

LGN0232 - Genética Molecular

# Identificação de Sequências por Hibridização e Sequenciamento

5ª aula

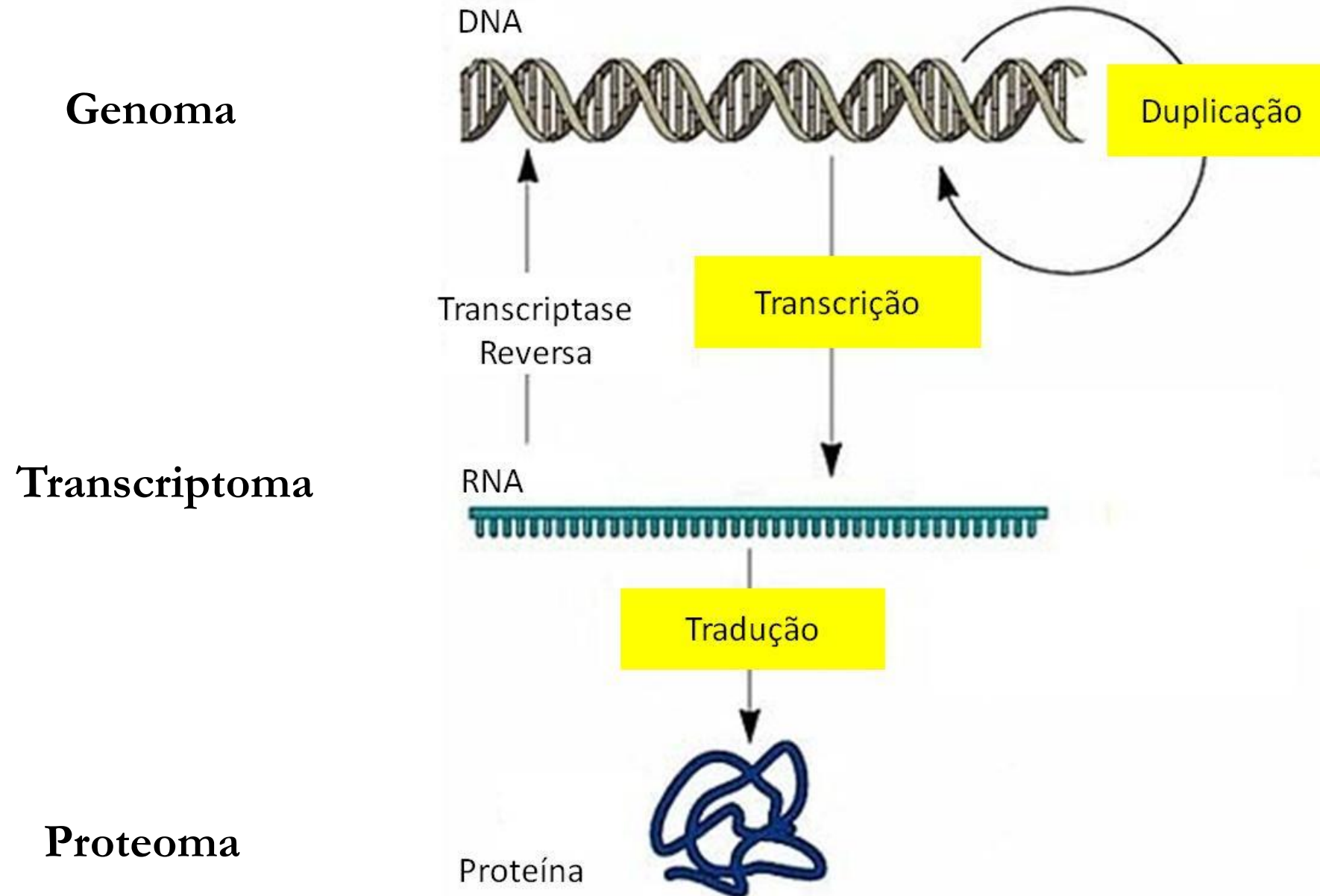
Antonio Figueira

CENA

[figueira@cena.usp.br](mailto:figueira@cena.usp.br)



# Dogma Central da Biologia Molecular





**Como conhecer as sequências presentes em um genoma e/ou quais estão sendo expressas (RNA e proteínas)?**

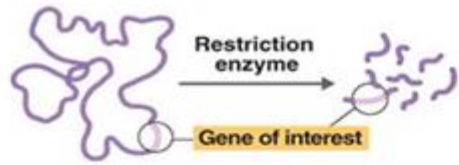
- Hibridização com sondas conhecidas
- Sequenciamento

