

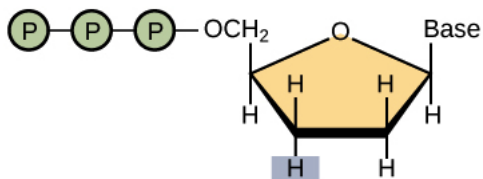
Advanced Molecular Methods Workshop

Sequencing Technologies

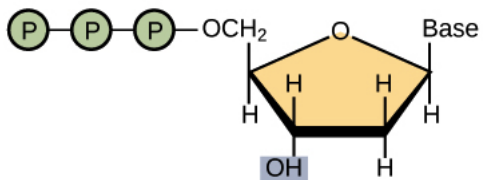
Hasan Huseyin Kazan, PhD

Department of Medical Genetics

Sanger Sequencing



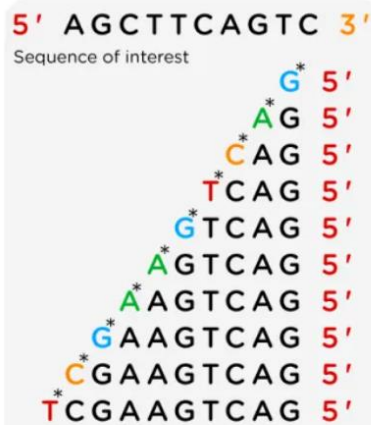
Dideoxynucleotide (ddNTP)



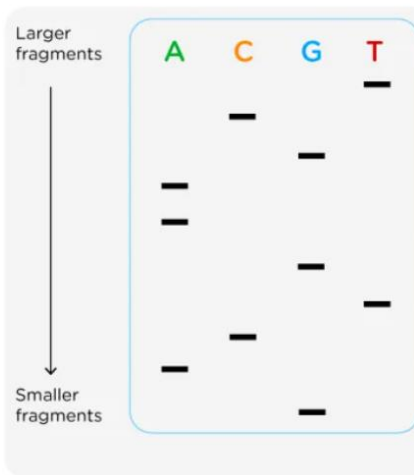
Deoxynucleotide (dNTP)

Sanger DNA Sequencing

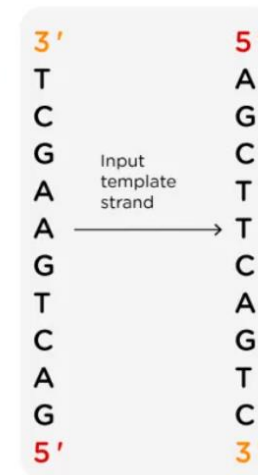
1. PCR with radioactive dideoxynucleotides*



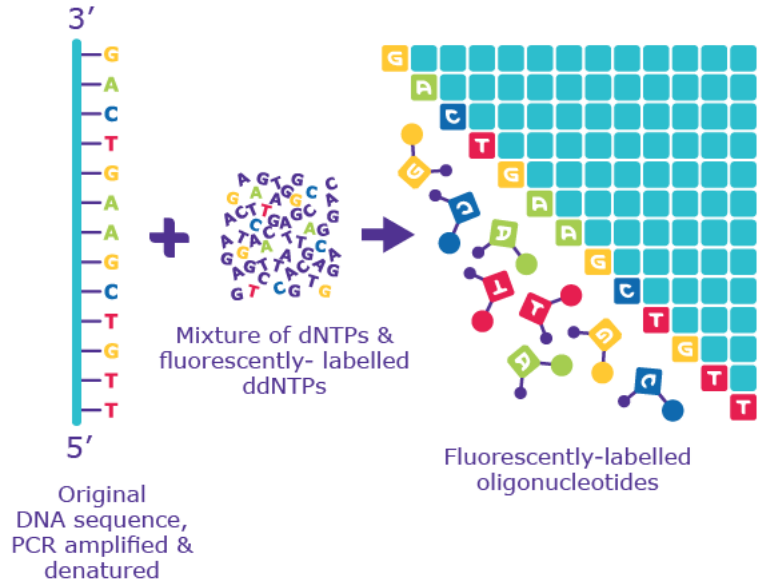
2. Gel electrophoresis to separate PCR products



