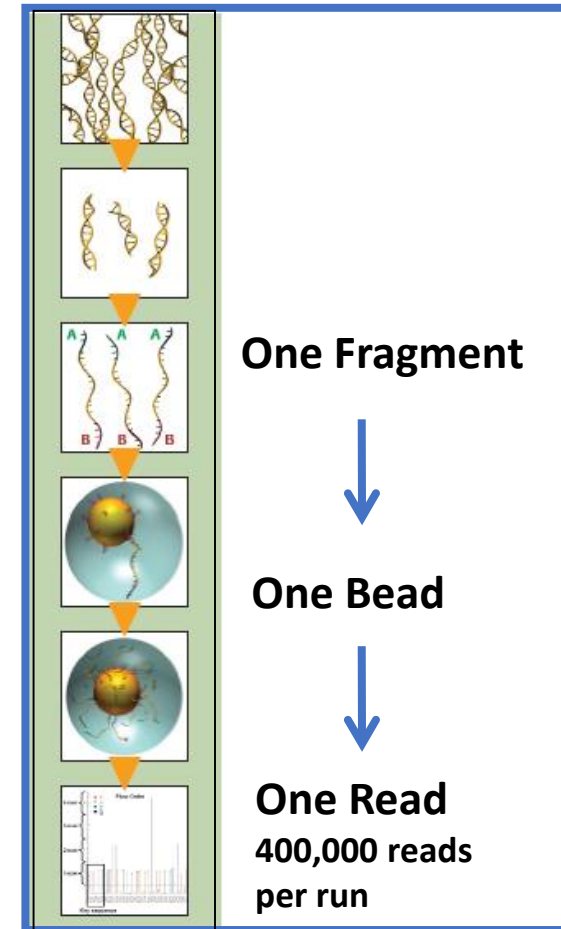
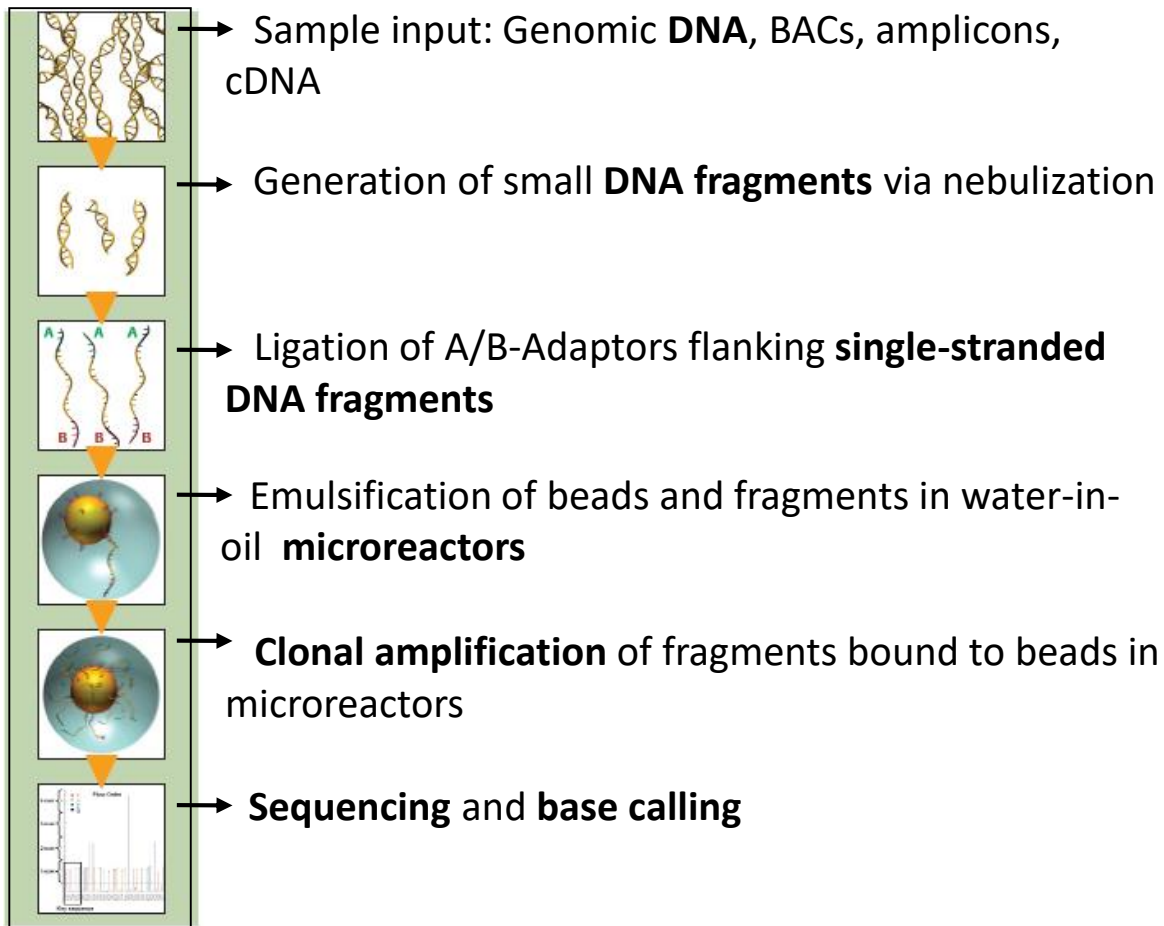


