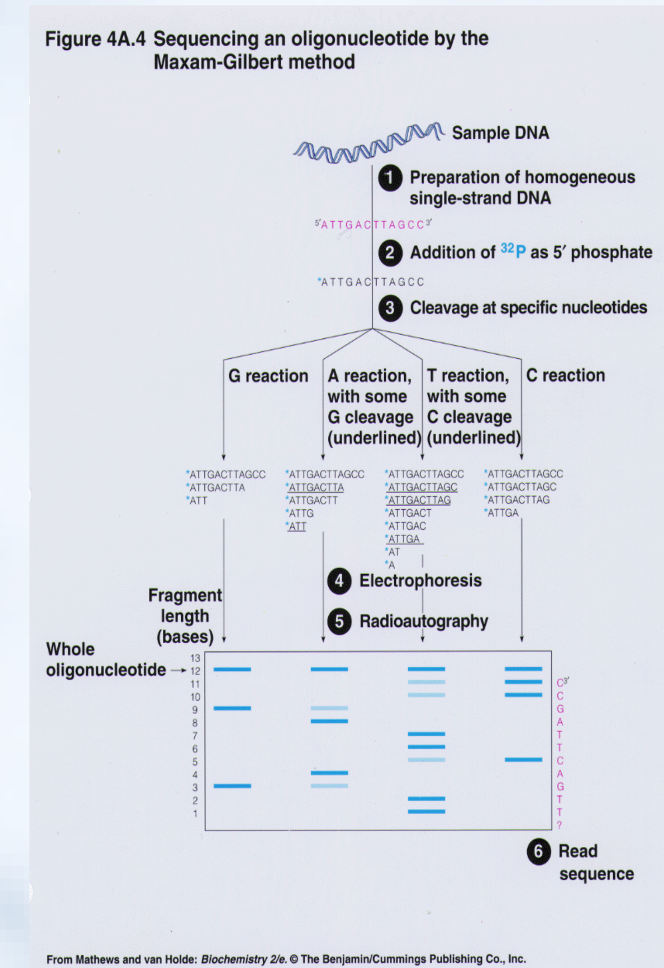
– Evolution of Genome Sequencing Techniques –

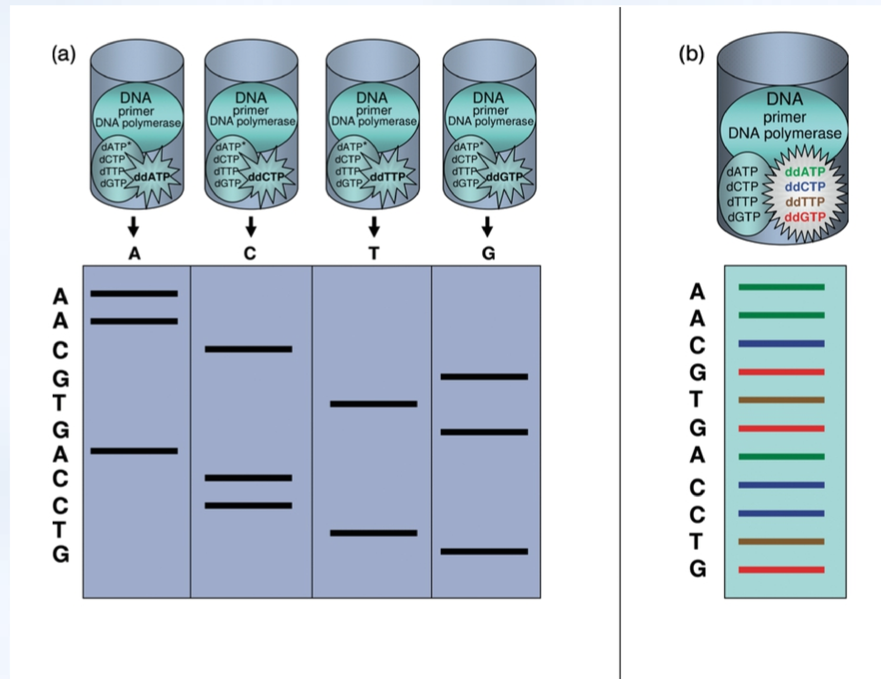
Luisa María Henau Mejía y Cristina Goebel Vázquez – Máster Oficial en Biología Celular y Molecular 2015/2016

