CIICAM
RESEARCH CENTER

Obteniendo información del DNA. De Sanger hasta el secuenciamiento de última generación

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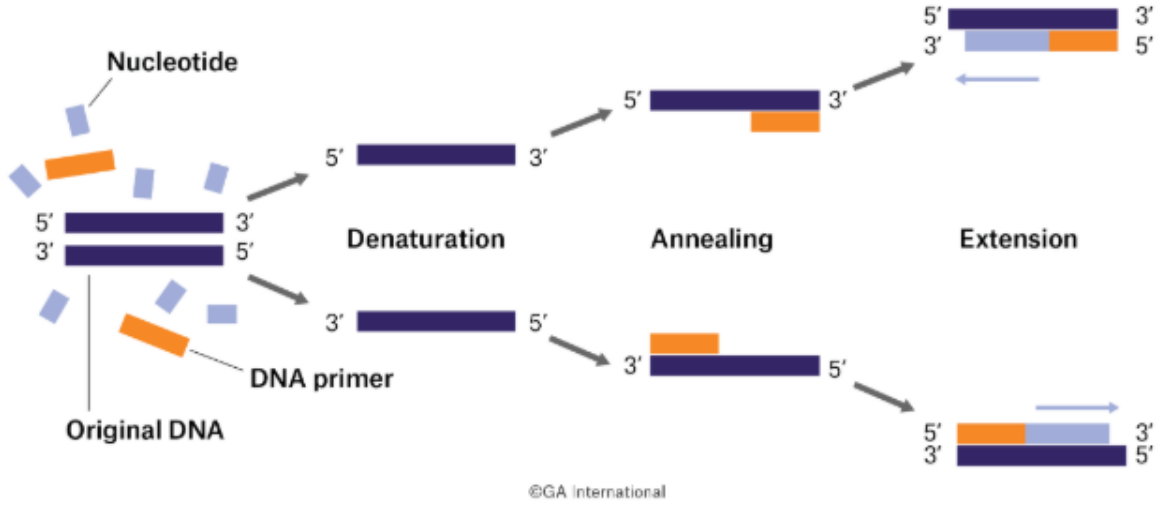
Amplificación de DNA

1983- Kary Mullis

- Creador de la PCR

https://i1.wp.com/procrastinafacil.com/wp-content/uploads/2018/03/Kary-B-Mullis-400x505.jpg?resize=400%2C505&ssl=1



http://blog.labtag.com/a-brief-history-of-pcr-and-its-derivatives

Reacción en cadena de la polimerasa - PCR

Buffer

- Mantener el pH de la reacción
- 1X = 10 mM

Cationes

- $MgCl_2$ – Mn - KCl
- 1,5 mM – 2,5mM

dNTP

- Deoxynucleótidos dATP - dTTP - dGTP - dCTP
- 200 µM

Reacción en cadena de la polimerasa - PCR

Primers

- Oligonucleótidos diseñados
- 0,1 – 0,5 µM

Taq

- De acuerdo al tipo de PCR
- 1 – 2 U

Template

- DNA puro A260/A280 =1,5 - 1,9
- Íntegro, a veces degradado.