# Técnicas de “Blotting”

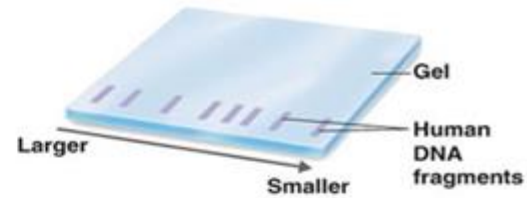
*Southern blot* diz respeito a uma **técnica** utilizada na Biologia Molecular pela qual é possível verificar se uma sequência de DNA específica (**gene de interesse**) está ou não presente na amostra em análise que contem uma mistura complexa (genoma inteiro de organismo digerido pro enzima de restrição).

*northern blot* é uma técnica usada na Biologia Molecular para estudar a expressão gênica, ou seja, verificar se um determinado gene de um genoma é ou não transcrito em RNA e quantificar isso. Essa técnica tem tal nome devido à similaridade de seu procedimento com o Southern blot (batizada pelo biólogo britânico Edwin Southern; com a diferença chave de que, em vez de DNA, a substância analisada por eletroforese com uma sonda hibridizadora é RNA).

*western blot* é um método em biologia molecular e bioquímica para detectar proteínas em um homogenato (células bem trituradas) ou um extrato de um tecido biológico. Essa **técnica** usa eletroforese em gel para separar as proteínas desnaturadas por massa.



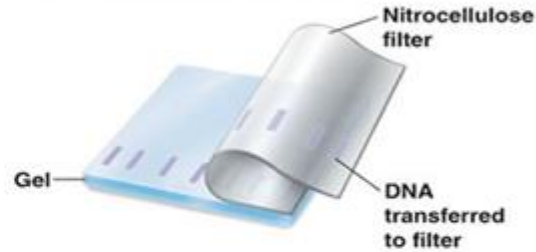
**1** DNA containing the gene of interest is extracted from human cells and cut into fragments by restriction enzymes.



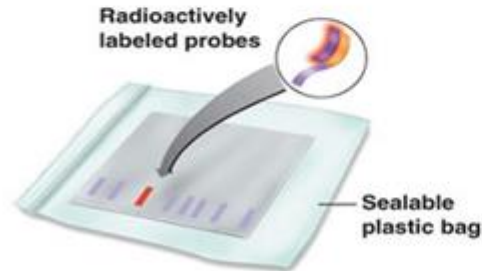
**2** The fragments are separated according to size by gel electrophoresis. Each band consists of many copies of a particular DNA fragment. The bands are invisible but can be made visible by staining.



**3** The DNA bands are transferred to a nitrocellulose filter by blotting. The solution passes through the gel and filter to the paper towels.



**4** This produces a nitrocellulose filter with DNA fragments positioned exactly as on the gel.



**5** The filter is exposed to a radioactively labeled probe for a specific gene. The probe will base-pair (hybridize) with a short sequence present on the gene.



**6** The filter is then exposed to X-ray film. The fragment containing the gene of interest is identified by a band on the developed film.

