


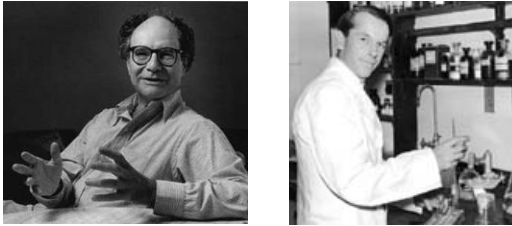
Seqüenciamento



Embrapa
 Pesquisa Científica e Inovação

RS
 INSTITUTO FEDERAL DE RESEARCH

1977: Walter Gilbert e Frederick Sanger seqüenciamento de DNA



Nobel 1980
 “pelas contribuições na determinação de seqüências de ácidos nucleicos”

Embrapa
 Felipe R. da Silva

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 11/04/2011, 17:56m, 20/22

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

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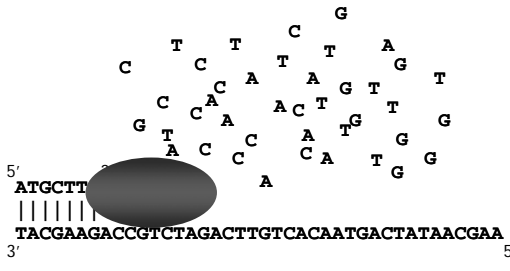
Procedimento enzimático (Sanger, 1977)

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

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Polimerização de DNA

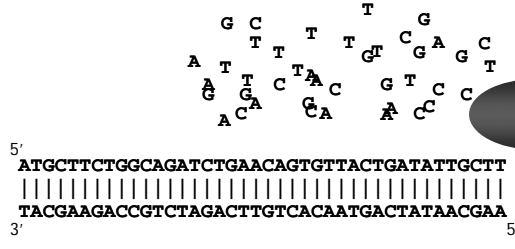


5' ATGCTT
 TACGAAGACCGTCTAGACTTGTACAATGACTATAACGAA 3'

