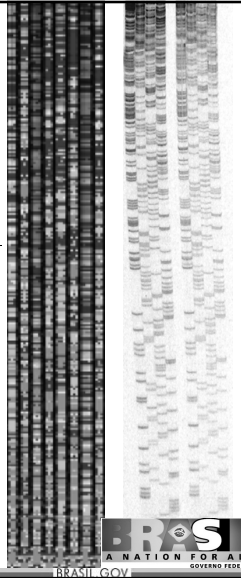
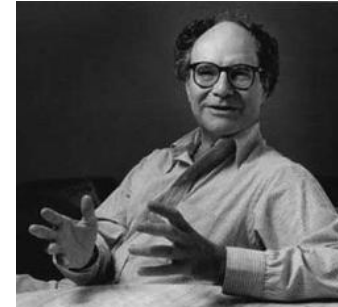


Seqüenciamento



1977: Walter Gilbert e Frederick Sanger seqüenciamento de DNA



Nobel 1980

“pelas contribuições na determinação de seqüências
de ácidos nucleicos”

Proc. Natl. Acad. Sci. USA
Vol. 74, No. 12, pp. 5463-5467, December 1977
Biochemistry

DNA sequencing with chain-terminating inhibitors

(DNA polymerase/nucleotide sequences/bacteriophage ϕ X174)

F. SANGER, S. NICKLEN, AND A. R. COULSON

Medical Research Council Laboratory of Molecular Biology, Cambridge CB2 2QH, England

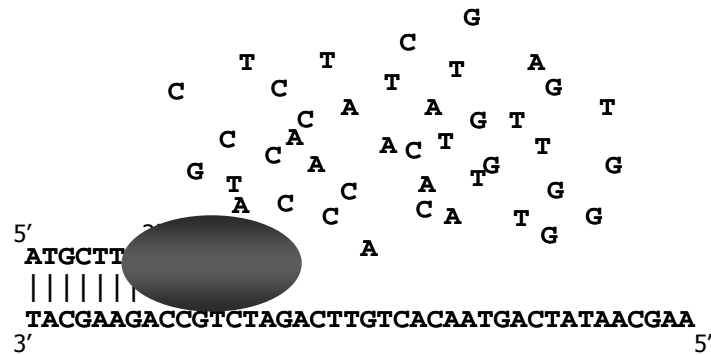
Contributed by F. Sanger, October 3, 1977

Procedimento enzimático

(Sanger, 1977)

A posição das bases é determinada pelo
tamanho dos fragmentos obtidos
através de reações de
polimerização
na presença de
dideoxynucleotídeos.

