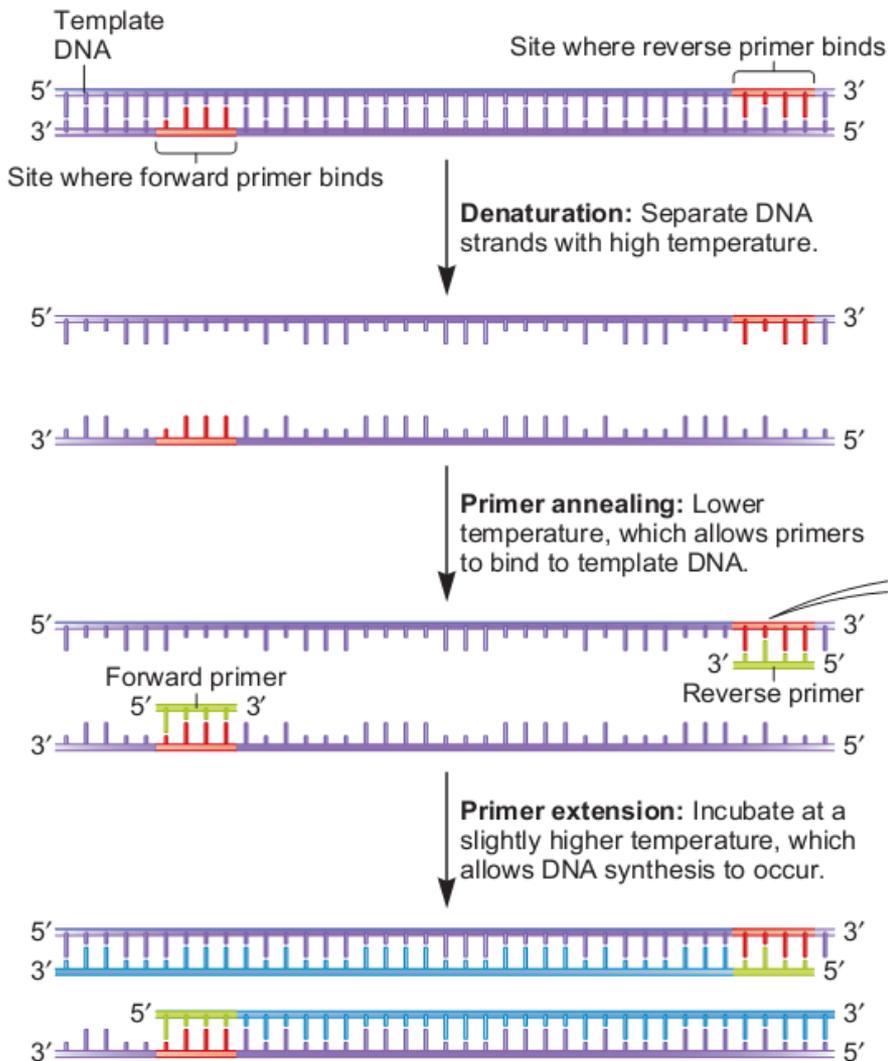


Sequenciamento de DNA

Lembrando.....Ciclo da reação de PCR



(b) The 3 steps of a PCR cycle

- Temperaturas:
Desnaturação → 94 – 96°C – 1min
Pareamento → 50 – 65°C- 45s
Extensão → 68 – 72°C 1 a 2min

Sequenciamento de DNA

Varição da técnica de PCR na presença de UM único primer e pequenas quantidades de nucleotídeos que apresentam uma **didesoxiribose** ao invés de desoxiribose.

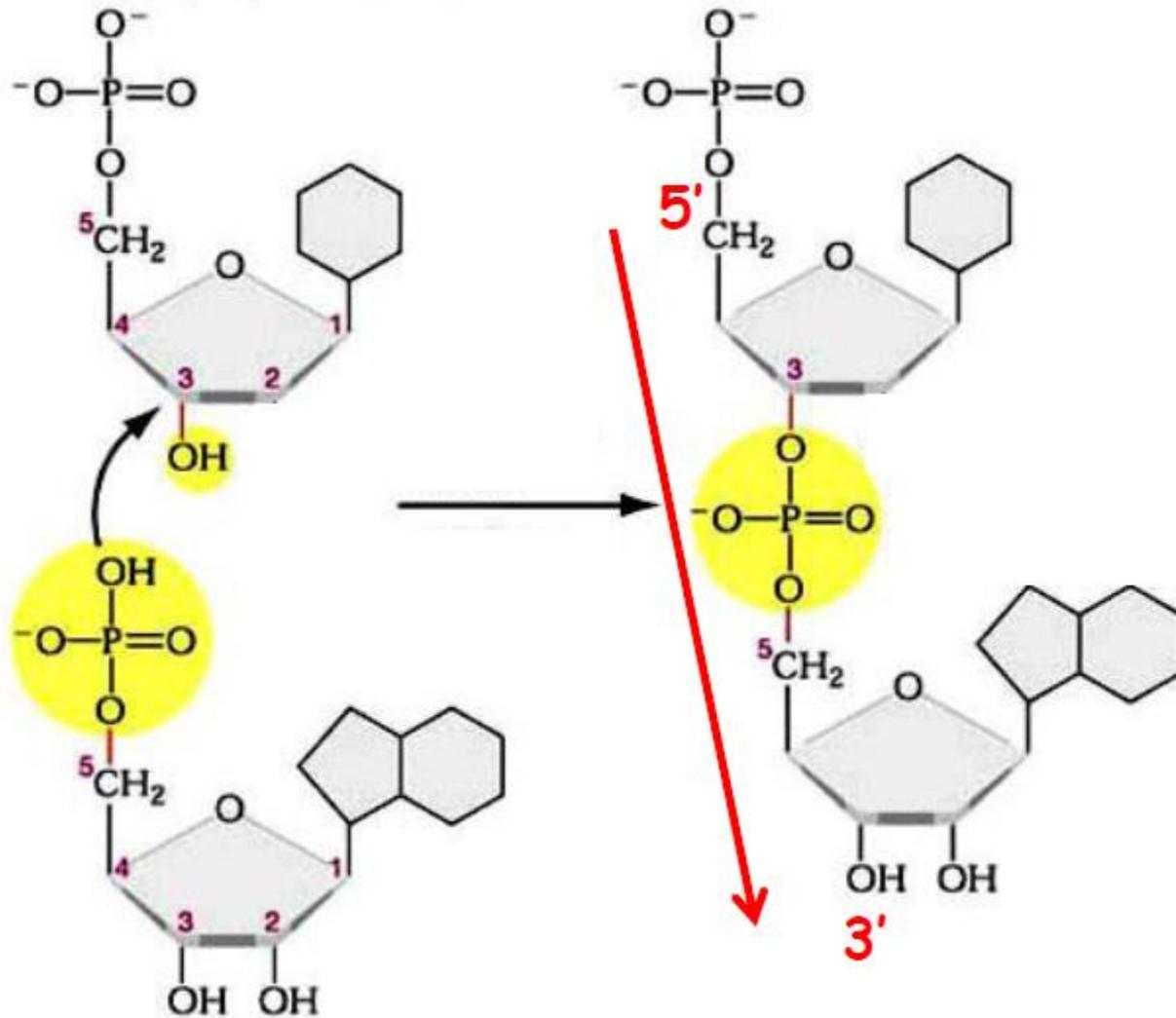


A didesoxiribose **NÃO** apresenta o grupamento 3'OH, que é essencial para a adição do próximo nucleotídeo durante a síntese de DNA