1º Generation Sequencing (1976-1977)

Alan Maxam & Walter Gilbert



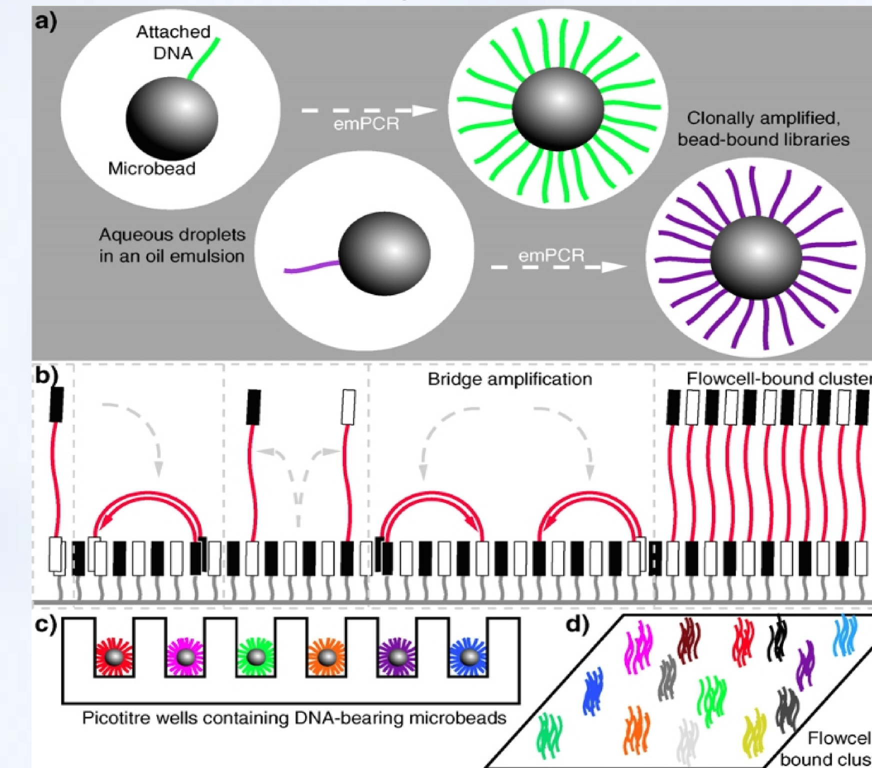
Frederick Sanger



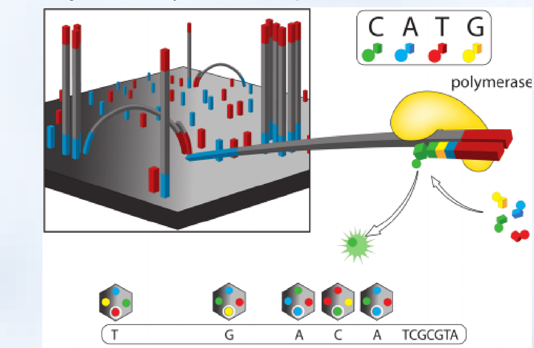
The first one is a chemical method to cleave DNA at specific points and the second one uses ddNTPs which synthesizes a copy from the DNA chain template. Both methods generate fragments of varying lengths that are further electrophoresed. Some advancements within the first generation include the replacement of radioactive labels by fluorescent labeled ddNTPs and cycle sequencing with thermostable DNA polymerase, which allows automation and signal amplification making the process cheaper, safer and faster.