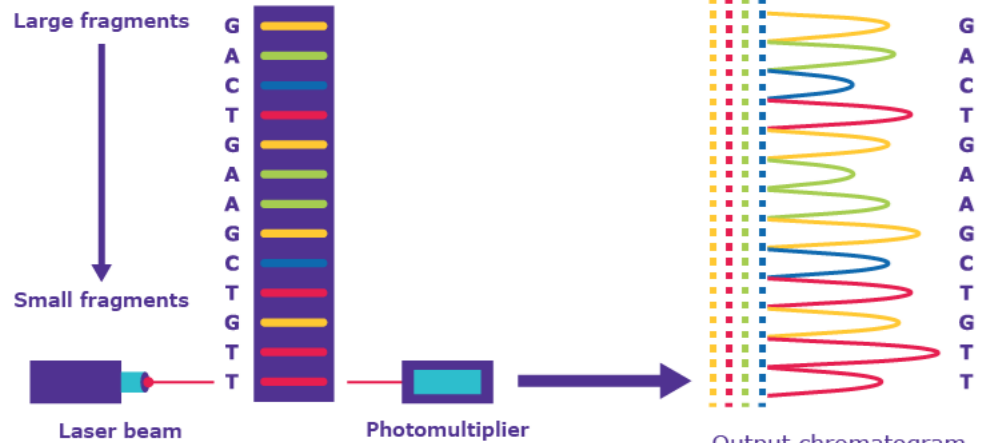
3. Decoding sequence of interest



1 PCR with fluorescent, chain-terminating ddNTPs

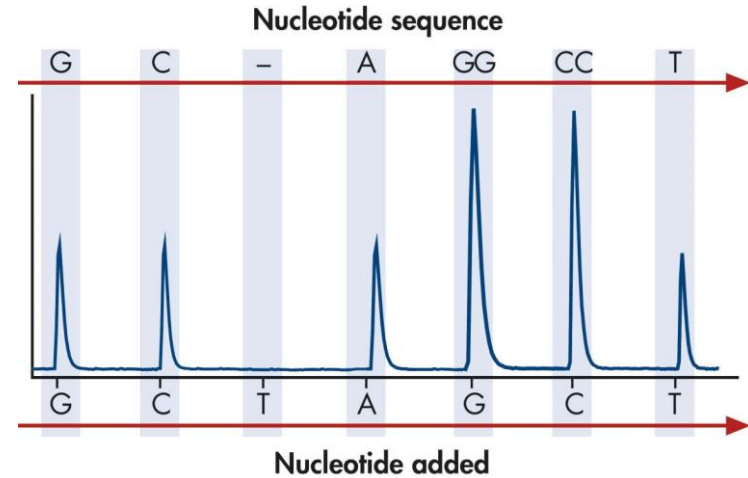
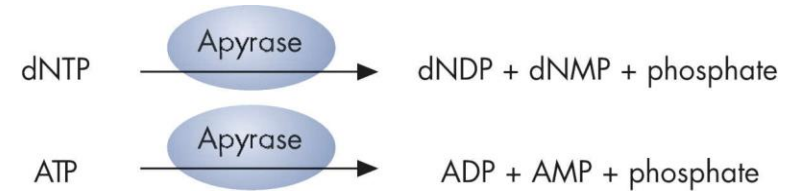
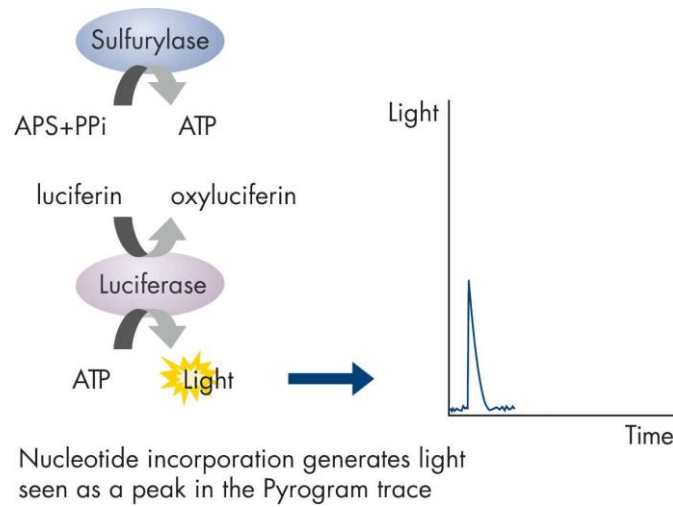
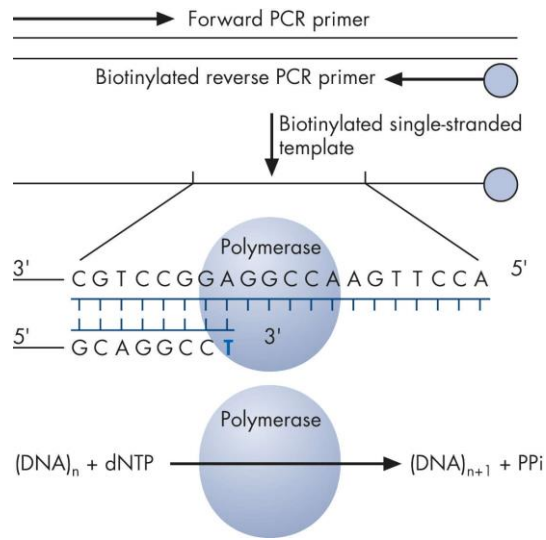