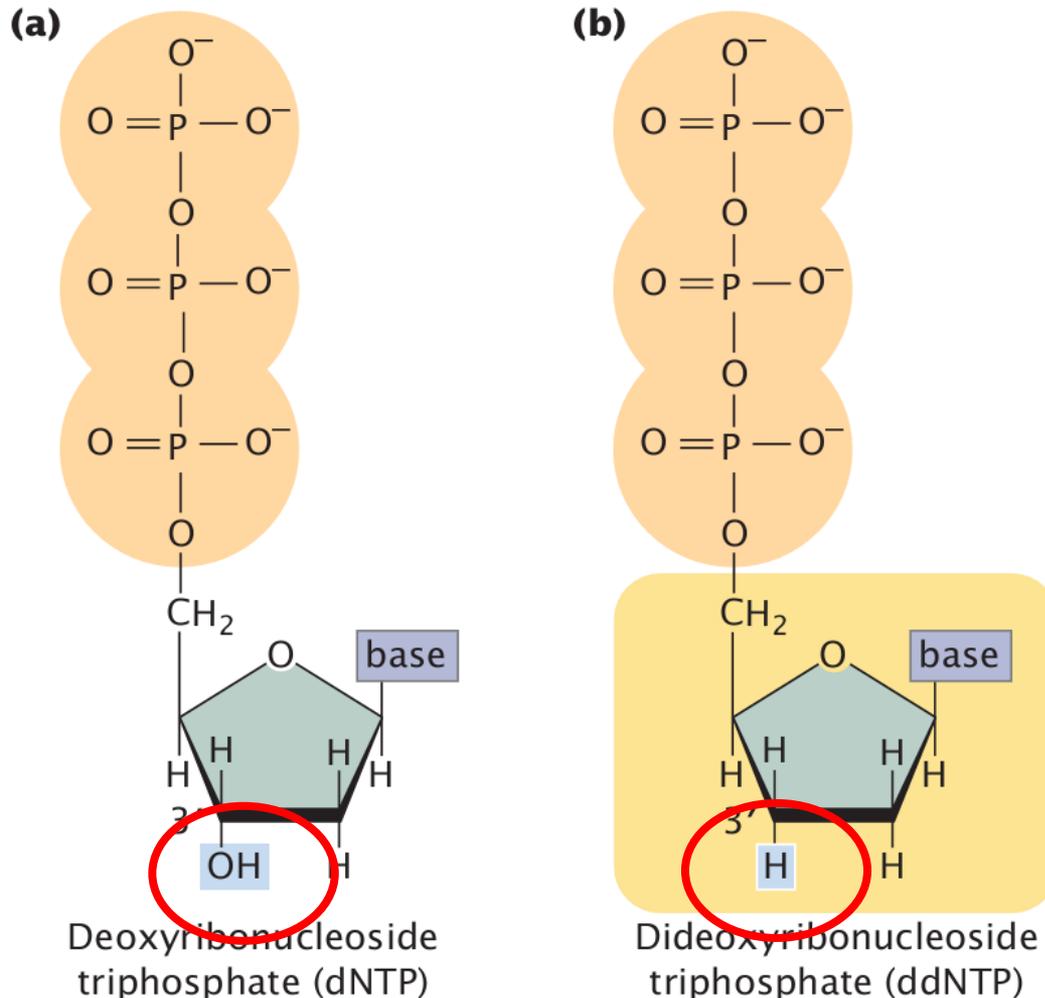
IMPEDEM O TÉRMINO DA EXTENSÃO DA FITA DE DNA

Lembrando o sentido da Replicação do DNA



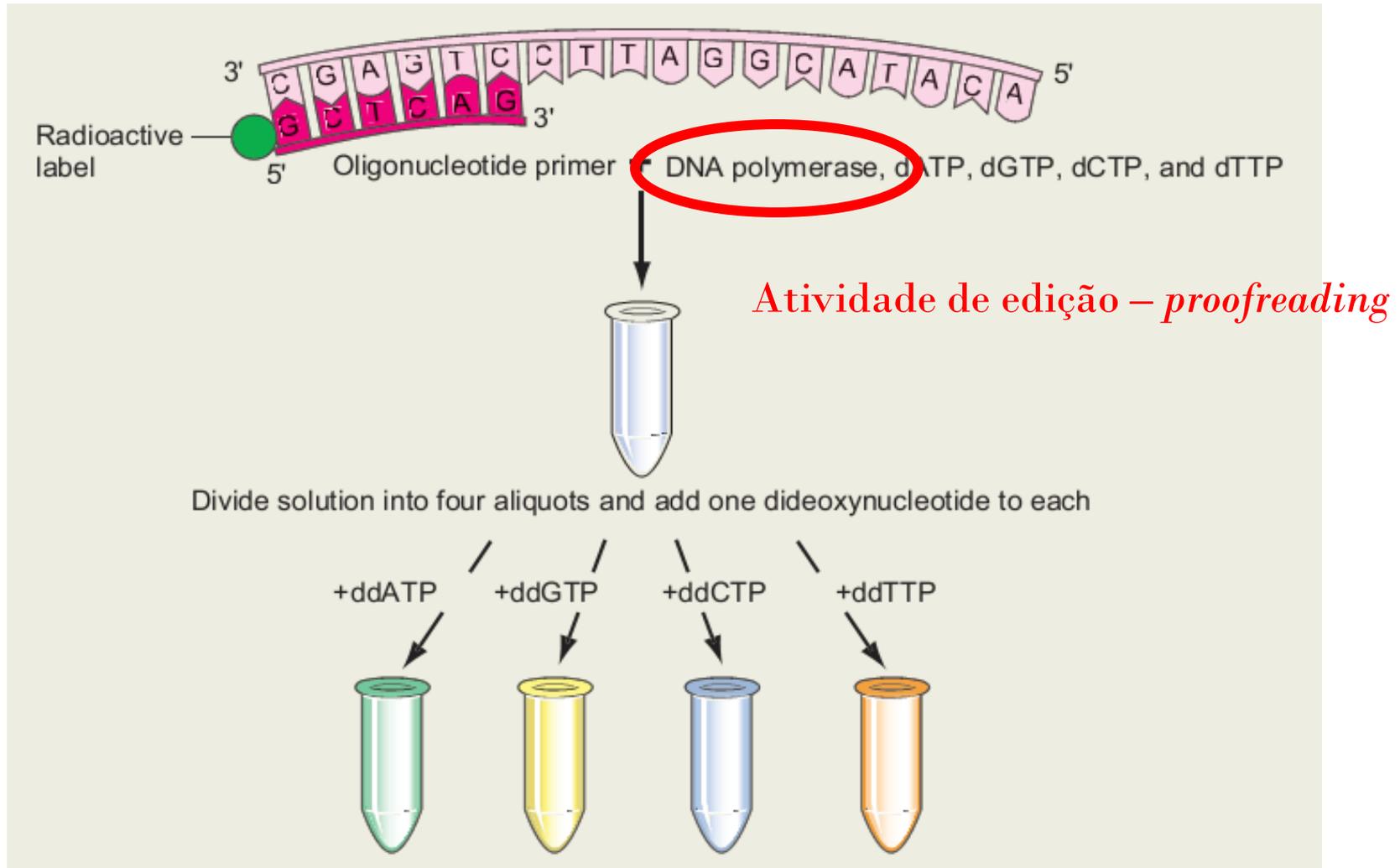
Qual a diferença entre dNTP x ddNTPS

Ausência da $-OH$ livre na posição 3' no ddNTP, o que impede a extensão da cadeia de DNA.