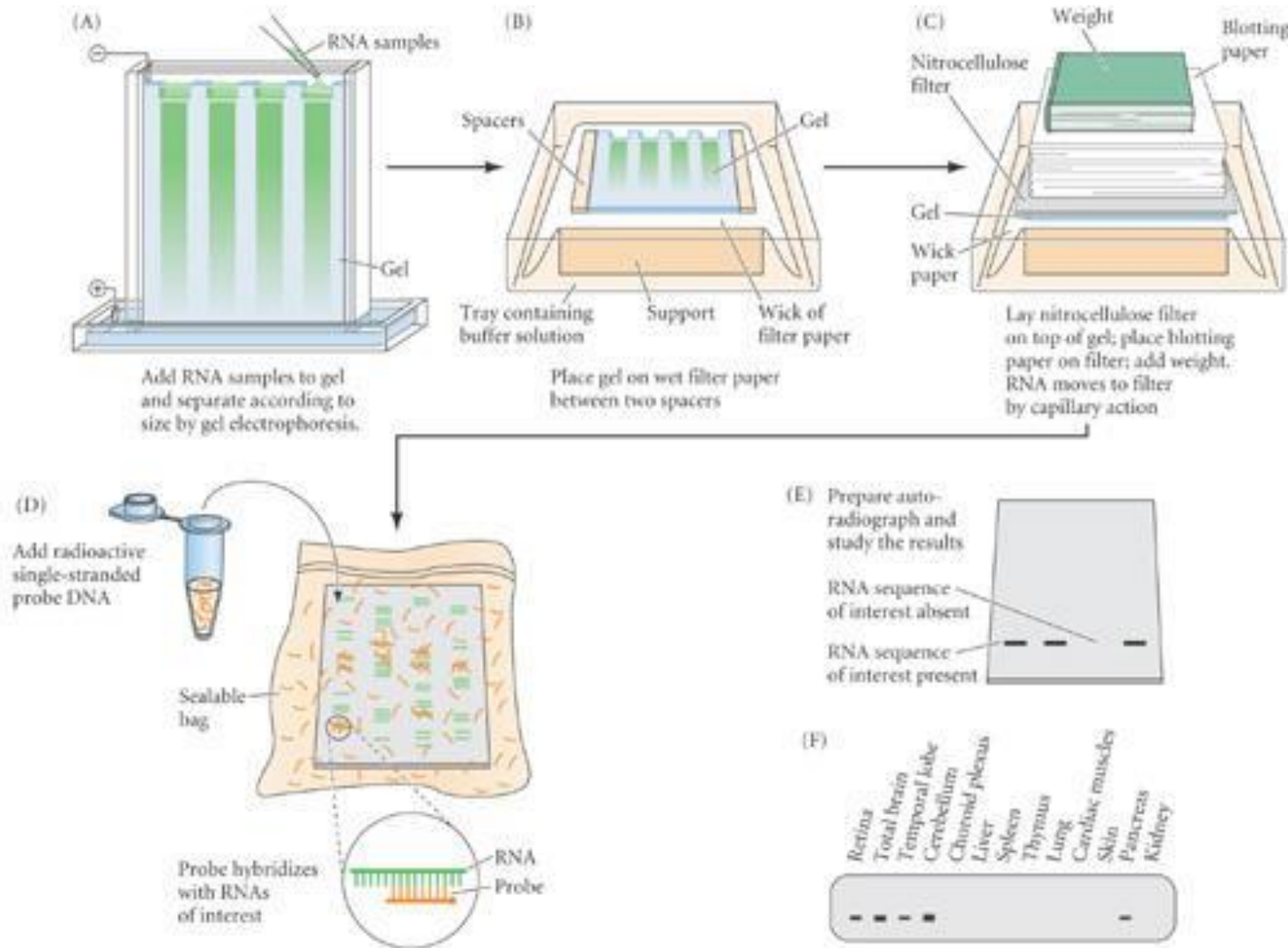
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# Southern blot