Polimerização de DNA

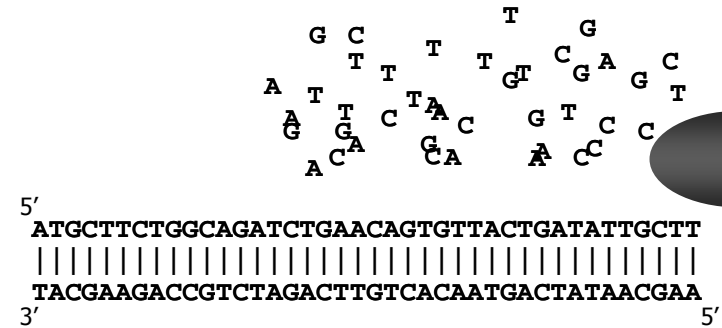


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Polimerização de DNA

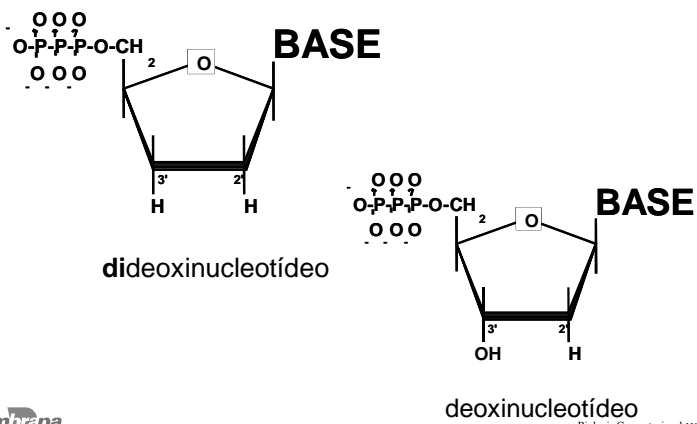


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Dideoxynucleotídeo

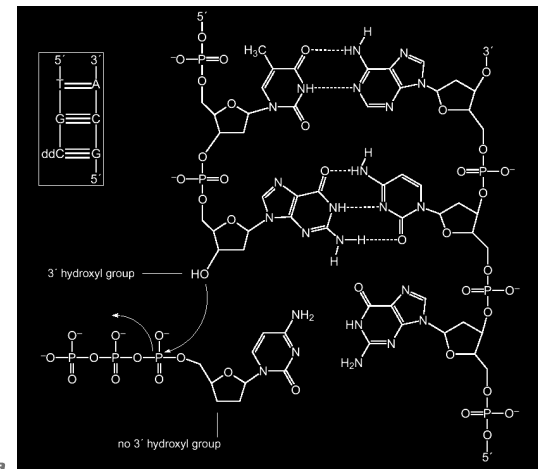


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Seqüenciamento de DNA



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Polimerização de DNA *o dideoxi*

5' ATGCTTCTGGCAGAT
3' TACGAAGACCGTCTAGACTTGT CACAATGACTATAACGAA

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Polimerização de DNA *o dideoxi*

5' ATGCTTCTGGCAGAT
3' TACGAAGACCGTCTAGACTTGT CACAATGACTATAACGAA

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Seqüenciamento de DNA

5' ATGCTTCTGGCAGAT
3' TACGAAGACCGTCTAGACTTGT CACAATGACTATAACGAA

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Seqüenciamento de DNA

- molde
- polimerase
- dNTPs

•ddGTPs

•ddATPs

•ddTTPs

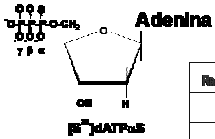
•ddCTPs

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Nucleotídeos Radioativos

[S³²]dNTP α S

A baixa energia de emissão β do S³² resulta em bandas mais nítidas o que aumenta a eficiência de leitura no tipo do gel. A menor energia permite também provocar menor número de clivagens permitindo o armazenamento das reações por tempos mais prolongados do que as reações com incorporação de P³².



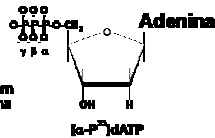
Radionúclido	Meia-vida	Energia (MeV)
S ³²	87,4 dias	0,157
P ³²	14,3 dias	1,710
P ³³	25,3 dias	0,240

[P³²]dNTP

A maior energia de emissão β do P³² apresenta a vantagem de menores tempos de exposição, no entanto apresenta menor resolução e estabilidade das reações que não devem ser armazenadas por tempos prolongados.

[P³³]dNTP

As partículas β emitidas por P³³ apresentam energia média 50% superior a de S³². As sequências geradas utilizando-se P³³ apresentam tempos de exposição mais curtos e de mesma resolução de que as obtidas com S³².



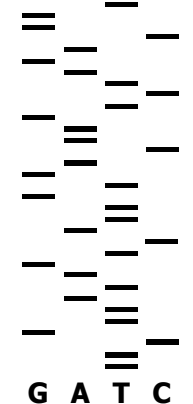
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Sequenciamento de DNA

```

ATGCTTCT
ATGCTCTG
ATGCTCTGG
ATGCTCTGGCA
ATGCTCTGGCAG
ATGCTCTGGCAGA
ATGCTCTGGCAGAT
ATGCTCTGGCAGATC
ATGCTCTGGCAGATCT
ATGCTCTGGCAGATCTG
ATGCTCTGGCAGATCTGA
ATGCTCTGGCAGATCTGAA
ATGCTCTGGCAGATCTGAAC
ATGCTCTGGCAGATCTGAACA
ATGCTCTGGCAGATCTGAACAG
ATGCTCTGGCAGATCTGAACAGT
ATGCTCTGGCAGATCTGAACAGTG
ATGCTCTGGCAGATCTGAACAGTGT
ATGCTCTGGCAGATCTGAACAGTGTG
ATGCTCTGGCAGATCTGAACAGTGTGA
ATGCTCTGGCAGATCTGAACAGTGTGAT
ATGCTCTGGCAGATCTGAACAGTGTGATG
ATGCTCTGGCAGATCTGAACAGTGTGATGG
ATGCTCTGGCAGATCTGAACAGTGTGATGGT
ATGCTCTGGCAGATCTGAACAGTGTGATGGCT
ATGCTCTGGCAGATCTGAACAGTGTGATGGCTT
    
```