Amplified template
captured by single
polymerase molecule
(PacBio)

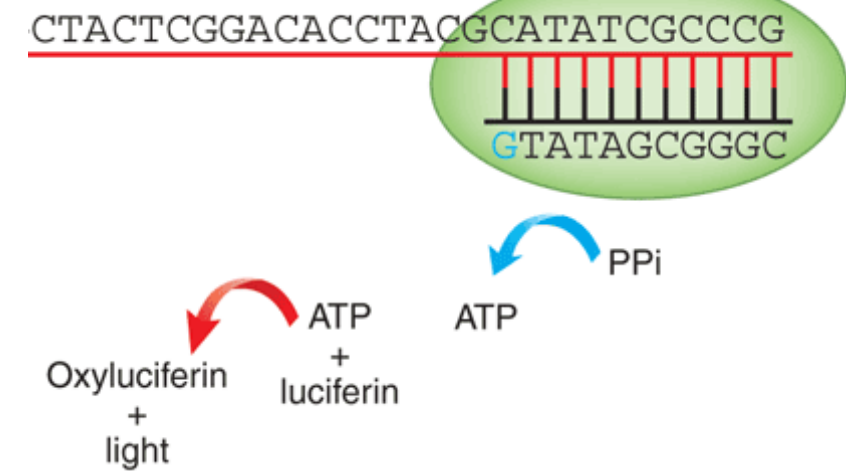
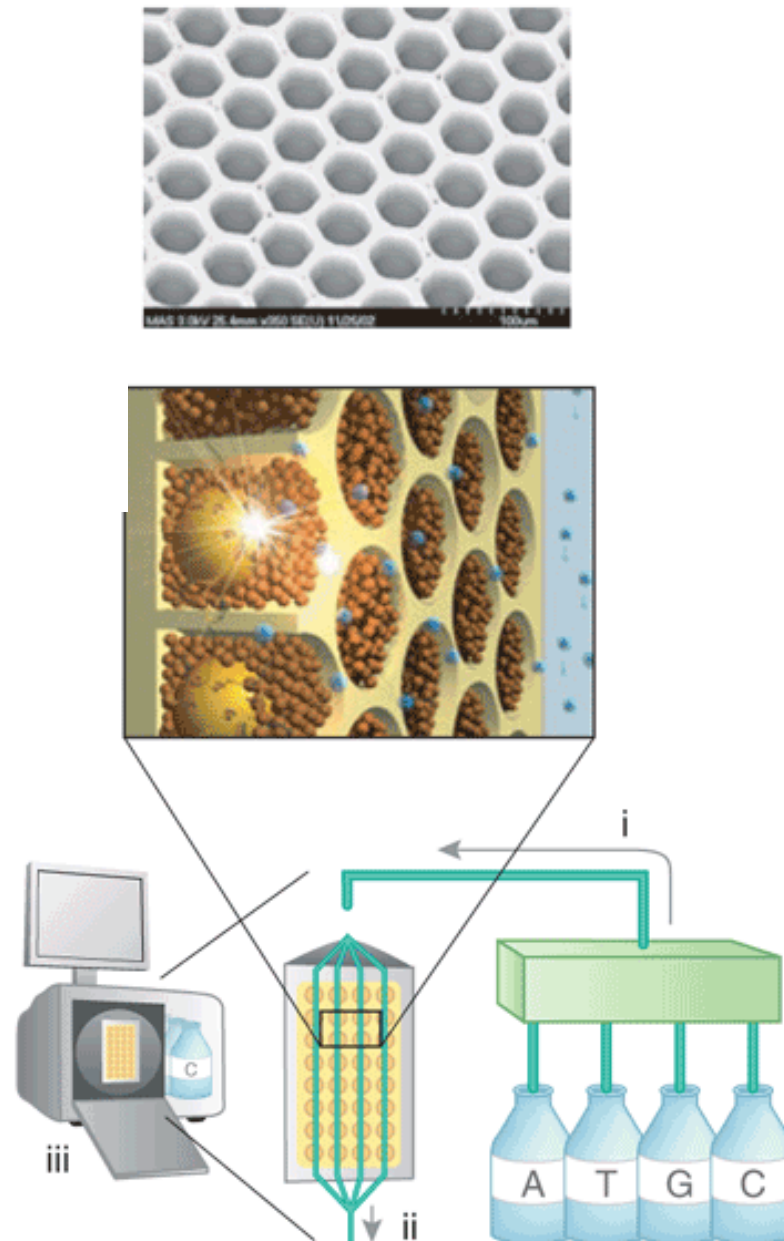
Ion Torrent



2nd Gen: 454 Sequencing (Roche; deprecated)