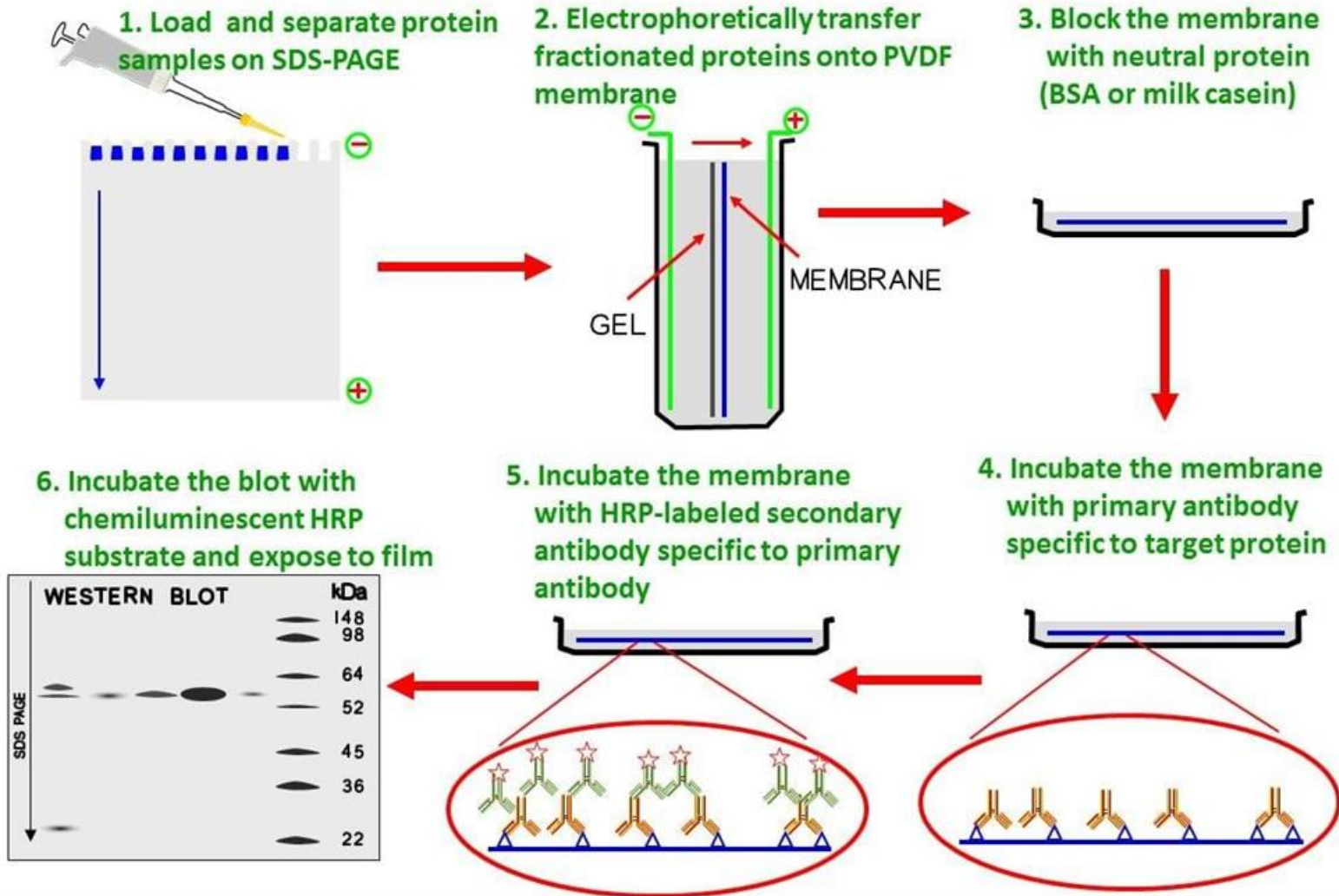
Por Edward Southern

[https://www.youtube.com/watch?v=3I9wzvj0b\\_A](https://www.youtube.com/watch?v=3I9wzvj0b_A)

# northern blot



# western blot



# Limitações das Técnicas

- Qualitativa ou semiquantitativa
- Uma sonda utilizada por vez
- Depende de uma sequência conhecida previamente (**sonda**)

**Ainda MUITO utilizadas para confirmação de transgênicos, validação de dados de transcritômicos proteômicos!**





Como obter as sequências??

# Tecnologias para o sequenciamento de DNA

## Tecnologias de primeira geração:

- **Método Maxam & Gilbert (1977)\***
  - Método de degradação química
- **Método Sanger (1977)**
  - Método enzimático, dideoxi ou de término da cadeia
  - Síntese enzimática de uma fita complementar de DNA, cujo crescimento é interrompido pela adição de um dideoxinucleotídeo (ddNTP)

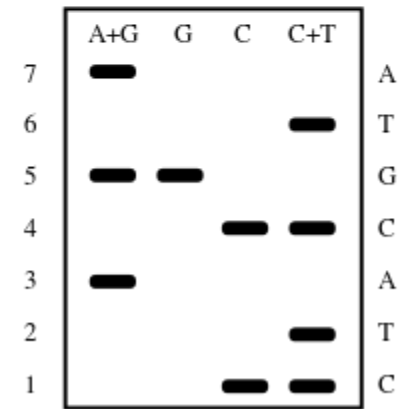
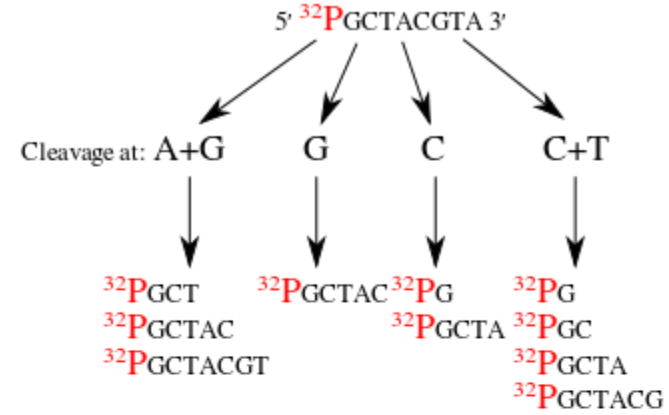
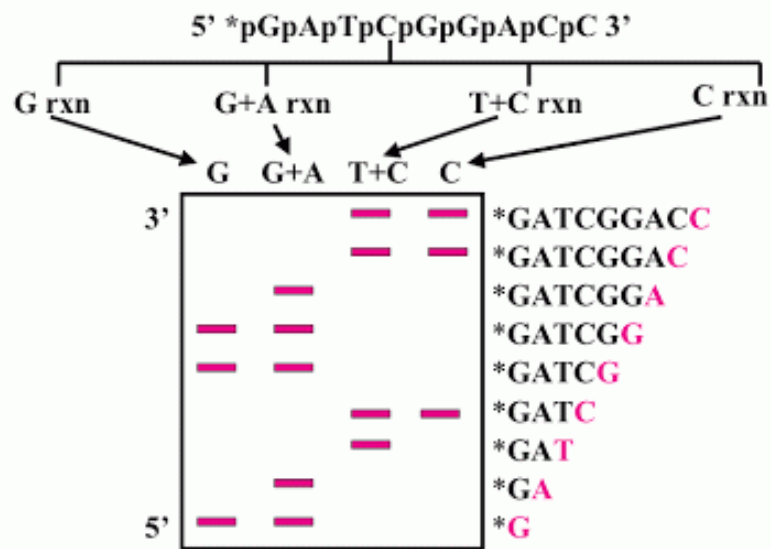