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Sequenciamento de DNA

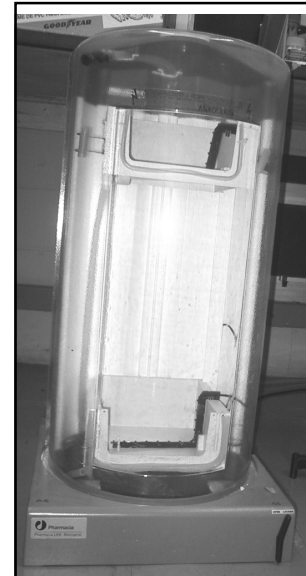
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ATGCTTCT
ATGCTCTG
ATGCTCTGG
ATGCTCTGGC
ATGCTCTGGCA
ATGCTCTGGCAG
ATGCTCTGGCAGA
ATGCTCTGGCAGAT
ATGCTCTGGCAGATC
ATGCTCTGGCAGATCT
ATGCTCTGGCAGATCTG
ATGCTCTGGCAGATCTGA
ATGCTCTGGCAGATCTGAA
ATGCTCTGGCAGATCTGAAC
ATGCTCTGGCAGATCTGAACA
ATGCTCTGGCAGATCTGAACAG
ATGCTCTGGCAGATCTGAACAGT
ATGCTCTGGCAGATCTGAACAGTG
ATGCTCTGGCAGATCTGAACAGTGT
ATGCTCTGGCAGATCTGAACAGTGTG
ATGCTCTGGCAGATCTGAACAGTGTGA
ATGCTCTGGCAGATCTGAACAGTGTGAT
ATGCTCTGGCAGATCTGAACAGTGTGATG
ATGCTCTGGCAGATCTGAACAGTGTGATGG
ATGCTCTGGCAGATCTGAACAGTGTGATGGT
ATGCTCTGGCAGATCTGAACAGTGTGATGGCT
ATGCTCTGGCAGATCTGAACAGTGTGATGGCTT
    