2 Size separation by capillary gel electrophoresis

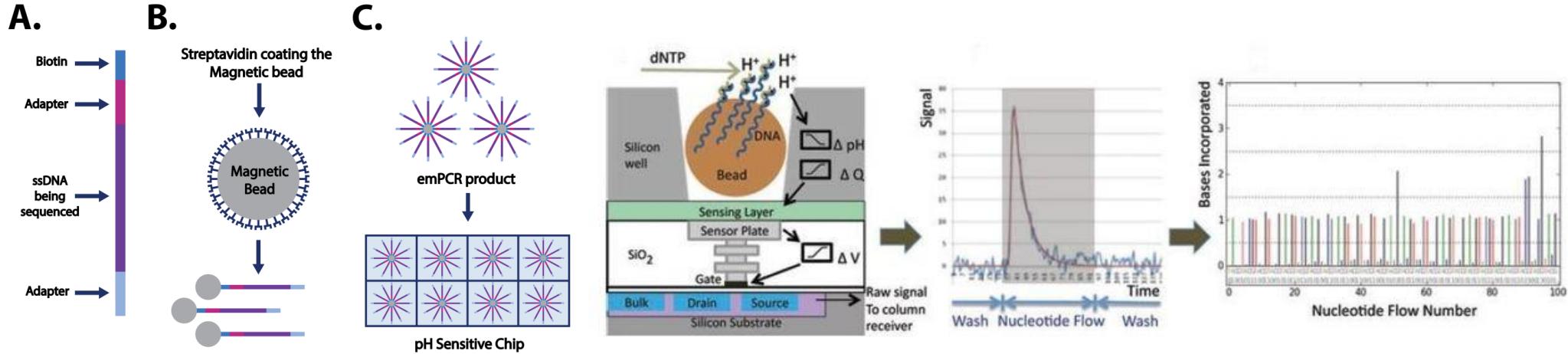


3 Laser excitation & detection