2º Generation Sequencing (Next Generation Sequencing)

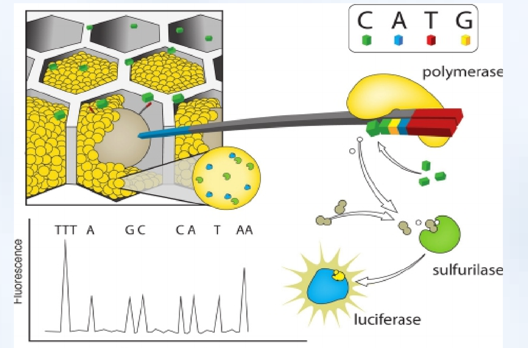
emulsion PCR & bridge PCR



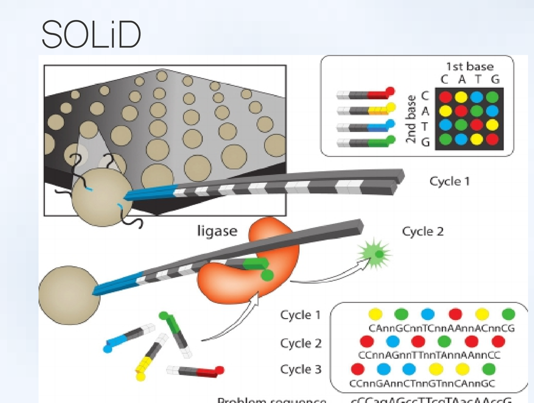
Pyrosequencing(Roche/454)



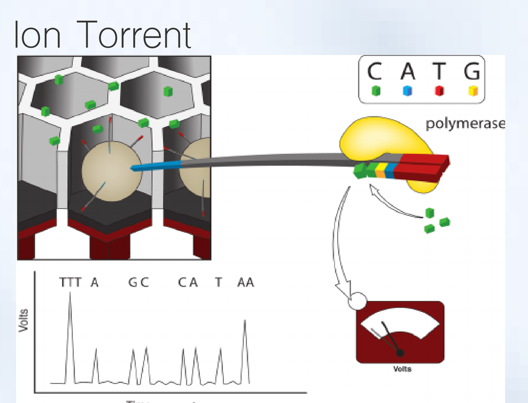
Illumina/Solexa



SOLiD



Ion Torrent



In the 2º generation sequencing electrophoresis was completely eliminated with many methods that can process multiple samples. The Pyrosequencing is based on the "sequencing by synthesis" principle. It differs from Sanger sequencing, in that it relies on the detection of pyrophosphate release on nucleotide incorporation. One of the obsolete methods but sometimes used is SOLiD, based on sequencing by ligation of fluorescently dye-labeled di-base probes which competes to ligate to the sequencing primer. Specificity of the di-base probe is achieved by interrogating every 1st and 2nd base in each ligation reaction. The widely used Solexa/Illumina method uses modified dNTPs containing so called "reversible terminators" which blocks further polymerization. The terminator also contains a fluorescent label, which can be detected by the camera. The previous step towards the third generation was in charge of Ion Torrent, who developed a technique that is based in a method of "sequencing-by-synthesis". Its main feature is the detection of hydrogen ions that are release during base incorporation.

1976-1977

(Pyrosequencing) 1996

1998 (Solexa)

2000 (454)

2006 (SOLiD)

2010 (Ion Torrent)