Técnica de sequenciamento de DNA

- Frederick Sanger e colaboradores (1974)

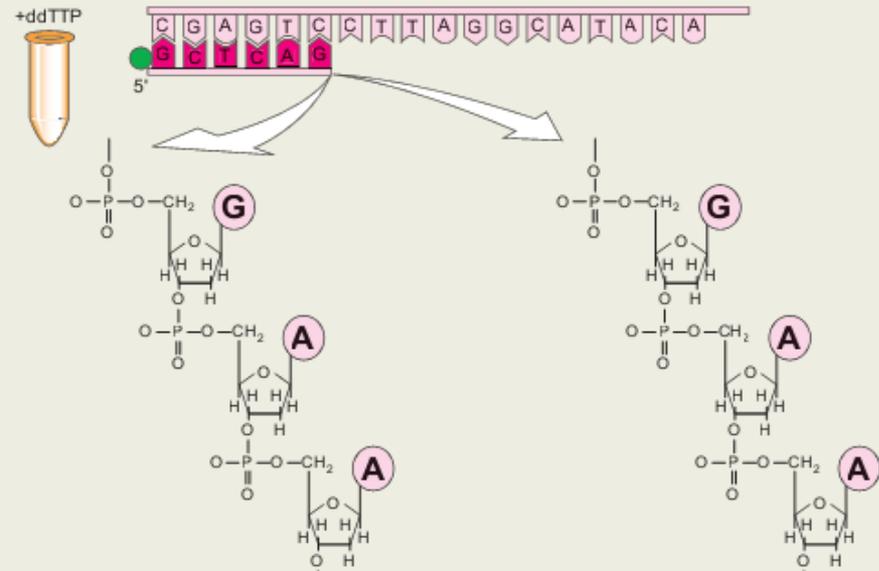


Temperaturas:

Desnaturação → 94 – 96°C – 2 min

Pareamento → 50°C - 15s

Extensão → 60°C - 2min

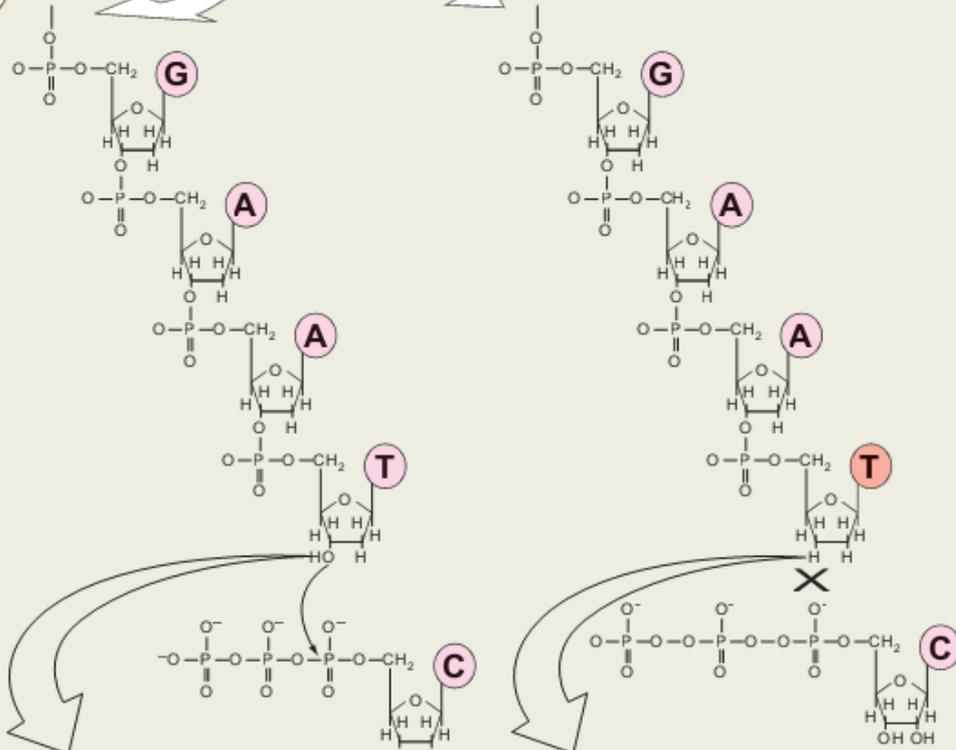


+ddTTP



C G A G T C C T T A G G C A T A C A

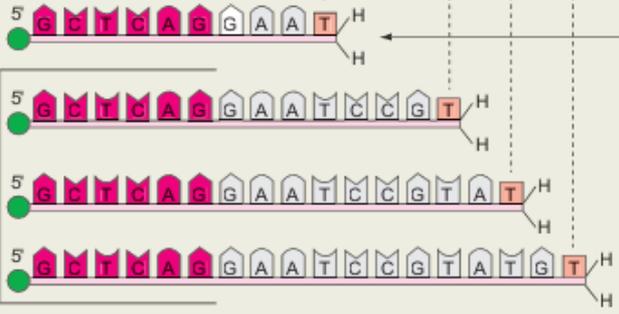
5' G C T C A G



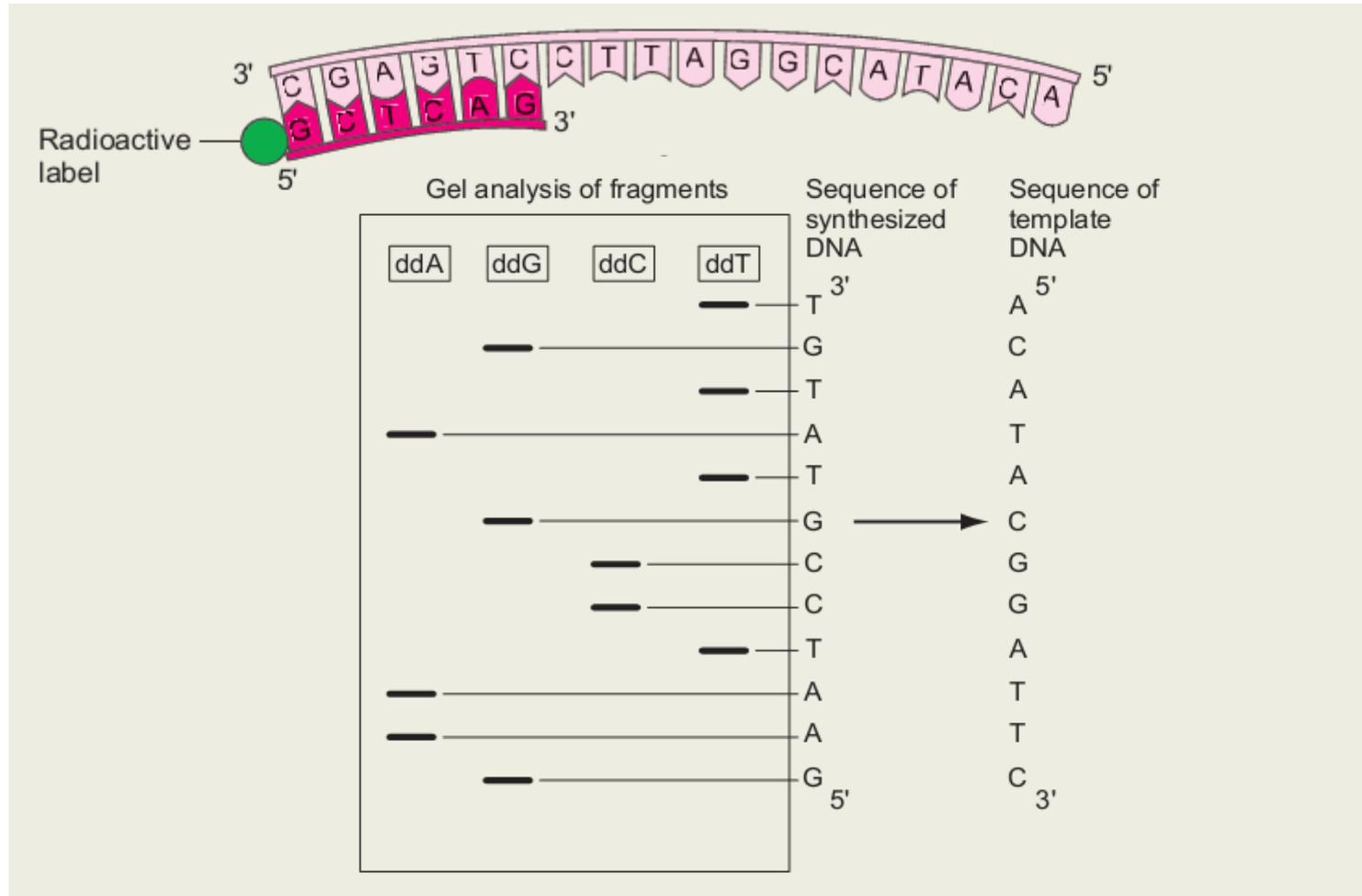
Incorporation of normal deoxy-T allows further chain elongation.

