

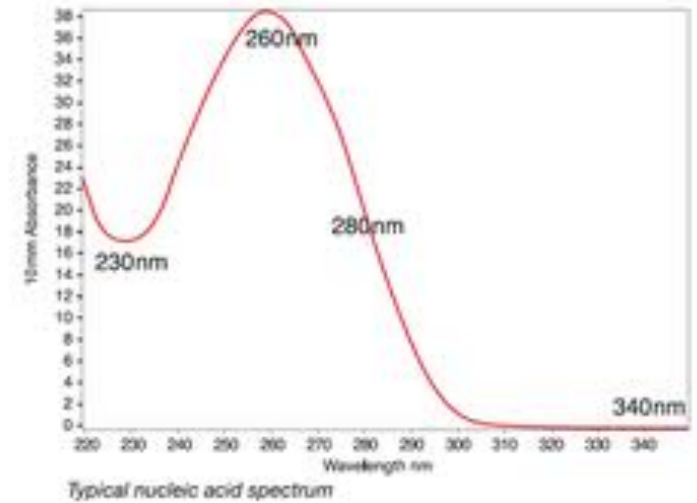
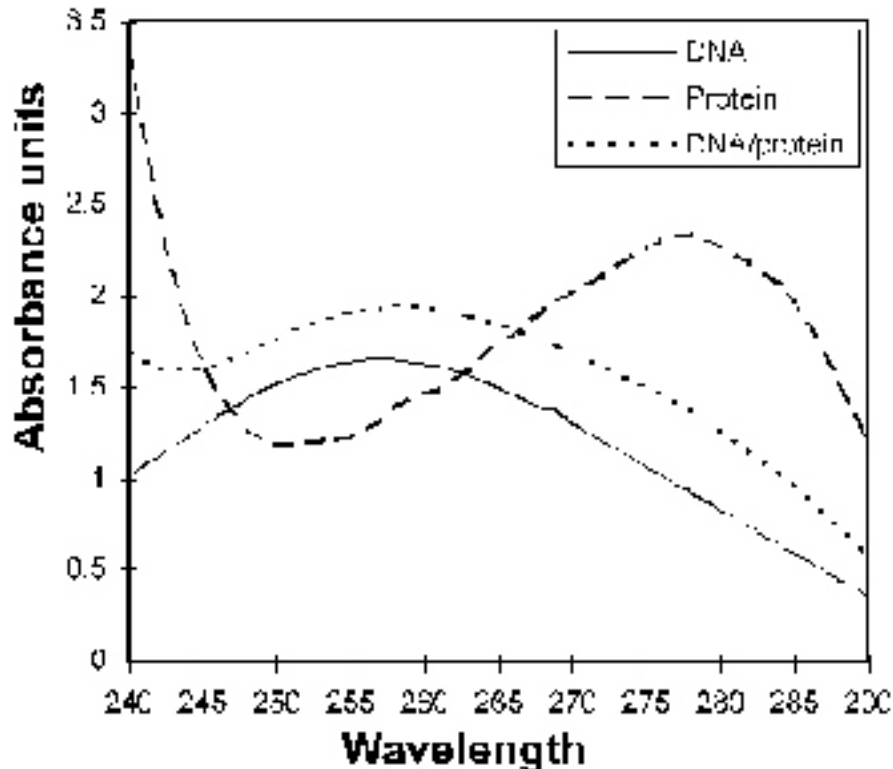
SUMMER INSTITUTE - ONLINE MODALITY CALENDAR 2022

SUN	MON	TUE	WED	THU	FRI	SAT
June 26	27	28	29	30	31	July 02
	9:00-10:00am Virtual Program Orientation for Summer Institute Online Modality					
July 03	04	05	06	07	08	09
	Holiday (Independence Day)	8:30-10:00am -Welcome Reception and Buddy Meet & Greet Event	Free Day	Classes begin! 8:30-11am: BIOL4905 INTRODUCTION 8-10:20pm: Afternoon course	8:30-11am: BIOL4905 DNA PREPARATION 8-10:20pm: Afternoon course	
10	11	12	13	14	15	16
	8:30-11am:BIOL4905 PROTEOMICS I 8-10:20pm: Afternoon course	8:30-11am:BIOL4905 PROTEOMICS II 8-10:20pm: Afternoon course	8:30-11am:BIOL4905 PROTEOMICS III 8-10:20pm: Afternoon course	8:30-11am: BIOL4905 RNA PREPARATION 8-10:20pm: Afternoon course	Virtual Independence Day Activity	
17	18	19	20	21	22	23
	8:30-11am:BIOL4905 qPCR / ROBOTS 8-10:20pm: Afternoon course	8:30-11am:BIOL4905 DNA Sequence Analysis 8-10:20pm: Afternoon course	Midterm Break		8:30-11am:BIOL4905 Next Gen. Sequencing 8-10:20pm: Afternoon course	
24	25	26	27	28	29	30
	8:30-11am:BIOL4905 Microarray I 8-10:20pm: Afternoon course	8:30-11am:BIOL4905 Nanostring 8-10:20pm: Afternoon course	8:30-11am:BIOL4905 Automated Microscopy /AFM 8-10:20pm: Afternoon course	8:30-11am:BIOL4905 Flow Cytometry 8-10:20pm: Afternoon course	FINALS	
31	August 01	02	03			
	9:00-10:00am: Closing Reception		Grades available in PAWS			

Legend:
Orange: Courses Blue: Activities



Absorbance of DNA and protein at various wavelengths



Nucleic acids absorb ultraviolet light in a specific pattern. In a spectrophotometer, a sample is exposed to ultraviolet light at 260 nm, and a photo-detector measures the light that passes through the sample. The more light absorbed by the sample, the higher the nucleic acid concentration in the sample.

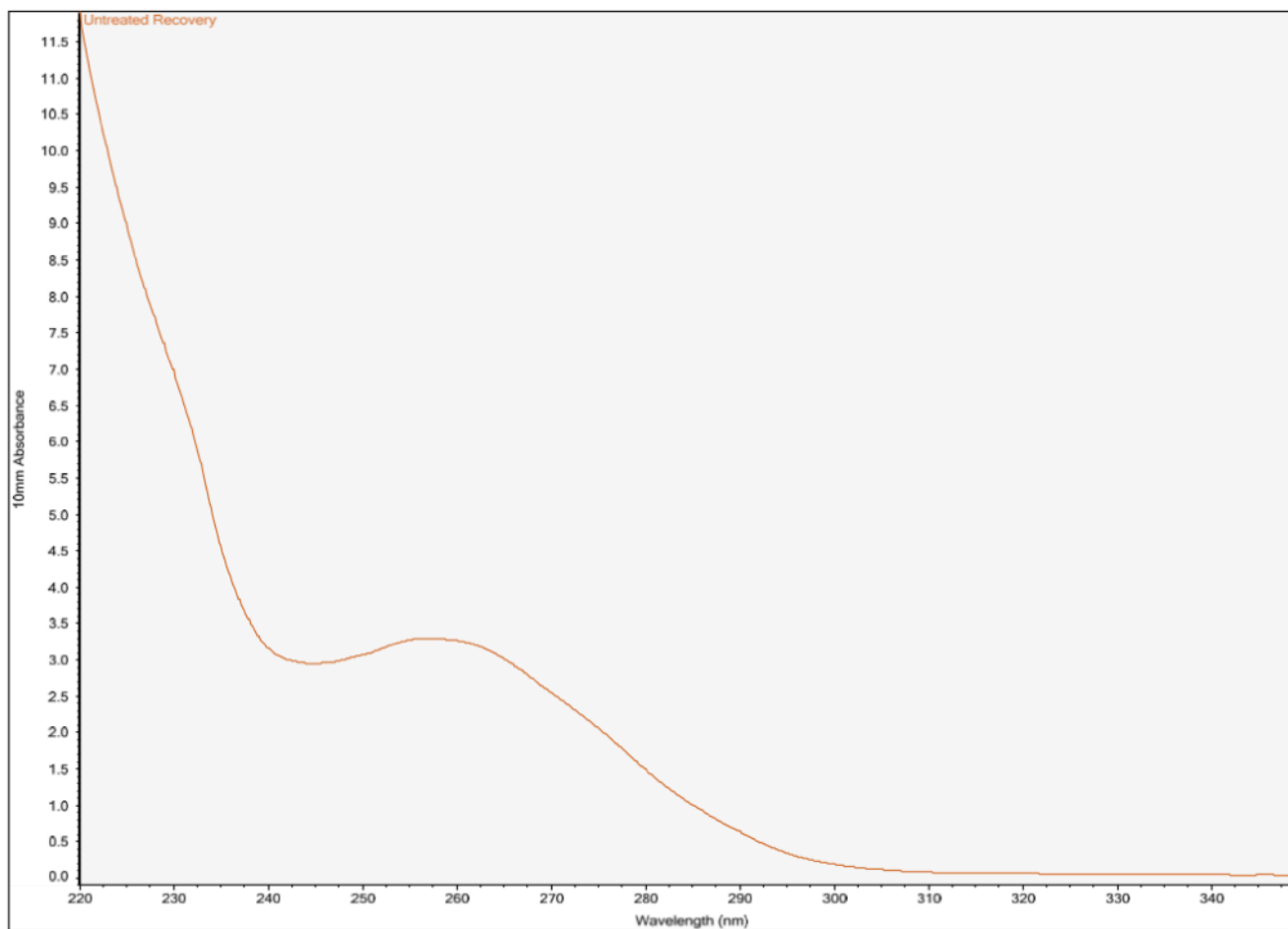
The ratio of absorbance at 260 nm and 280 nm is used to assess the purity of DNA and RNA.

A ratio of ~1.8 is generally accepted as “pure” for DNA;

a ratio of ~2.0 is generally accepted as “pure” for RNA.

Unless there is something strange in the base-composition of the RNA, I would say that your sample is contaminated with a bit of residual Trizol, which is a phenol and guanidium thiocyanate based reagent. I don't know for guanidium thiocyanate, but for sure phenol is known to affect measurements of nucleic acids OD ratios.

#	Sample ID	User name	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type	Factor
1	Untreated 0 Min	Alex Leach	7/20/2022 1:57:52 PM	329.9	ng/μl	8.248	3.691	2.23	1.86	RNA	40.00
2	Untreated 60 Min	Alex Leach	7/20/2022 1:59:17 PM	215.6	ng/μl	5.390	2.411	2.24	1.76	RNA	40.00
3	Untreated Recovery	Alex Leach	7/20/2022 2:00:19 PM	129.0	ng/μl	3.224	1.454	2.22	0.46	RNA	40.00
5	Cadmium 0	Alex Leach	7/20/2022 2:02:11 PM	155.2	ng/μl	3.879	1.750	2.22	0.57	RNA	40.00
6	Cadmium 60	Alex Leach	7/20/2022 2:03:07 PM	492.1	ng/μl	12.301	5.644	2.18	0.72	RNA	40.00
7	Cadmium Recovery	Alex Leach	7/20/2022 2:04:22 PM	782.2	ng/μl	19.556	8.720	2.24	1.91	RNA	40.00



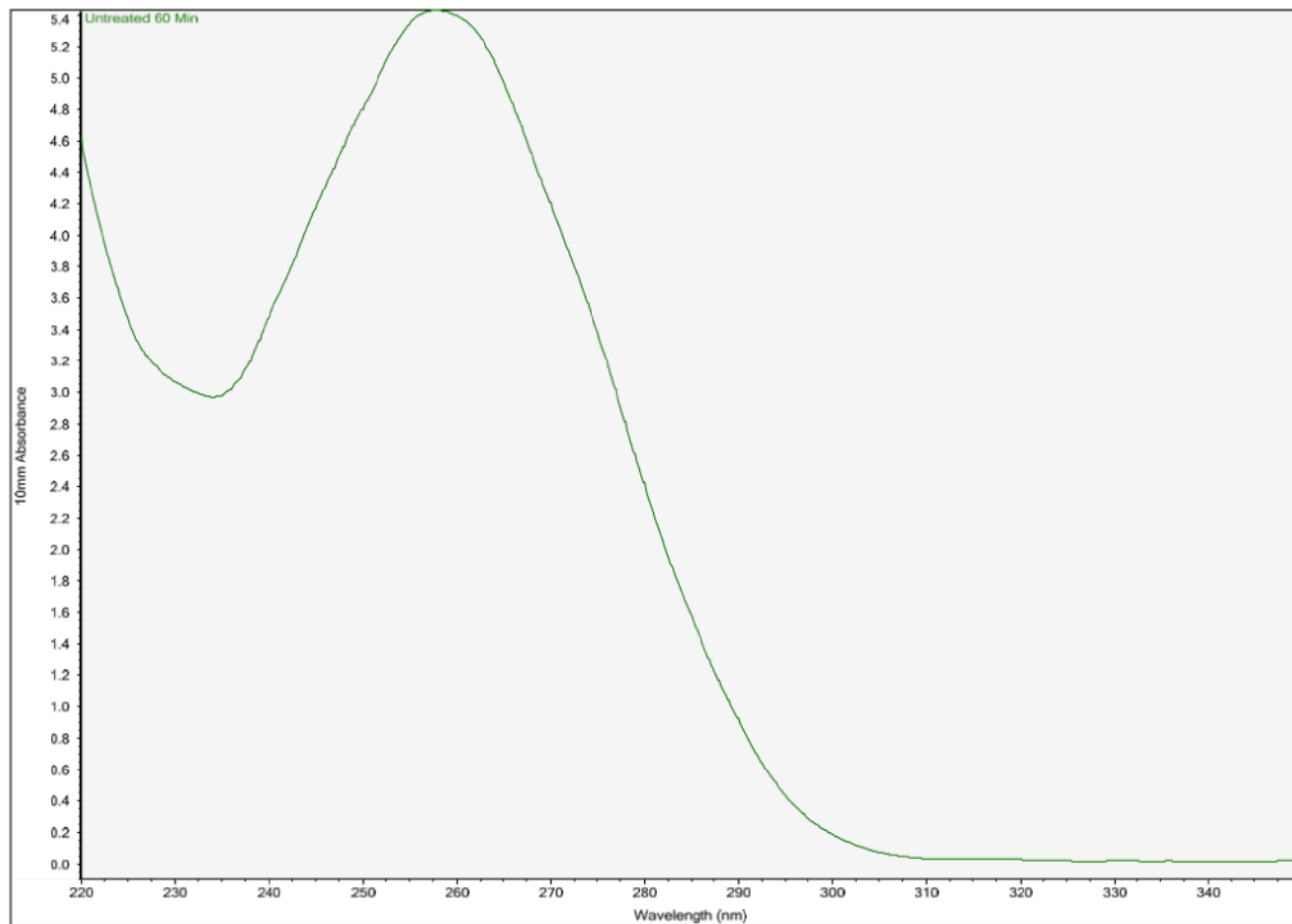
Principle and procedure

RNA purification using RNeasy technology

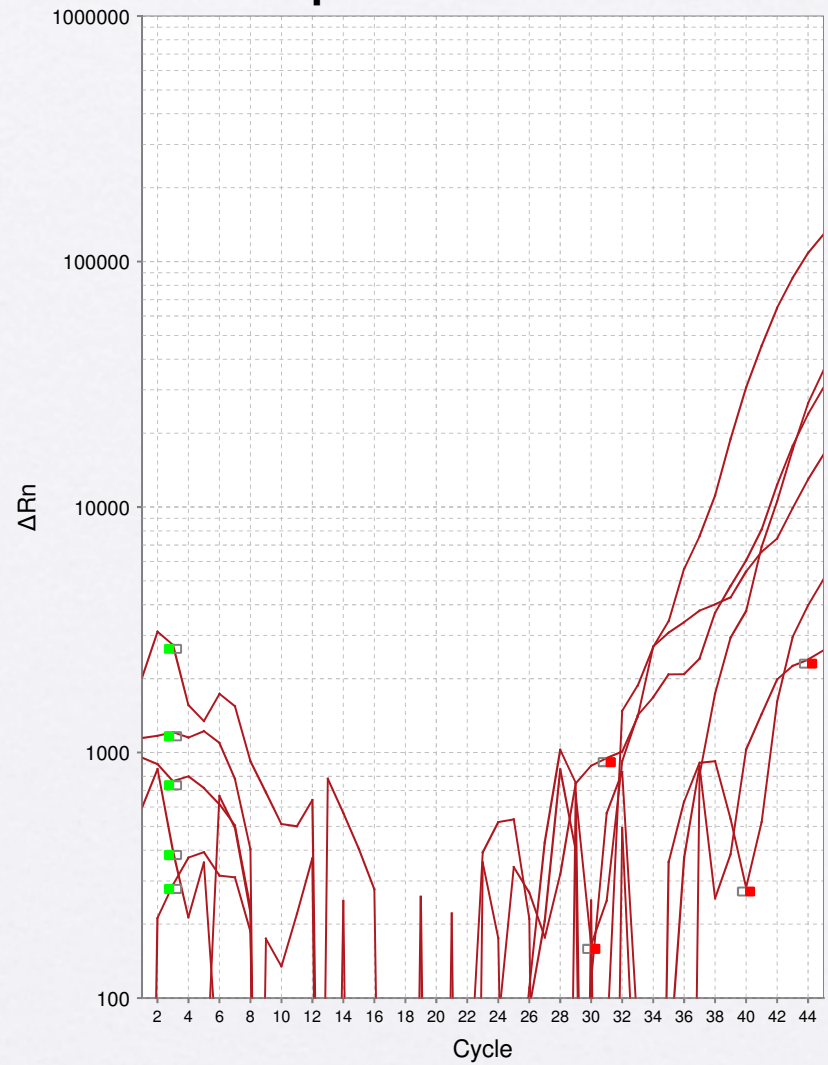
The RNeasy procedure represents a well-established technology for RNA purification. This technology combines the selective binding properties of a silica-based membrane with the speed of microspin technology. A specialized high-salt buffer system allows up to 100 µg of RNA longer than 200 bases to bind to the RNeasy silica membrane. Biological samples are first lysed and homogenized in the presence of a highly denaturing guanidine-thiocyanate-containing buffer, which immediately inactivates RNases to ensure purification of intact RNA. Ethanol is added to provide appropriate binding conditions, and the sample is then applied to an RNeasy Mini spin column, where the total RNA binds to the membrane and contaminants are efficiently washed away. High-quality RNA is then eluted in 30–100 µl water.

For an OD > 2.0, when purified using the RNeasy technology the RNA sample probably contains higher bits of **residual Trizol**, which is a phenol and **guanidium thiocyanate** based reagent. I don't know for guanidium thiocyanate, but for sure phenol is known to affect measurements of nucleic acids OD ratios.

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Amplification Plot

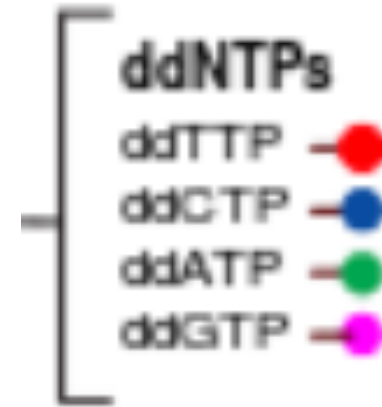
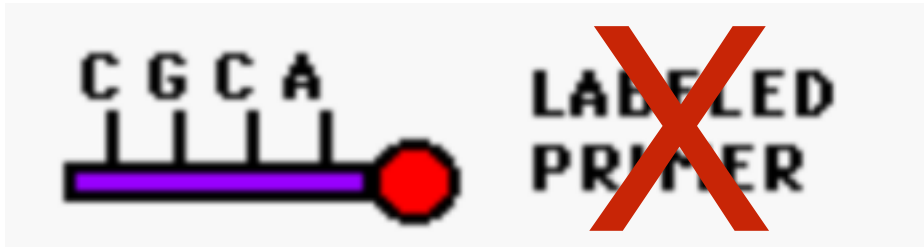


■ Genes PCR plots ...Low RNA / DNA template concentrations

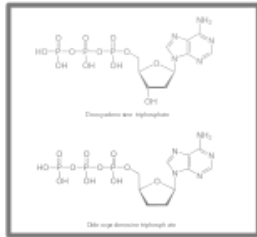


Genetic Analyzer
(ABI/ *Life technologies*) Model 3500xl

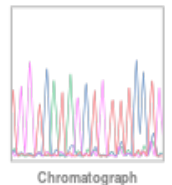
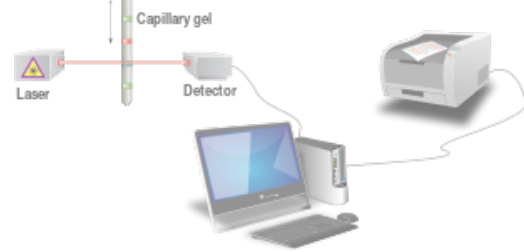
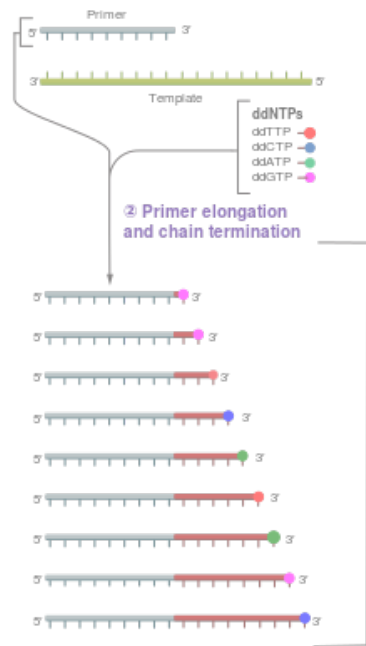
Sanger di-deoxy Sequence Analysis



- ① Reaction mixture
- ▶ Primer and DNA template
 - ▶ DNA polymerase
 - ▶ ddNTPs with flouochromes
 - ▶ dNTPs (dATP, dCTP, dGTP, and dTTP)