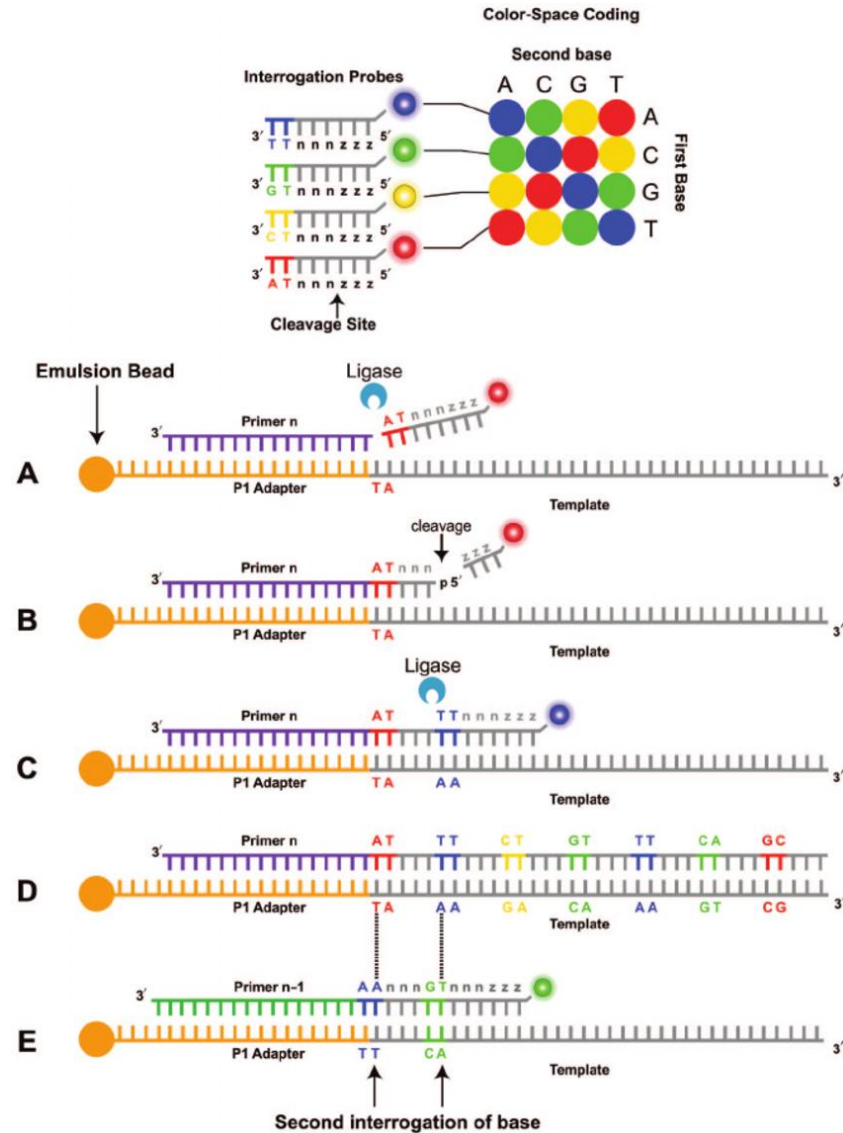
2nd Gen: 454 Sequencing (Roche; deprecated)



CSB2008 August 2008

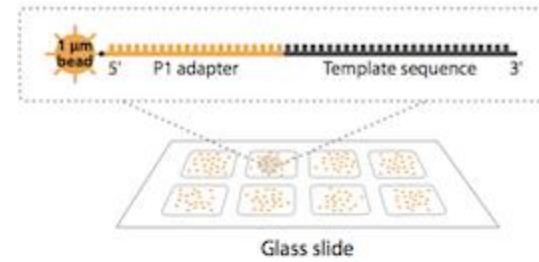
Rothberg & Leomon 2008

SOLiD Sequencing (ABI)