Incorporation of dideoxy-T causes chain termination.

C G A G T C C T T A G G C A T A C A



Sequenciamento manual do DNA



The Nobel Prize in Chemistry 1980



Paul Berg

Prize share: 1/2



Walter Gilbert

Prize share: 1/4



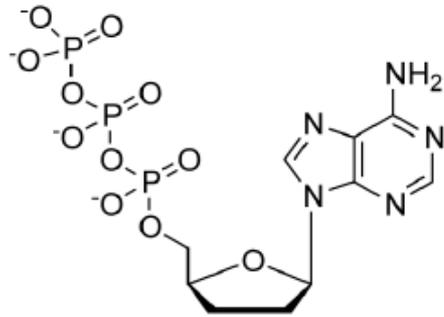
Frederick Sanger

Prize share: 1/4

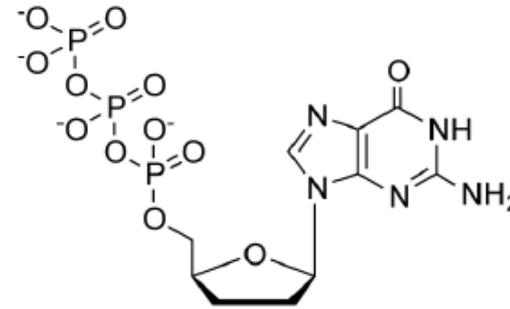
Sequenciamento Automático do DNA



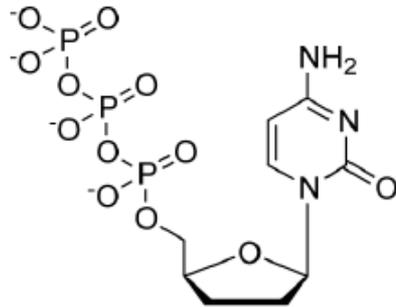
Sequenciamento Automático do DNA



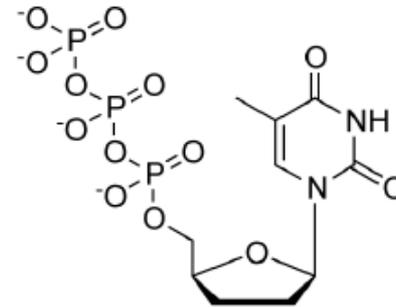
ddATP



ddGTP



ddCTP



ddTTP

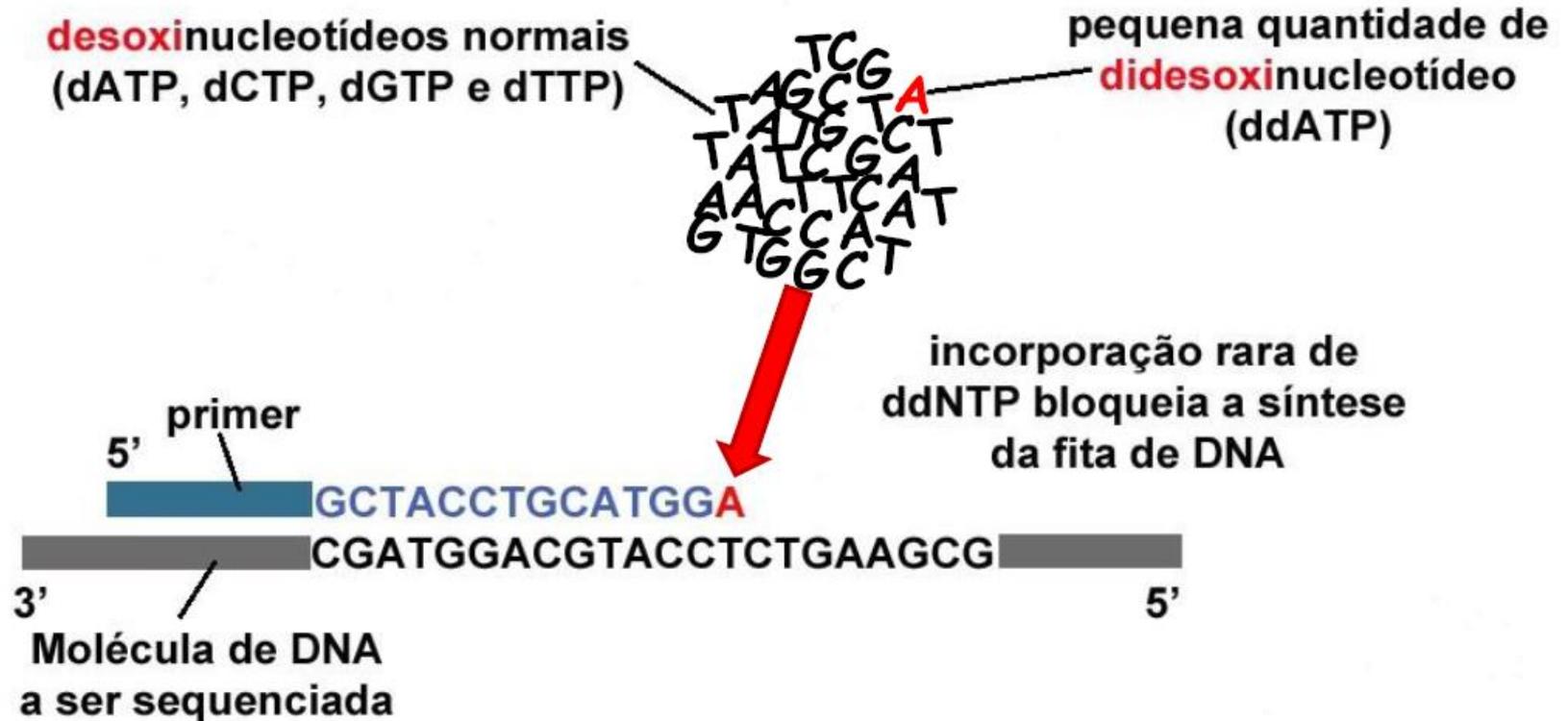
● ddATP

● ddCTP

● ddGTP