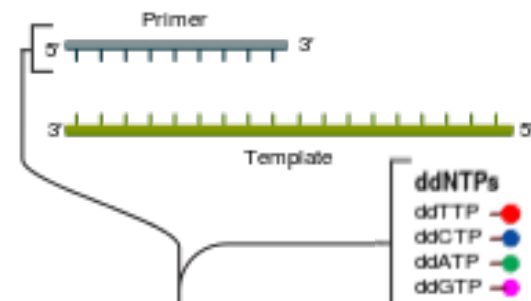
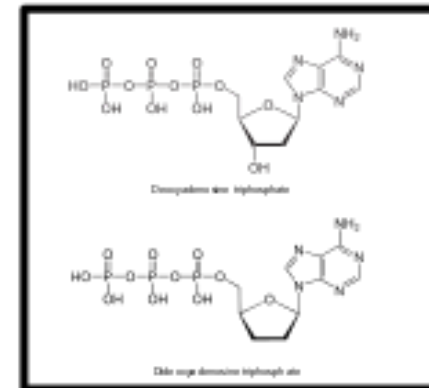
③ Capillary gel electrophoresis separation of DNA fragments



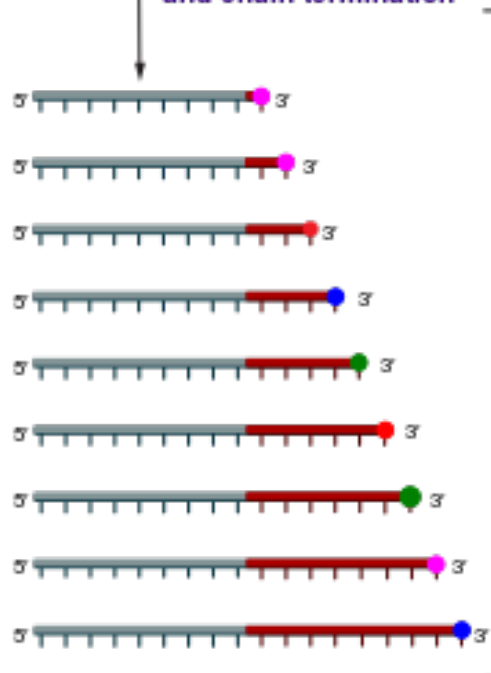
④ Laser detection of flouochromes and computational sequence analysis

① Reaction mixture

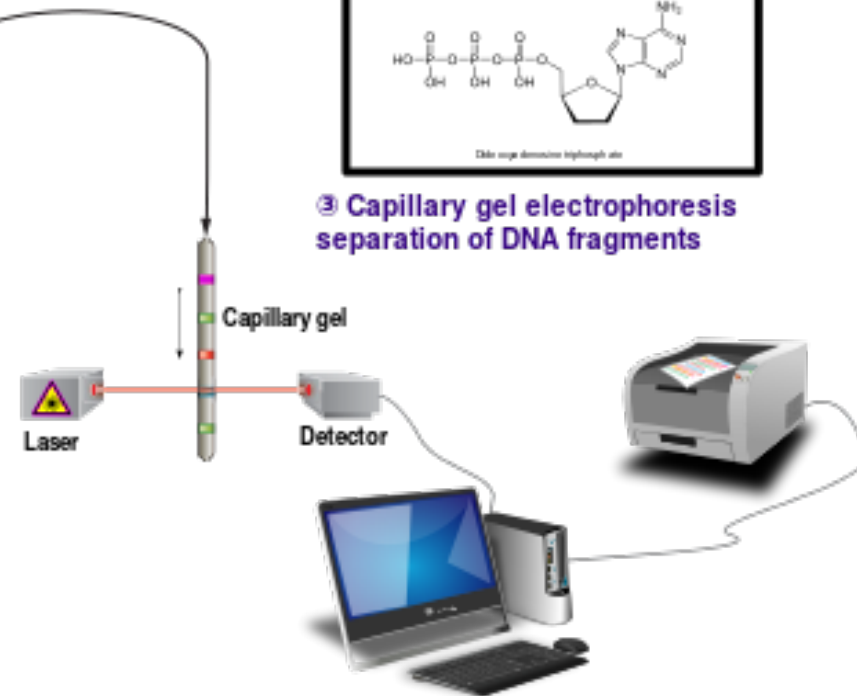
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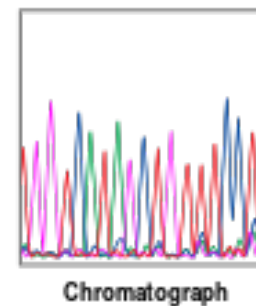
② Primer elongation and chain termination



③ Capillary gel electrophoresis separation of DNA fragments



④ Laser detection of flouochromes and computational sequence analysis

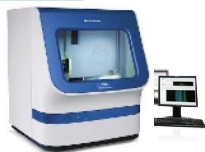


Sanger Sequencing

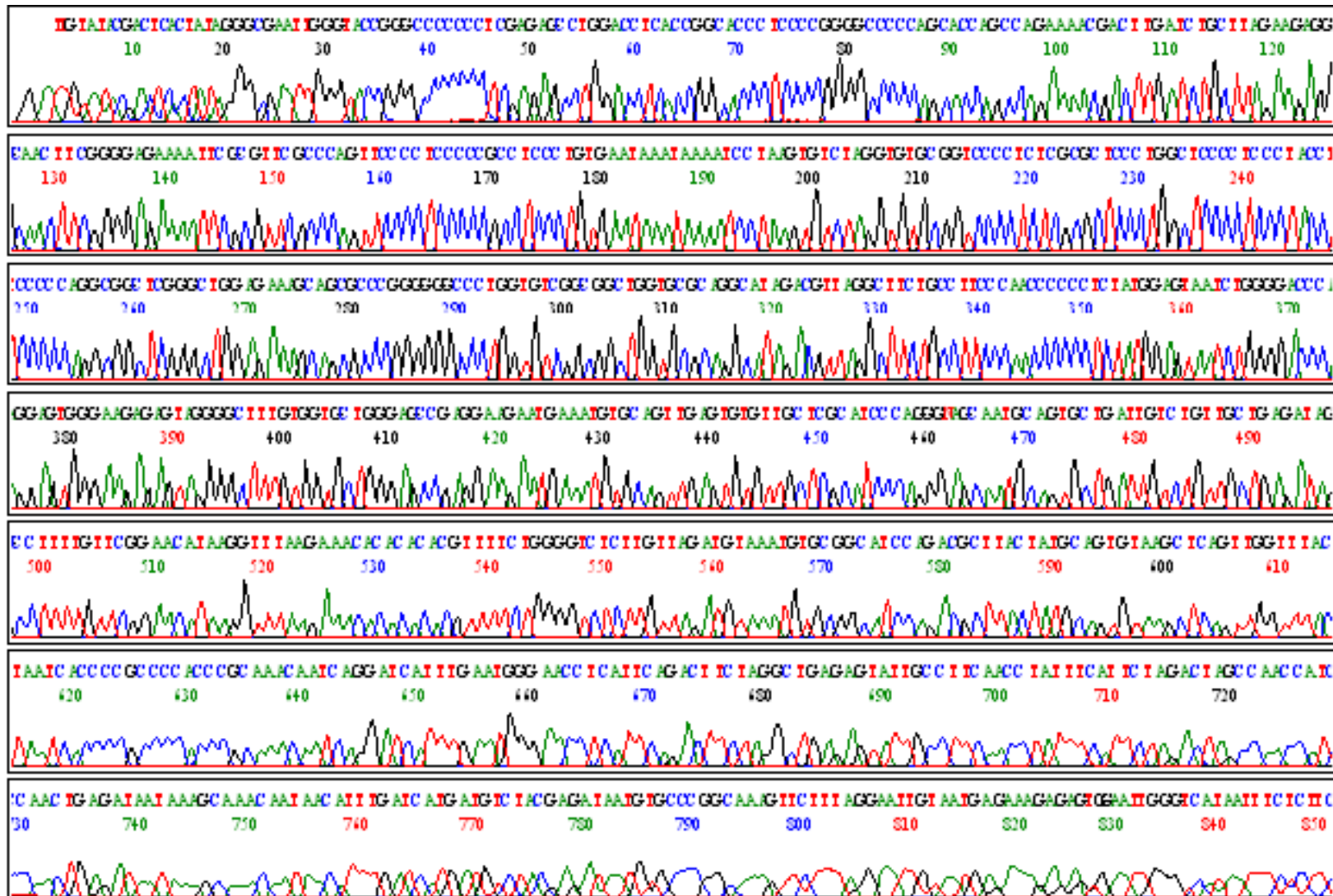
- Ideal for single gene assays
- Target gene candidates

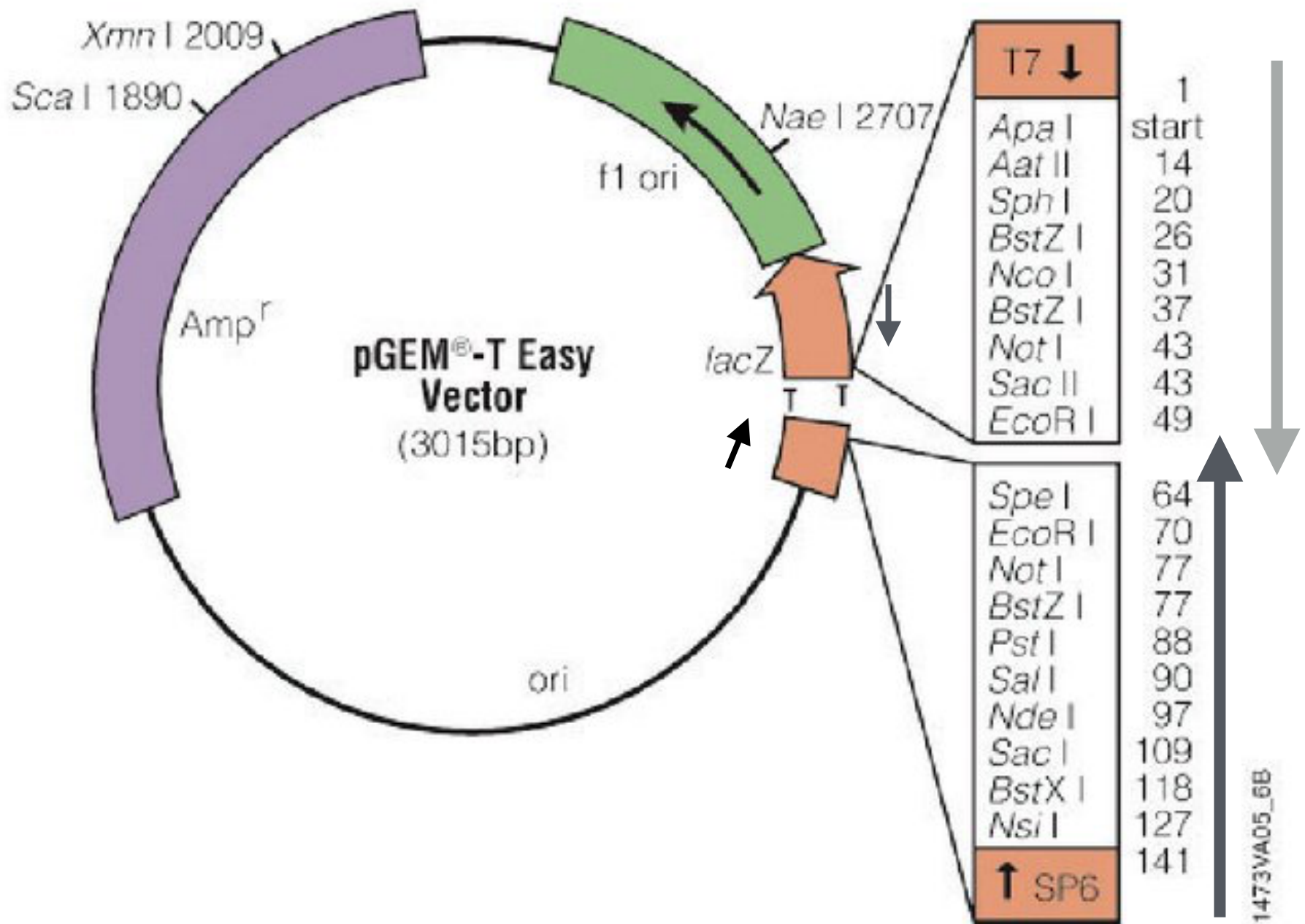
Single-gene (few amplicons)

p53



- Few amplicons, few samples
- Bidirectional sequencing
- Can be used to confirm variants from PGM







Sall #R31 RR 37° 165' CpG

We are excited to announce that all reaction buffers are now BSA-free. NEB began switching our BSA-containing reaction buffers in April 2021 to buffers containing **Recombinant Albumin (rAlbumin)** for restriction enzymes and some DNA modifying enzymes. Find more details at www.neb.com/BSA-free.

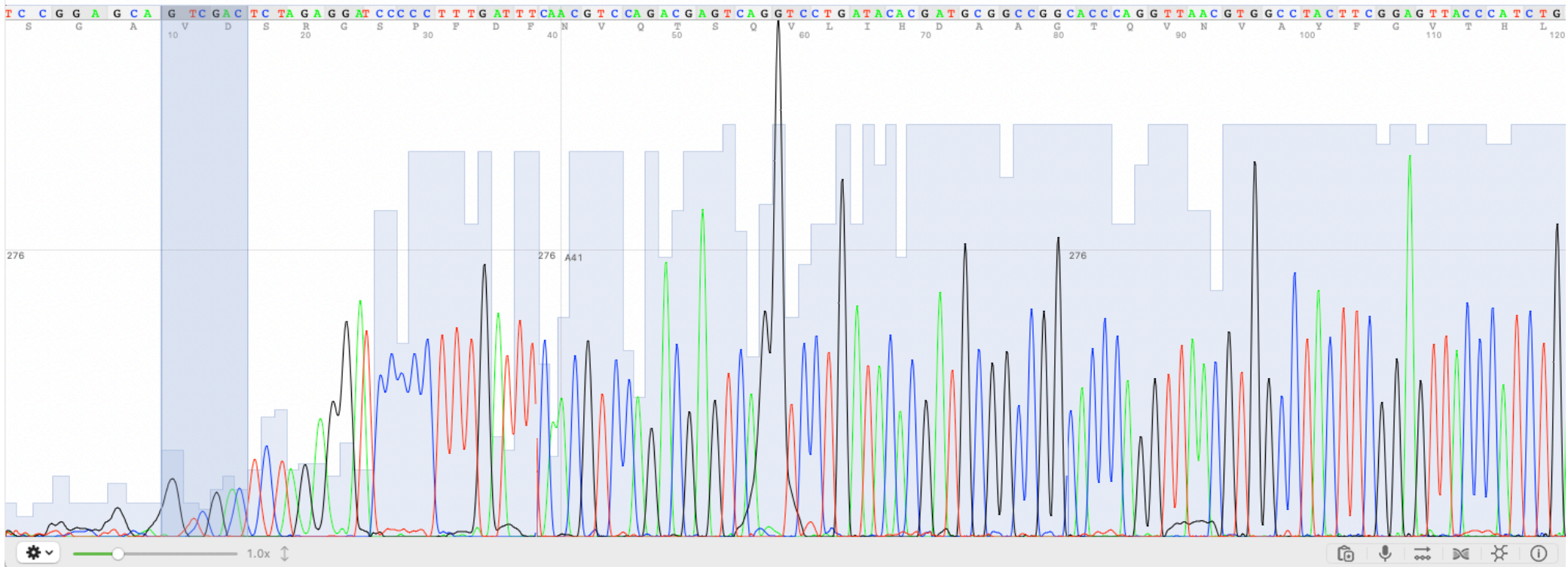


Isoschizomers | Single Letter Code | Pronunciation:

- Time-Saver™ qualified for digestion in 5-15 minutes
- High Fidelity (HF®) version available (NEB #R3138) supplied with rCutSmart™ Buffer
- Supplied with 1 vial of Gel Loading Dye, Purple (6X)
- Restriction Enzyme Cut Site: G/TCGAC

[75 Product Citations](#)

Q GTCGAC



Sall

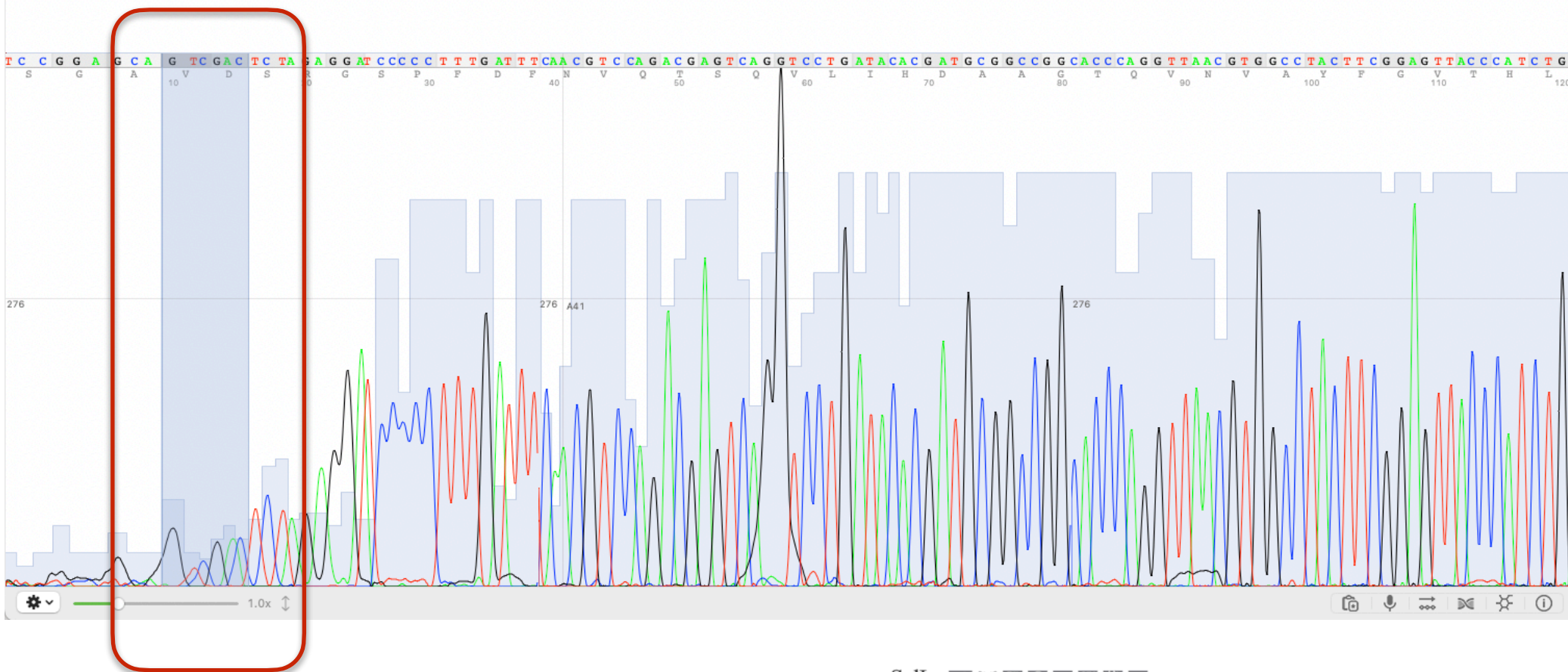
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75 Product Citations



SaI 

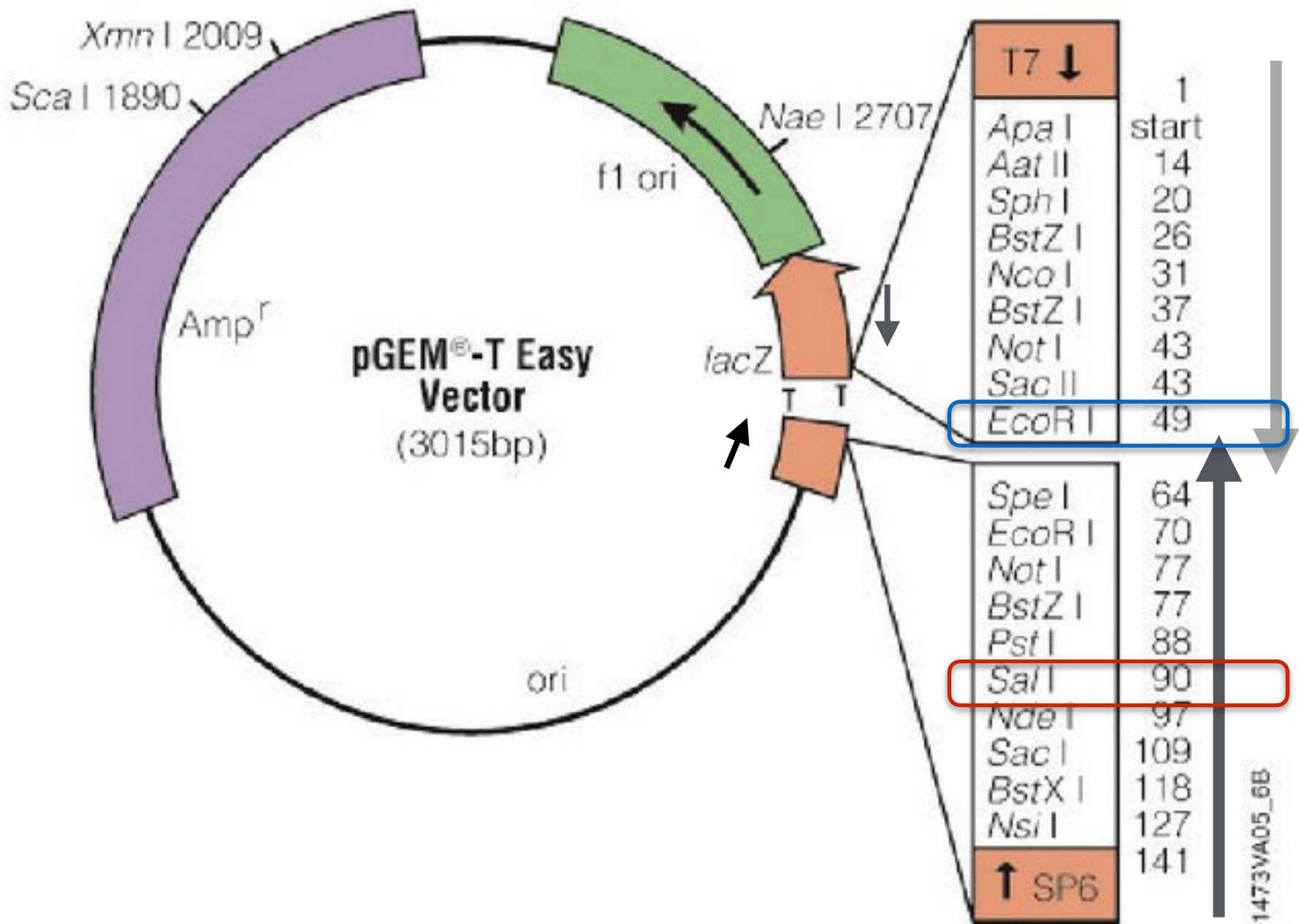
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5'... G^TTCGAC... 3'
3'... CAGC_TG... 5'