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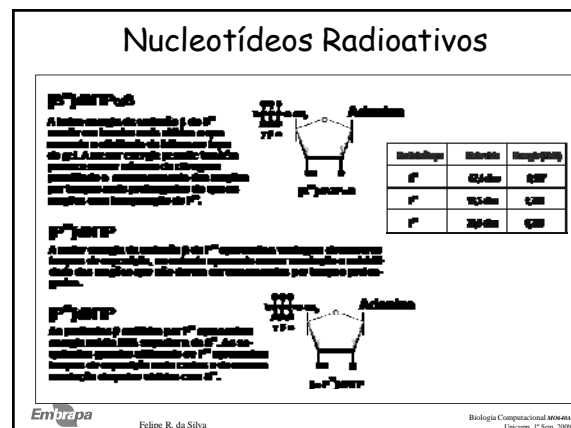
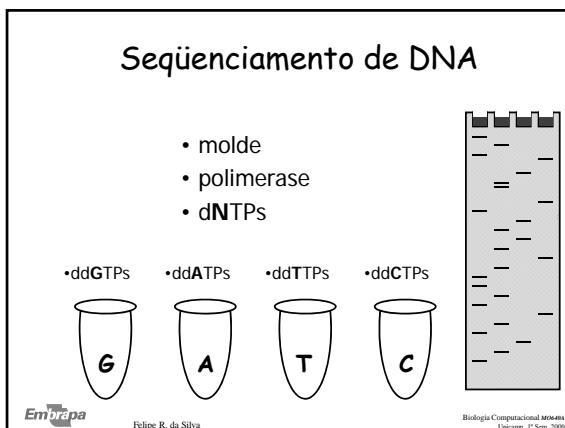
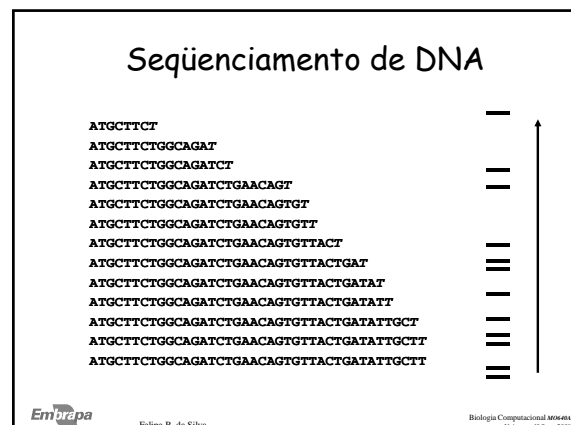
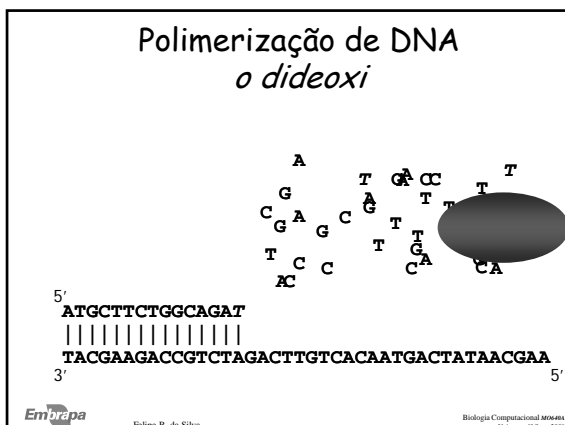
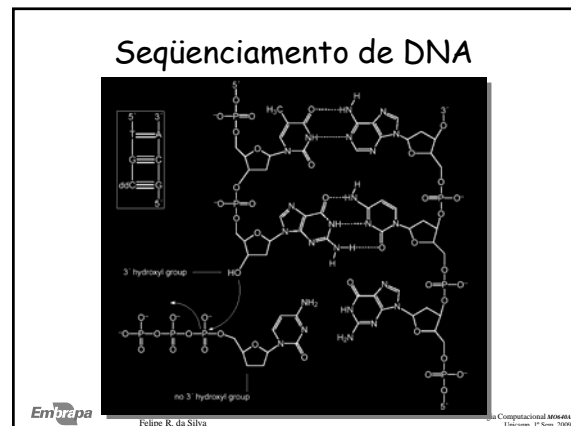
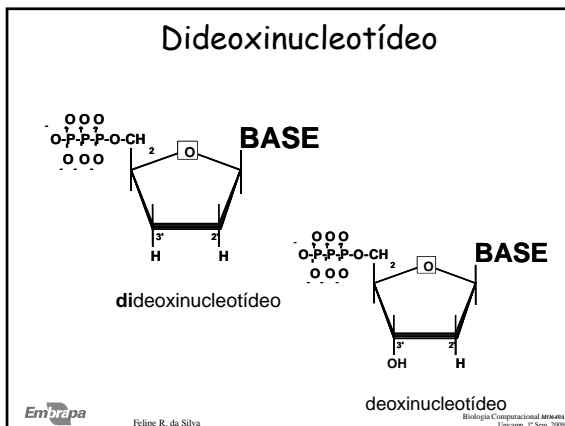
Polimerização de DNA



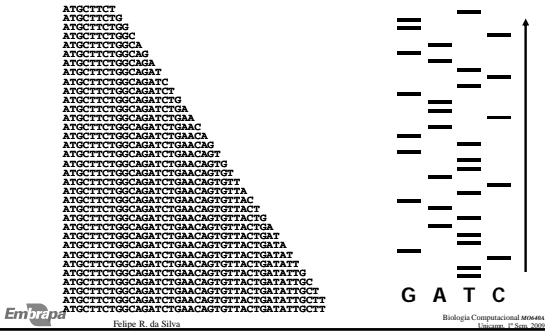
5' ATGCTTCTGGCAGATCTGAACAGTGTACTGATATTGCTT
 TACGAAGACCGTCTAGACTTGTACAATGACTATAACGAA 3'