● ddTTP

Sequenciamento Automático do DNA



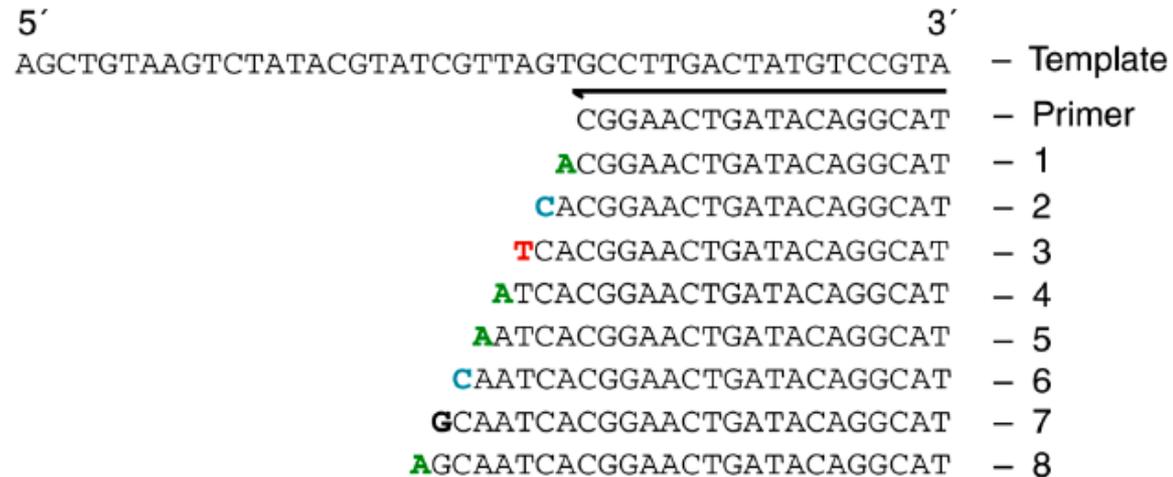
Sequenciamento Automático do DNA

 ddATP

 ddCTP

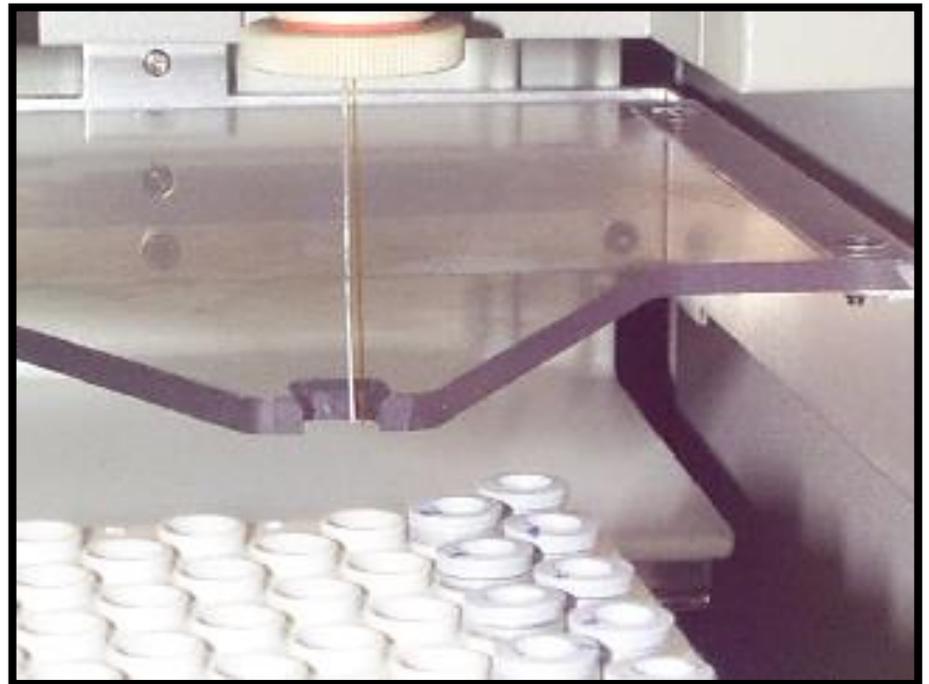
 ddGTP

 ddTTP

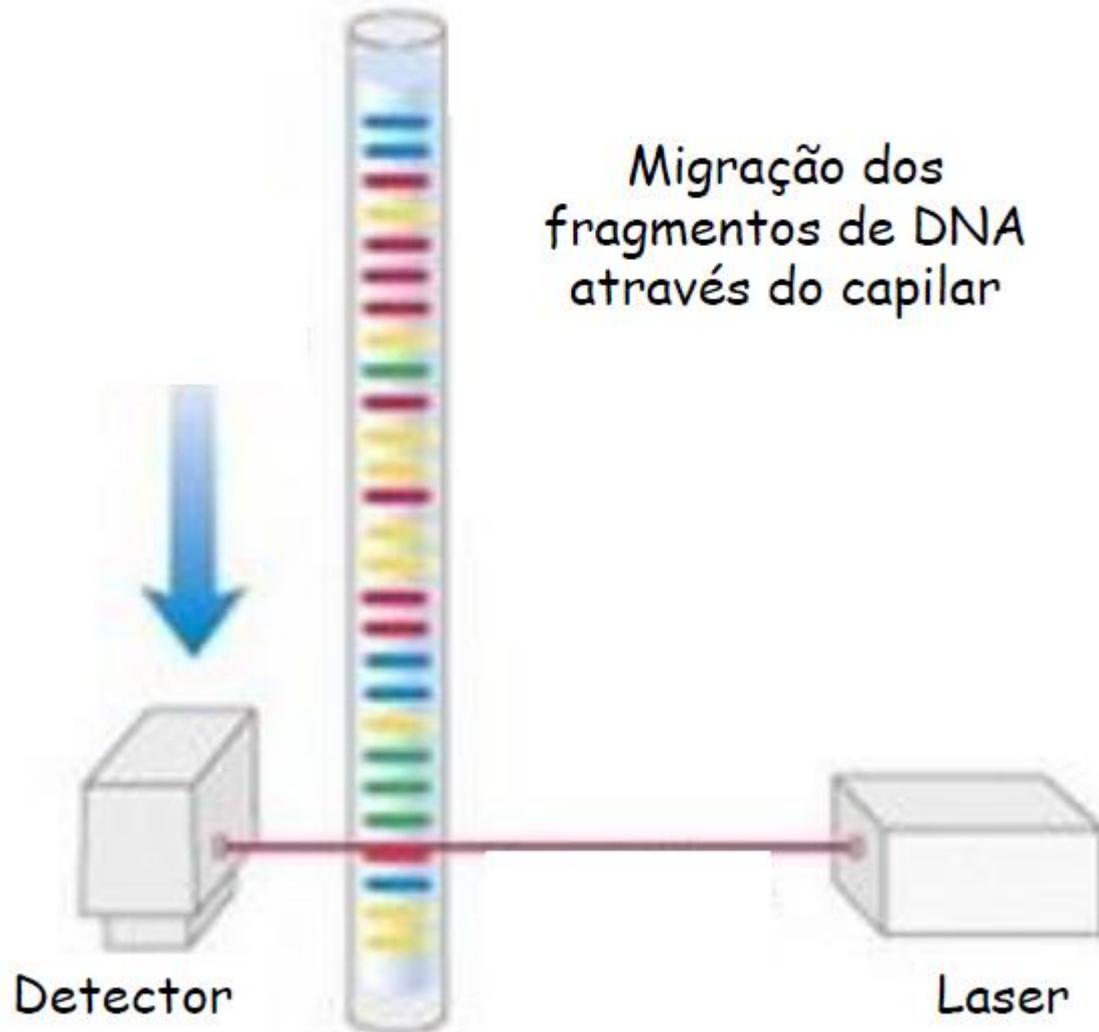


Etapas seguintes: precipitação e purificação

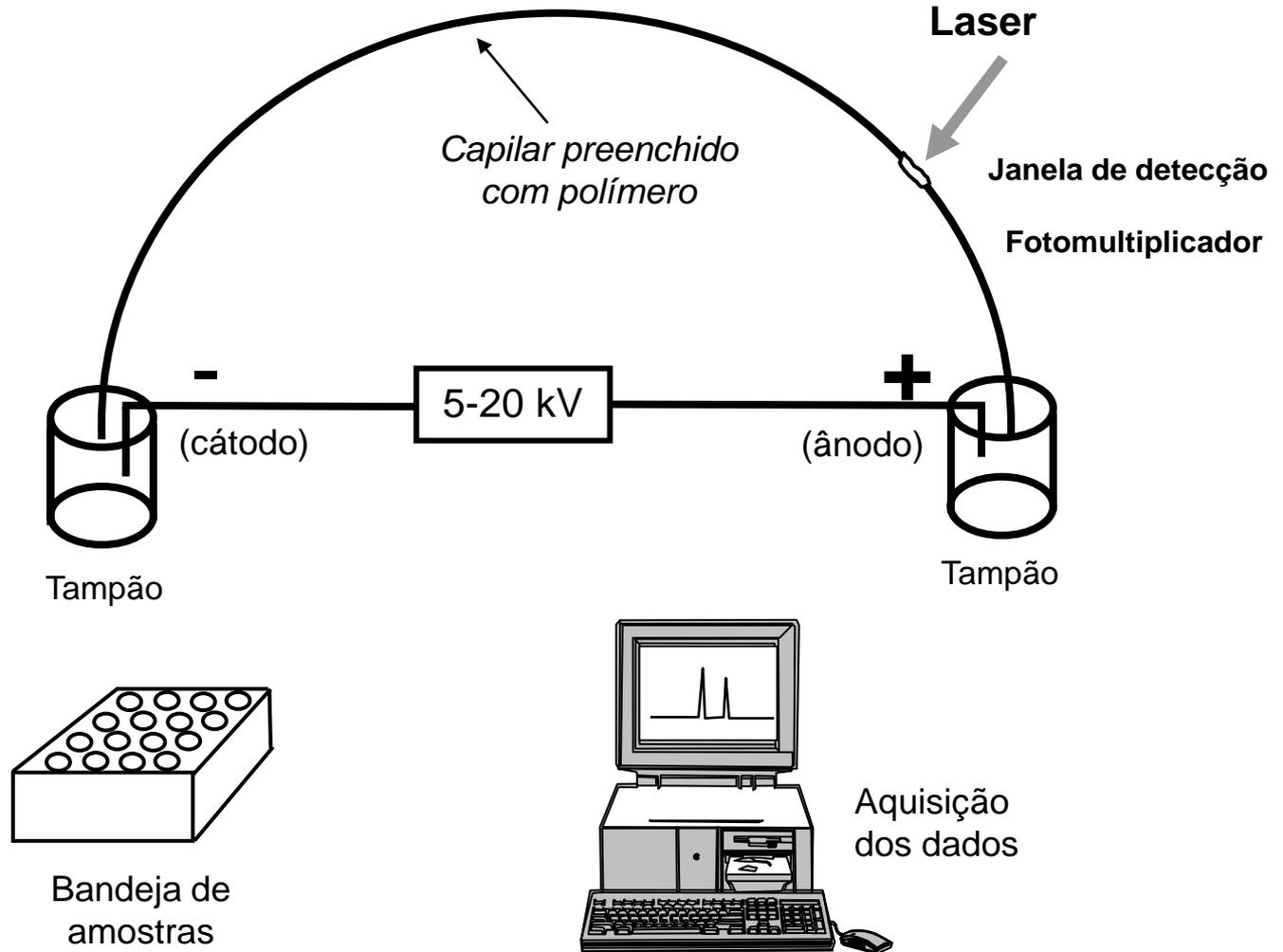
Sequenciamento Automático do DNA



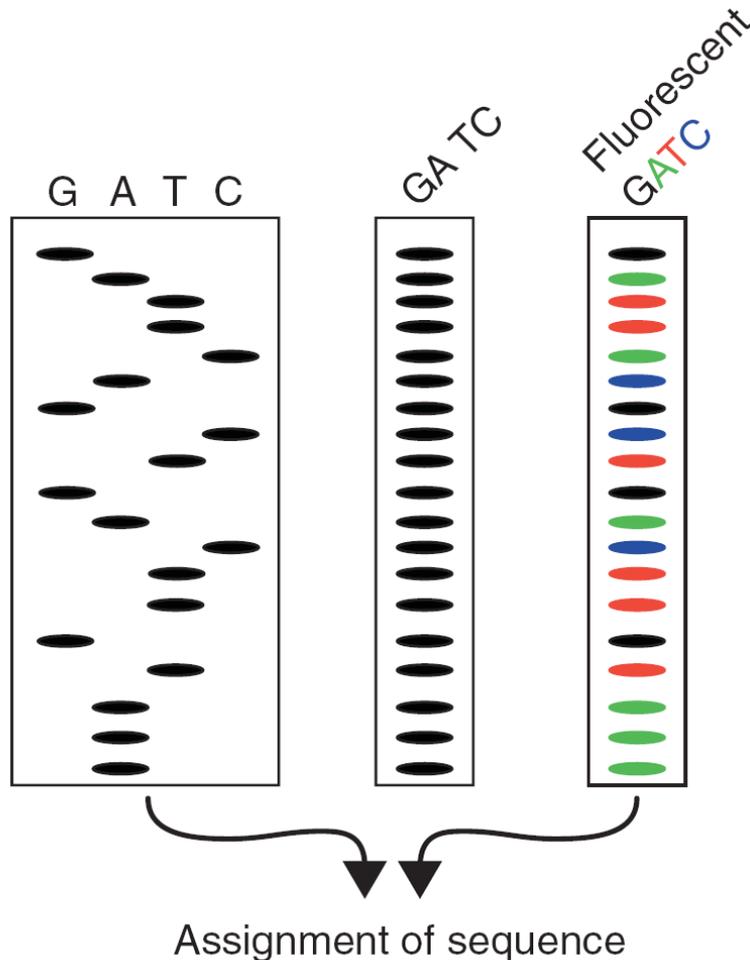
Sequenciamento Automático do DNA



Sequenciamento Automático do DNA



Sequenciamento Automático do DNA



Os ddNTPs que terminam a síntese são marcados com corantes fluorescentes