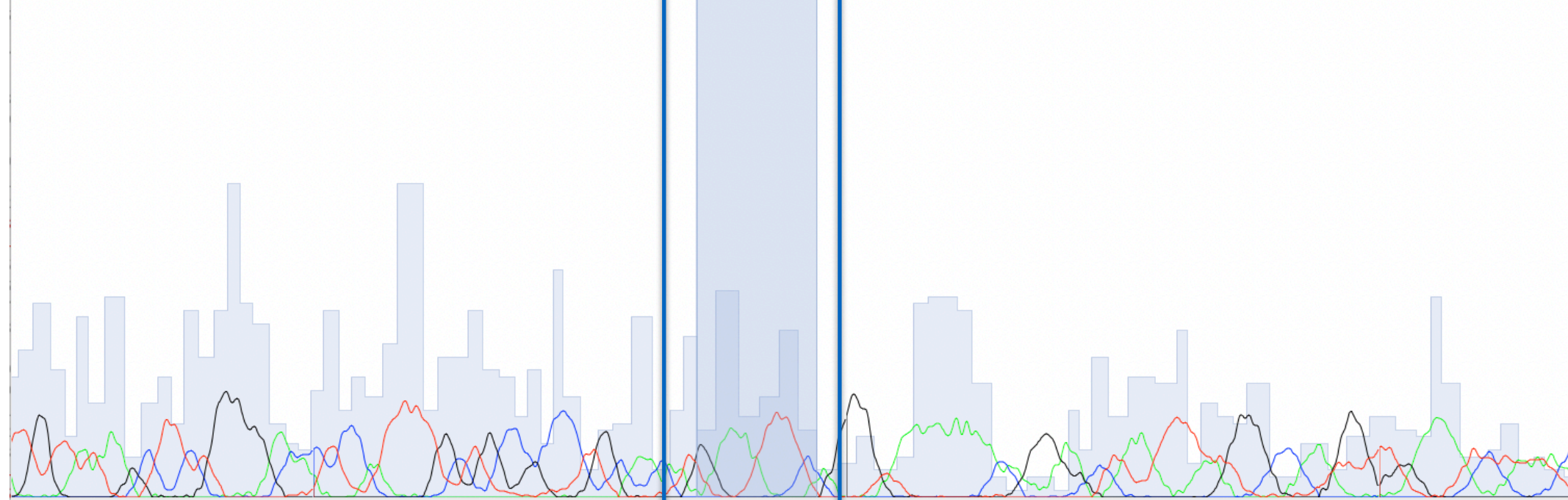
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T G T T A T A G C T T C T G G G G A C A C T C C A T T T T G C T G C C G C C C T G C A C A T G A A T T C A G G T A A A A A A C A G A A G C T A T T T A T G G C A T G T G A A A T C C T A T A



EcoRI

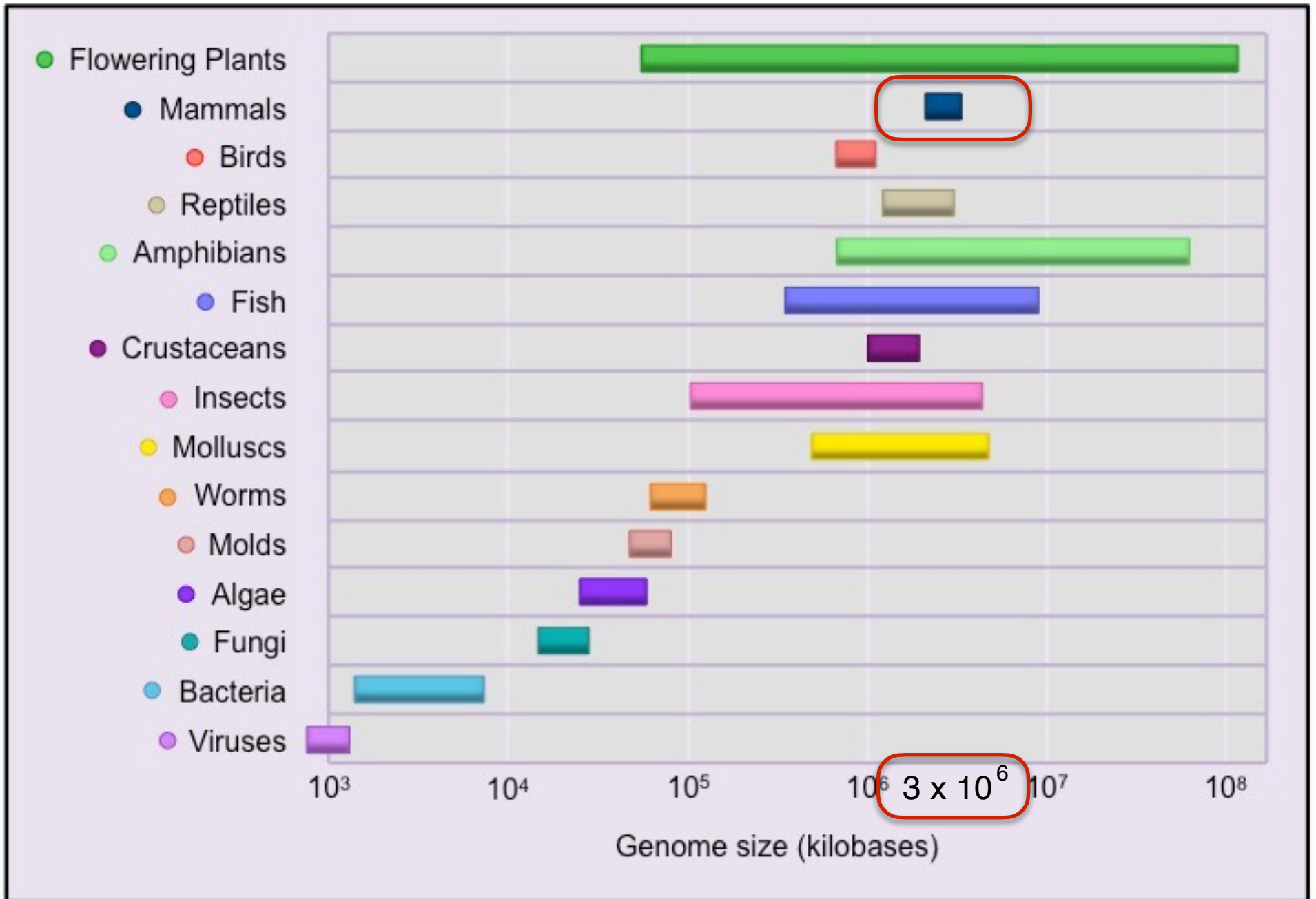
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- Now comes supplied with 1 vial of Gel Loading Dye, Purple (6X)
- Restriction Enzyme Cut Site: G/AATTC

2,332 Product Citations



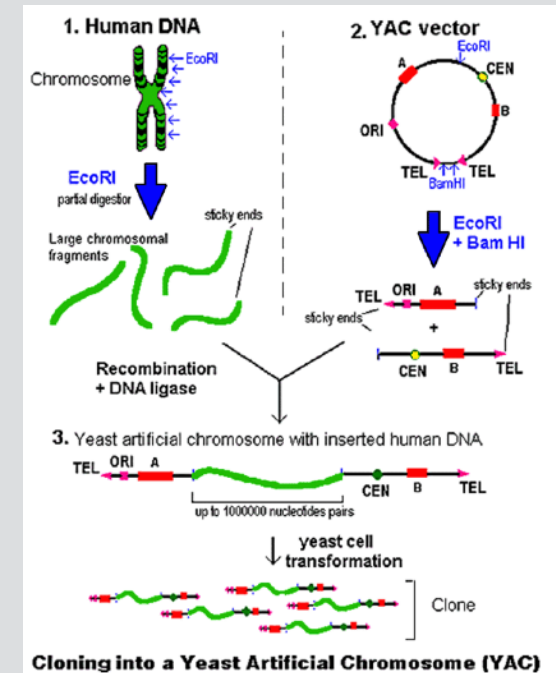
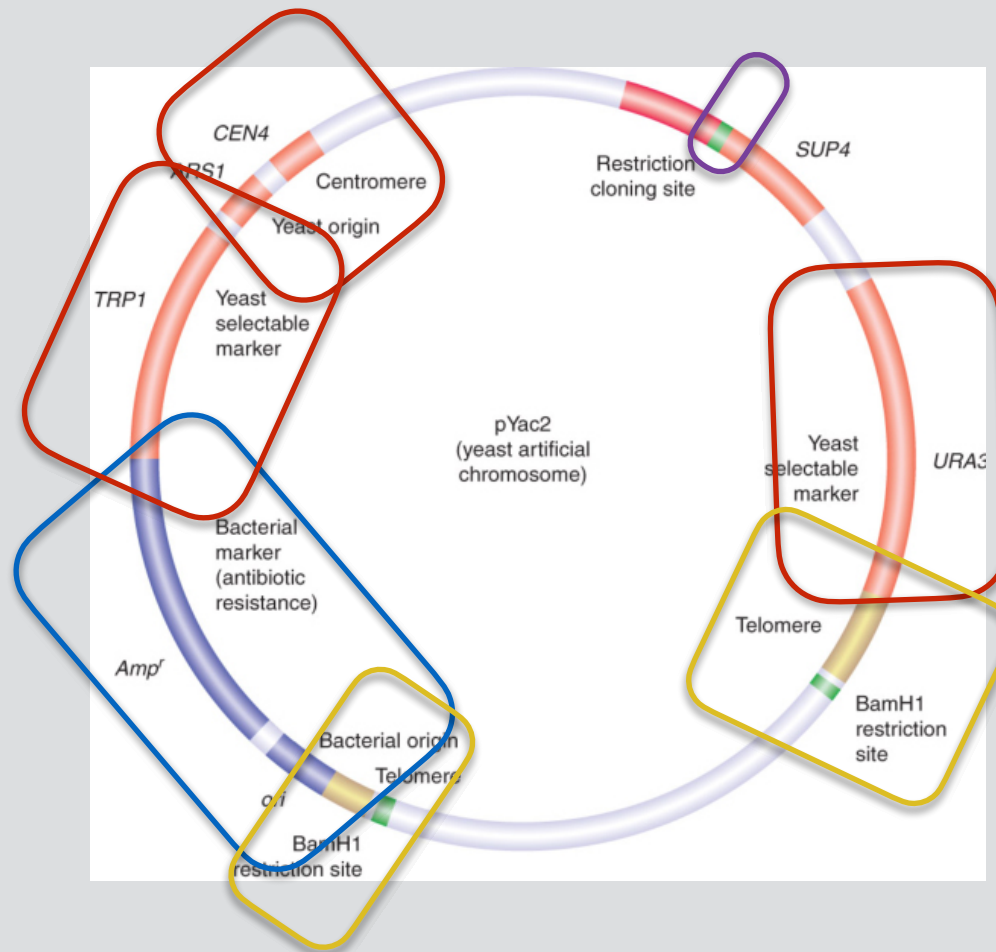
3×10^9 bp

Potential problems:

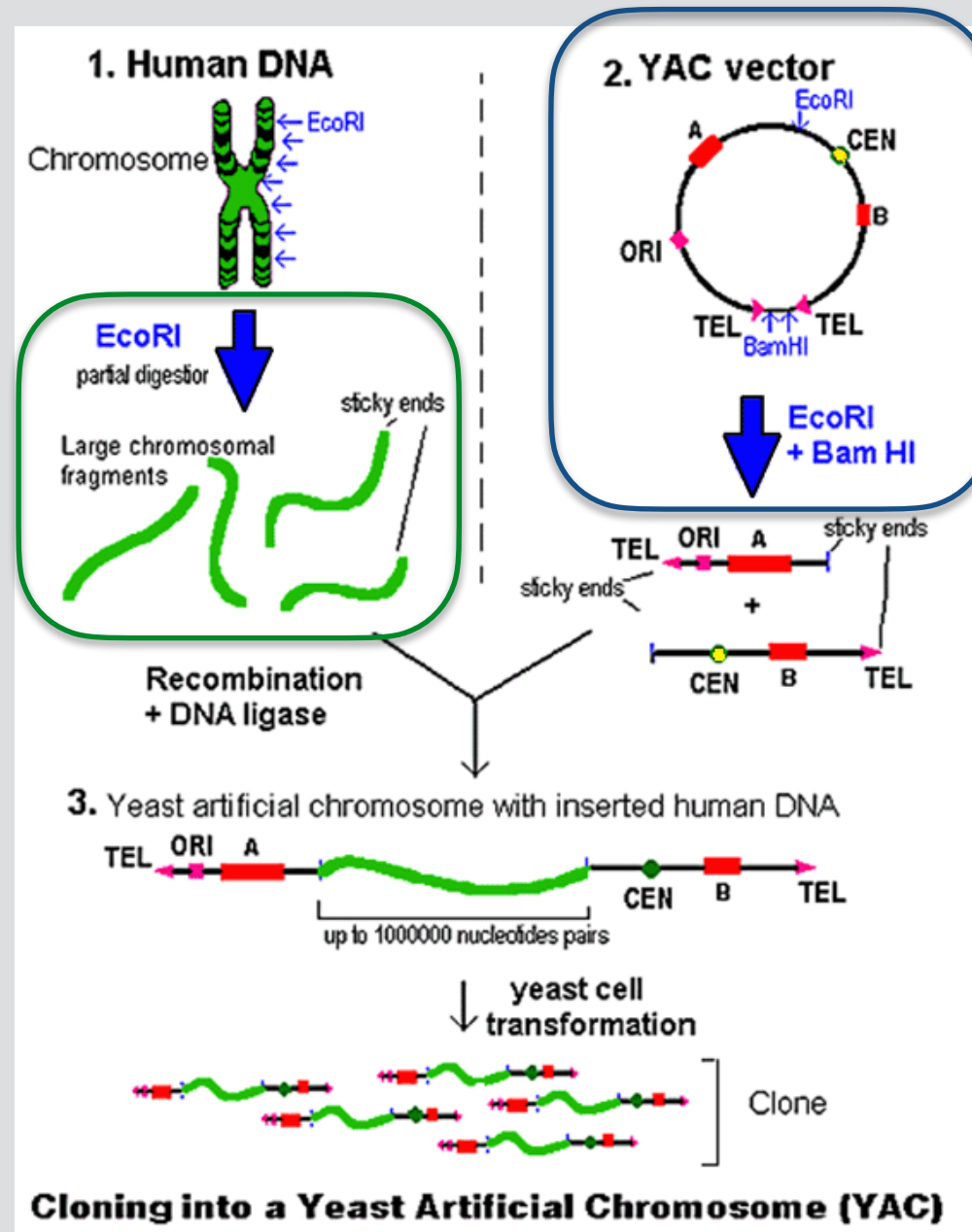
Size of genomes vary among the different 'karyotes', causing logistical problems.

Complexity and Gene structure.

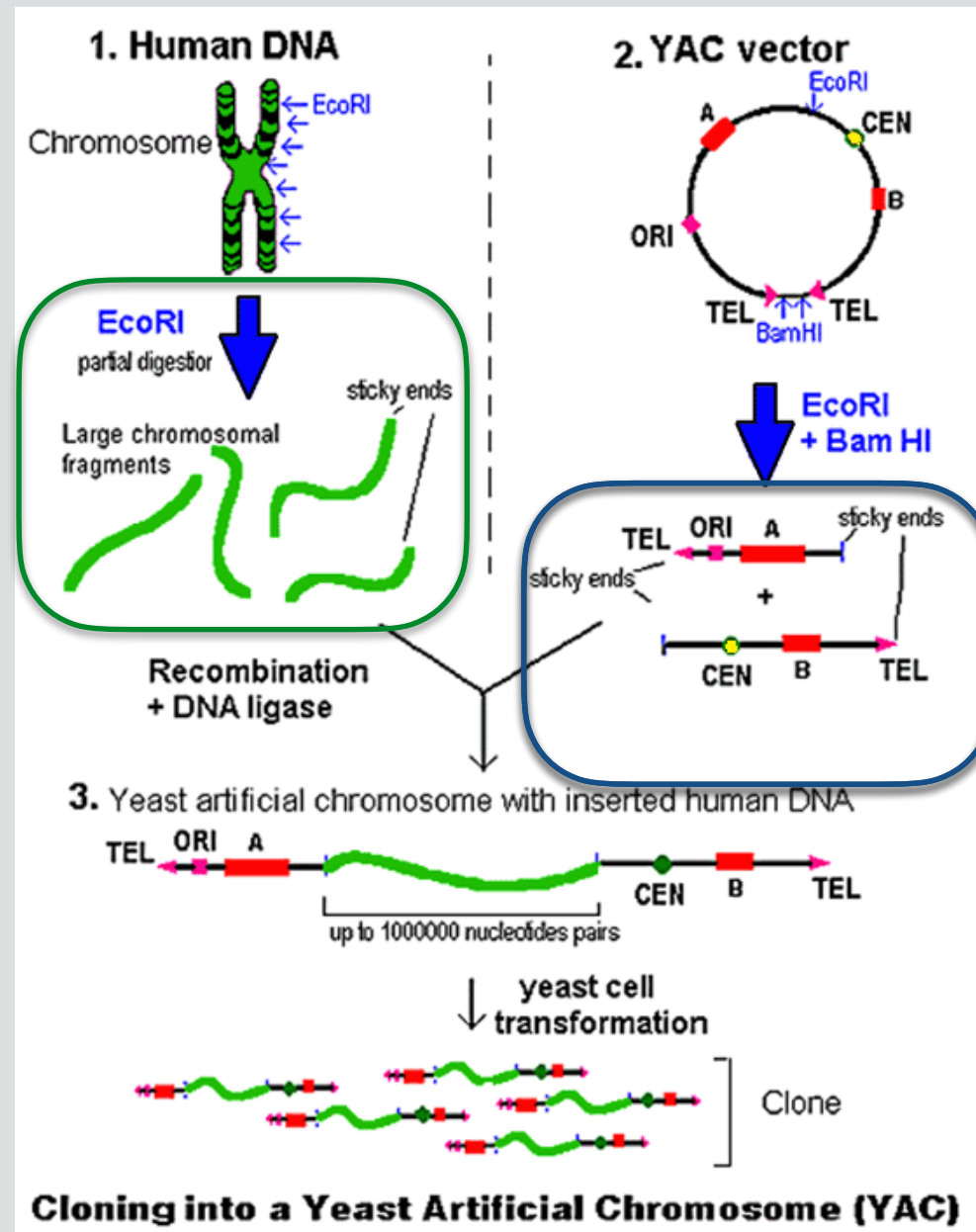
Use of **other cloning vehicles**, such as **YAC's** (**Y**east **A**rtificial **C**hromosomes) can enhance this capacity even further....to approximate **300 kbp** to **> 1 Mbp** of unknown sequence.