Frederick Sanger

Prêmio Nobel em Química (1980)

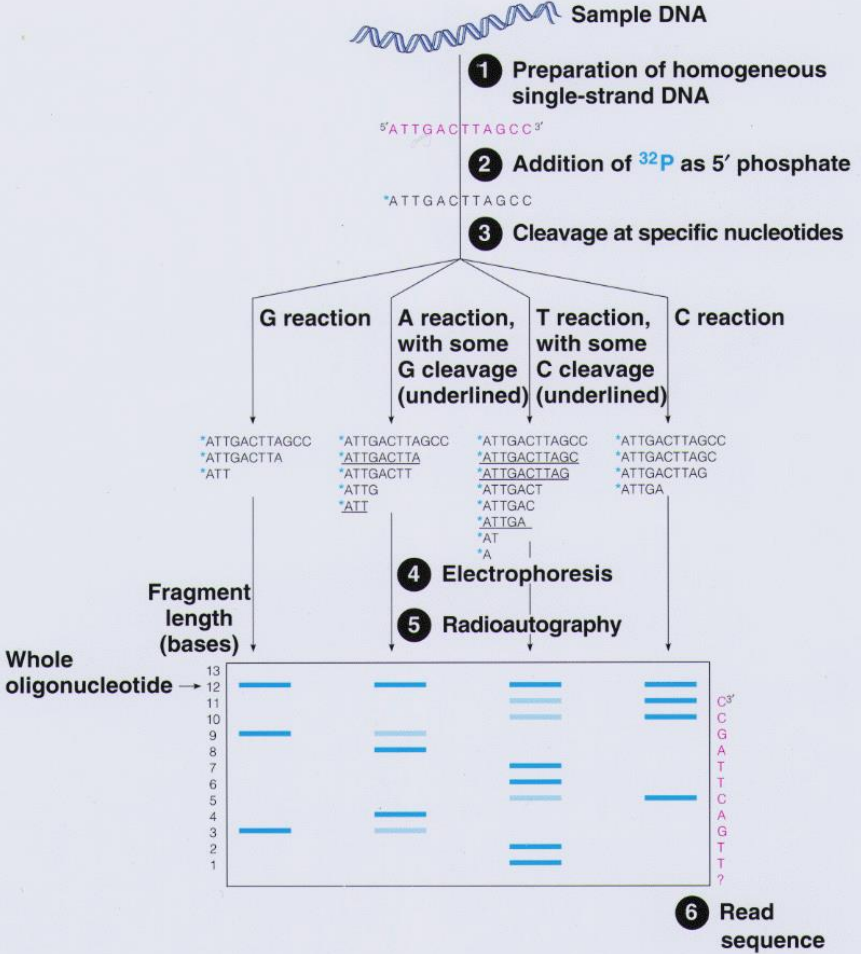
\*Produtos tóxicos e perigosos à saúde, além da dificuldade de automatização, essencial para o sequenciamento de um genoma completo.

# Maxam-Gilbert sequencing



Sequencing Gel

**Figure 4A.4 Sequencing an oligonucleotide by the Maxam-Gilbert method**



From Mathews and van Holde: *Biochemistry 2/e*. © The Benjamin/Cummings Publishing Co., Inc.