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 Felipe R. da Silva

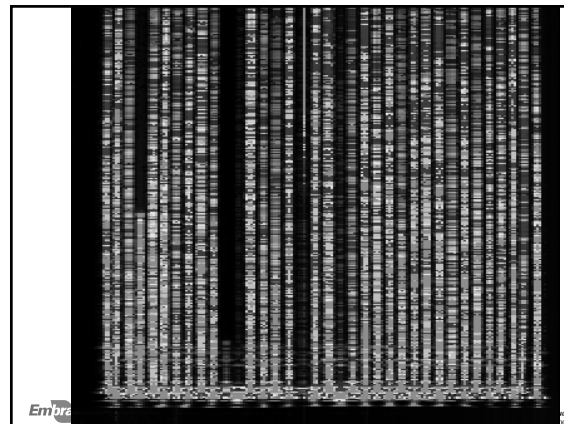
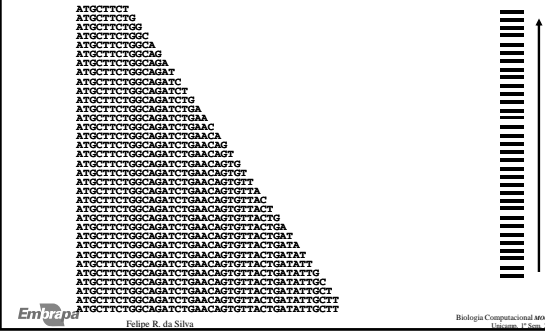
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Sequenciamento de DNA



Sequenciamento de DNA



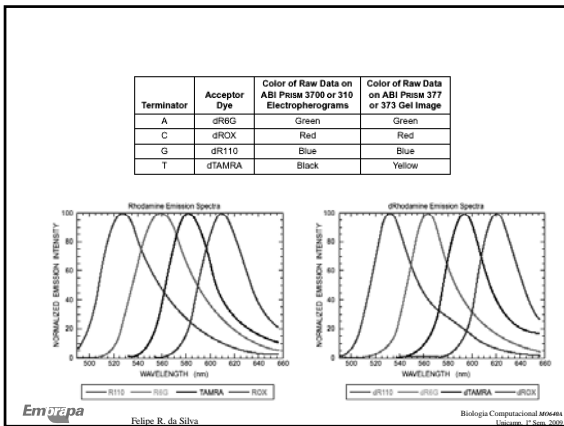
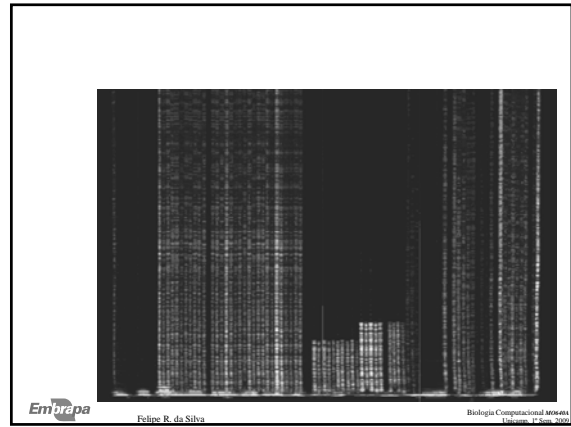
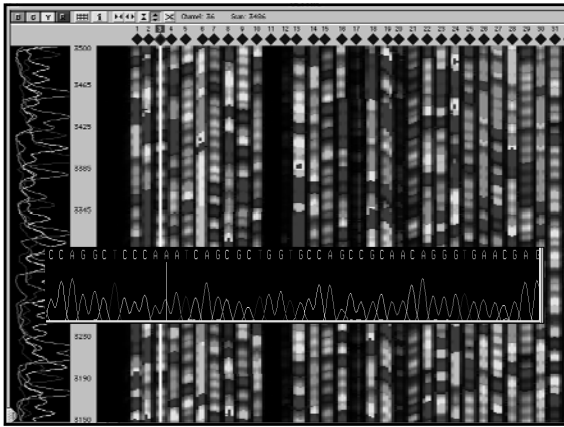