```



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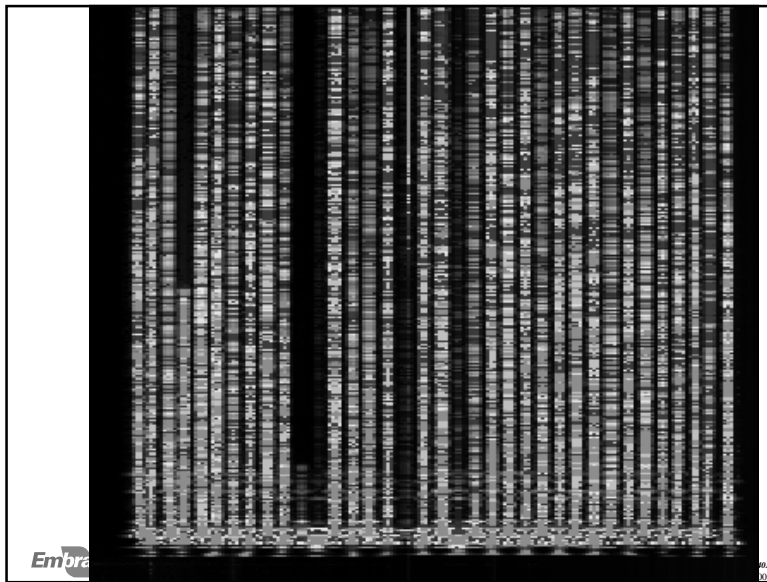
Biologia Computacional M0660A
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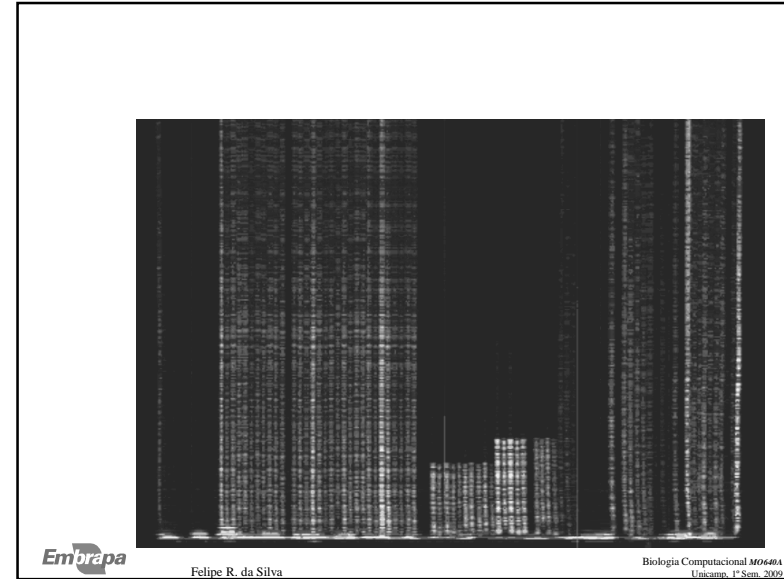
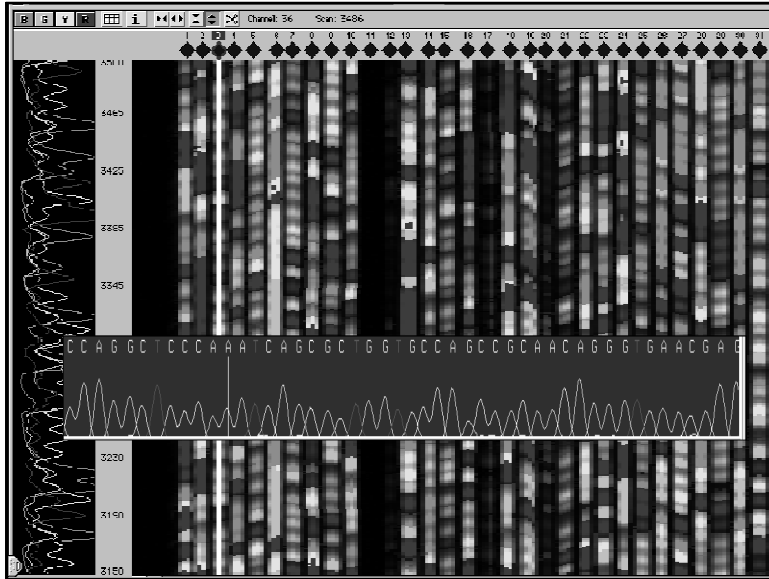
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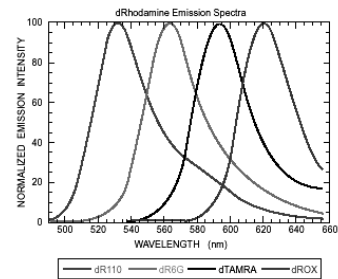
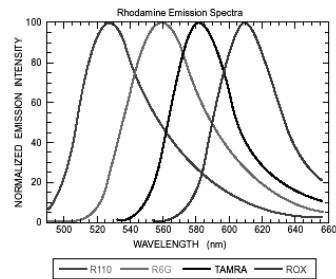
02



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Terminator	Acceptor Dye	Color of Raw Data on ABI PRISM 3700 or 310 Electroferograms	Color of Raw Data on ABI PRISM 377 or 373 Gel Image
A	dR6G	Green	Green
C	dROX	Red	Red
G	dR110	Blue	Blue
T	dTAMRA	Black	Yellow



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Table 2-6 ABI PRISM 310, ABI 373 with BigDye Filter Wheel, and ABI PRISM 377 Chemistry Recommendations

	dRhodamine Terminator	BigDye Terminator	BigDye Primer
DNA Sequencing Application			
<i>De novo</i> sequencing—high throughput	S	R ^a	R
<i>De novo</i> sequencing—mid-to-low throughput	S	R	S
Comparative sequencing (germline mutations 50:50 heterozygotes)	S	R	R
Comparative sequencing (somatic mutations 30:70 heterozygotes)	N	S	R
Comparative sequencing (somatic mutations 10:90 heterozygotes)	N	N	S
Gene walking (custom primers)	S	R	N
Shotgun sequencing (universal primers, M13)	S	R	R
Deletion clone sequencing (universal primers)	S	R	R
Gap closure (custom primers)	S	R	N
DNA Sequence Context			
GC-rich >65%	S	R	S
AT-rich >65%	R	R	R
GT-rich regions	R	N	R
Homopolymer A or T >25 bp ^b	R	N	R
Template			
Plasmid (<15 kb)	R	R	R
M13	R	R	R
BAC, cosmid, lambda, large PCR product	S	R	S
Bacterial genomic DNA	N	R	N
PCR amplicon	R	R	R
PCR amplicon (heterozygous 50:50)	S	R	R
PCR amplicon (heterozygous 30:70)	N	S	R