# ETAPAS DO SEQUENCIAMENTO DE DNA

Preparação do DNA



Reação de sequenciamento

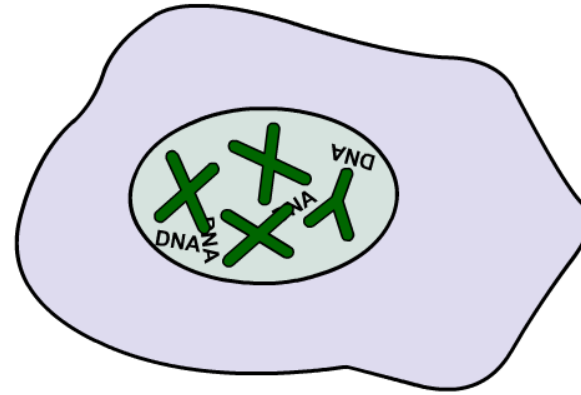


Eletroforese capilar

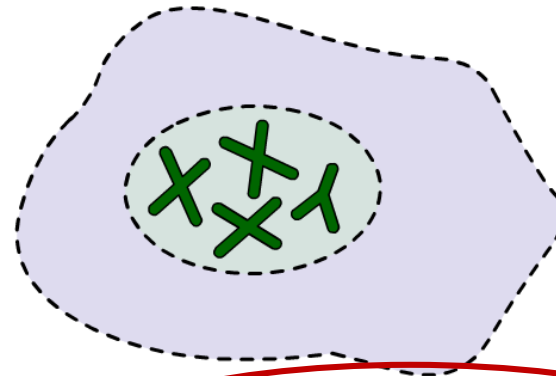


Análise computacional

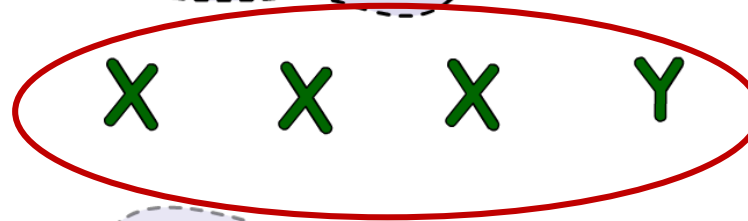