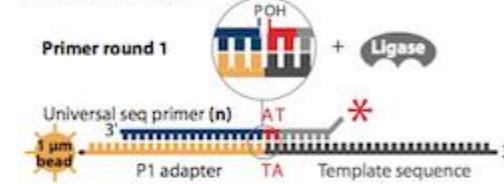


a

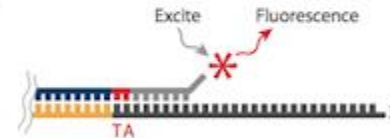
SOLiD™ substrate



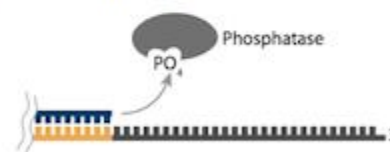
1. Prime and ligate



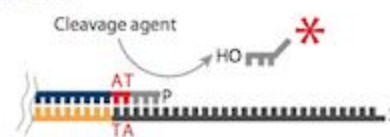
2. Image



3. Cap unextended strands

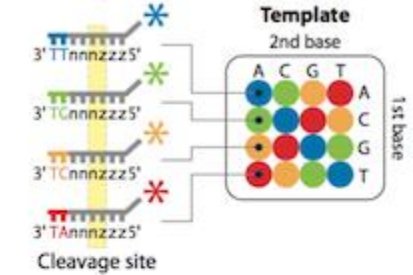


4. Cleave off fluor



8. Repeat Reset with , n-2, n-3, n-4 primers

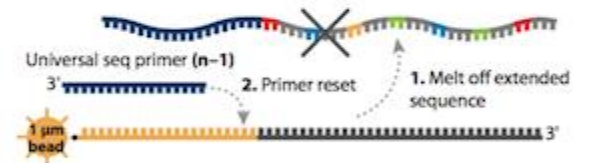