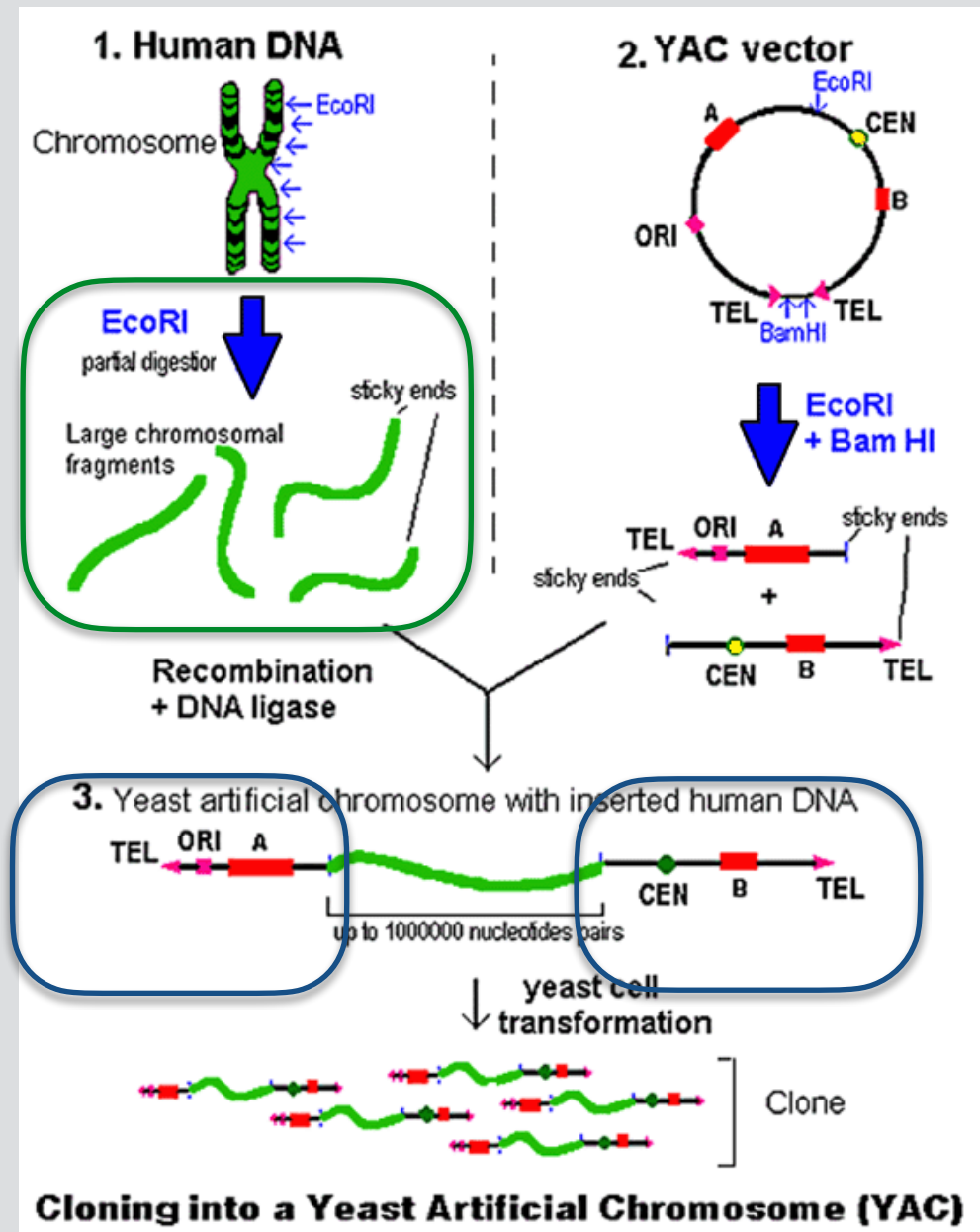
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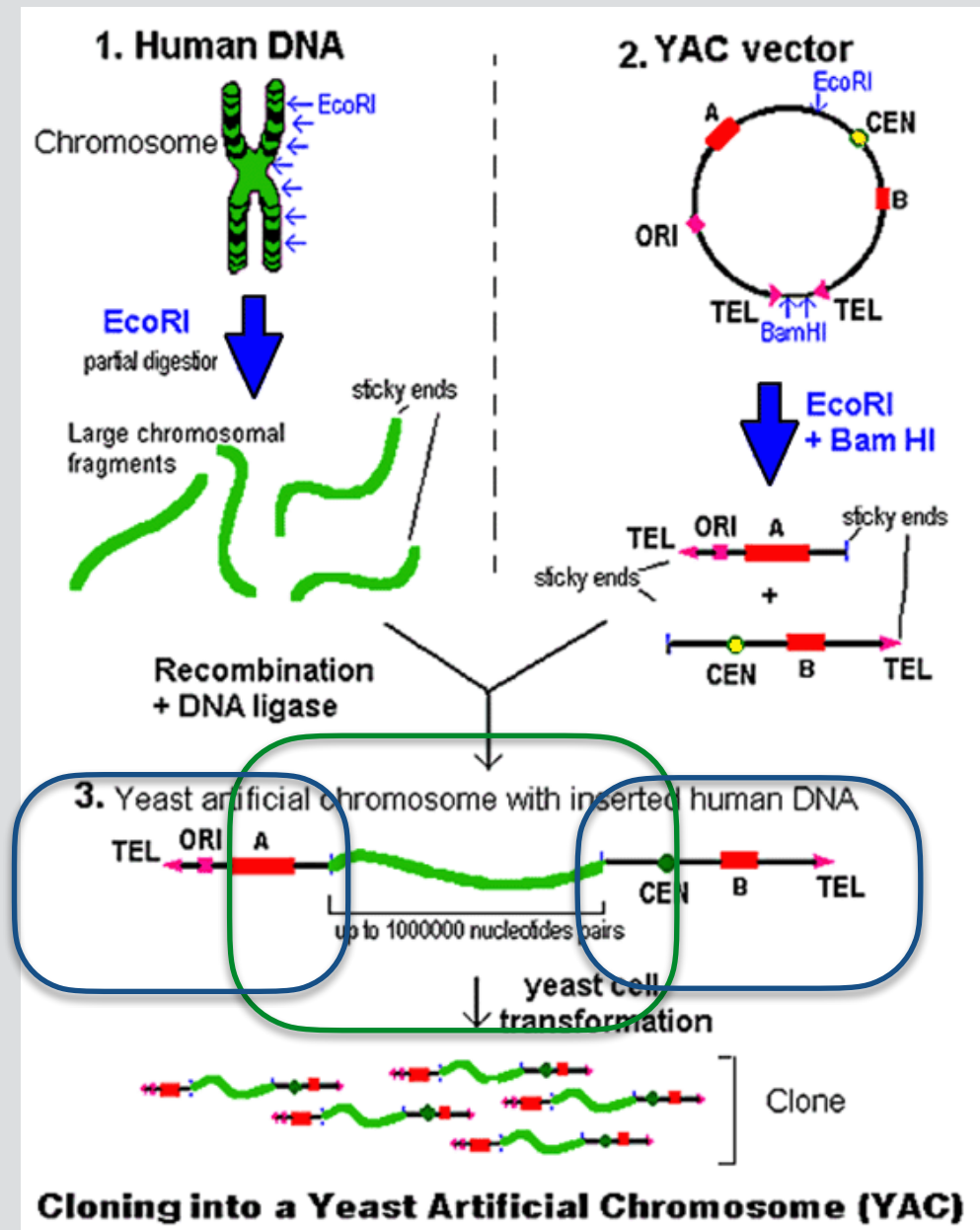
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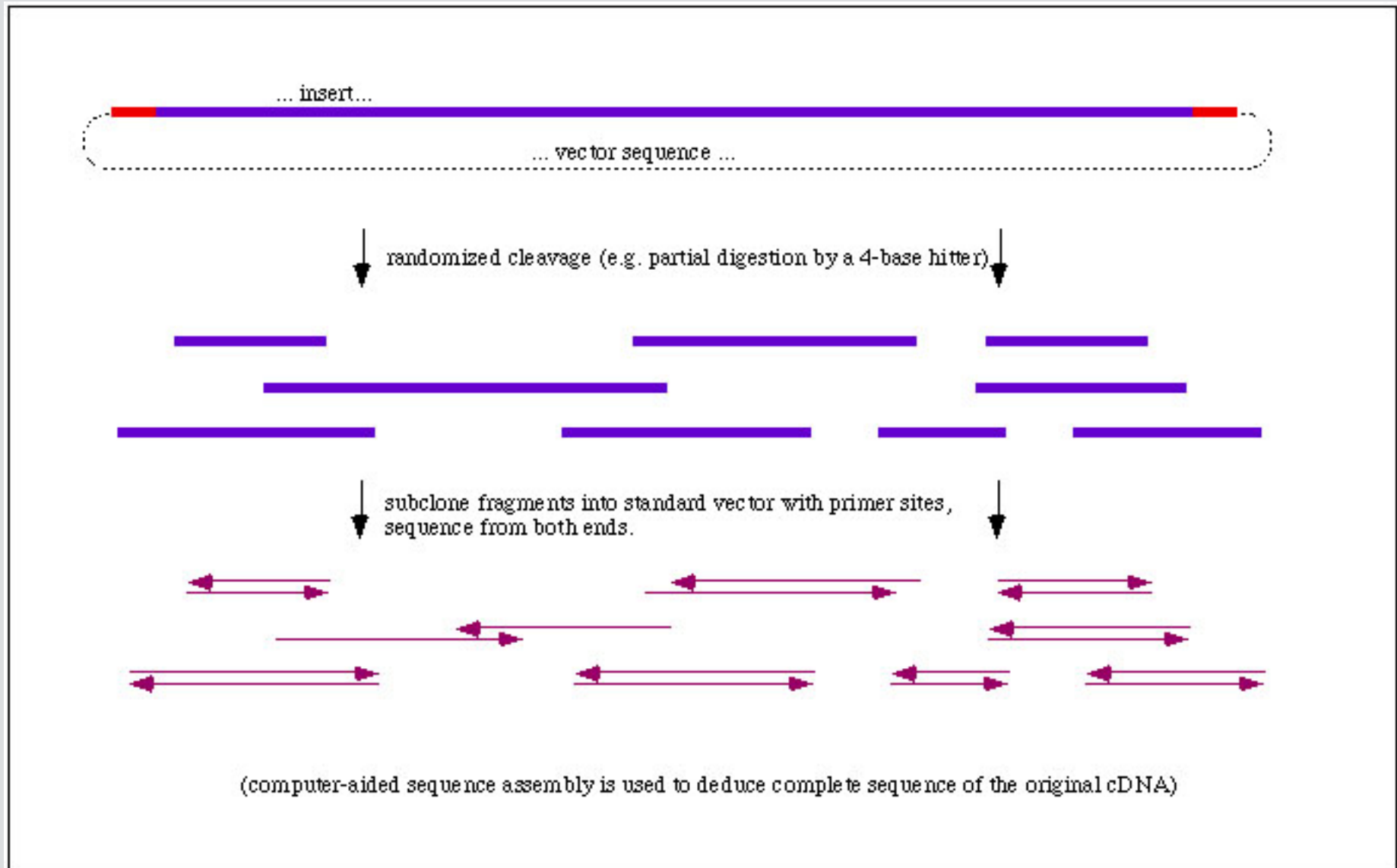
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Sanger sequencing throughput

- Run up to 384 samples
- 700 base reads
- Up to 1 million bases per day
- Human genome is 6 billion bases
- Covered 7 times
- Over 100 years on one machine

Next-Generation, Deep Sequencing

Illumina sequencers



	MiSeq	HiSeq	NovaSeq	Sanger
Reads (millions)	30	3,000	13,000	0.0004
Gigabases/day	7	500	4000	0.001

Next-Generation, Deep Sequencing

Illumina sequencers



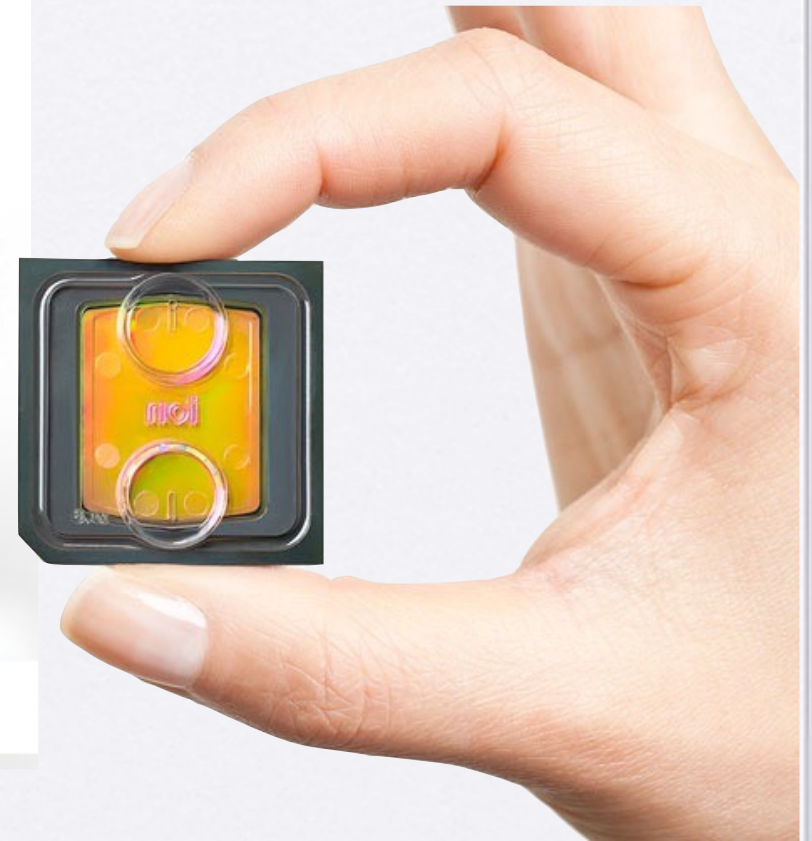
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Ion Proton Next-Gen, Deep Sequencing
(ABI / Life technologies)

Template Preparation

Genomic DNA or cDNA

Library preparation

Fragmentation of DNA

Adapter ligation

Library amplification

Emulsion PCR

Cluster generation

DNA is amplified onto microbeads

DNA is bridge amplified onto flow cell

Sequencing and imaging

Ion Torrent PGM

MiSeq

ATAGTACTTACTA

TAT↑

C

pH change

ATAGTACTTACTA

TAT↑

C

Fluorescence

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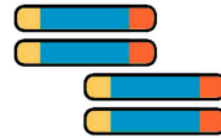
Genomic DNA or cDNA



Library preparation



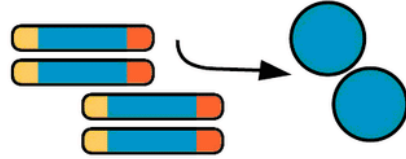
Fragmentation of DNA



Adapter ligation

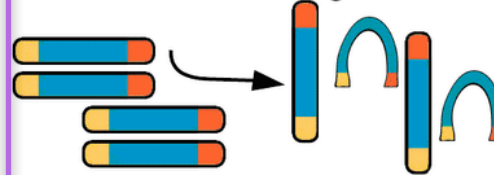
Library amplification

Emulsion PCR



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Cluster generation



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TAT ↑
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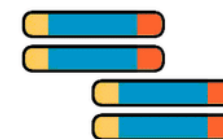
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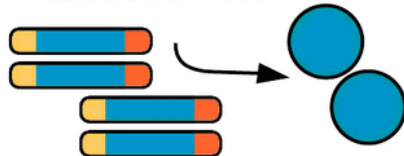
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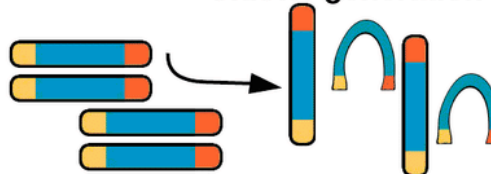
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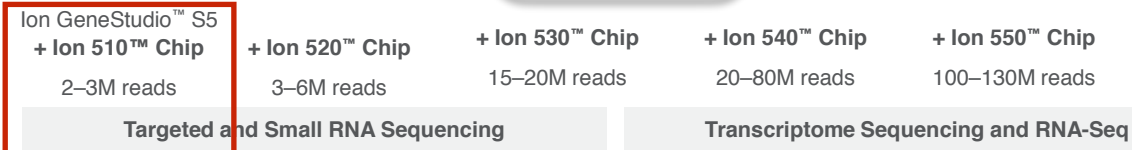
Fluorescence

Data Analysis

Ion GeneStudio S5 Series | One Platform For All Your RNA Sequencing Needs

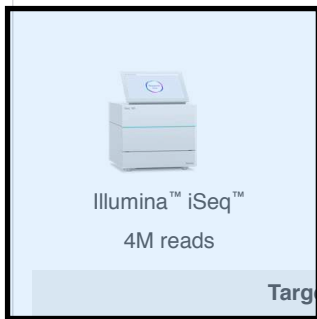
Ion Torrent

Sequence all RNA applications with only **one** Ion Torrent system



Illumina

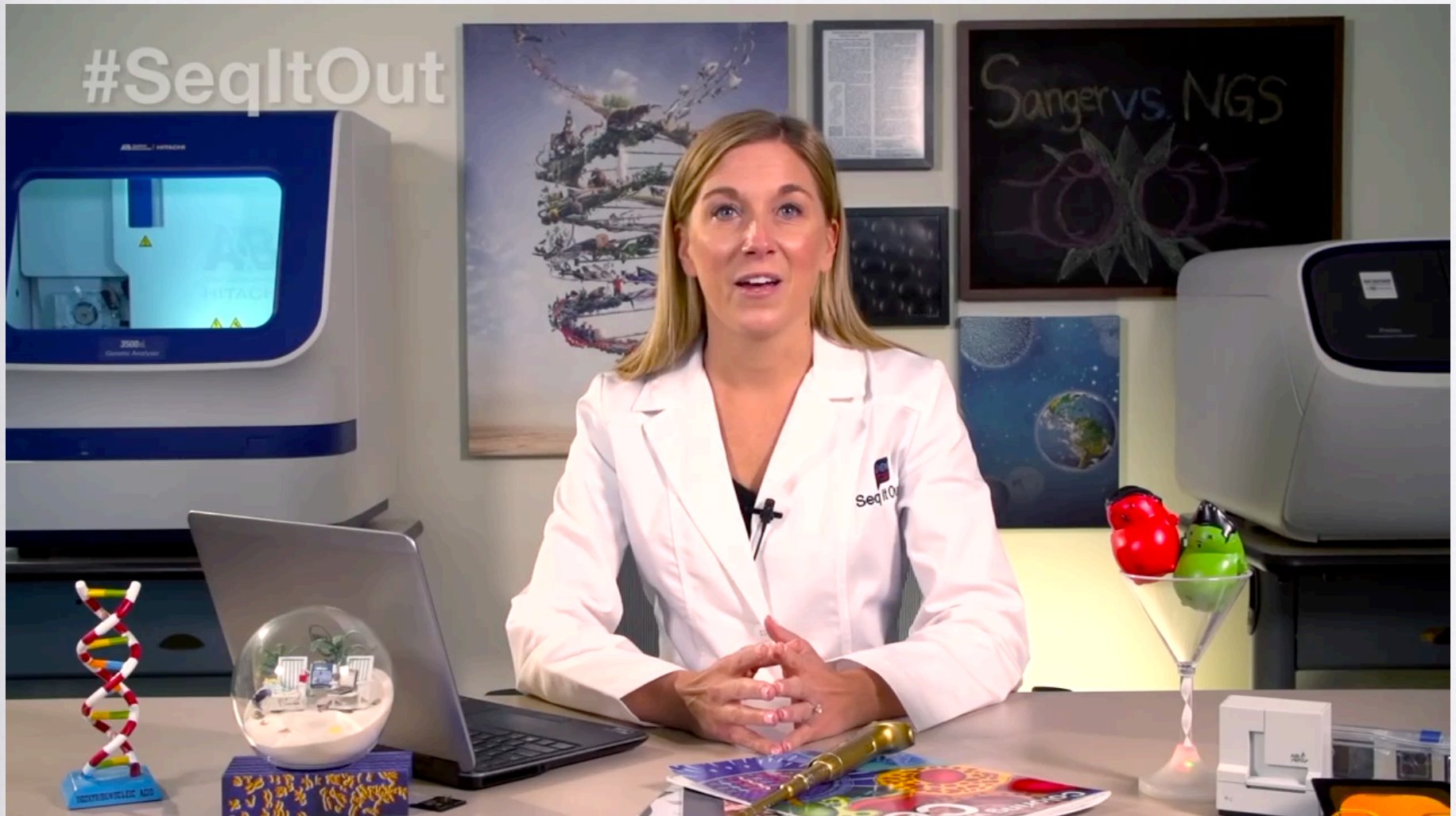
Sequence all RNA applications, with **four different** Illumina™ systems



Illumina sequencers



	MiSeq	HiSeq	NovaSeq	Sanger
Reads (millions)	30	3,000	13,000	0.0004
Gigabases/day	7	500	4000	0.001



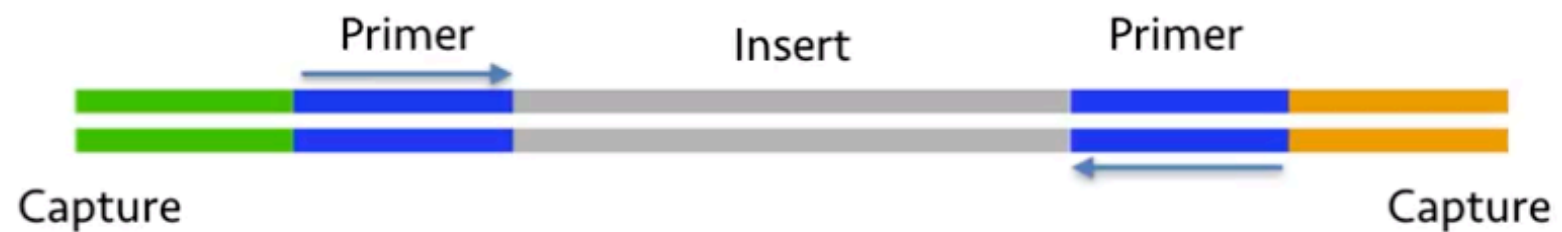
Automation, Ion Chef
(ABI / Life technologies)

Illumina sequencers



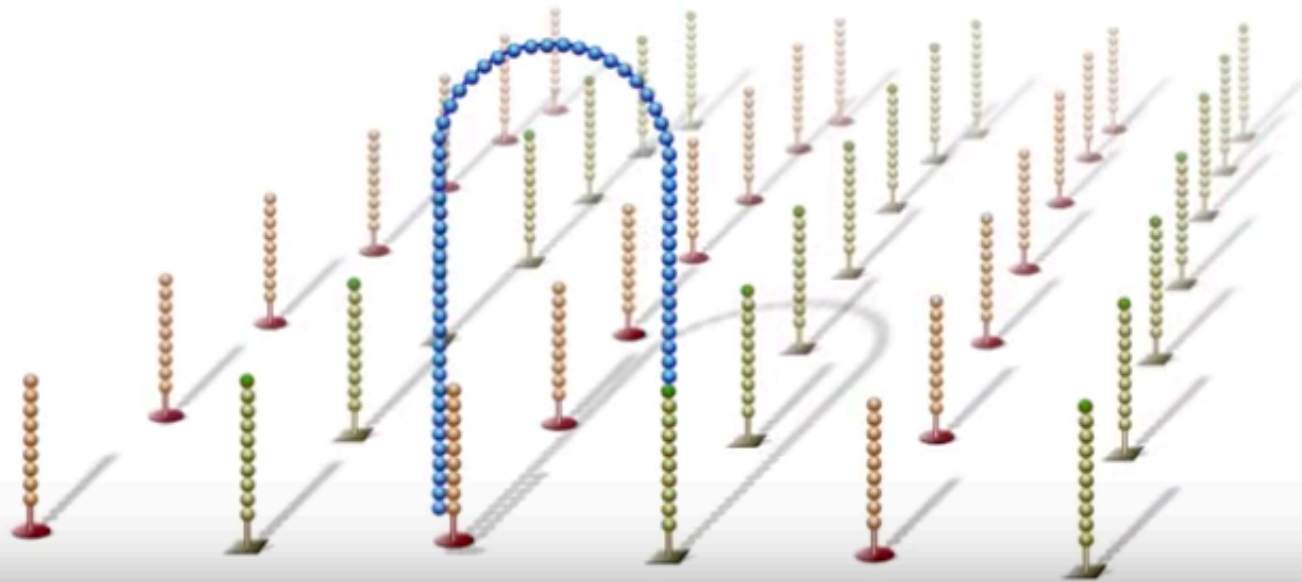
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Illumina Sequencing Libraries