by sequencing machine

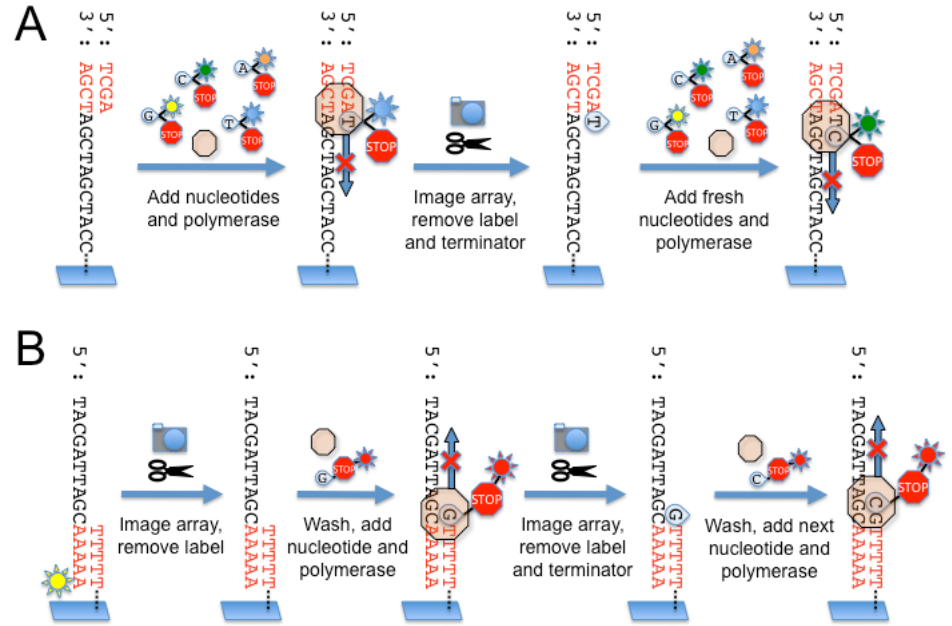
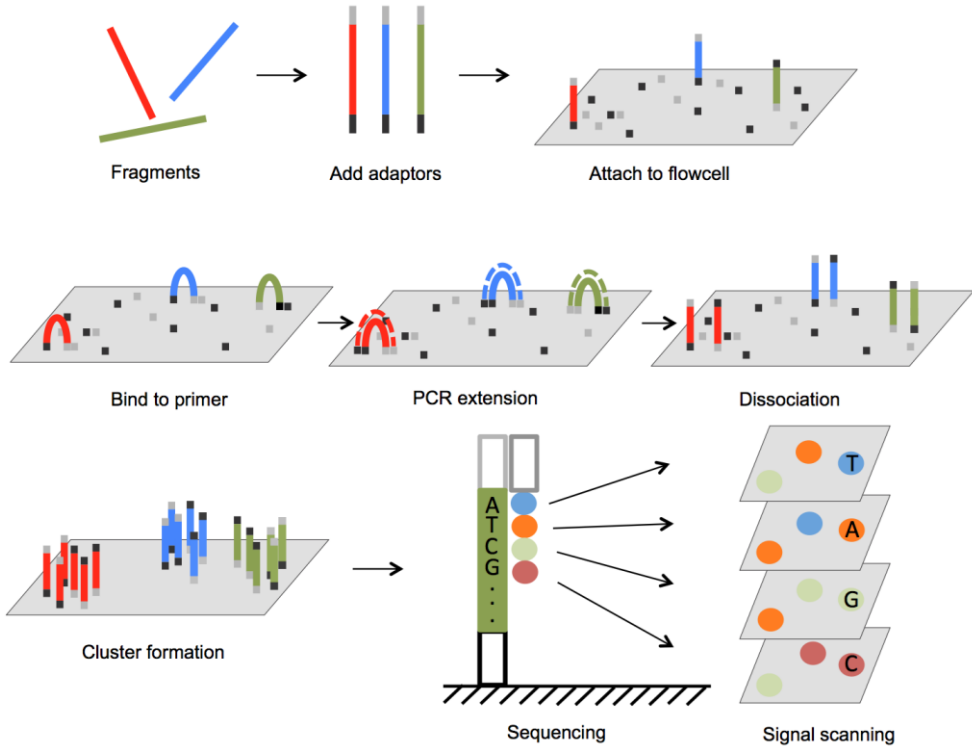
Pyrosequencing-Roche