2011

2012

Comparison Sequencing Technology

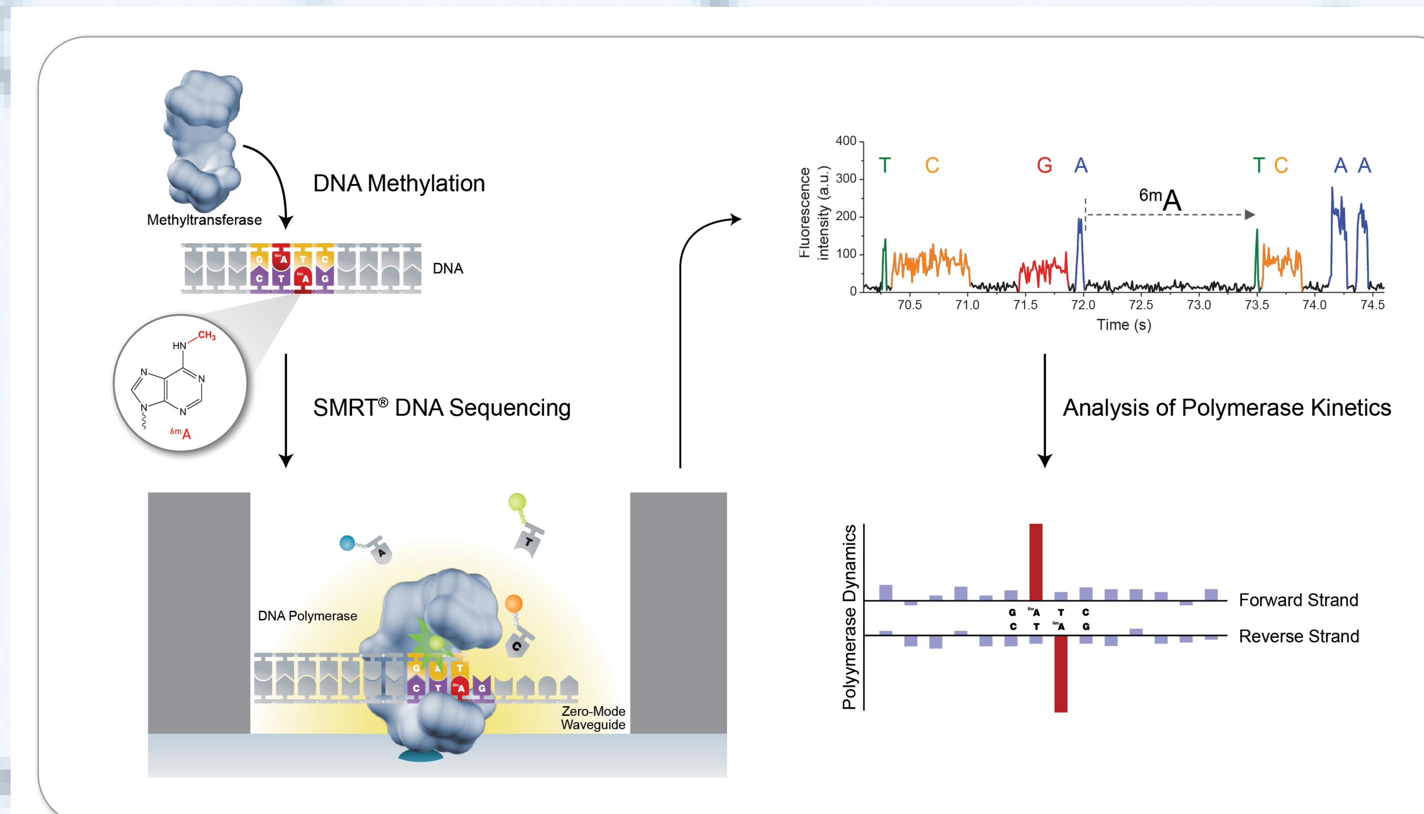
Table 1 Performance comparison of sequencing platforms of various generations