# Preparação do DNA



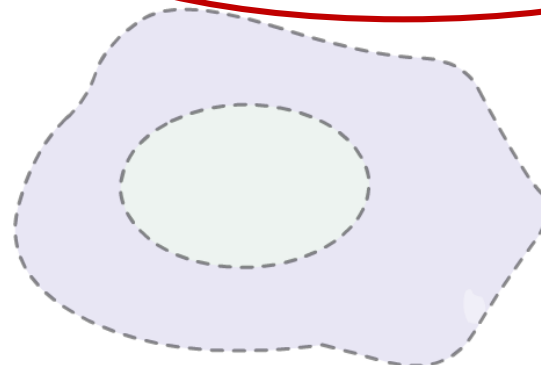
Célula



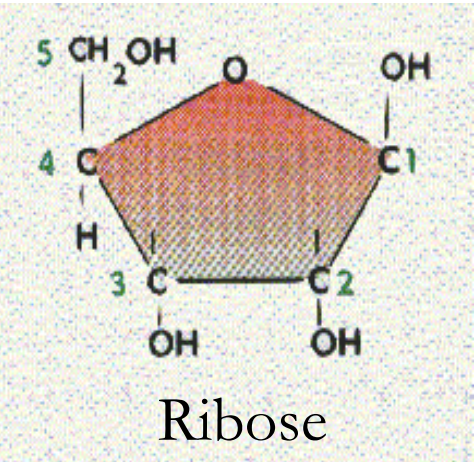
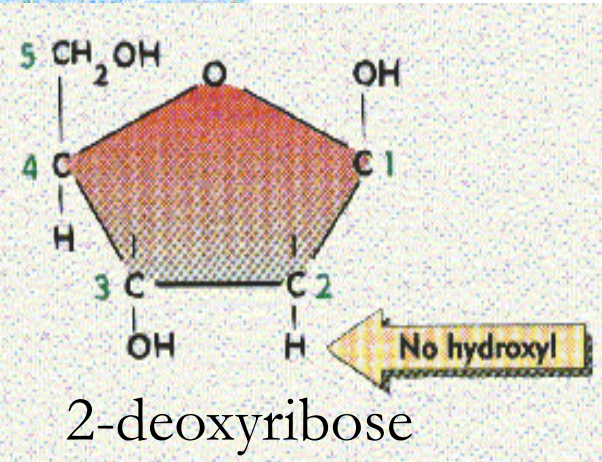
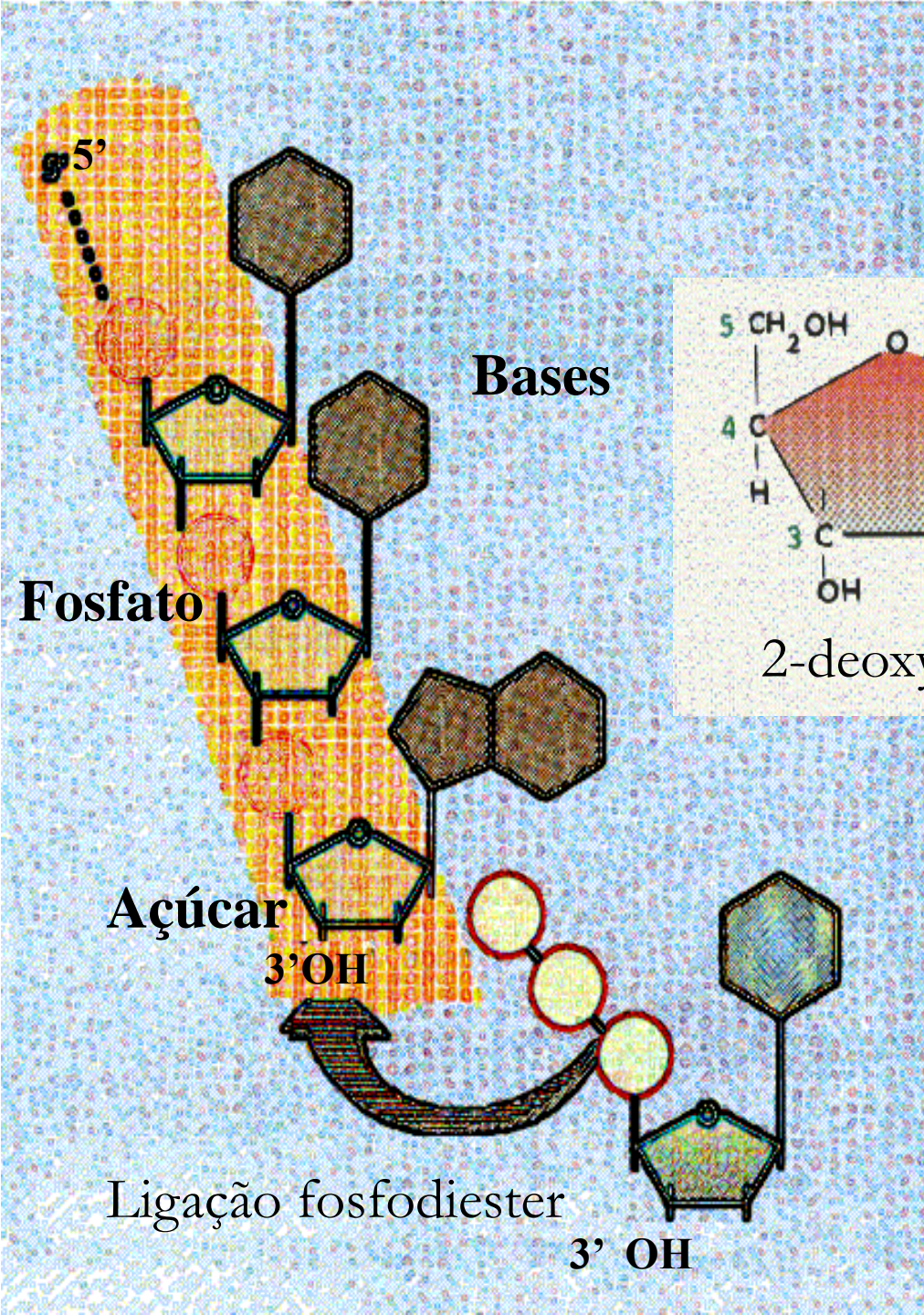
Membrana Plasmática e  
Parede Celular