1 A single-stranded DNA fragment whose base sequence is to be determined (the template) is isolated.

2 Each of the four ddNTPs is tagged with a different fluorescent dye, and the Sanger sequencing reaction is carried out.

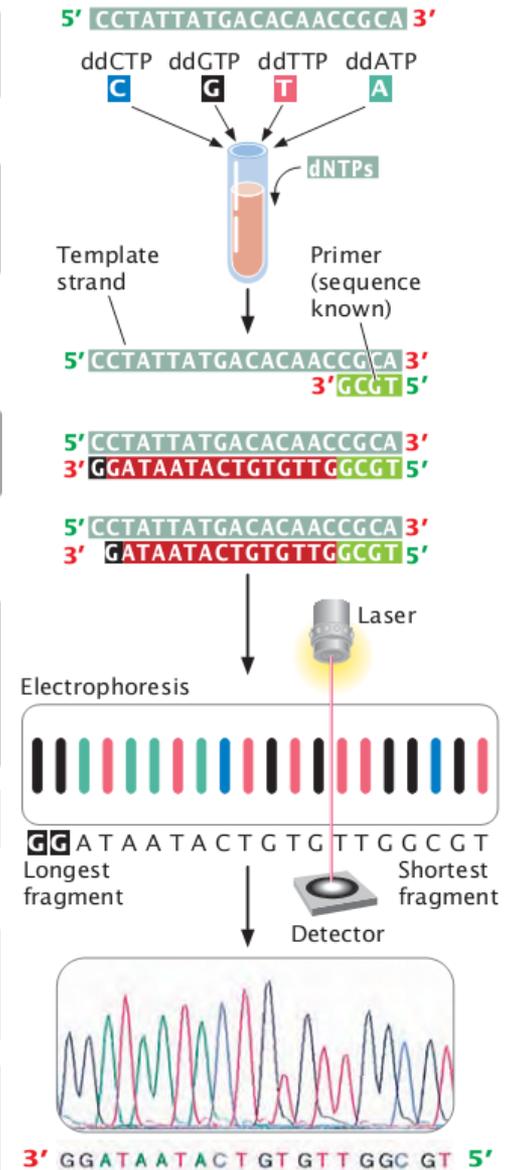
3 The fragments that end in the same base have the same colored dye attached.

4 The products are denatured, and the DNA fragments produced by the four reactions are mixed and loaded into a single well on an electrophoresis gel. The fragments migrate through the gel according to size,...

5 ...and the fluorescent dye on the DNA is detected by a laser beam.