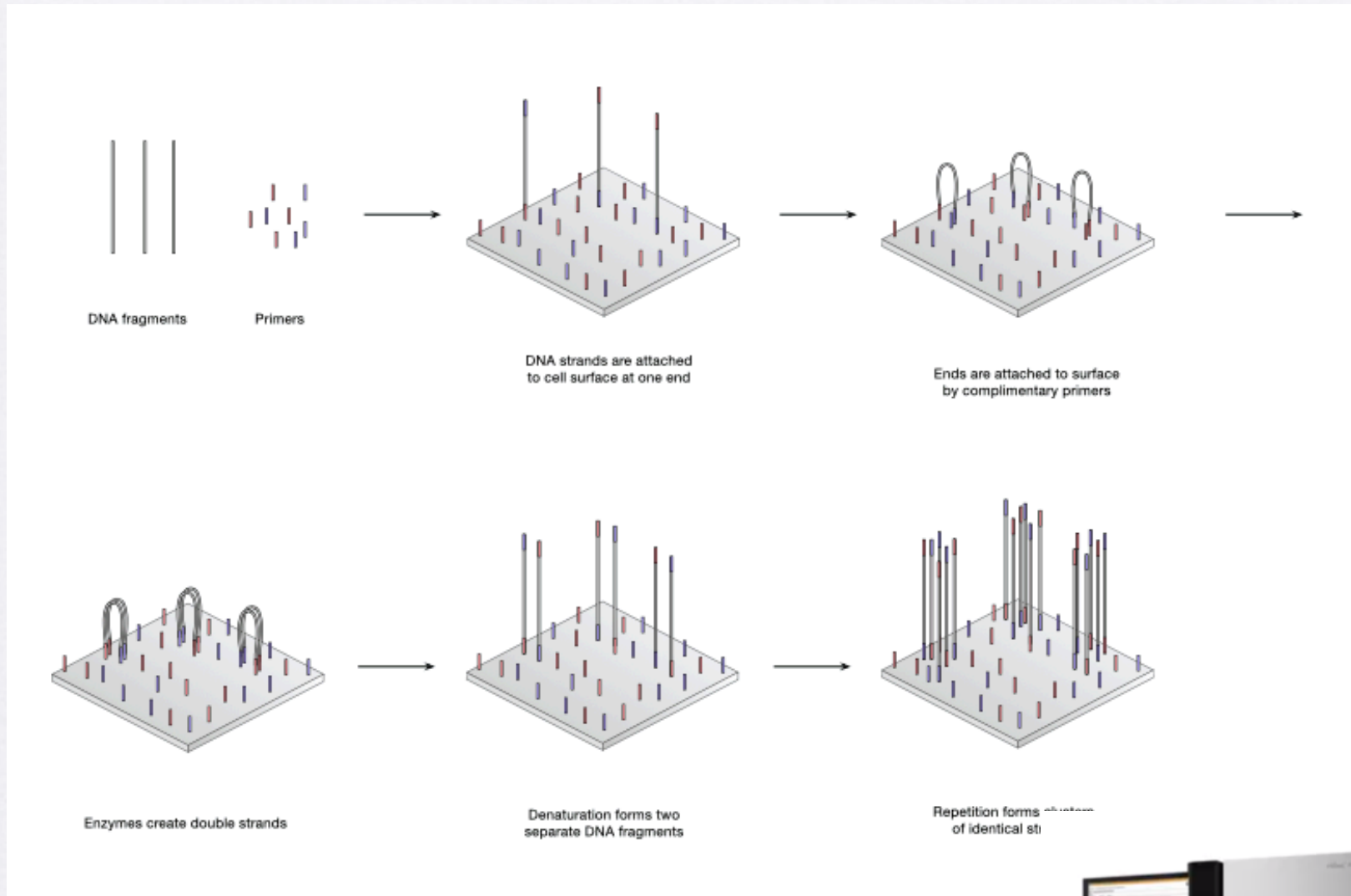


- Adapters required for sequencing
- Adapter sequence includes primer binding sites and capture sequences.

Flow cell clustering



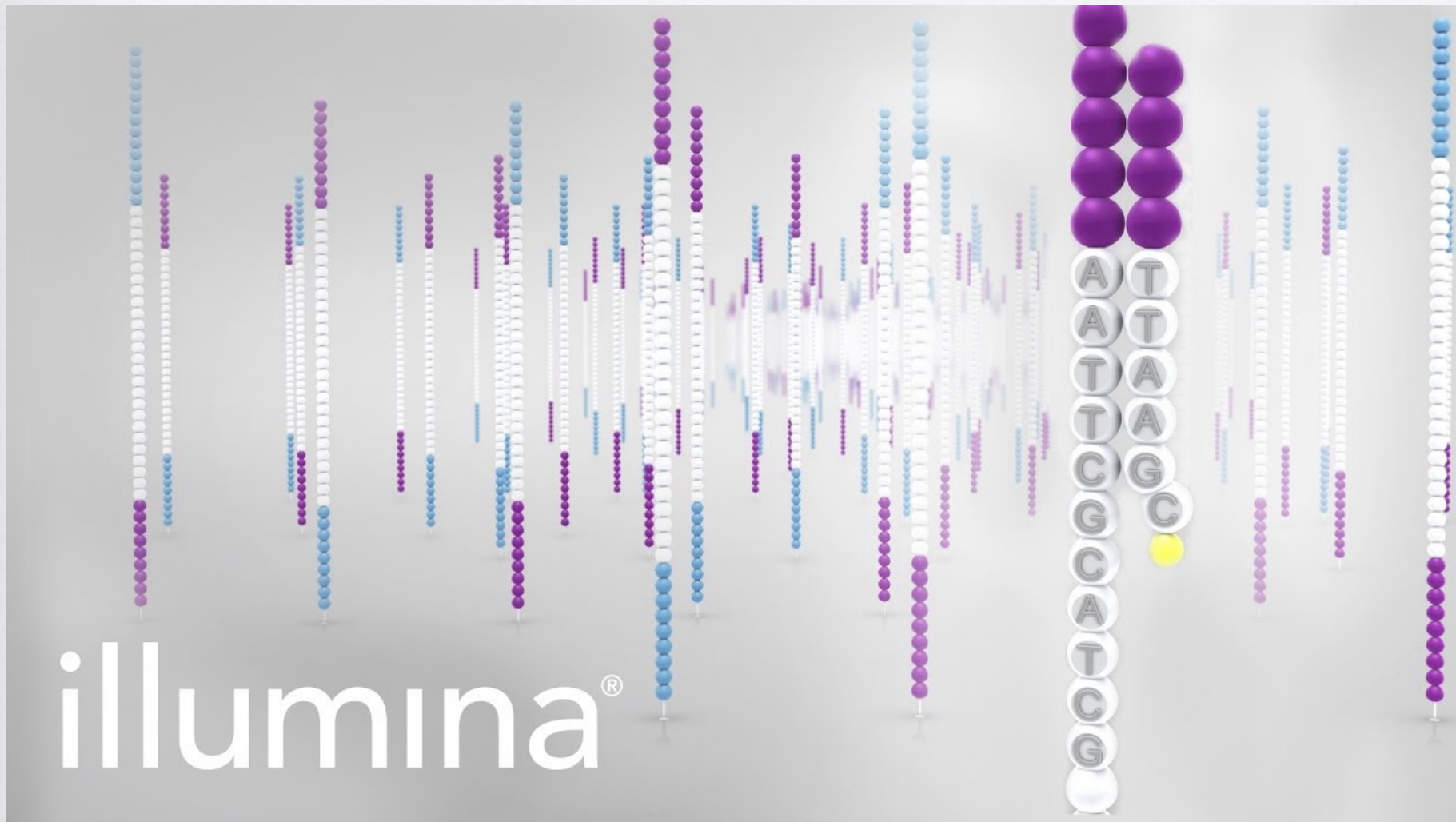
d



Bridge PCR amplification

Illumina Sequence Analysis





Bridge PCR amplification

Illumina Sequence Analysis



Ion GeneStudio S5 System | Simple. Fast. Scalable. Versatile.



	Ion GeneStudio™ S5	iSeq™	MiniSeq™	MiSeq™	NextSeq™
Scalability¹	2M, 5M, 20M, 80M, and 130M	4M only	8M and 25M	1M, 4M, 15M, and 25M	130M and 400M
Max throughput per week²	1,300M reads	20M reads	125M reads	125M reads	1,200M reads
Workflow simplicity³ (hands-on-time)	45 minutes Automated library prep on Chef	255 minutes Manual library prep	255 minutes Manual library prep	255 minutes Manual library prep	255 minutes Manual library prep
End-to-end support⁴	Library prep, Sequencing, Bioinformatics, Reporting	Library prep, Sequencing only	Library prep, Sequencing only	Library prep, Sequencing only	Library prep, Sequencing only
Max read length	Up to 600 bp	Up to 2x150 bp	Up to 2x150 bp	Up to 2x300 bp	Up to 2x150 bp

1. Reads per run
2. Maximum throughput per run multiplied by number of runs per week at highest output: assumes 10 Ion GeneStudio S5 runs of Ion 550 chip per week; 5 runs per week of iSeq™, MiniSeq™, and MiSeq™ sequencers; and 3 runs per week for NextSeq™ sequencer
3. Ion AmpliSeq™ library chemistry is automated on Ion Chef™ instrument
4. Field support provided to portfolio products

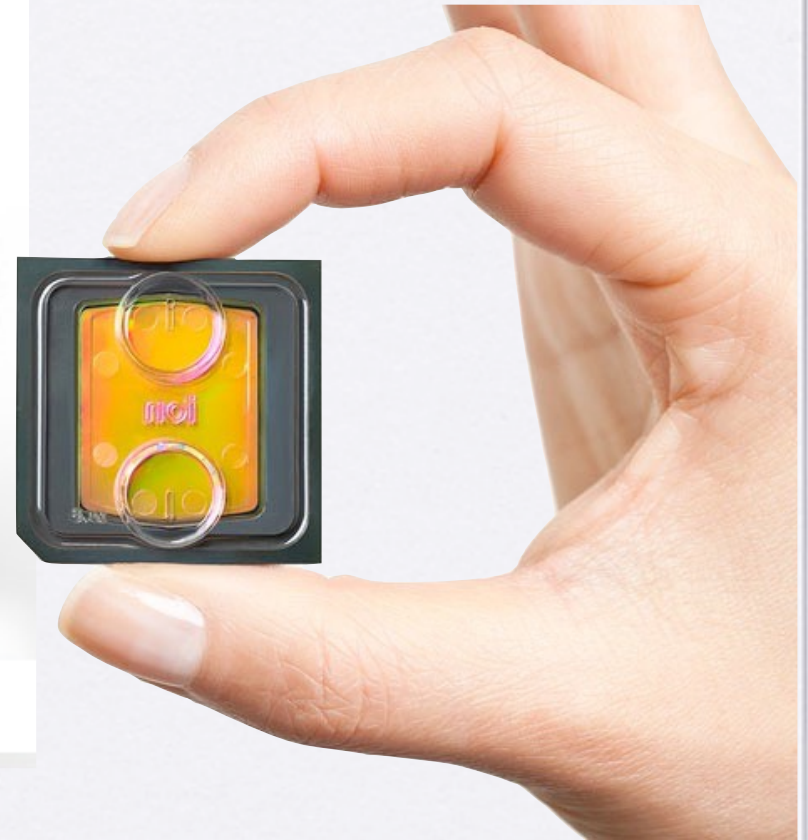
16 For Research Use Only. Not for use in diagnostic procedures.

ThermoFisher
SCIENTIFIC

Bridge PCR amplification

Illumina Sequence Analysis

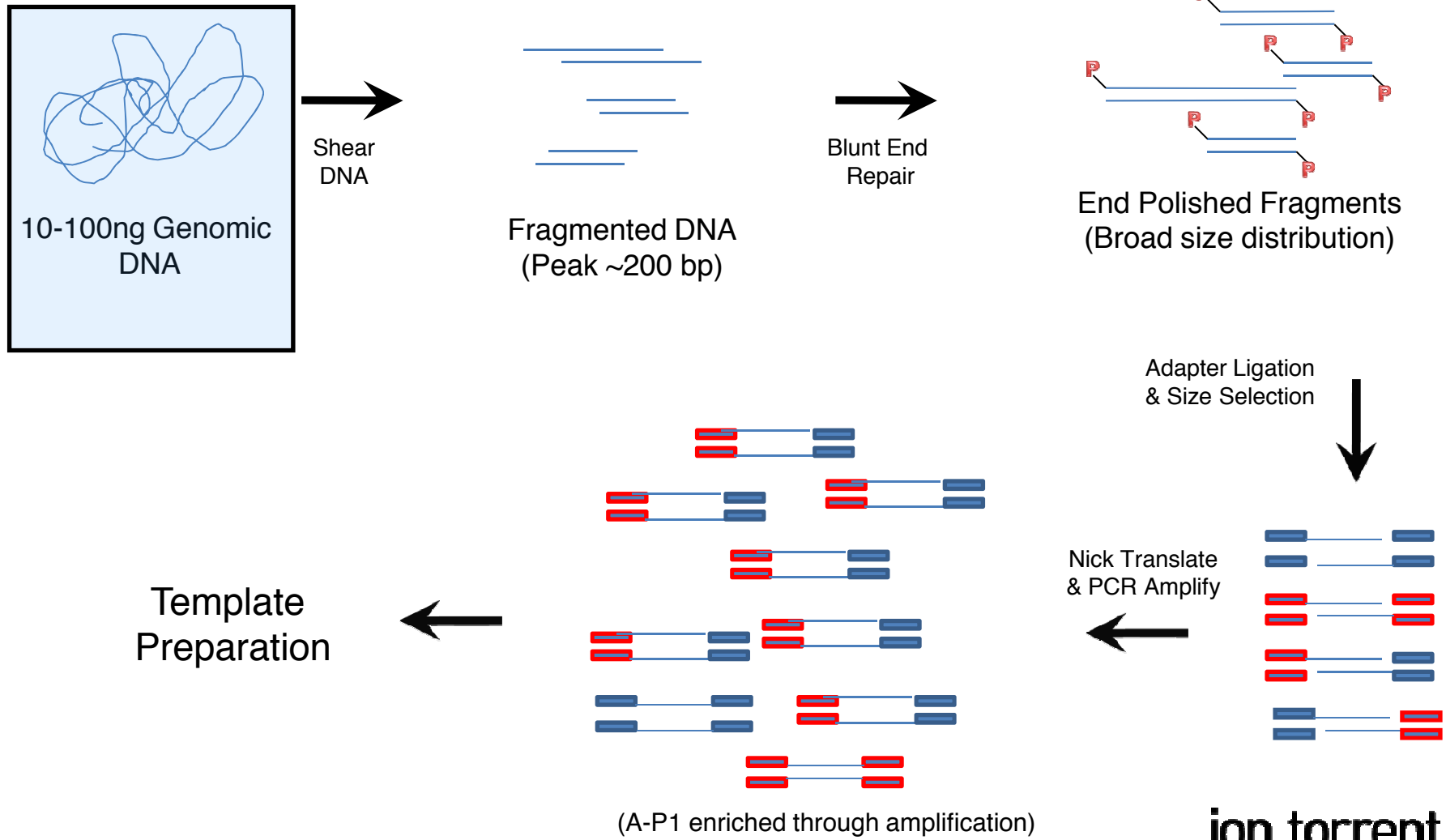




Ion Proton Next-Gen, Deep Sequencing
(ABI / Life technologies)

Ion Fragment Library Kit

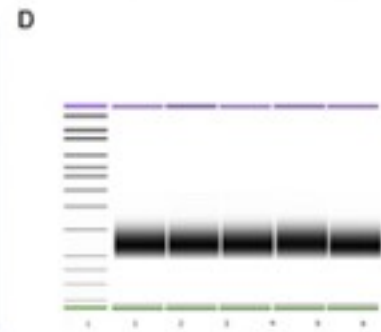
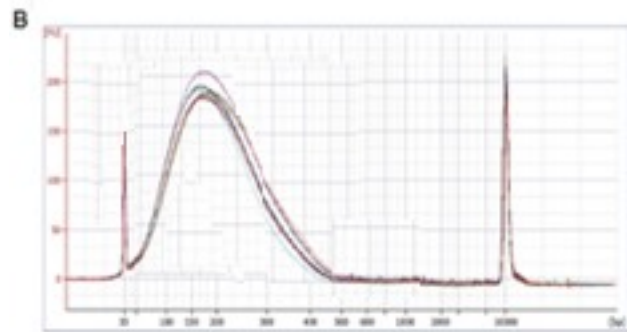
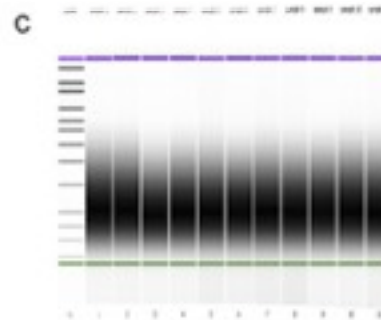
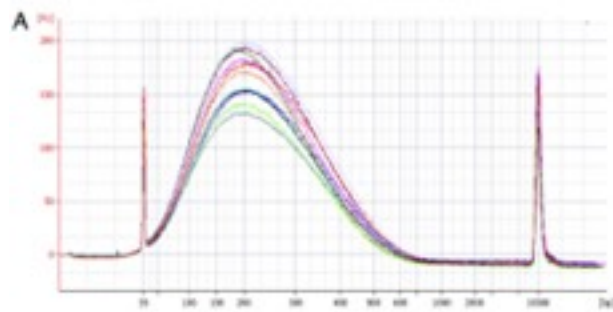
Fragment library Workflow



“Vortex”



“Sonicator”



“Shear”