Reacción en cadena de la polimerasa – PCR

Puntos críticos de optimización de la PCR

Mastermix

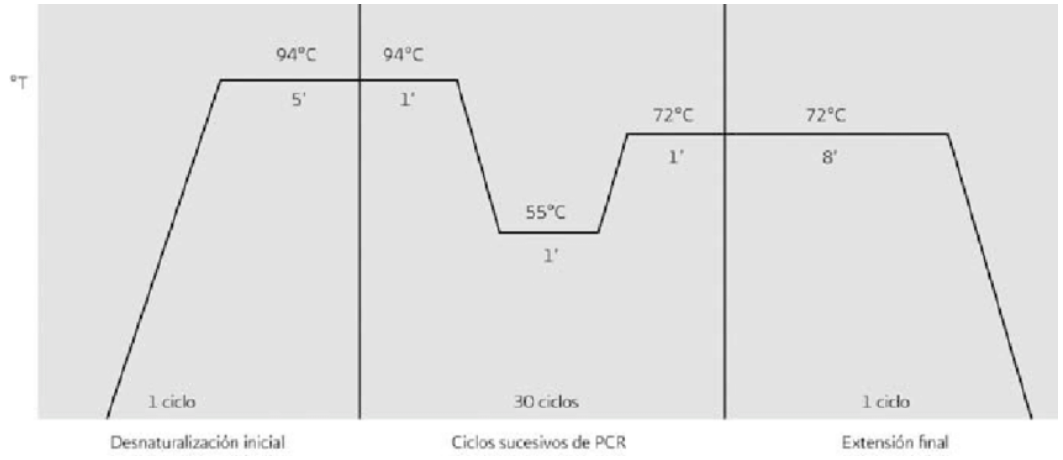
- Calidad y cantidad del DNA (Eucariotas 1 μg, Levaduras 10 ng, Bacterias 1 ng, Plásmidos 1 pg)
- Cationes, no exceder 2,5 mM.
- Taq. Tipo de taq y la cantidad.

Reacción en cadena de la polimerasa – PCR

Puntos críticos de optimización de la PCR

Reacción en temociclador

- Anillamiento: específico para cada primer. A mayor temperatura más astringencia de la reacción.
- Número de ciclos: Dependerá del número de copias del gen en el genoma.
- Extensión: depende de la taq y del protocolo establecido.



https://www.researchgate.net/figure/Figura-3-Etapas-de-la-PCR-temperaturas-tiempos-y-ciclos-estandares_fig3_266856169

Reacción en cadena de la polimerasa - PCR

TABLE 8-2 Theoretical Number of Cycles Required for PCR

	Targets					
Y	1	10	100	1,000	10,000	100,000
1.00	34	30	27	24	20	17
0.95	35	32	28	25	21	18
0.90	36	33	29	26	22	18
0.85	38	34	30	27	23	19
0.60	40	36	32	28	24	20
0.75	42	38	33	29	25	21
0.70	44	40	35	31	27	22
0.65	46	42	37	33	28	23
0.60	49	45	40	35	30	25
0.55	53	48	43	37	32	27
0.50	57	52	46	40	35	29
0.45	62	56	50	44	38	31
0.40	69	62	55	48	42	35
0.35	77	70	62	54	47	39
0.30	88	79	71	62	53	44
0.25	104	93	83	73	62	52
0.20	127	114	102	89	76	64
0.15	165	149	132	116	99	83
0.10	242	218	194	170	145	121

Reprinted, with permission, from Rameckers et al. (1997 [©Springer-Verlag]).

Number of PCR cycles (rounded) required to reach 10 ng of DNA (based on a 200-bp PCR product) at various efficiency levels (Y) and various target numbers (targets).

Aplicaciones de la PCR

Carcinogenesis vol.19 no.1 pp.233–235, 1998

SHORT COMMUNICATION

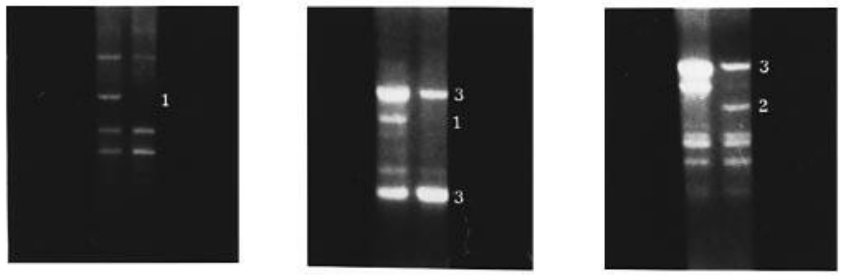
Detection of genomic instability in lung cancer tissues by random amplified polymorphic DNA analysis

Tong-man Ong[1,3], Bi Song[1], Hong-wei Qian[1], Zhong Liang Wu[2] and Wen-zong Whong[1]

[1]Toxicology and Molecular Biology Branch, Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Morgantown, WV 26505, USA and [2]Guangzhou Medical College, Guangzhou, China

instability in lung cancer tissues ranges from 45 to 76% (6,8,9) for small cell lung cancer (SCLC*) and only from 2 to 34% (5,6,10–12) for non-small cell lung cancer (NSCLC). The varying results may be due, in part, to differences in the type and number of primers (6 to 36 pairs) used.