Di base probes



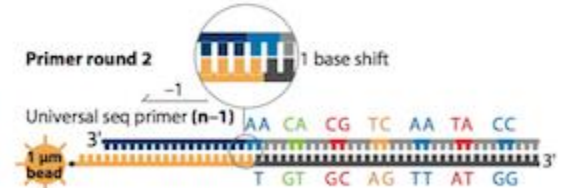
5. Repeat steps 1-4 to extend sequence



6. Primer reset



7. Repeat steps 1-5 with new primer

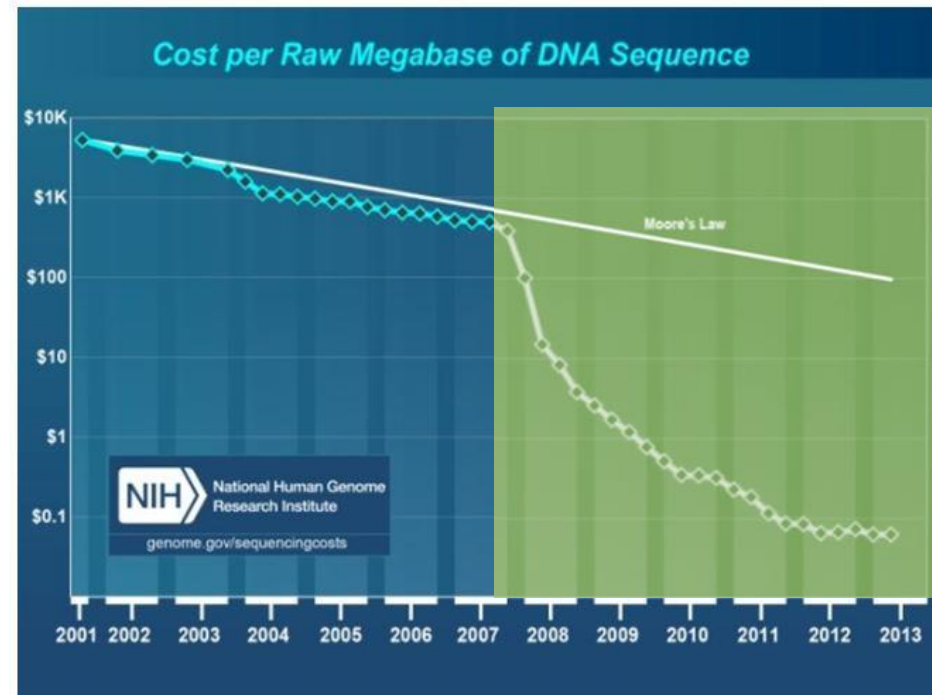


Illumina Devices

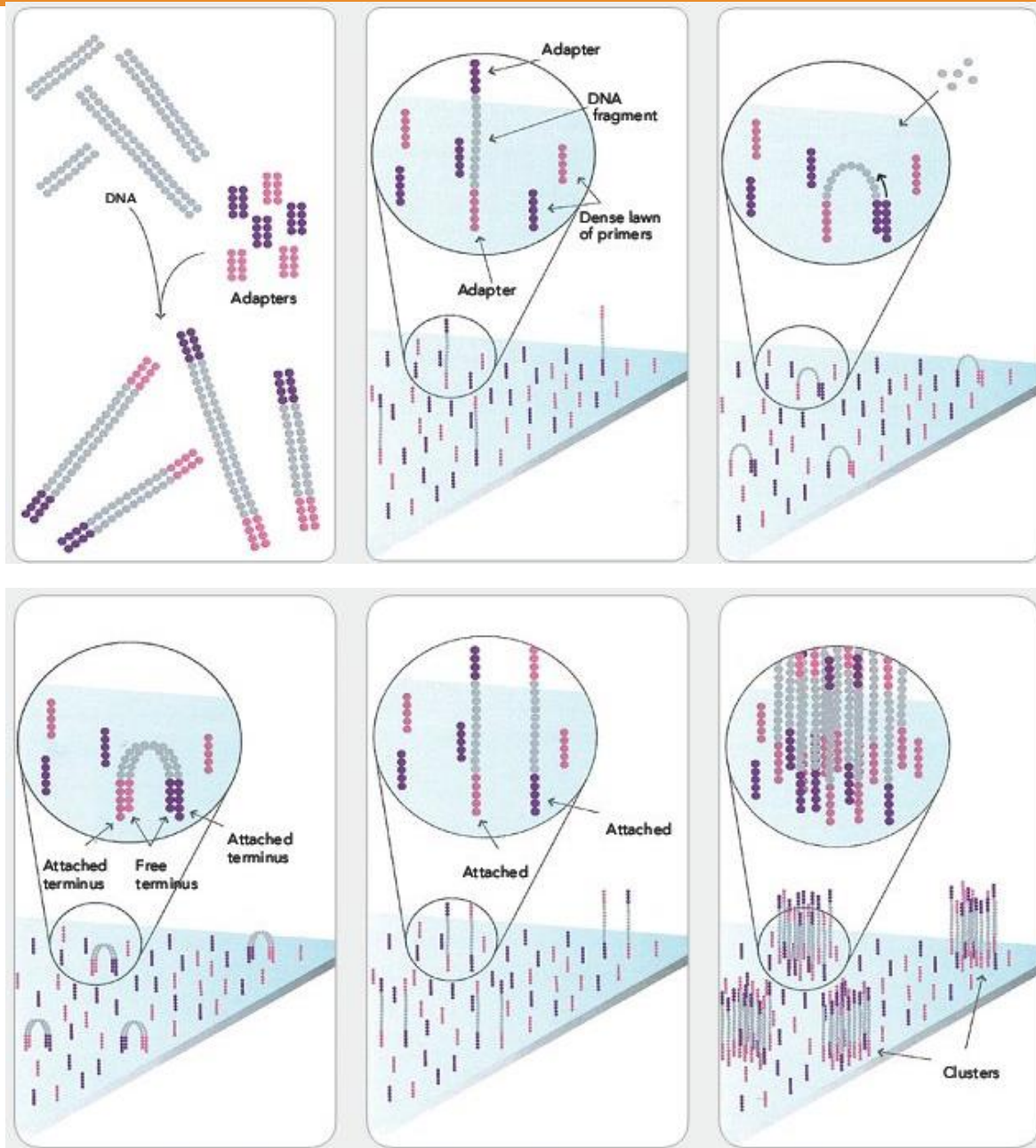


MiniSeq System

Cost per Raw Megabase of DNA Sequence

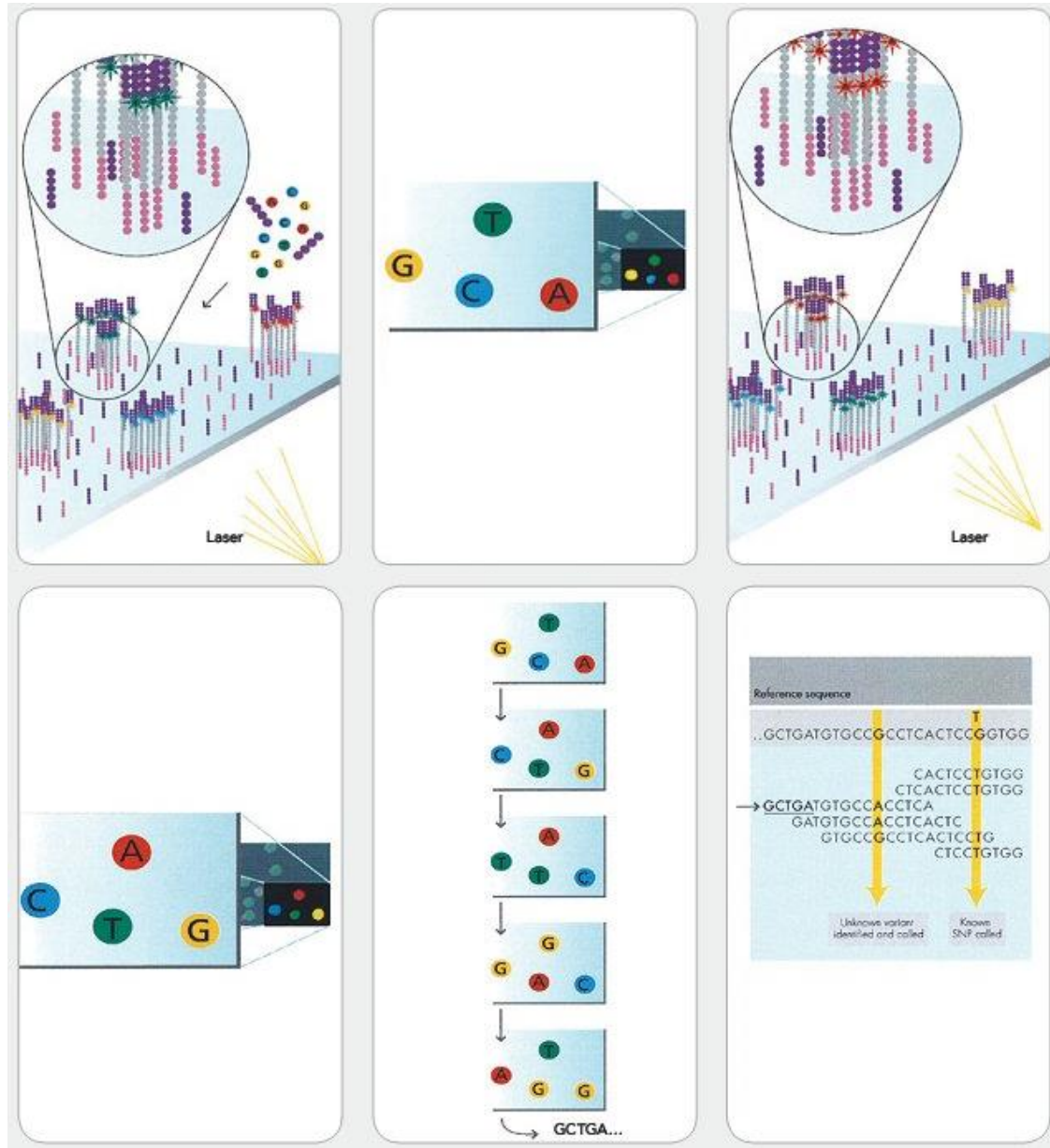


Illumina sequencing (formerly Solexa)