Ion Workflow

Library Prep



Physical Shearing
Ion* or Other Vendors

1 Fragmentation

2 End repair

3 Adapter ligation

4 Size selection

5 Nick Translation and
Amplification

6 Quantification

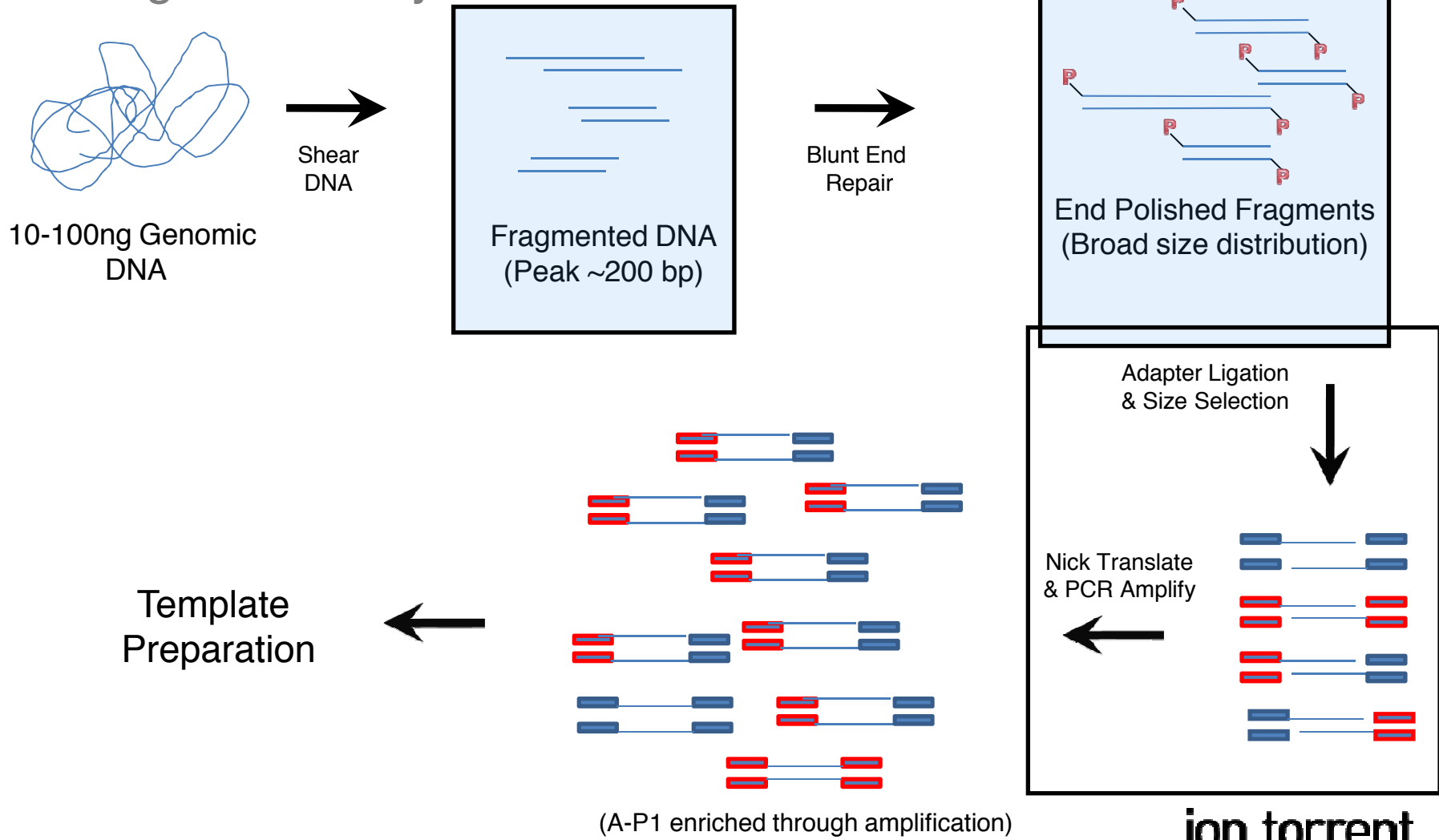
Total Time 2 - 6 hours

ion torrent

by life technologies

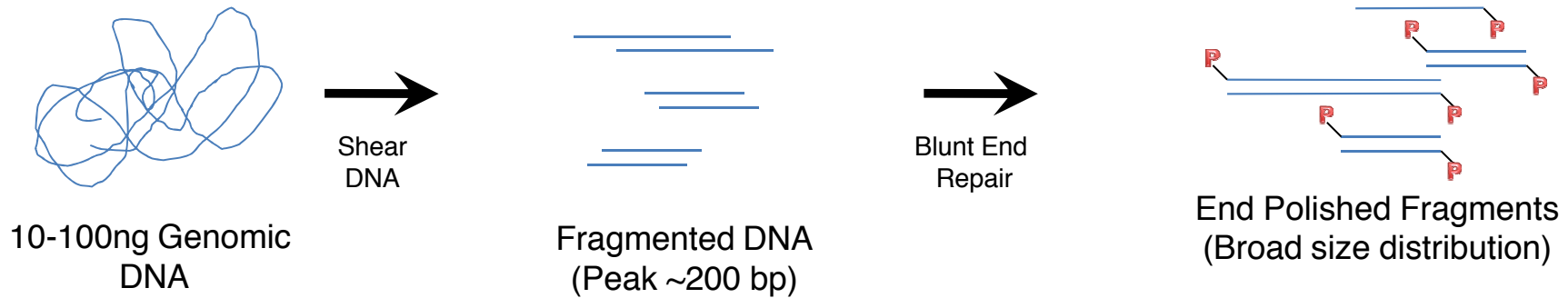
Ion Fragment Library Kit

Fragment library Workflow

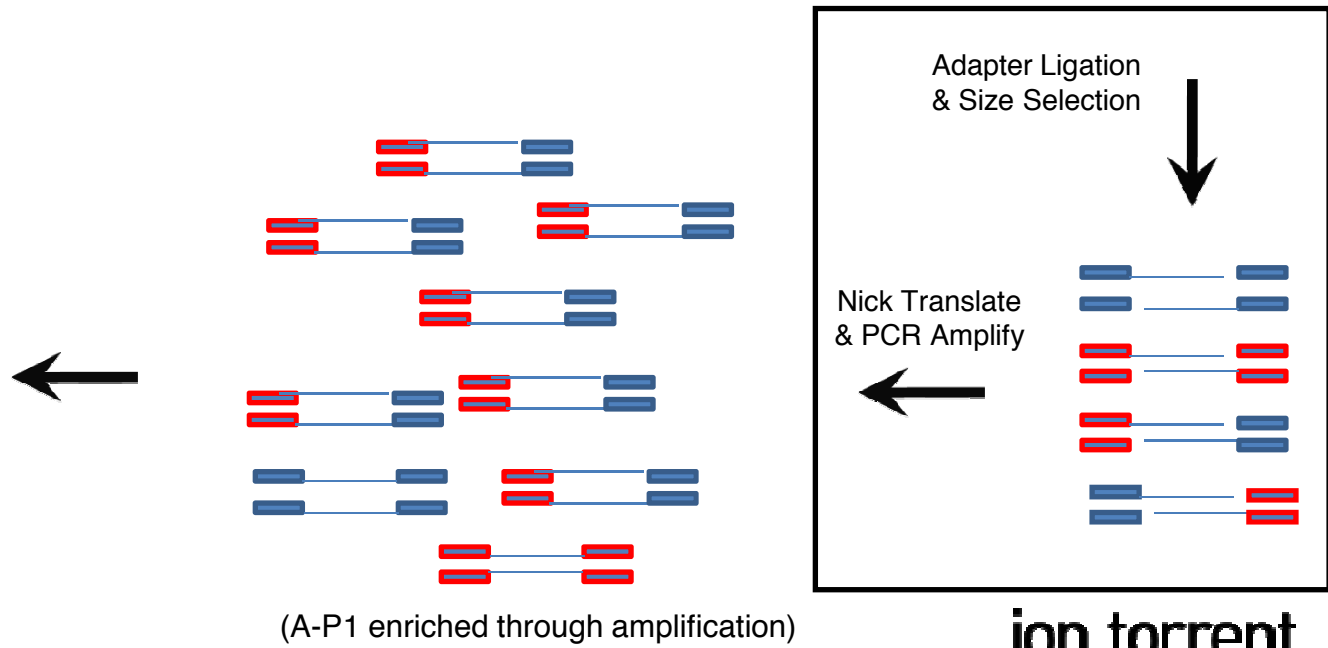


Ion Fragment Library Kit

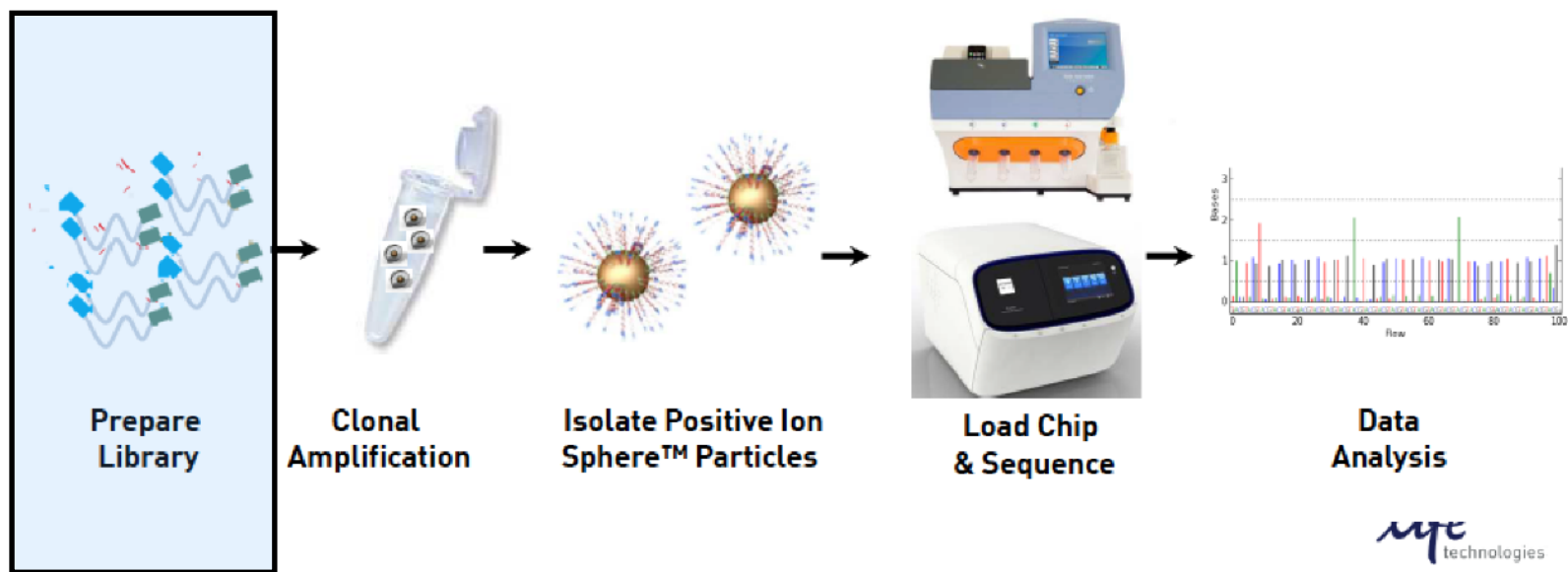
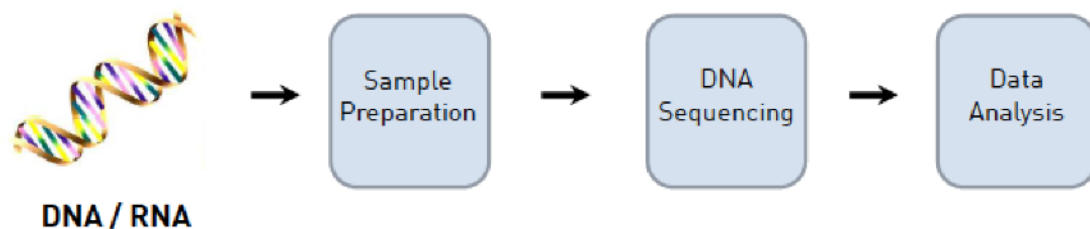
Fragment library Workflow



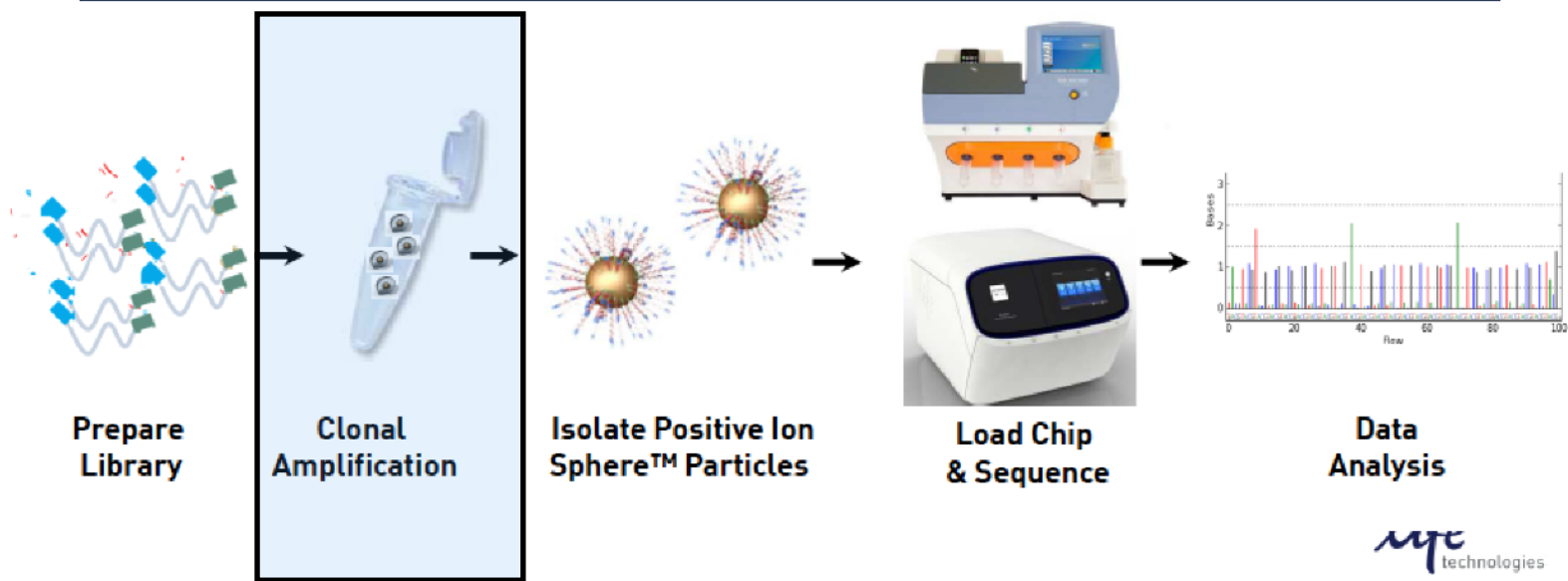
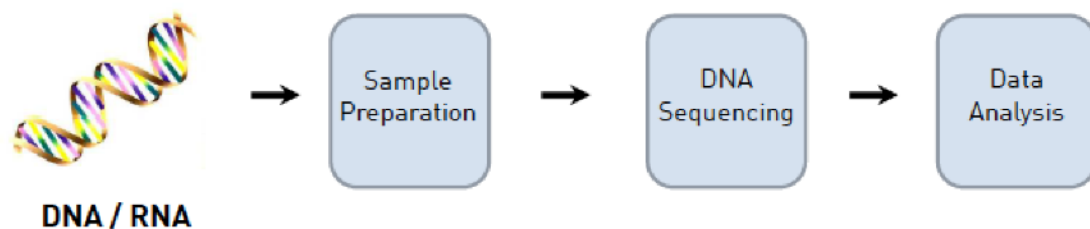
Template Preparation



Ion Workflow Overview

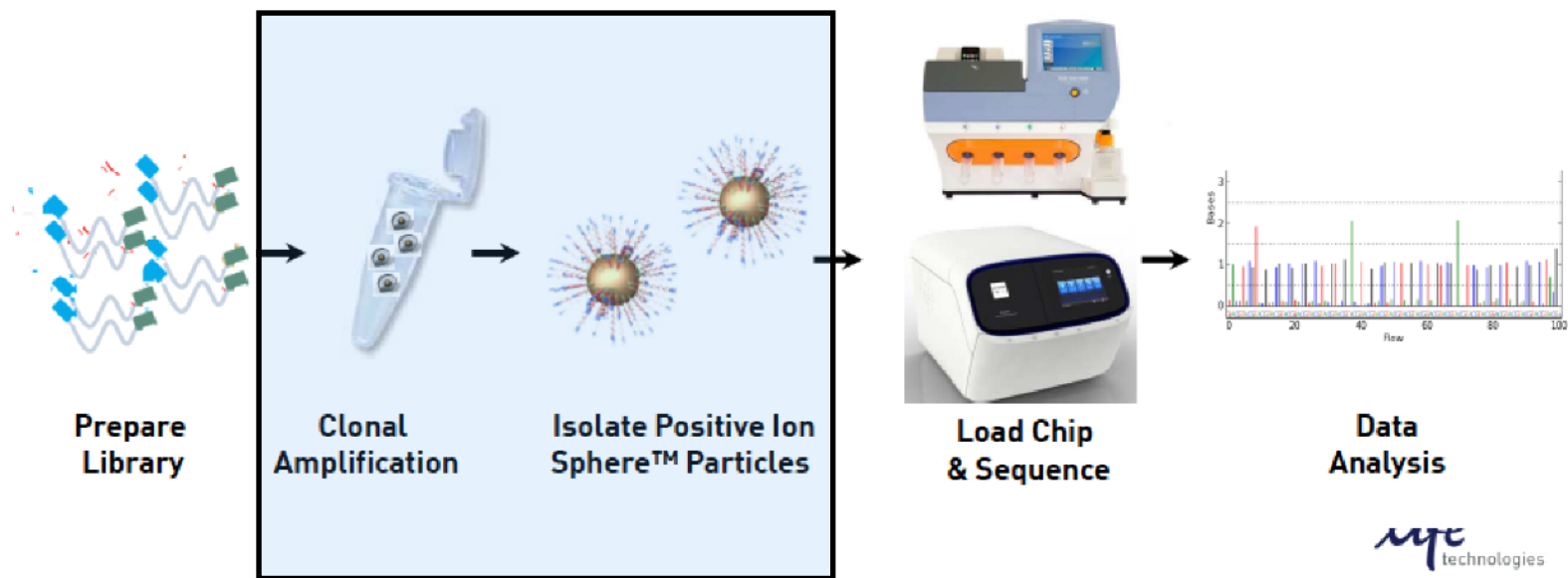
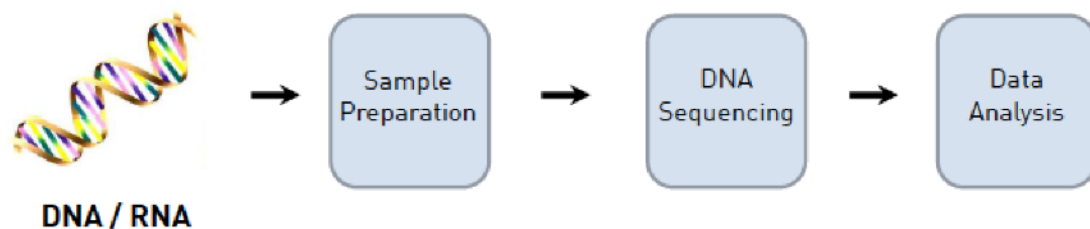


Ion Workflow Overview



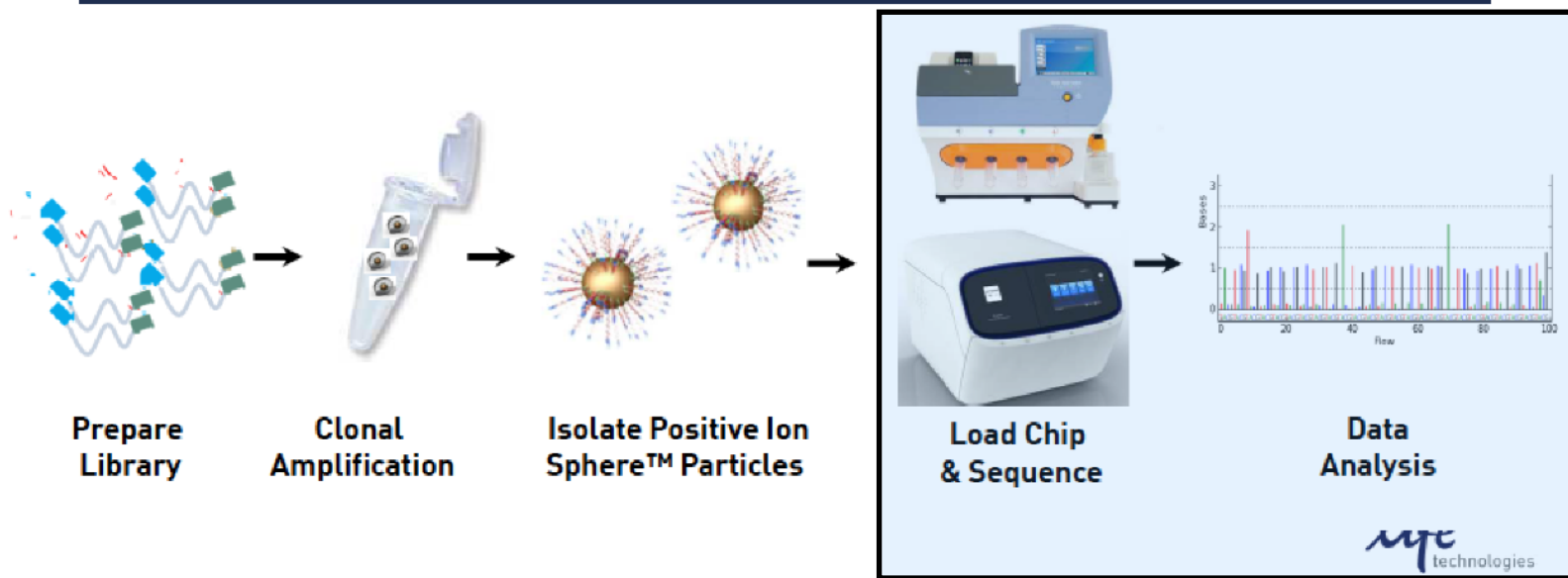
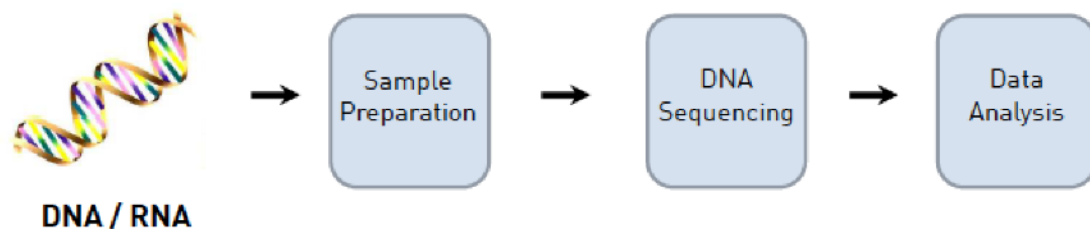
myc technologies