Method	Generation	Read length (bp)	Single pass error rate (%)	No. of reads per run	Time per run	Cost per million bases (USD)
Sanger ABI 3730×1	1st	600–1000	0.001	96	0.5–3 h	500
Ion Torrent	2nd	200	1	8.2×10^7	2–4 h	0.1
454 (Roche) GS FLX +	2nd	700	1	1×10^6	23 h	8.57
Illumina HiSeq 2500 (High Output)	2nd	2 × 125	0.1	8×10^9 (paired)	7–60 h	0.03
Illumina HiSeq 2500 (Rapid Run)	2nd	2 × 250	0.1	1.2×10^9 (paired)	1–6 days	0.04
SOLiD 5500×1	2nd	2 × 60	5	8×10^8	6 days	0.11
PacBio RS II: P6-C4	3rd	$1.0–1.5 \times 10^4$ on average	13	$3.5–7.5 \times 10^4$	0.5–4 h	0.40–0.80
Oxford Nanopore MinION	3rd	$2–5 \times 10^3$ on average	38	$1.1–4.7 \times 10^4$	50 h	6.44–17.90

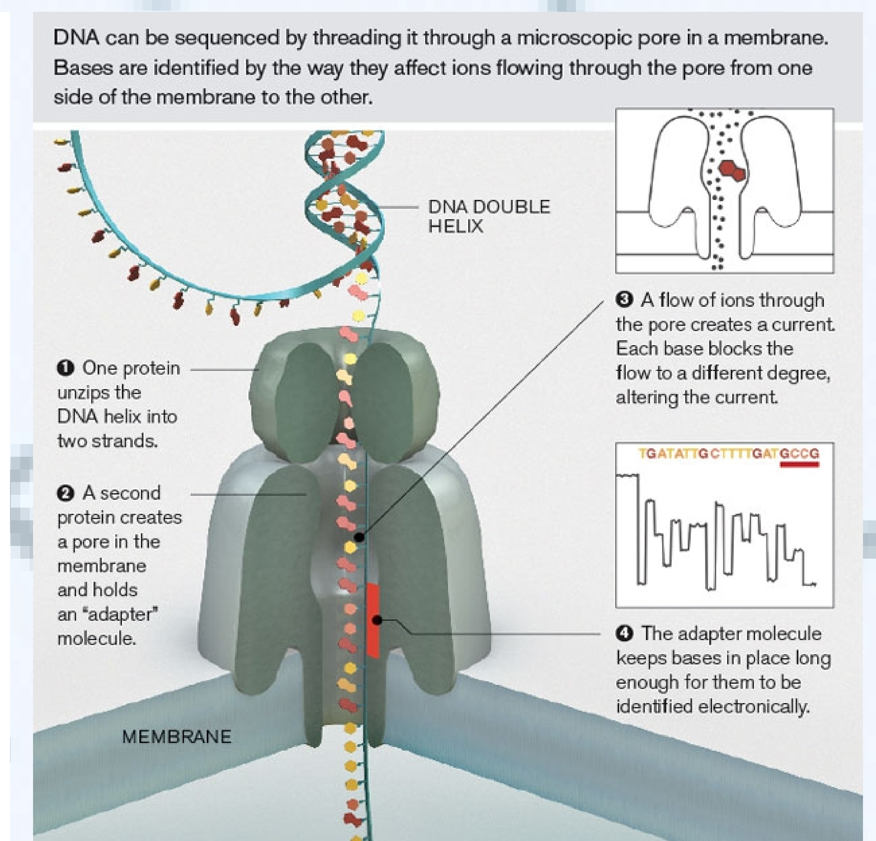
A new cohort of techniques has since been developed using single molecule sequencing and single real time sequencing, removing the need for clonal amplification. This reduces errors caused by PCR, simplifies library preparation and, most importantly, gives a much higher read length using higher throughput platforms. The third generation takes into account nanotechnology advancements for the processing of unique DNA molecules to a real time synthesis sequencing system like PacBio; and finally, the NANOPORE, projected since 1995, also uses Nano-sensors forming channels obtained from bacteria that conducts the sample to a sensor that allows the detection of each nucleotide residue in the DNA strand.

3º Generation Sequencing

PacBio



NanoPore



How do we imagine the next generation?