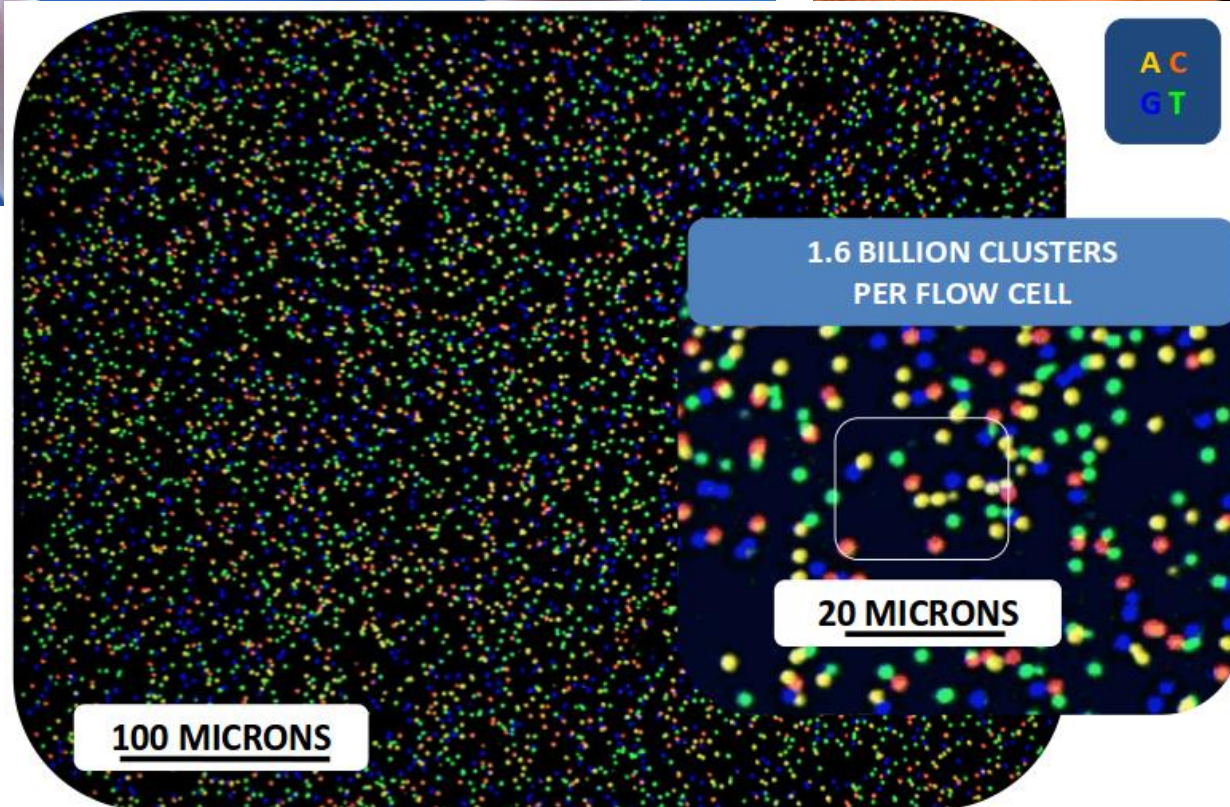
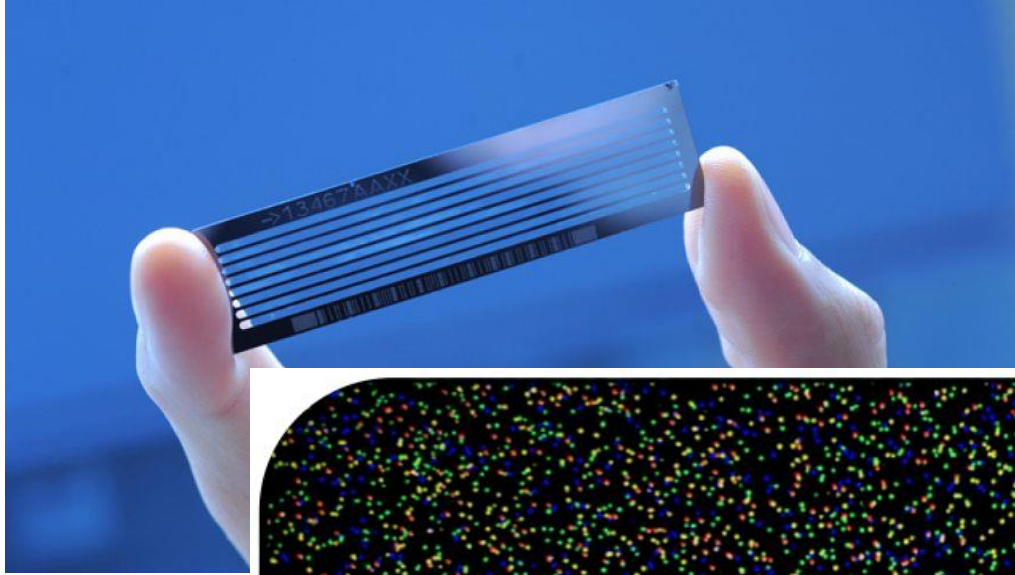
1. Prepare genomic DNA
2. Attach DNA to surface
3. Bridge amplification
4. Fragment become double stranded
5. Denature the double stranded molecules
6. Complete amplification

Illumina sequencing



7. Determine first base
8. Image first base
9. Determine second base
10. Image second base
11. Sequence reads over multiple cycles
12. Align data

Illumina flow cells



Working in groups (15 mins):

Stretch and divide into working groups

Summarize sequencing-by-synthesis (SBS) with Illumina

Review key concepts:

1. How a flow cell works
2. Contrast Sanger with SBS sequencing

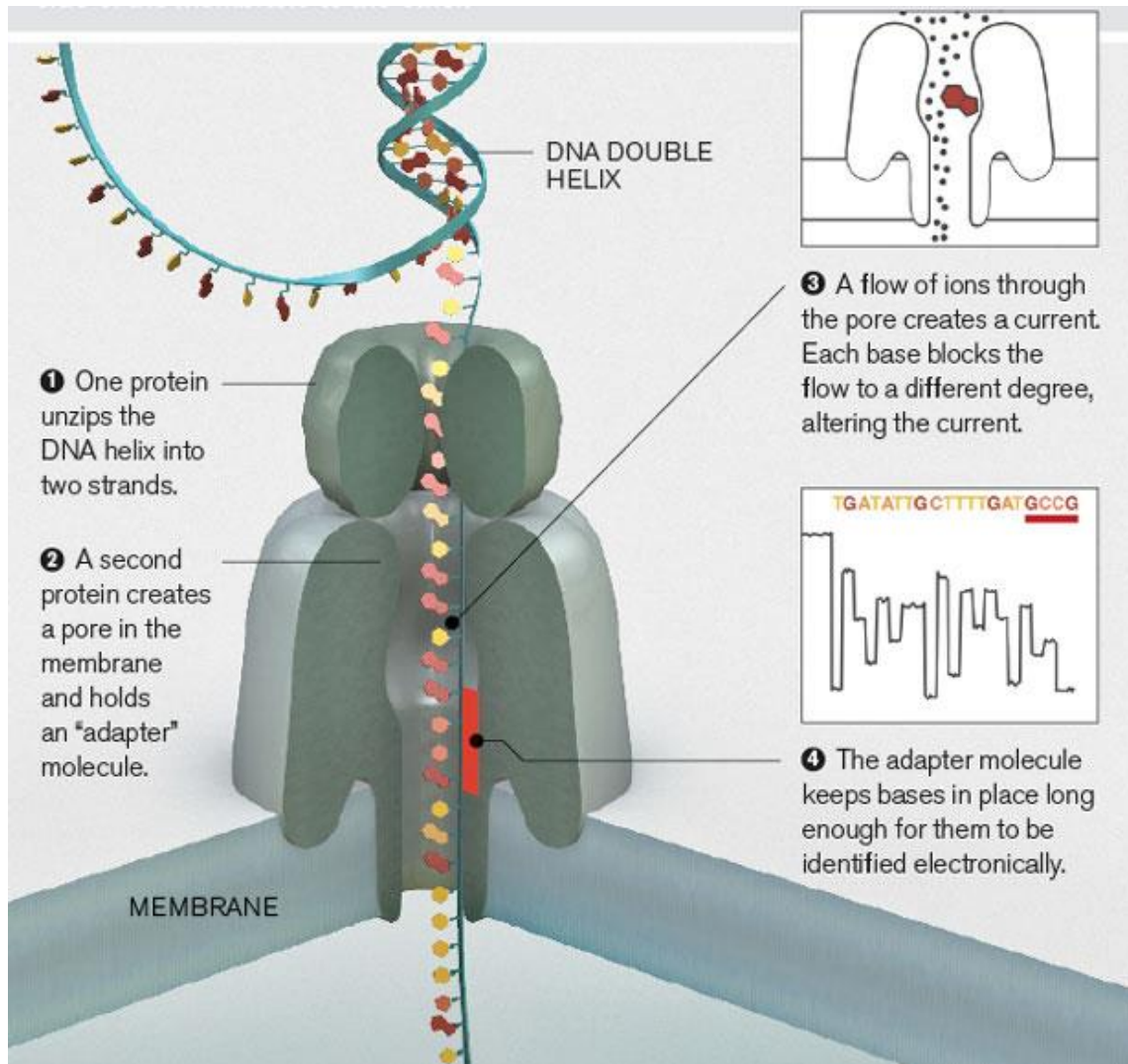
Try flowcharts or cartoons to simplify & summarize

BRAINSTORM:

What are the main benefits & limitations of each technology?

Why is coding valuable for 2nd generation sequencing?