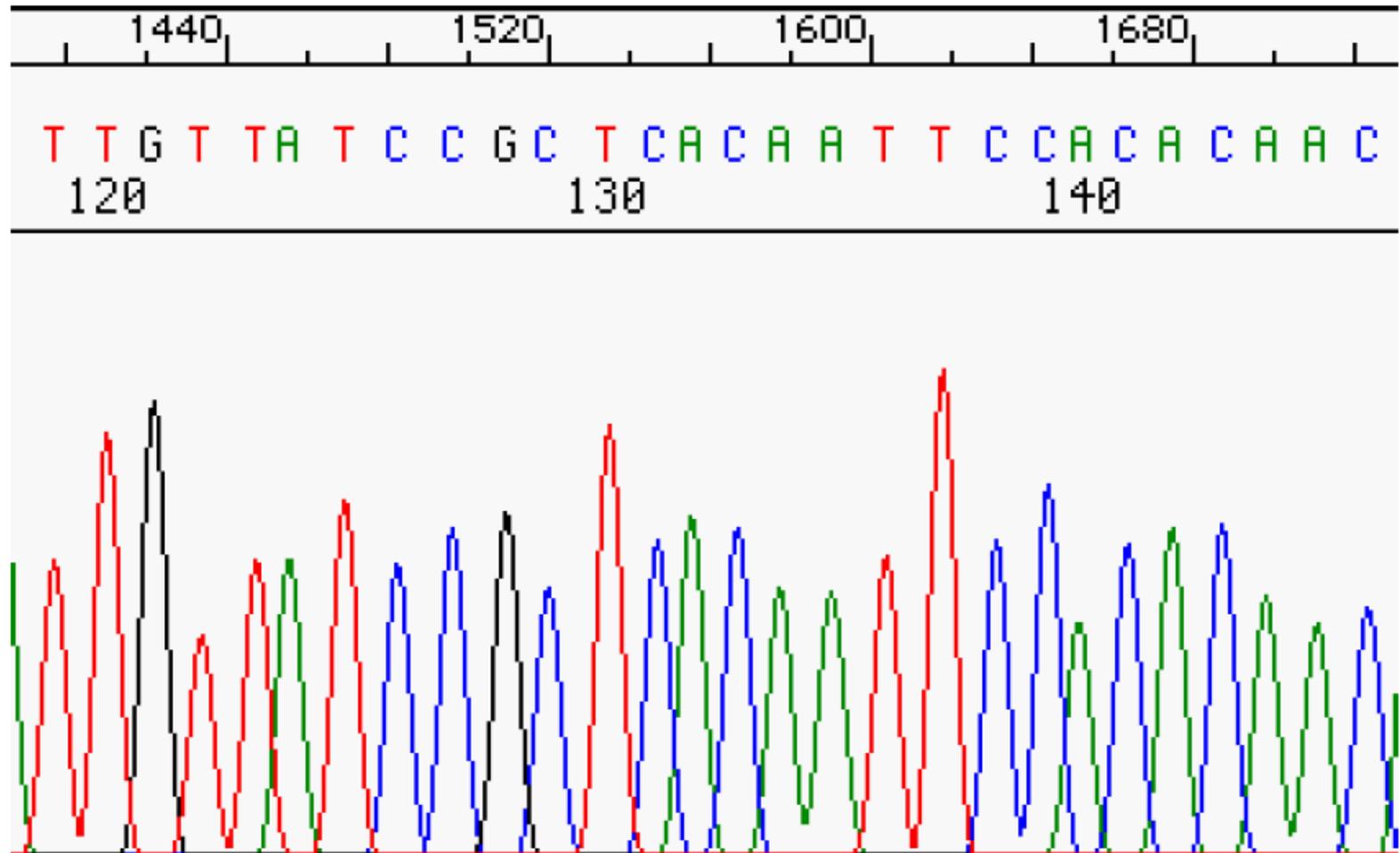
6 Each fragment appears as a peak on the computer printout; the color of the peak indicates which base is present.