Random amplified polymorphic DNA (RAPD) is a PCR-



Ramdom Amplified Polymorphism of DNA

JOURNAL OF CLINICAL MICROBIOLOGY, June 2000, p. 2076–2080 Vol. 38, No. 6
0095-1137/00/$04.00+0

Use of PCR with Universal Primers and Restriction Endonuclease Digestions for Detection and Identification of Common Bacterial Pathogens in Cerebrospinal Fluid

JANG-JIH LU,[1]* CHERNG-LIH PERNG,[1] SHIH-YI LEE,[1] AND CHIH-CHIENG WAN[2]

Division of Clinical Pathology, Department of Pathology,[1] and Department of Pediatrics,[2] Tri-Service General Hospital and National Defense Medical Center, Taipei, Taiwan, Republic of China

Received 3 November 1999/Returned for modification 23 December 1999/Accepted 23 March 2000

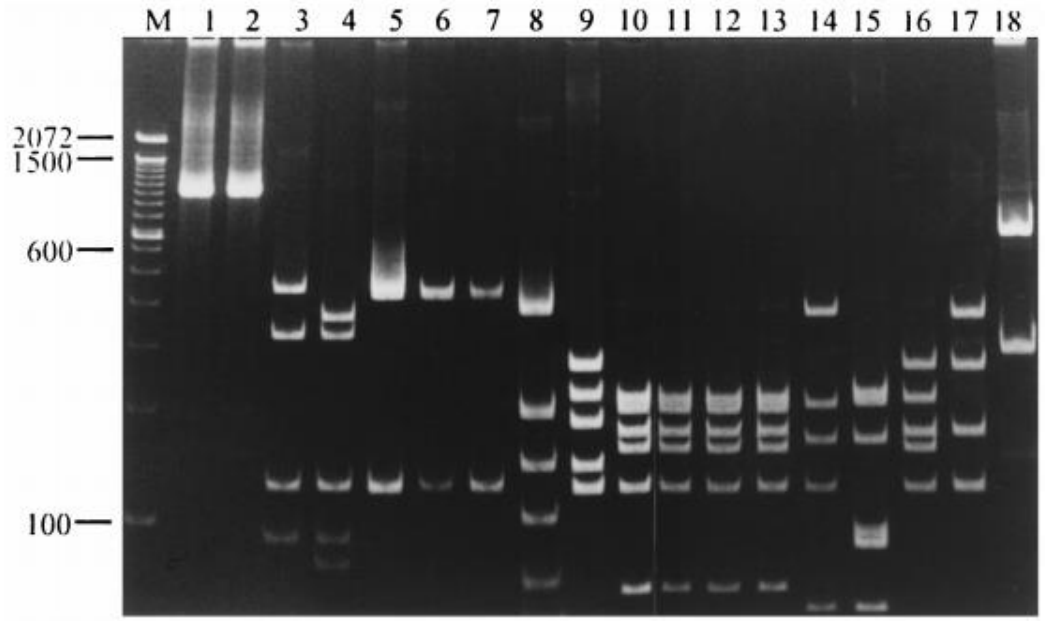


FIG. 1. *Hae*III digestion patterns of universal PCR products. Samples in different lanes were *Hae*III-digested PCR products from the following bacteria: lane 1, *S. aureus*; lane 2, *S. epidermidis*; lane 3, *S. pyogenes*; lane 4, *S. agalactiae*; lane 5, *S. pneumoniae*; lane 6, *E. faecium*; lane 7, *E. faecalis*; lane 8, *M. tuberculosis*; lane 9, *L. pneumophila*; lane 10, *E. coli*; lane 11, *K. pneumoniae*; lane 12, *S. marcescens*; lane 13, *E. cloacae*; lane 14, *P. aeruginosa*; lane 15, *A. baumannii*; lane 16, *P. mirabilis*; lane 17, *H. influenzae*; lane 18, *N. meningitidis*. Lane M contained molecular size standards (base pairs). The sizes of the molecular size standards are marked on the left of the gel.