3rd Gen: Nanopore Sequencing

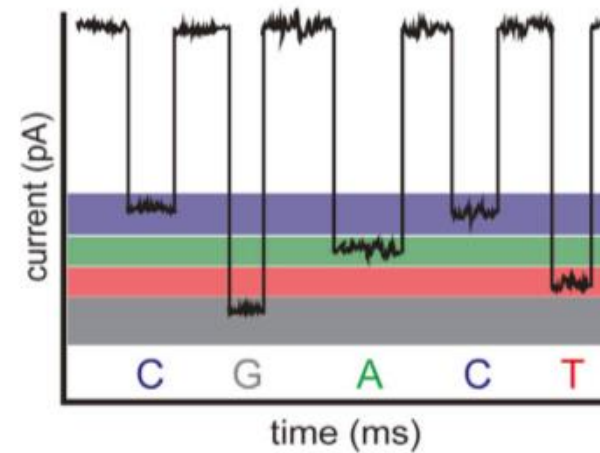
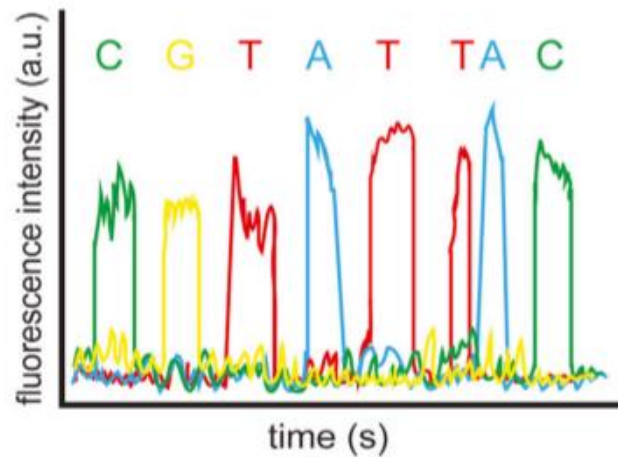
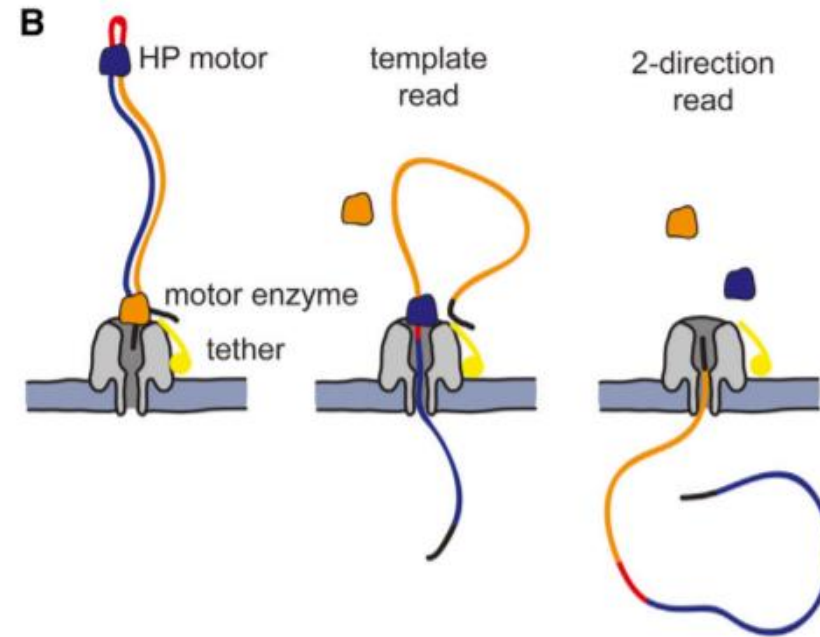
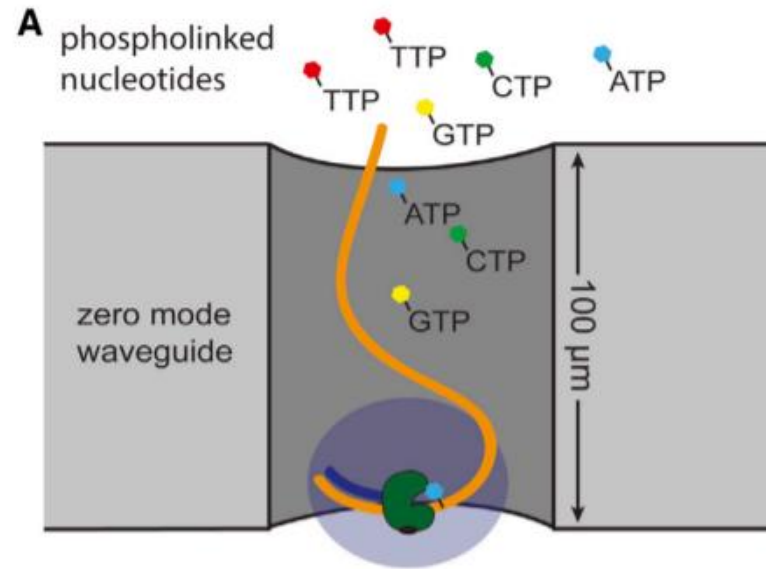


MinION



* Coming soon

3rd Gen: Nanopore Sequencing



Nanopore Sequencing Comparison

Platform	Instrument	Mreads	Length (bp)	Gbp	Type
Illumina	NovaSeq 6000 S4	10000	300	3000	SR & PE
Illumina	NextSeq 500 High-Output	400	300	120	SR & PE
Illumina	HiSeq X	375	300	112.5	PE
Illumina	HiSeq High-Output v4	250	250	62.5	SR & PE
Illumina	MiSeq v3	25	600	15	SR & PE
Illumina	MiniSeq High-Output	25	300	7.5	SR & PE
Oxford Nanopore	MinION	1M+	20 SR		
Oxford Nanopore	PromethION			1000	SR
Ion	Proton I	60	200	12	SR
Ion	PGM 318	4	400	1.6	SR
Ion	PGM 316	2	400	0.8	SR
Ion	PGM 314	0.4	400	0.16	SR
PacBio	PacBio Sequel	0.37	20000	7.4	SR
PacBio	PacBio RS II (P6)	0.055	15000	0.825	SR
Roche 454	GS FLX+ / FLX	0.7	700	0.49	SR
SOLiD	5500xl W	267	100	26.7	SR & PE