7 The sequence information is read directly into the computer, which converts it into the complementary target sequence.



Sequenciamento Automático do DNA

Cromatograma



Cromatograma

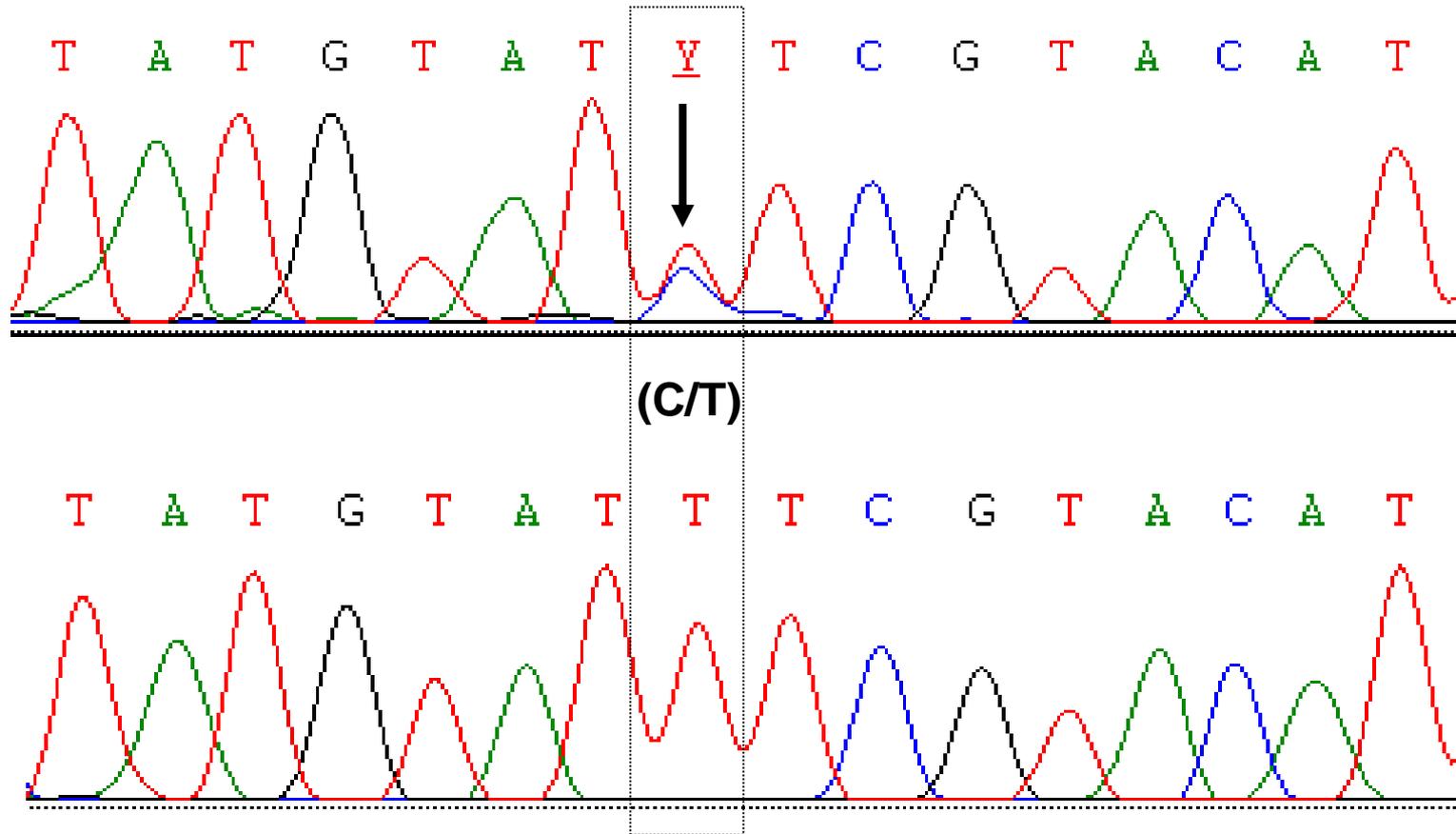
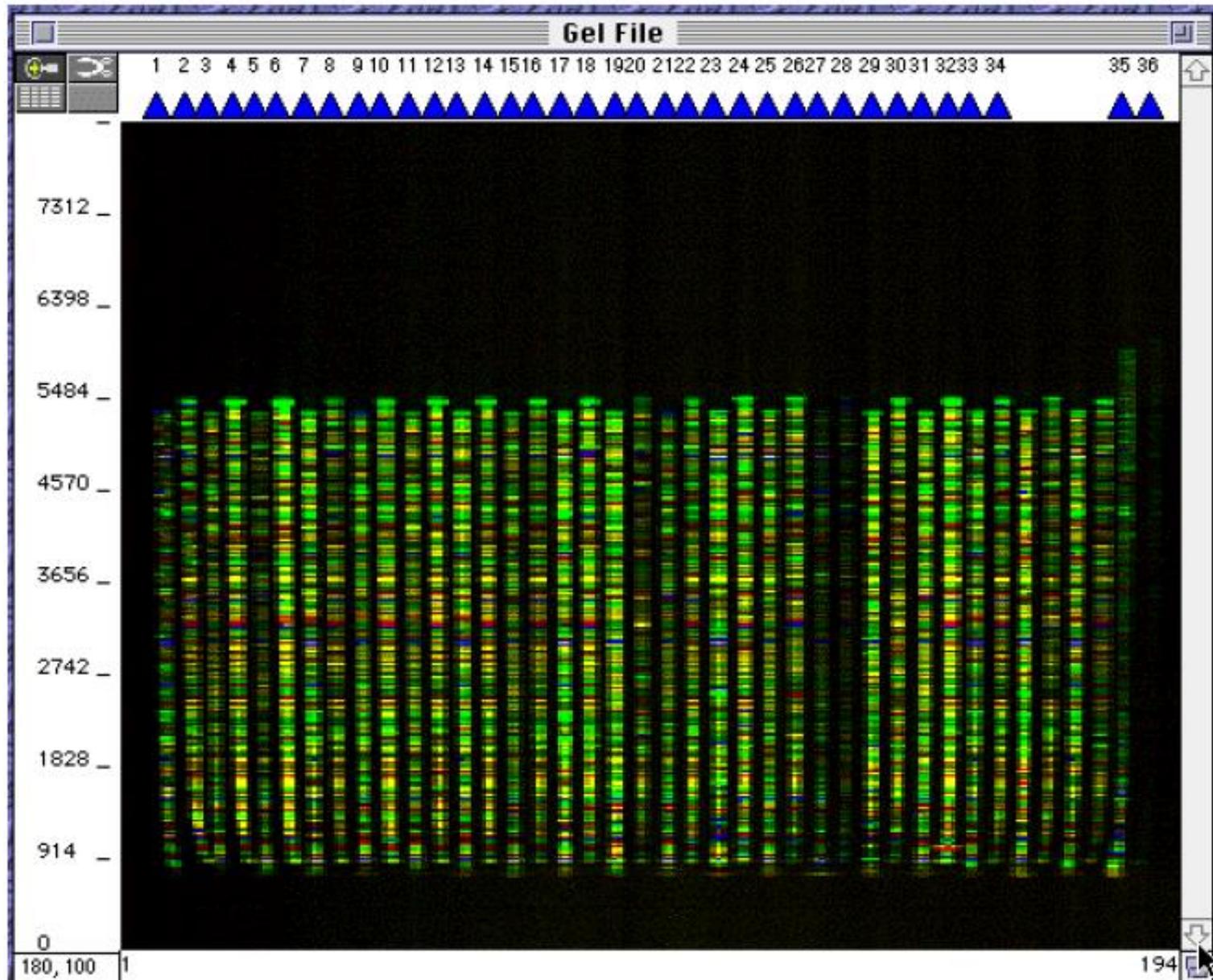
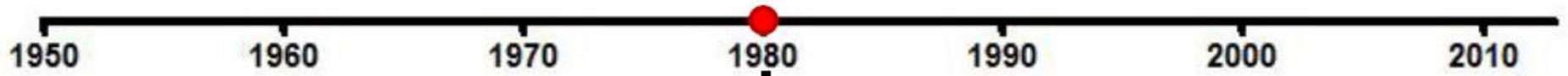
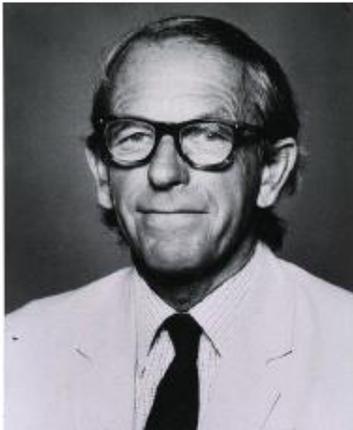


Imagem de um gel de sequenciamento com detecção por fluorescência





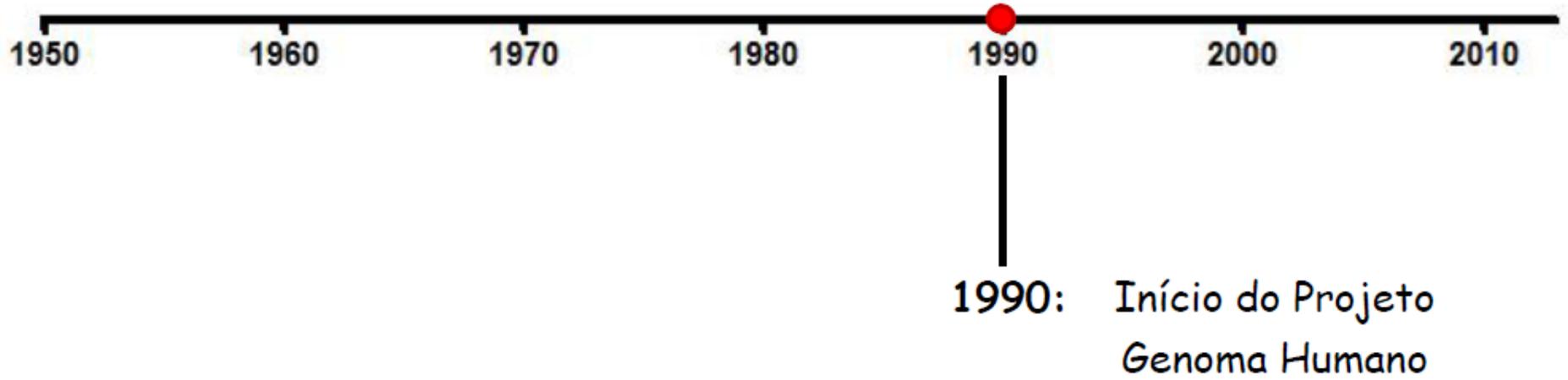
1980: Prêmio Nobel de Química



Frederick
Sanger



Walter
Gilbert



✓ Obter a sequência dos **3 bilhões de pares de bases nitrogenadas** presentes no genoma humano;

1950 1960 1970 1980 1990 2000 2010



2001:
Publicação do
Genoma Humano

The sequence of the human genome. Venter, J.C. et al. Science 291, 1304-1351 (2001)
Initial sequencing and analysis of the human genome. Lander, E.S. et al. Nature 409, 860-921 (2001)

Sequenciamento de nova geração

Sequenciamento de DNA

1982	Fago Lambda – 49 mil pb
1995	<i>Haemophilus influenzae</i> – 1,8 milhão de pb
1996	<i>Saccharomyces cerevisiae</i> – 12 milhões de pb
1997	<i>Escherichia coli</i> – 5.5 milhões de pb
1998	<i>Caenorhabditis elegans</i> – 100 milhões de pb
2000	<i>Drosophila melanogaster</i> – 122 milhões de pb
2001	<i>Homo sapiens</i> – 3,3 bilhões de pb
2008	<i>Mammuthus primigenius</i> - > 4 bilhões de pb
2010	<i>Homo neanderthalensis</i> – 4 bilhões de pb
2011	Genômica Pessoal