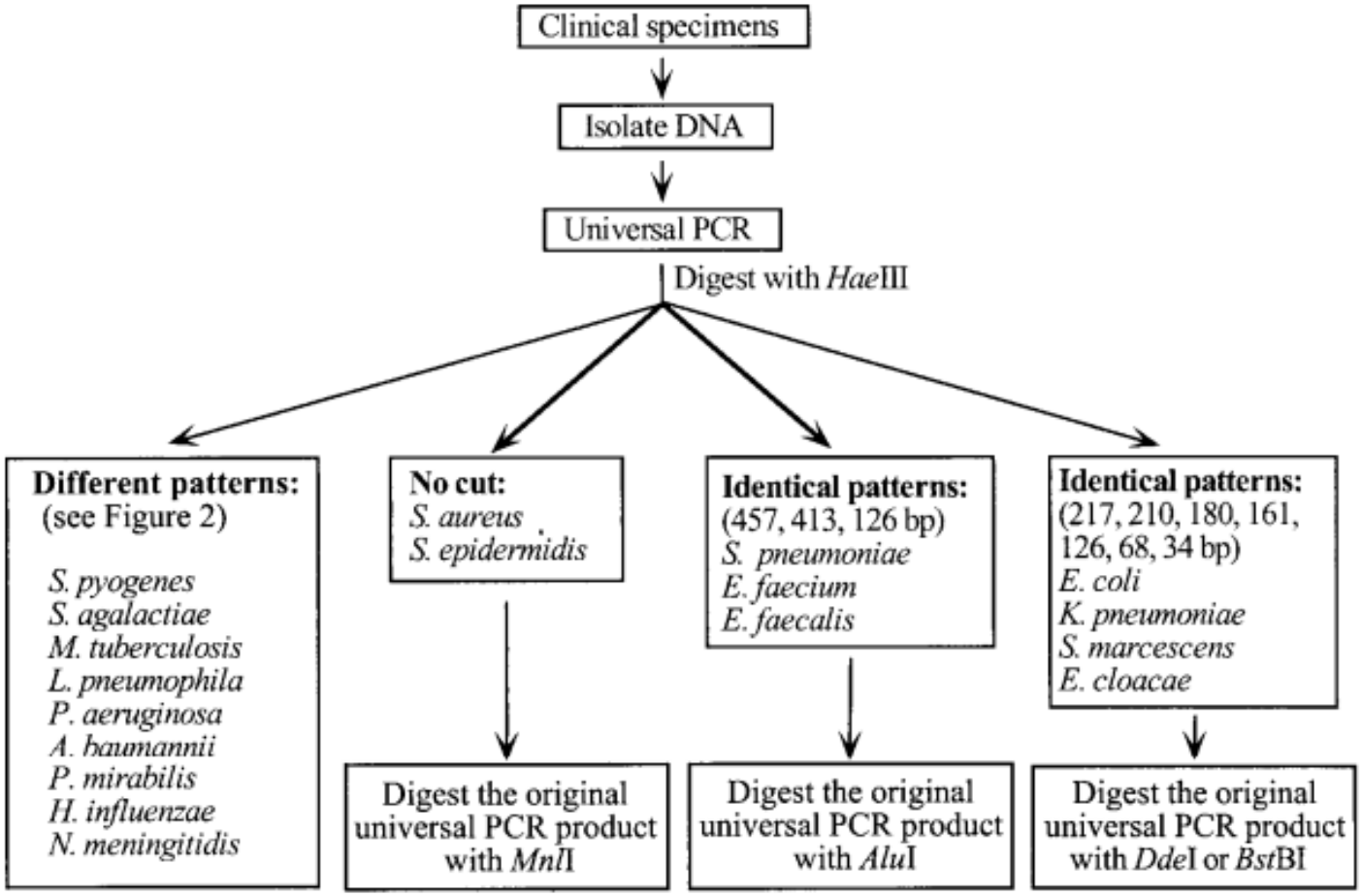
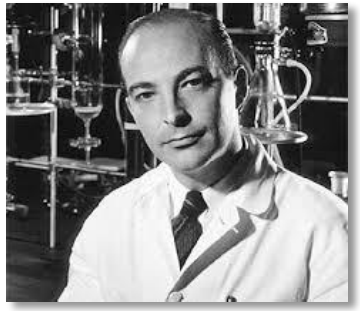