Table 2-6 ABI Prism 310, ABI 373 with BigDye Filter Wheel, and ABI Prism 377 Chemistry Recommendations

DNA Sequencing Application	dRhodamine Terminator	BigDye Terminator	BigDye Primer
De novo sequencing—high throughput	S	R	R
De novo sequencing—mid/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 (normal mutations 50-50 heterozygotes)	N	N	S
Gene walking (custom primers)	S	R	N
Shotgun sequencing (universal primers, M13)	S	R	R
Cloning clone sequencing (universal primers)	S	R	R
Gap closure (custom primers)	S	R	N
DNA Sequence Context			
GC-rich >65%	S	R	S
All non-ATGC	R	R	R
GC-rich regions	R	N	R
Homopolymer A or T >25 bp ^a	R	N	R
Template			
Plasmid (<15 kb)	R	R	R
M13	R	R	R
BAC, cosmid, lambda, large PCR product	S	R	S
Substrate genomes 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

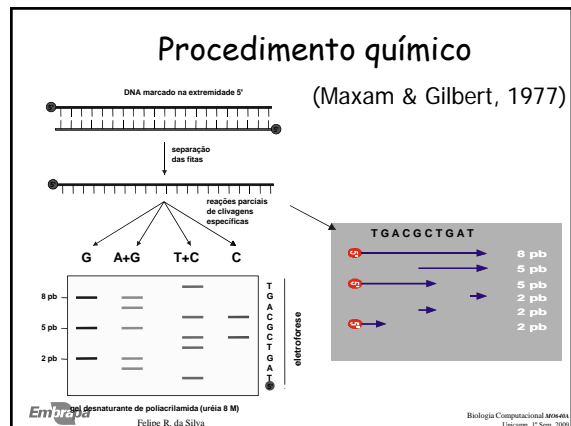
^a R = recommended, S = suboptimal, N = not recommended
^b All cycle sequencing chemistries can have difficulty with homopolymers >40 bp.

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

A new method for sequencing DNA

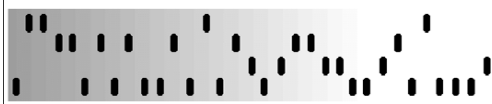
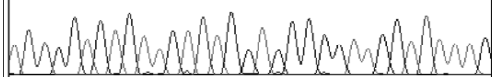
(DNA chemistry/dimethyl sulfate cleavage/hydrazine/piperidine)

ALLAN M. MAXAM AND WALTER GILBERT
 Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts 02138
 Contributed by Walter Gilbert, December 9, 1976



Seqüenciamento de DNA

ATTCCACACACATACGAGCCGGAGCATAAAG
180 190 200



Embrapa

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Tubiana, F. Sem. 2002

Seqüenciamento de DNA

1996, tese de mestrado:

Q..S..S..P..L..P..A..L..M..A..A..Q..L..A..Q..Q..L..T..M.. 57
AGTCGTCACCGCTCCCGGCGCTGATGGGGCGCAGAAAGCACAGCAACTGAGGCTATGCT 207
AGCCGCTC.....CCGCTGATGGGGCGCAGAAAGCACAGCAACTGAGGCTATGCT 423
AGCCGCTC.....CCGCTGATGGGGCGCAGAAAGCACAGCAACTGAGGCTATGCT 559
Q..P..L.....A..L..M..A..A..Q..L..A..Q..L..T..M.. 50

2000, tese de doutorado

440 445 450 455 460 465 470 475 480 485
ACTTGGGATTTTACAGAGGACCTTACTTCAGAGGACACACAGCTTACAGAGCTTGGG
ACTTGGGATTTTACAGAGGACCTTACTTCAGAGGACACACAGCTTACAGAGCTTGGG



Embrapa

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Tubiana, F. Sem. 2002