PCR amplicon (heterozygous 10:90)	N	N	S

a. R = recommended, S = satisfactory, N = not recommended

b. All cycle sequencing chemistries can have difficulties with homopolymers >40 bp.

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Proc. Natl. Acad. Sci. USA
 Vol. 74, No. 2, pp. 560-564, February 1977
 Biochemistry

A new method for sequencing DNA

(DNA chemistry/dimethyl sulfate cleavage/hydrazine/piperidine)

ALLAN M. MAXAM AND WALTER GILBERT

Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts 02138

Contributed by Walter Gilbert, December 9, 1976

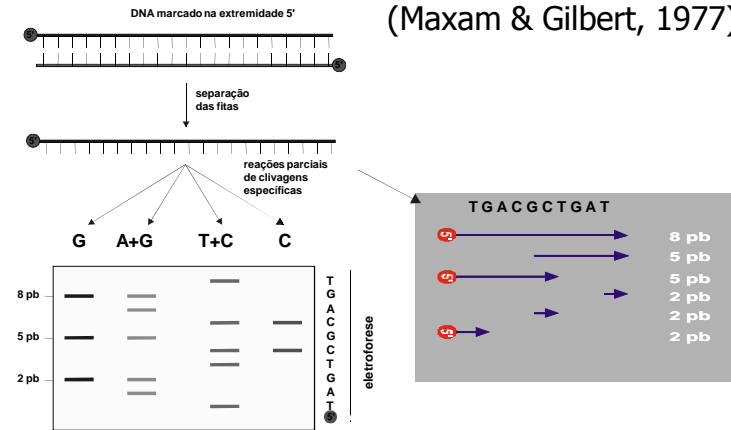


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Procedimento químico

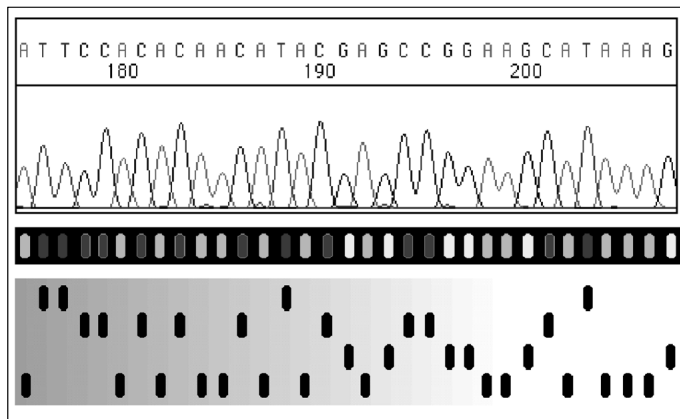
(Maxam & Gilbert, 1977)



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Seqüenciamento de DNA



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Seqüenciamento de DNA

1996, tese de mestrado:

Q. .S. .S. .P. .L. .P. .A. .L. .M. .A. .A. .Q. .I. .A. .Q. .Q. .L. .T. .Q. .M. . 57
 AGTCGTCACCGCTCCCGGCGCTGATGGGGCCSAAA TAGCACAGCAACTGACCGCGATGT 2075
 AGCCCGCTC GCGCTGATGGCGGCG?AAATAGDE?AGCAACTGACCGGATGT 420
 AGCCCGCTC GCGCTGATGGCGGCGSAAA TAGCGAGCAACTGACCGCTGATGT 559
 Q. .P. .L. A. .L. .M. .A. .A. .Q. .I. .A. .Q. .Q. .L. .T. .Q. .M. . 50

2000, tese de doutorado:

440 445 450 455 460 465 470 475 480 485
 A C T G G A A T T T A C * A A G A A C T T A C T C * A A G A A A C * A G C T C A C A G A G C T G G G *
 A C T G G A A T T T A C * A A G A A C T T A C T C * A A G A A A C * A G C T C A C A G A G C T G G G *
 A C T G G A A T T T A C * A A G A A C T T A C T C * A A G A A A C * A G C T C A C A G A G C T G G G *



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