FIG. 4. Flow chart of the universal PCR and RFLP for detection and identification of common bacterial pathogens in body fluids.

Secuenciamiento de primera generación

1956- Arthur Kornberg

- DNA polimerasa



https://www.biografiasyvidas.com/biografia/k/fotos/kornberg.jpg

1965 Robert Holley y col.

- Primera sequencia tRNA alanine
- *Saccharomyces cerevisiae*

https://www.ars.usda.gov/ARSUserFiles/80620000/images/RobertHolley.jpg

Fred Sanger y col colleagues

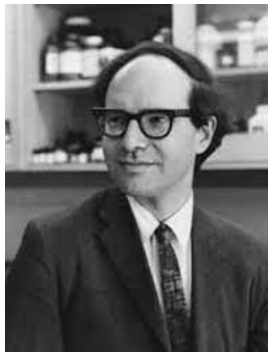
- Fragmentos parcialmente digeridos
- Marcados con radioisótopos
- Fraccionamiento 2-D
- Minus plus

1975-Allan Maxam y Walter Gilbert

- Secuenciamiento de DNA

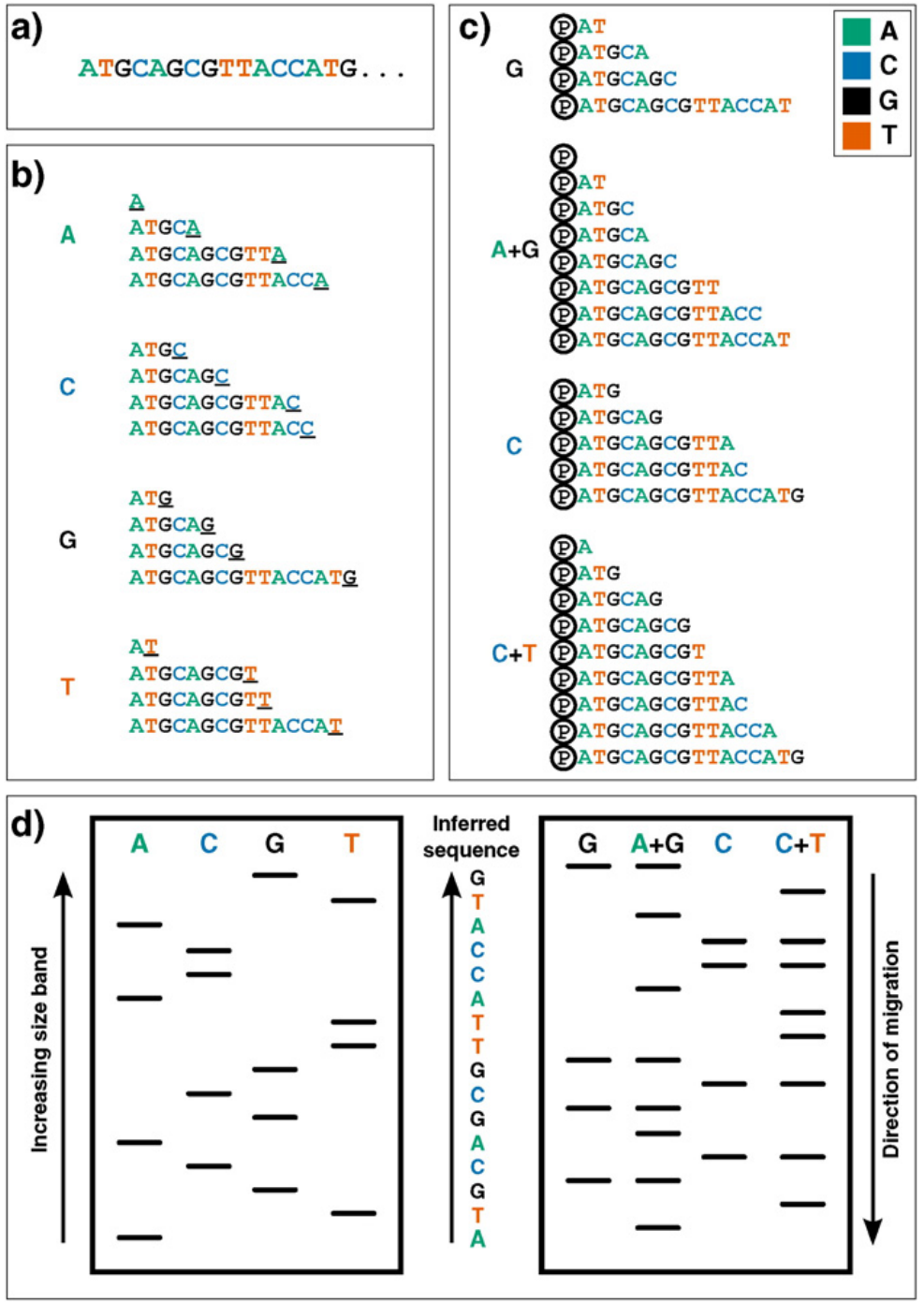
1977-Frederick Sanger

- Terminación de la cadena.
- Dideoxinucleótidos



https://www.nobelprize.org/images/sanger-13123-portrait-mini-2x.jpg

https://www.nndb.com/people/634/000100334/walter-gilbert-1.jpg

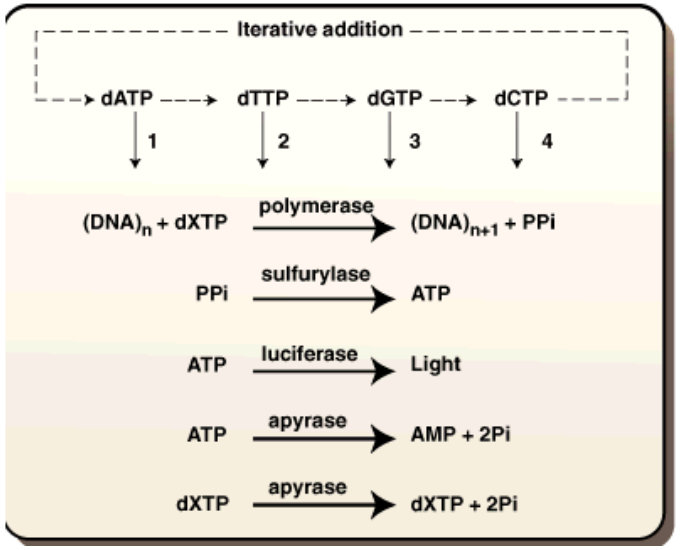


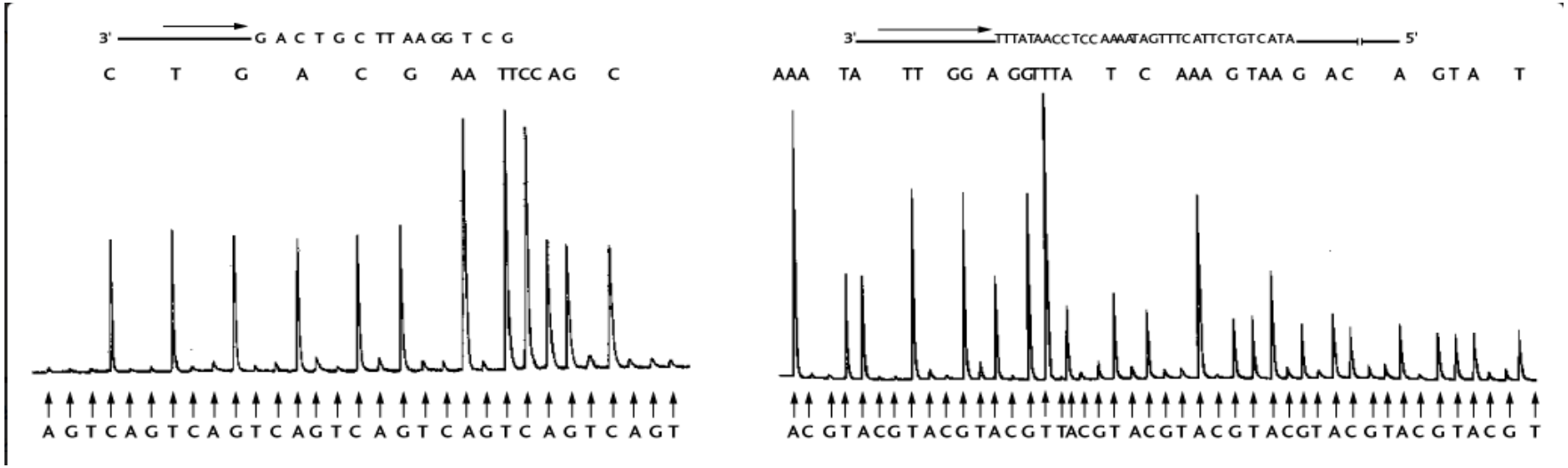
https://www.youtube.com/watch?v=oeJoTZCRrvU&t=242s