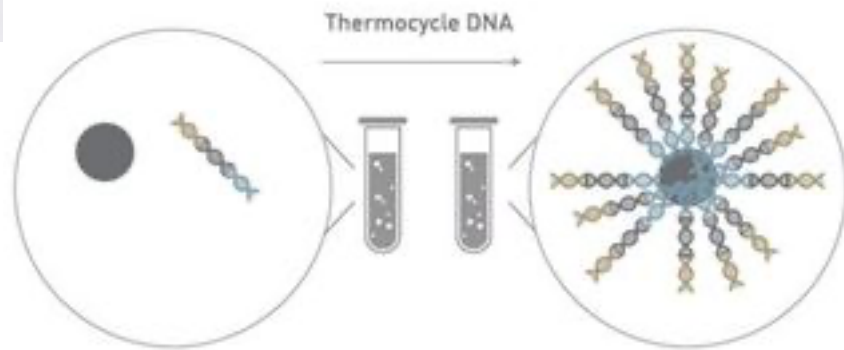
Ion Workflow Overview



myc
technologies

Ion Workflow Overview





Overview



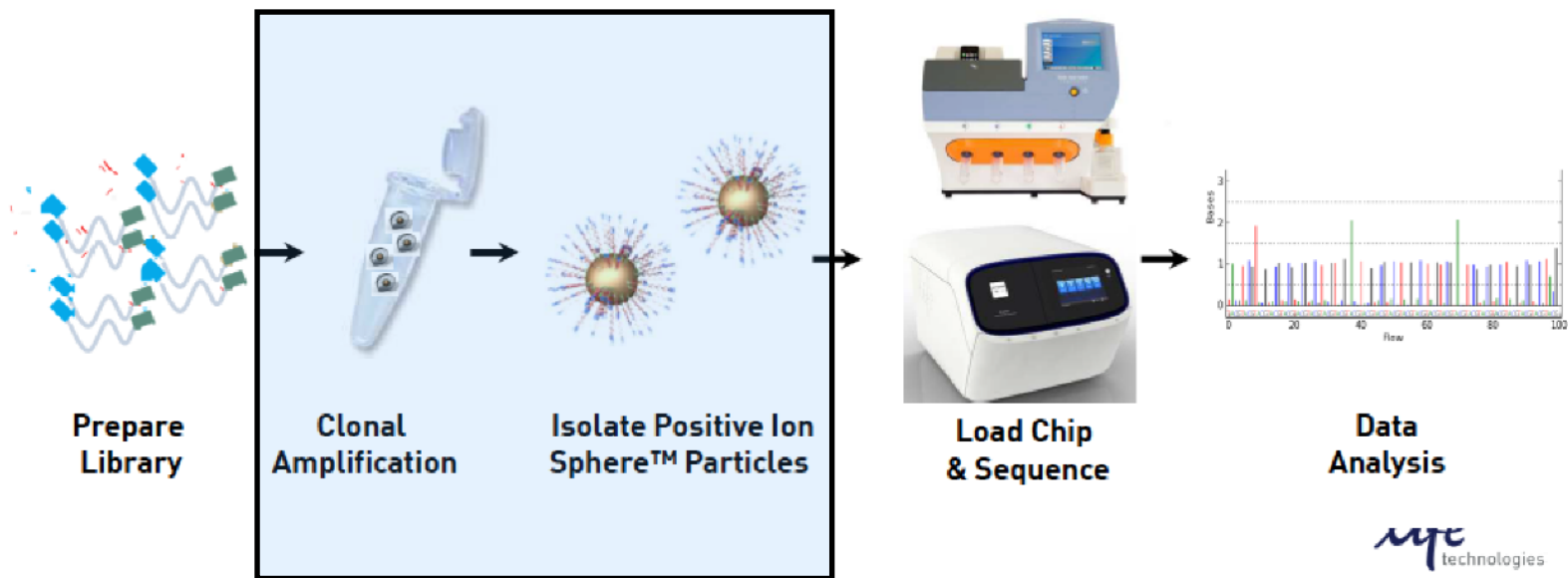
Sample
Preparation

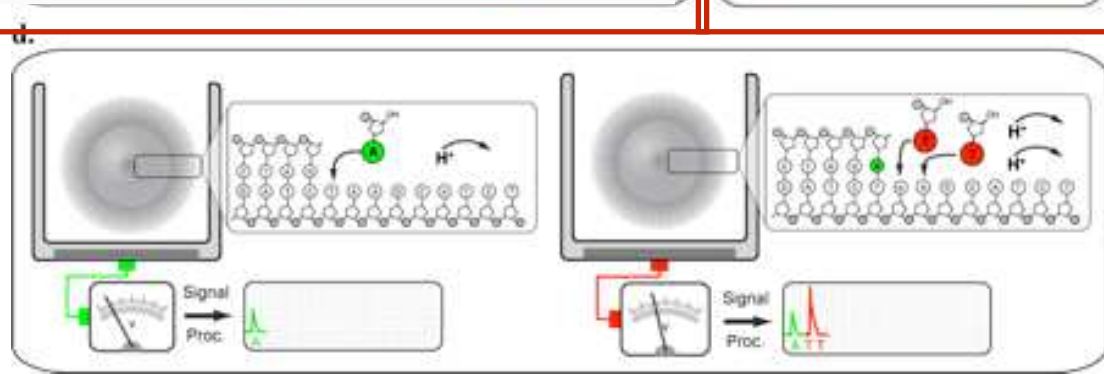
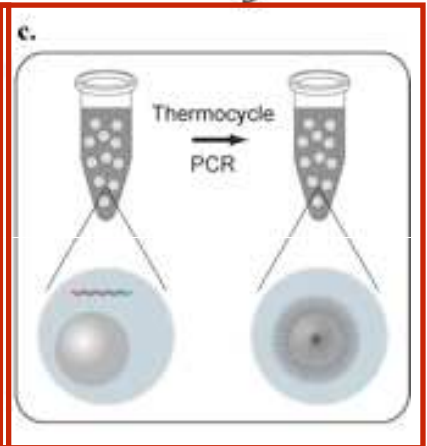
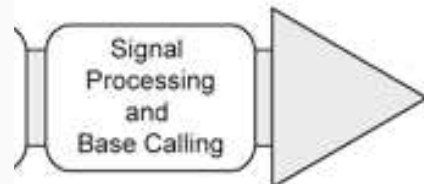
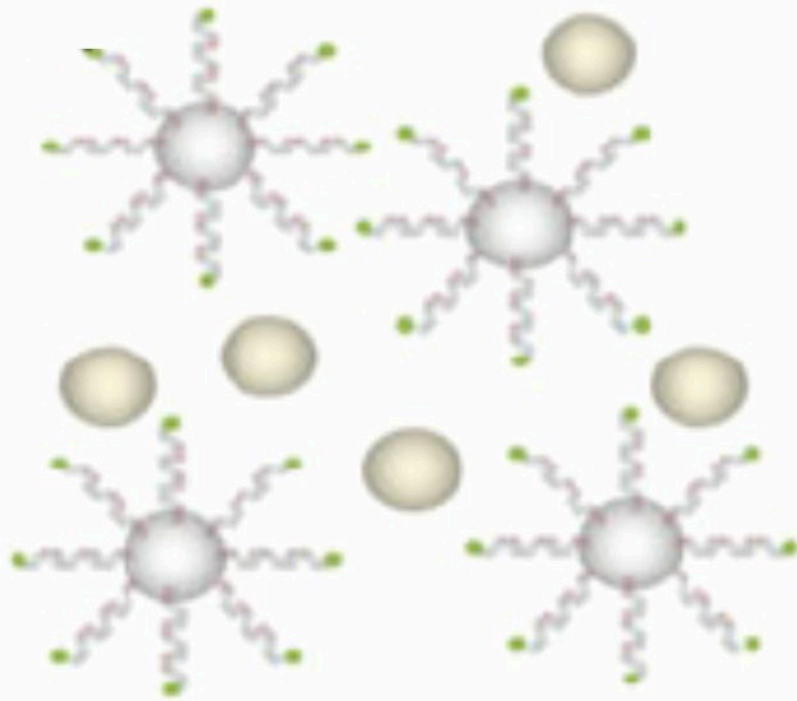


DNA
Sequencing



Data
Analysis





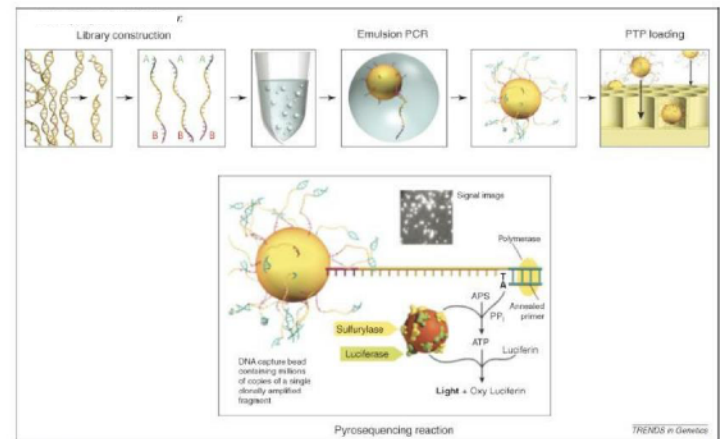
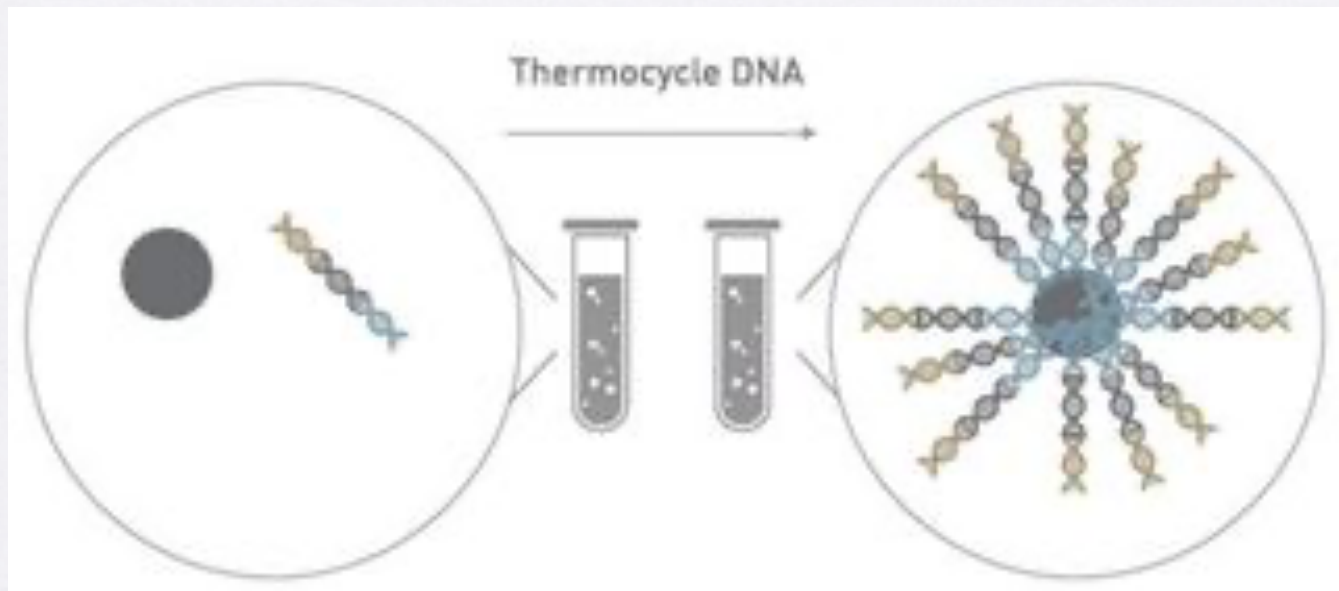


Figure 1. 454 Workflow: library construction ligates 454-specific adapters to DNA fragments and couple amplification beads with DNA in an emulsion PCR to simplify fragments before sequencing. The beads are loaded into the piostter plate (PTP). The bottom panel illustrates the pyrosequencing reaction that occurs on nucleotide incorporation to report sequencing by synthesis.

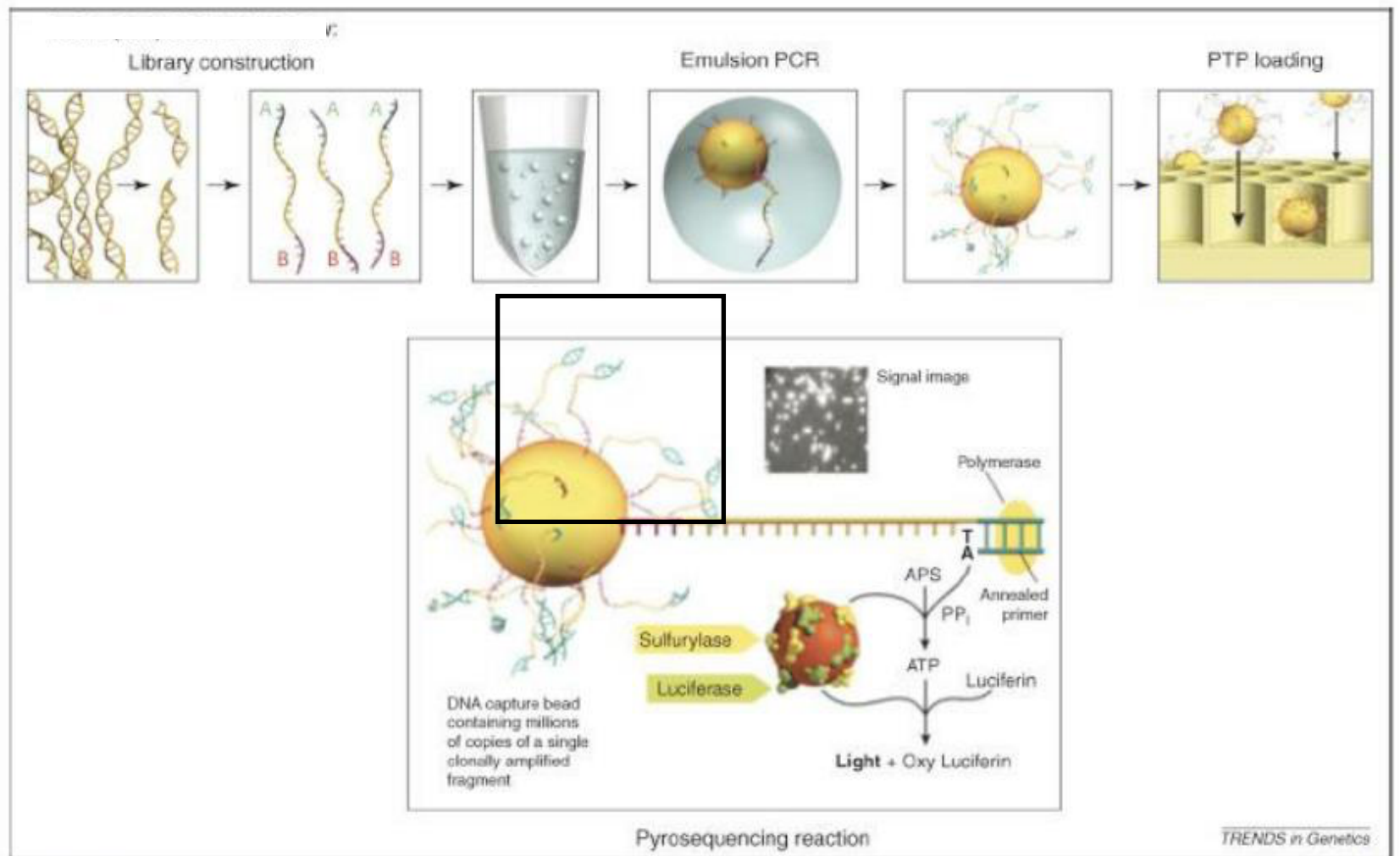
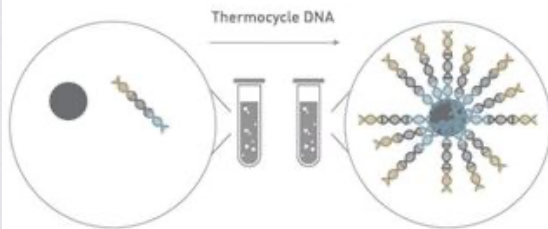
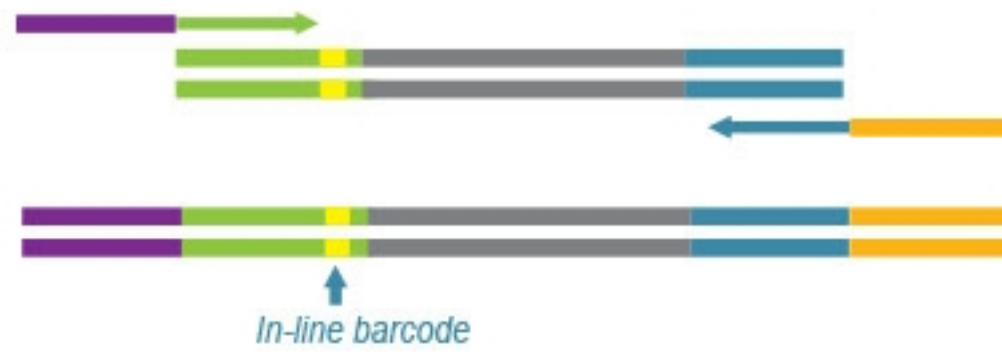


Figure 1. 454 Workflow: library construction ligates 454-specific adapters to DNA fragments and couples amplification beads with DNA in an emulsion PCR to amplify fragments before sequencing. The beads are loaded into the picotiter plate (PTP). The bottom panel illustrates the pyrosequencing reaction that occurs on nucleotide incorporation to report sequencing by synthesis.





Automation, Ion Chef
(ABI / Life technologies)

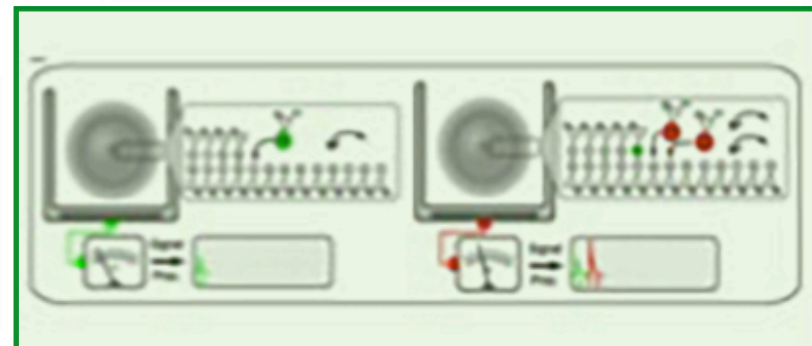
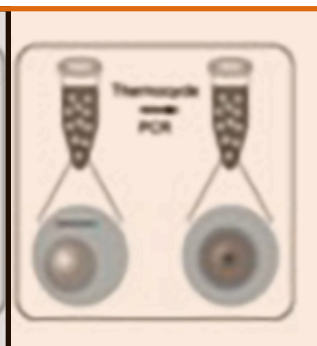
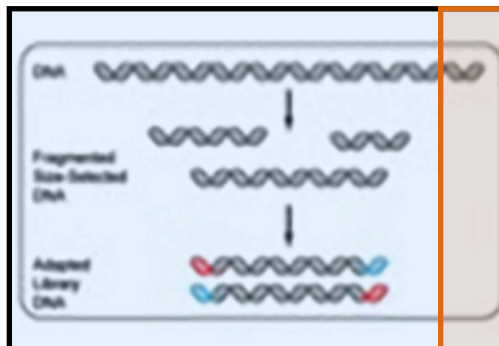
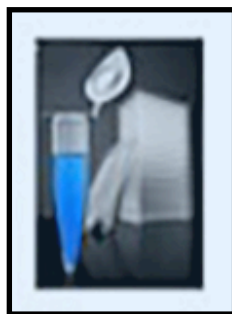
SELECTED TARGETS

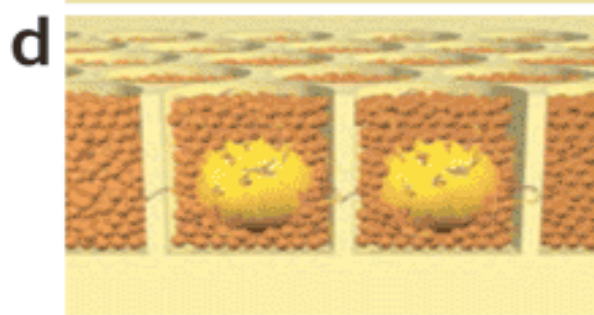
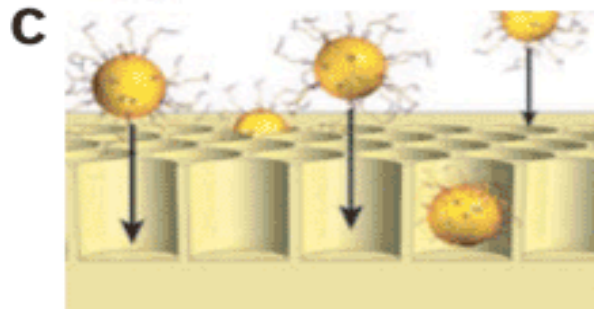
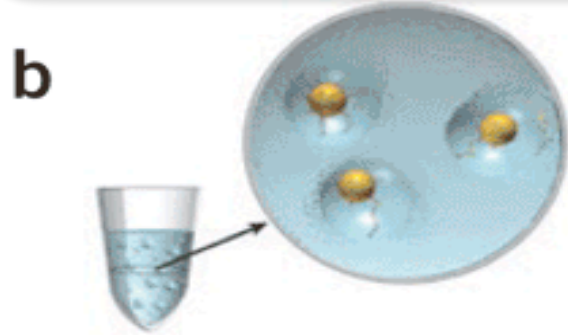
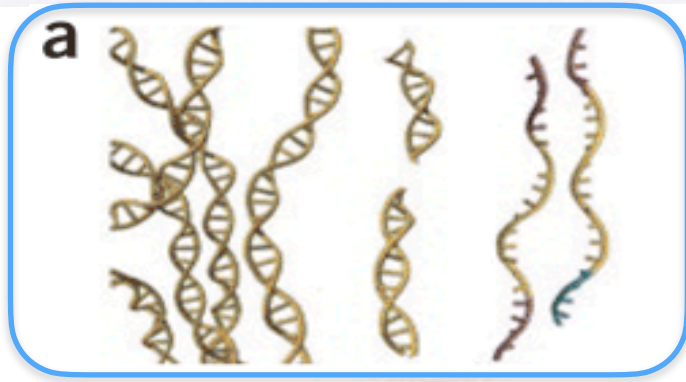
CONSTRUCT LIBRARY

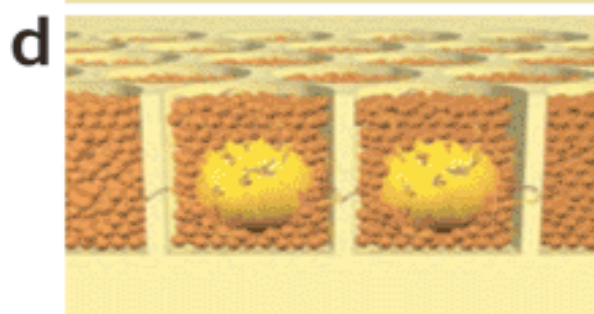
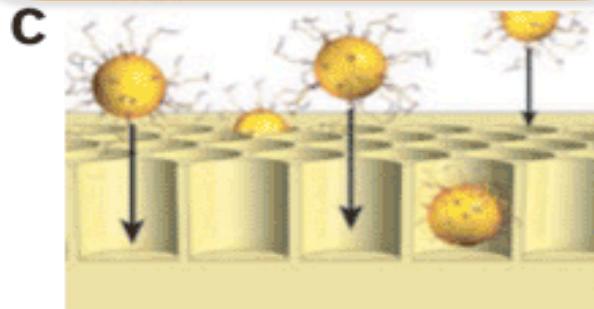
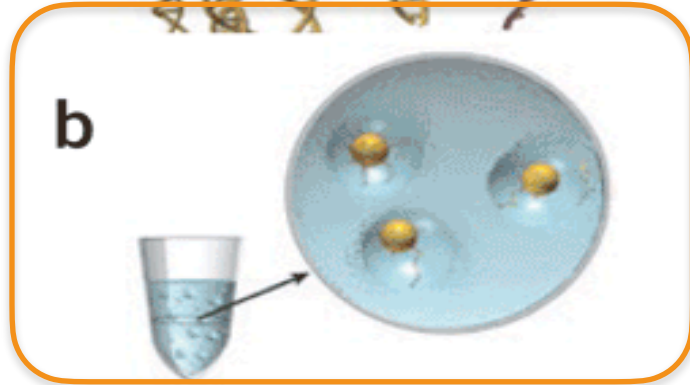
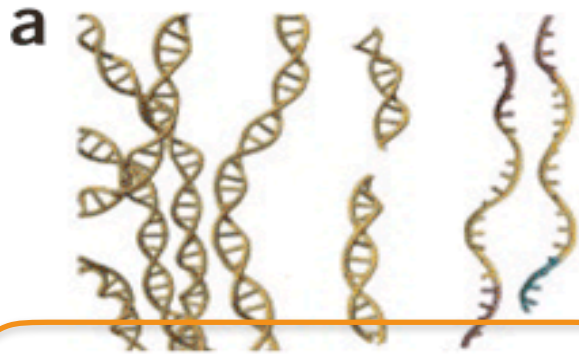
PREPARE TEMPLATE