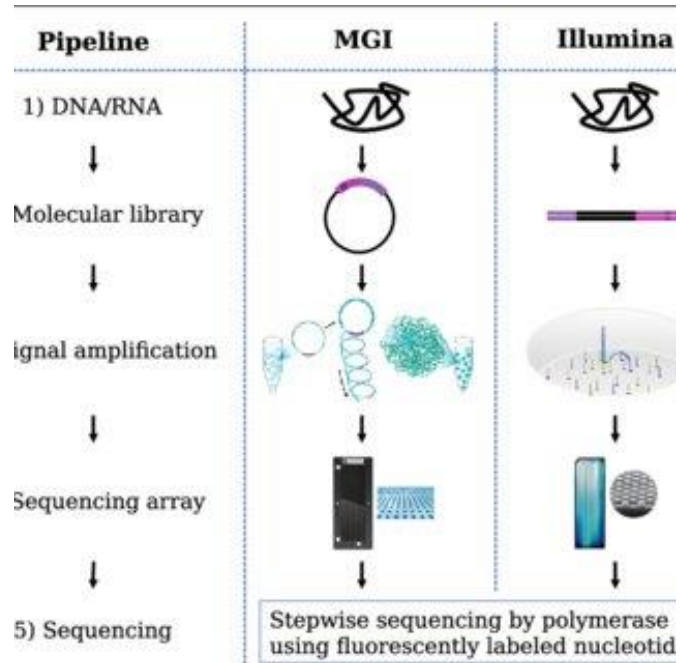
Ion Torrent-Thermo Scientific



Illumina



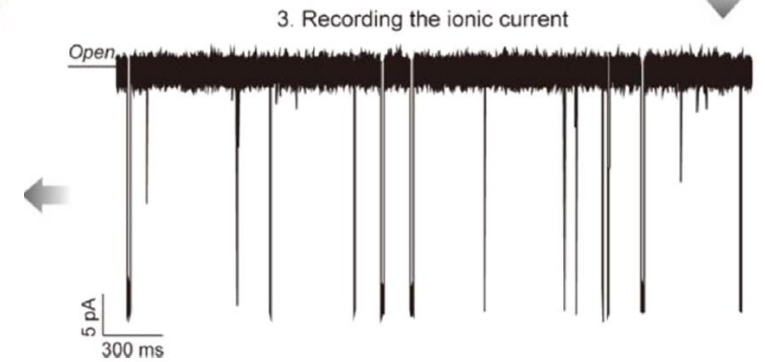
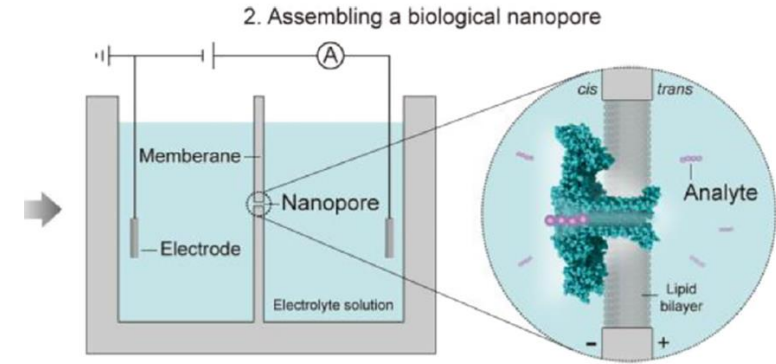
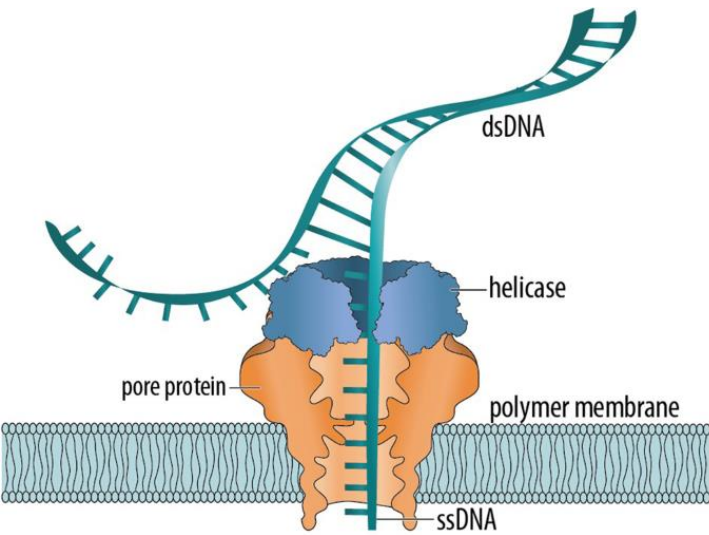
MGI/BGI



Next Generation Sequencing Pipeline



Oxford Nanopore



Platform	Mode	Read-Length	Reads Passing Filter per Run	Output	Run Time	Quality	Cost/Run	Instrument Price
Illumina HiSeq 2000/2500	High-Output	1 × 36–2 × 125	4 B	128 GB–1 TB	1–6 days	Q30 ≥ 80%	~\$29K	\$740K
	Rapid	1 × 36–2 × 150	600 M	18 GB–300 GB	7–60 h	Q30 ≥ 75%	~\$8K	
Illumina HiSeq X ten	X ten	2 × 150	5.3–6 B	1.6–1.8 TB	<3 days	Q30 ≥ 75%	~\$12K	\$1M*
Roche 454 FLX system	Titanium XL+	700	1 M	700 MB	23 h	99.997%	~\$6K	~\$500K
Life Technologies Ion Torrent	Proton I	200	165 M	~10 GB	2–4 h		~\$1000	\$149K
	Proton II	100	660 M	~32 GB	2–4 h			
Intelligent Biosystems (Qiagen)	MAX-Seq	2 × 55	75 M/lane	132 GB	2.5 days		~\$1200	~\$270K \$125K
	Mini-20	2 × 100	20 M/lane	80 GB		~\$150–300/sample		
PacBio RS	RS II	10–15 KB	50 K	500 MB–1 GB	4 h	>99.999%	~\$400	~\$700K
Oxford Nanopore	miniON	>200 KB	no fixed run time (~1 bp per nanosecond)				≤\$900	~\$1000

Thank you...