Secuenciamiento de segunda generación

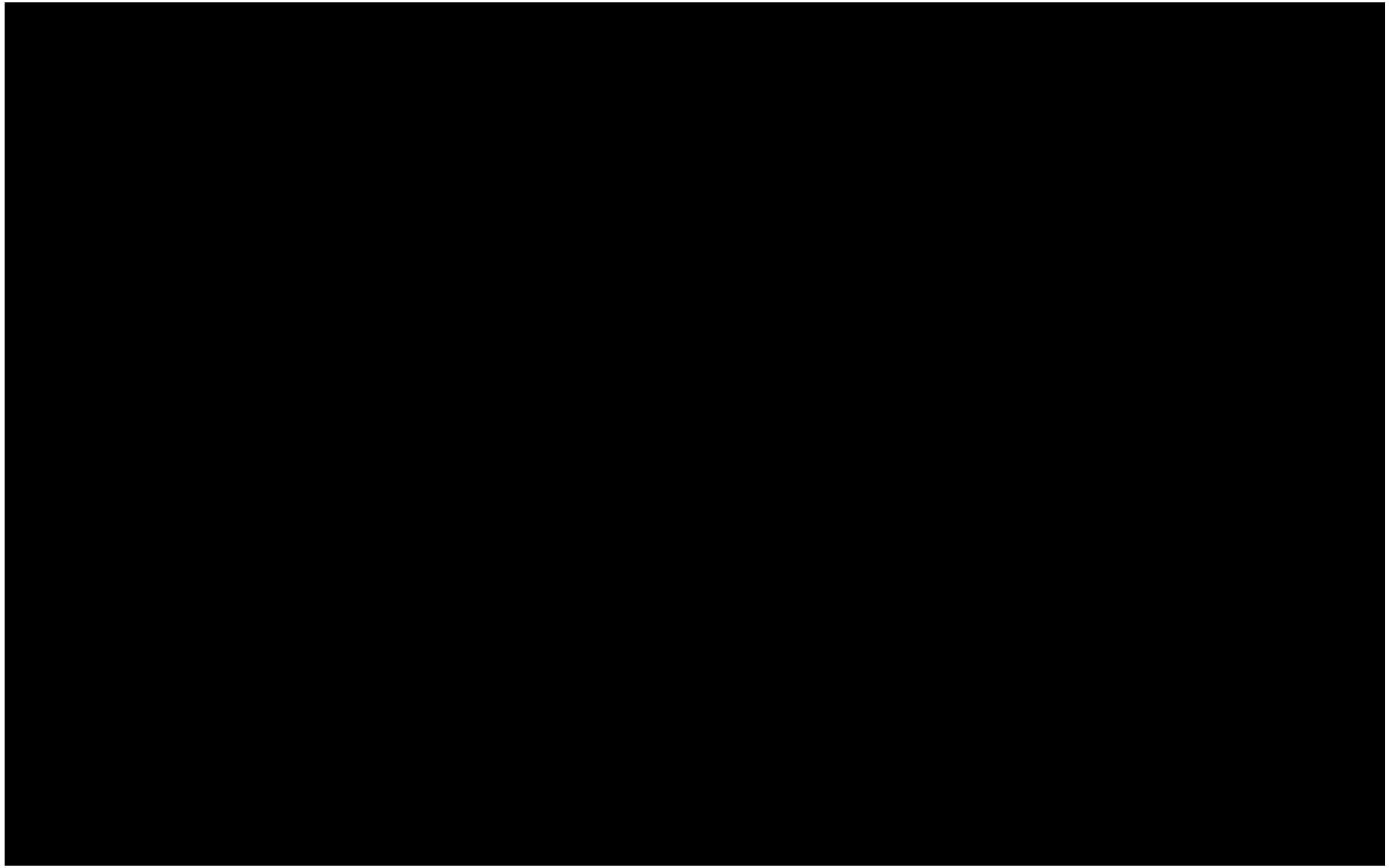
1998- Pål Nyrén

- Pirosequenciamiento





https://science.sciencemag.org/content/281/5375/363



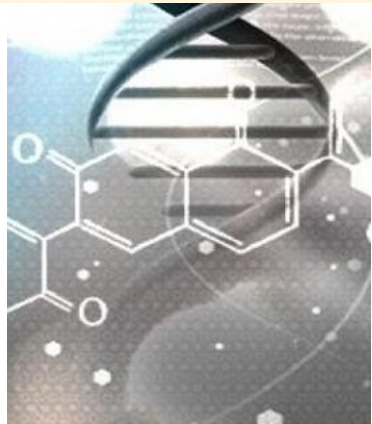
https://www.youtube.com/watch?v=fCd6B5HRaZ8

Table 1 Second-generation DNA sequencing technologies

	Feature generation	Sequencing by synthesis	Cost per megabase	Cost per instrument	Paired ends?	1° error modality	Read-length	References
454	Emulsion PCR	Polymerase (pyrosequencing)	~$60	$500,000	Yes	Indel	250 bp	14,20
Solexa	Bridge PCR	Polymerase (reversible terminators)	~$2	$430,000	Yes	Subst.	36 bp	17,22
SOLiD	Emulsion PCR	Ligase (octamers with two-base encoding)	~$2	$591,000	Yes	Subst.	35 bp	13,26
Polonator	Emulsion PCR	Ligase (nonamers)	~$1	$155,000	Yes	Subst.	13 bp	13,20
HeliScope	Single molecule	Polymerase (asynchronous extensions)	~$1	$1,350,000	Yes	Del	30 bp	18,30

The pace with which the field is moving makes it likely that estimates for costs and read-lengths will be quickly outdated. Vendors including Roche Applied Science, Illumina, and Applied Biosystems have major upgrade releases currently in progress. Estimated costs-per-megabase are approximate and inclusive only of reagents. Read-lengths are for single tags. Subst., substitutions; indel, insertions or deletions; del, deletions.

https://www.nature.com/articles/nbt1486.pdf



Secuenciamiento de tercera generación

2009- Jonas Korlach y col

- Secuenciamiento de una sola molécula de DNA.
- Tecnología de nanoporo.

https://mendelspod.com/sites/default/files/JonasKorlach.png


SMRT™ Cell

Dogma central de la biología molecular

1957: Francis Crick