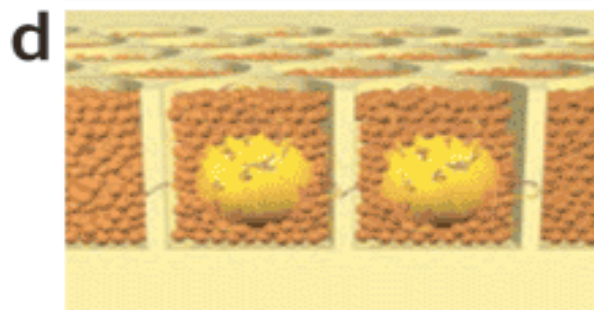
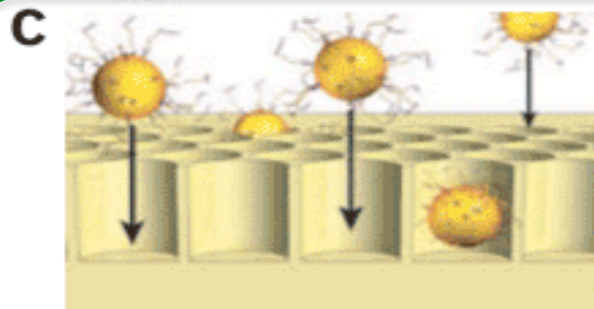
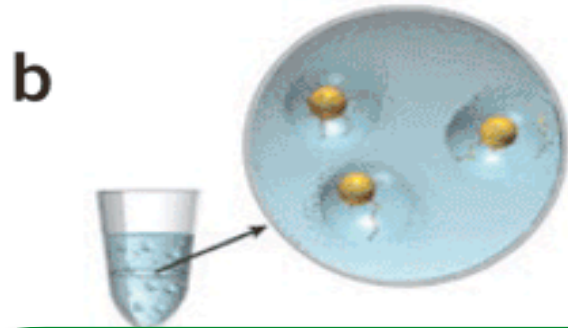
RUN SEQUENCE

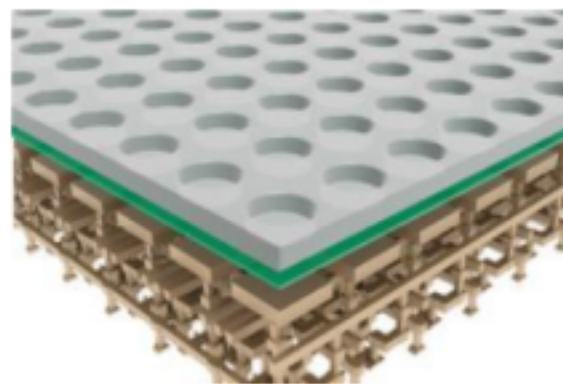
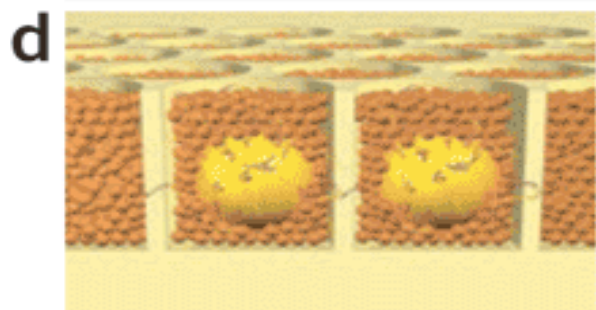
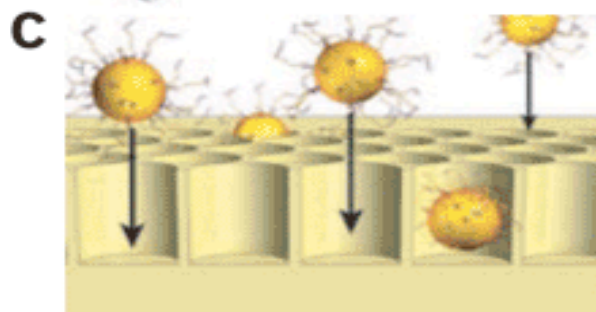
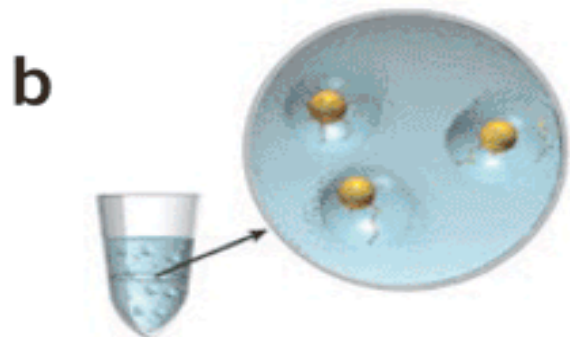
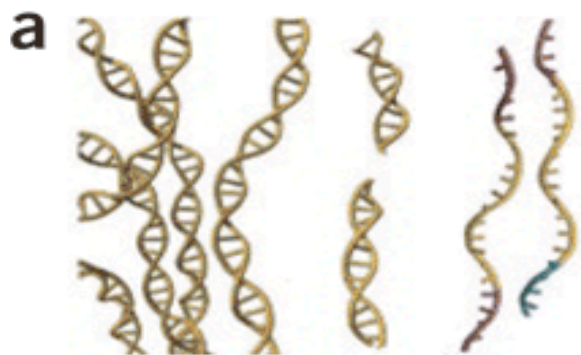
ANALYSE DATA



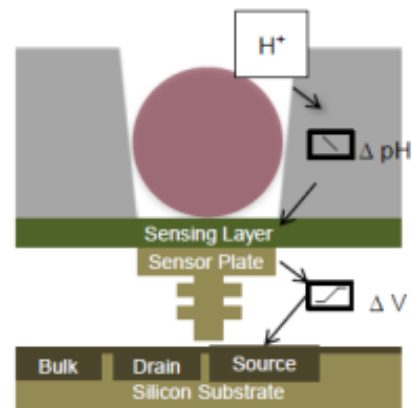








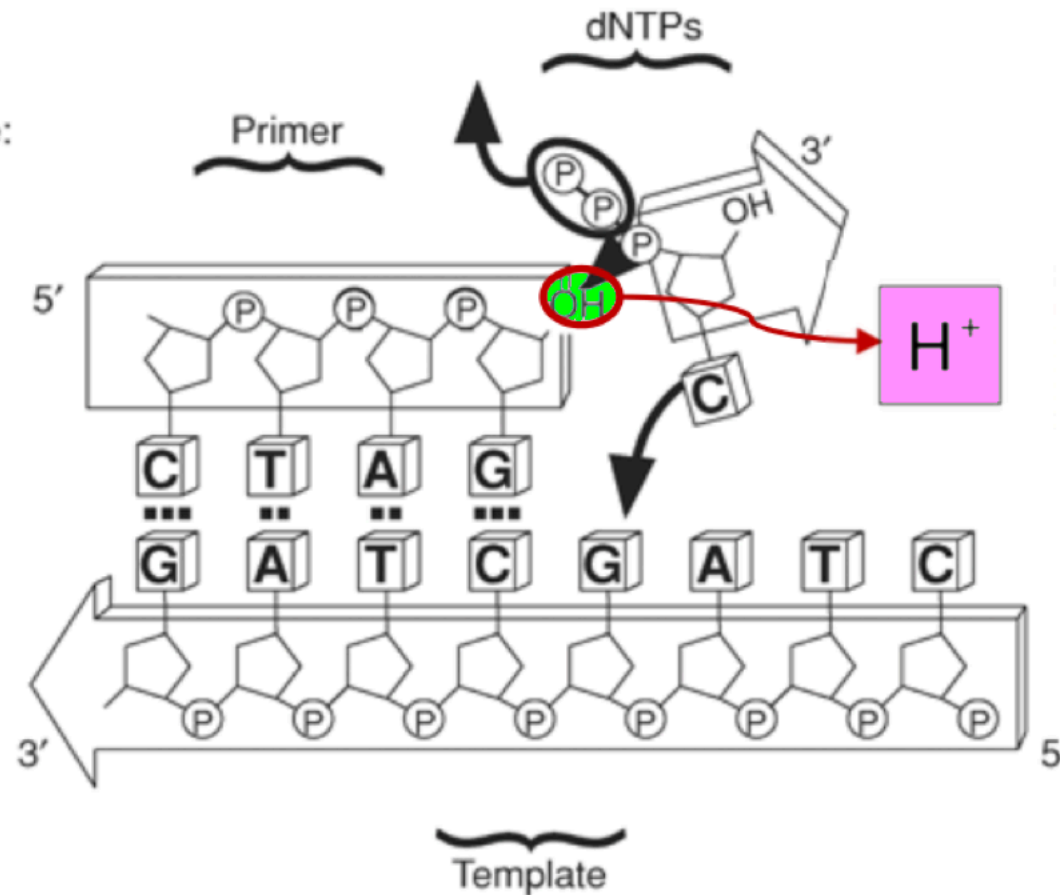
Millions of Sensors
Semiconductor Design



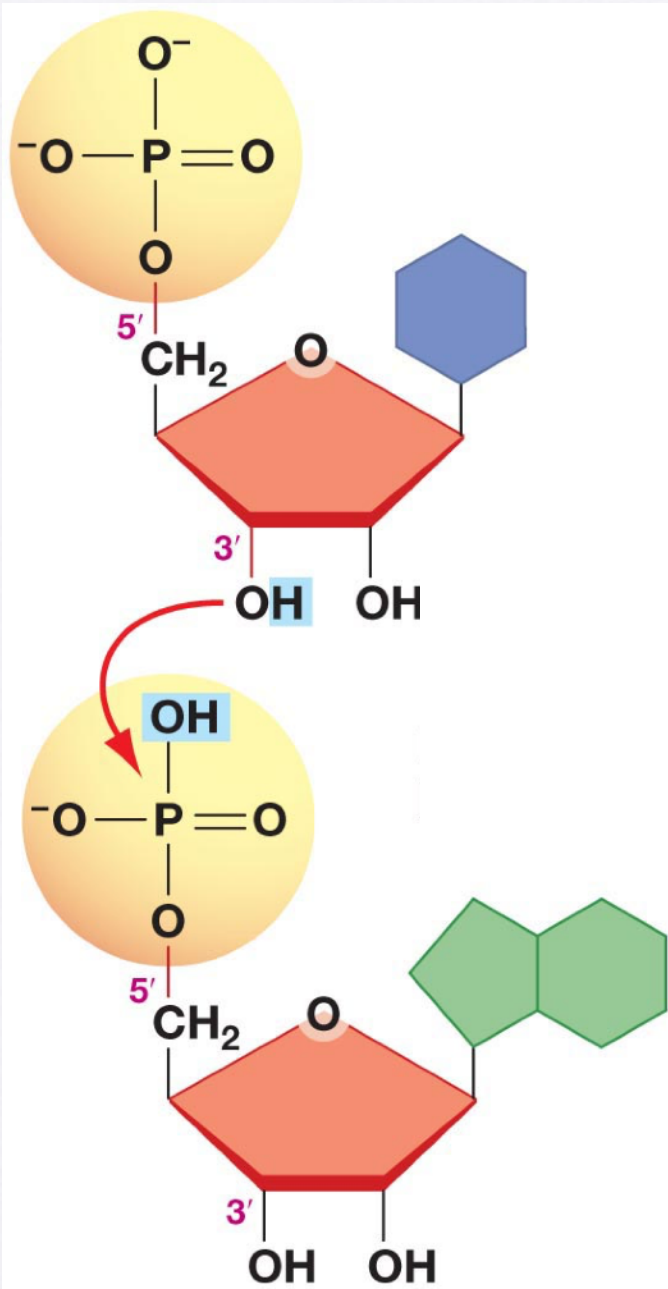
Simple, Natural Chemistry



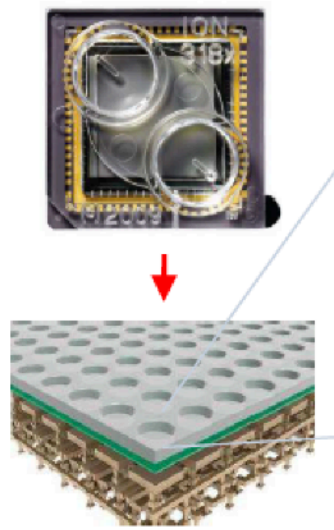
Example:



life
technologies



Rapid Direct Signal Detection



Sequencing: Flows

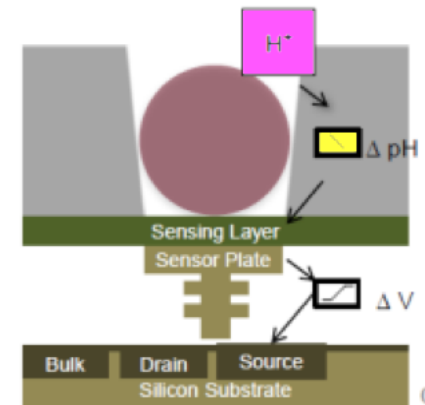
- A “flow” is the event of exposing the chip to one particular dNTP (T, A, C, or G), followed by a washing step
- The flow order repeats with pattern:
 - ‘TACGTACGTCTGAGCATCGATCGATGTACAGC’

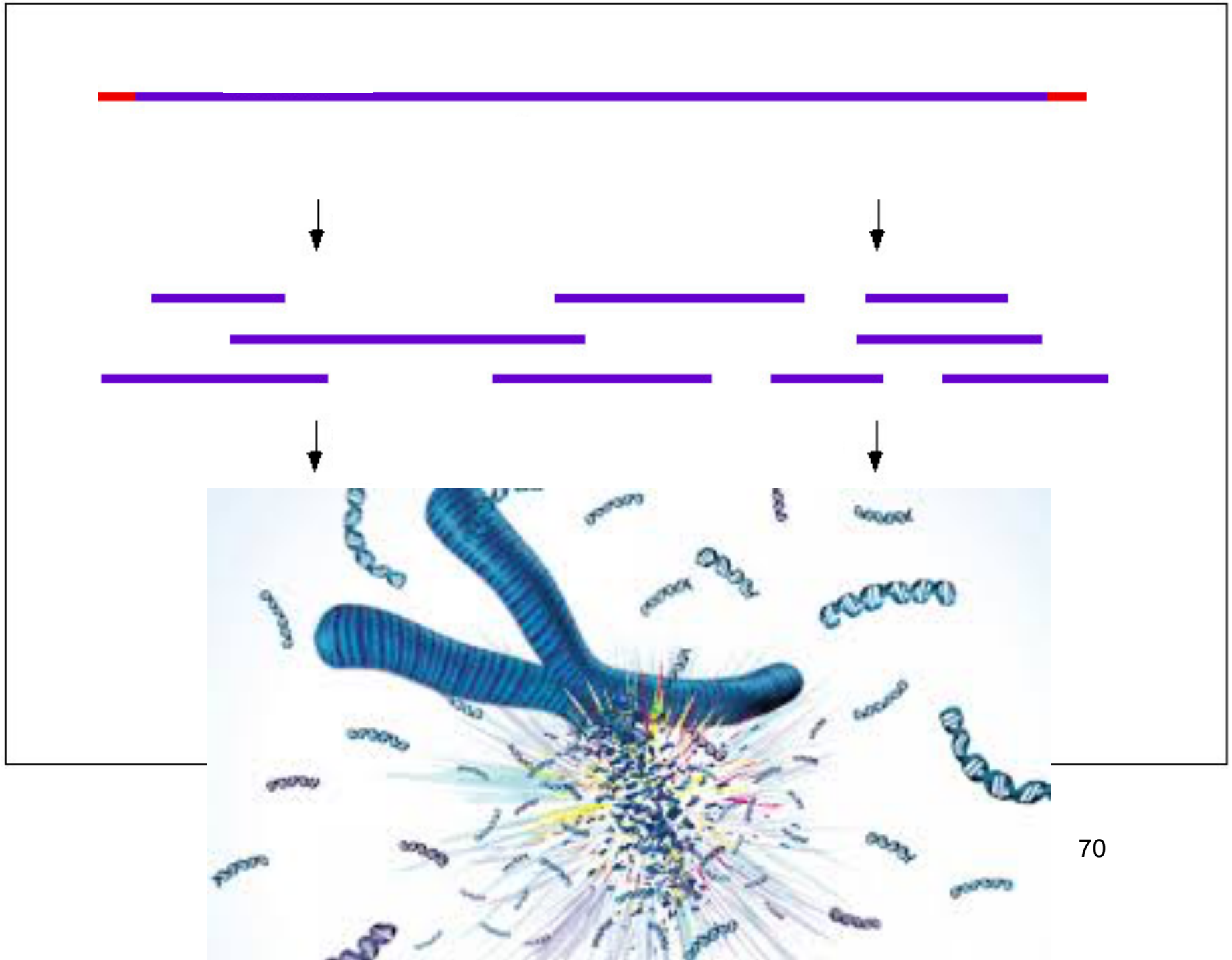


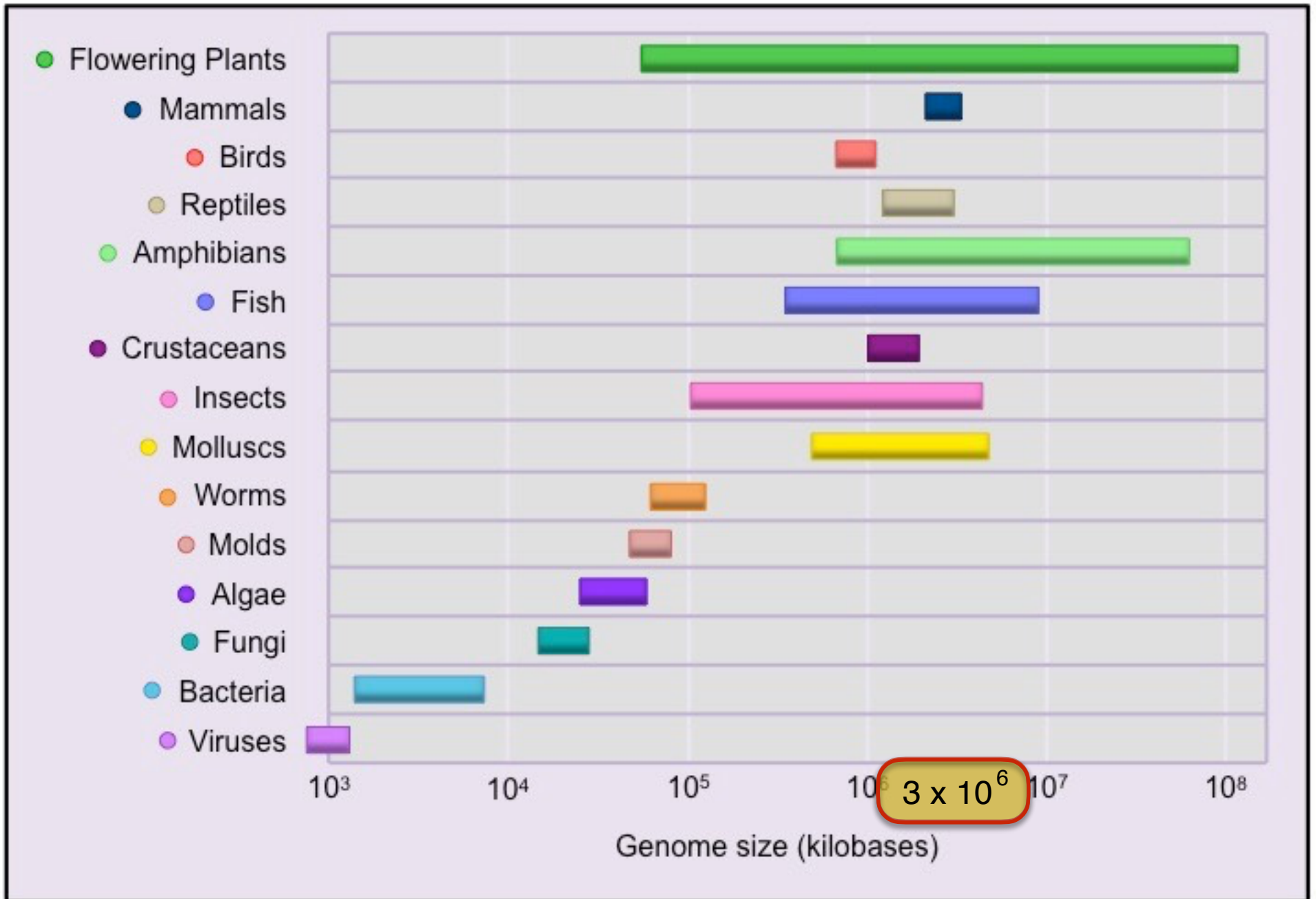
Flows 1-4

-----Primer----- **A G T C** A A G C G T C C C A T G
 Key Sequence Sequence of Inter

A “cycle” is four consecutive dNTP flows: for instance, T-A-C-G = 1 cycle









Ion PGM™ Sequencer



ionS5 Sequencer



	Ion Torrent PGM			Ion Torrent Proton	
Chip Type	PGM 314	PGM 316	PGM 318	Proton I	ionS5
# of sensors	1.3M	6.3M	11M	165M	660M
Total output	10-40Mb	100-400Mb	~1Gb	~10Gb	~100Gb
Run time	1-2 hrs	1-2 hrs	1-2 hrs	2.5 hrs	2.5 hrs
Read length	up to 400bp	~200bp	up to 400bp	~200bp	~200bp
Total reads	up to 0.6M	up to 3M	up to 6M	60-80M	240-330M

Covid-19 Genomic Sequence Analysis

@GSU



Equipment/Reagents

- ABS IonTorrent S5 with chef for ISP
- 540 chips offer 80 million reads allowing for- **80 samples per chip**
- Ion ampliseq technology for building library
 - 2 pools of ~1,200 primers each
 - Fragment the sequence into roughly 200 bp segments
- Ioncode Barcode adaptors
 - Unique beginning / ending sequences allow for multiple sequences on the same chip



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Pango Lineage Reports

- Sequences that were >98% complete were run through the Pangolin COVID-19 Lineage Assigner (~200 sequences)
 - <https://pangolin.cog-uk.io/>



Incidence of C-19 Lineages 4/2020-7/2021