Purificação do DNA  
total

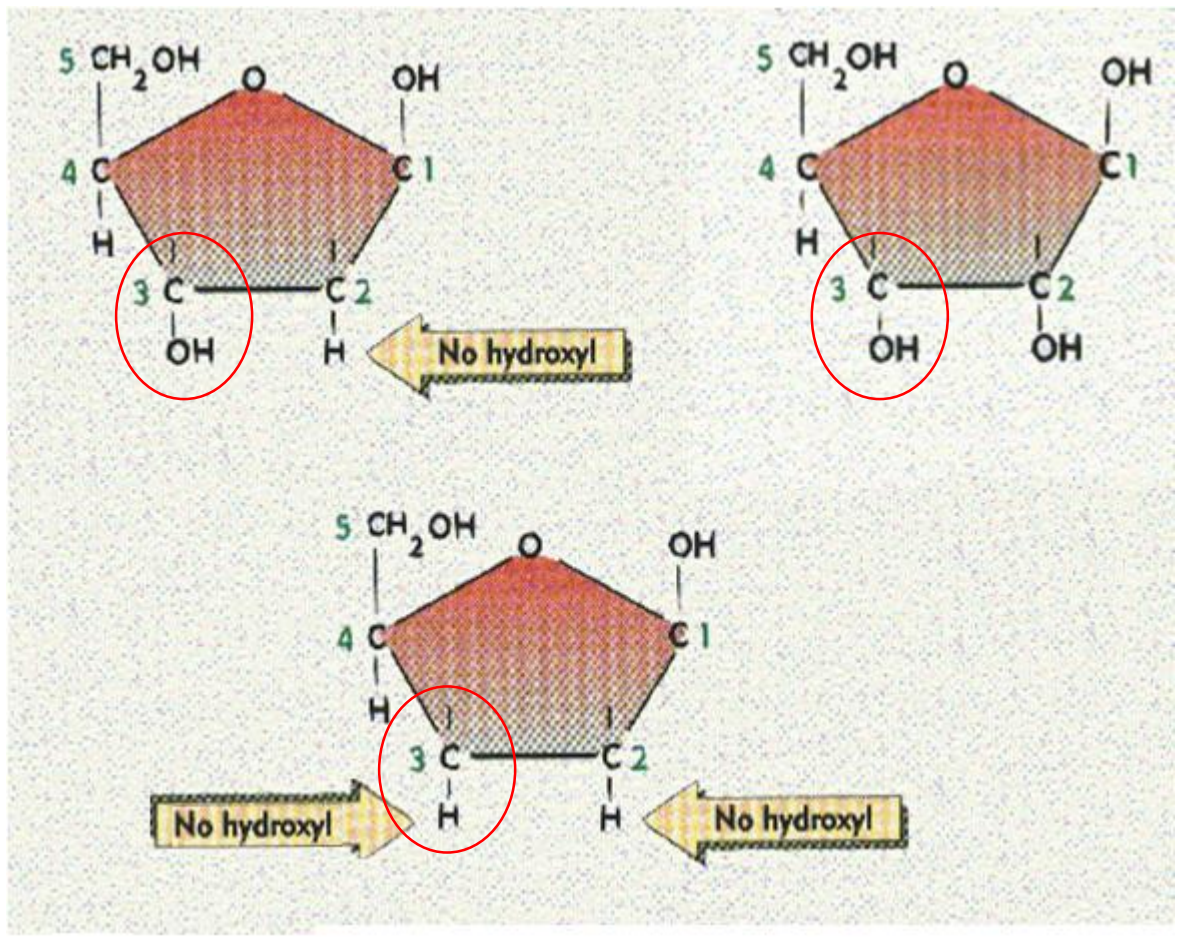


Separação dos restos  
celulares



DESOXIRIBOSE

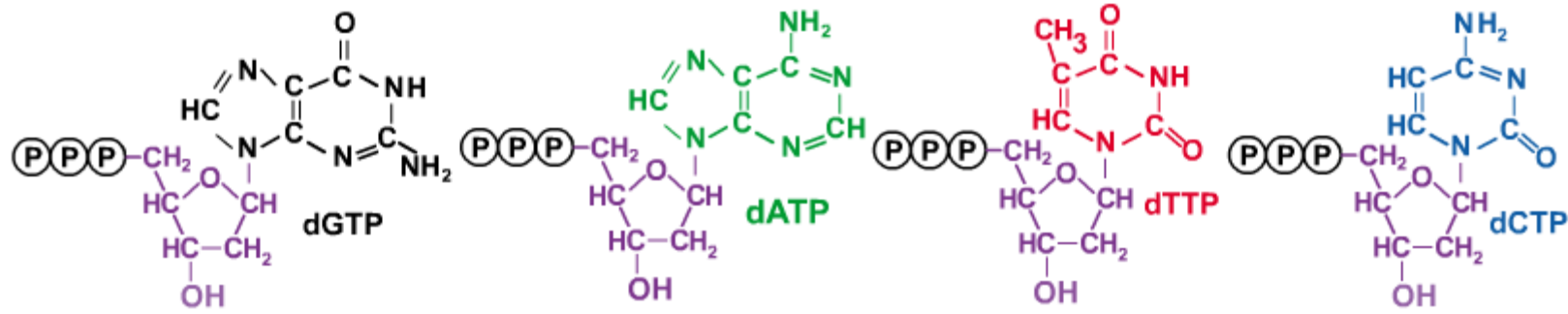
RIBOSE



DIDESOXIRIBOSE



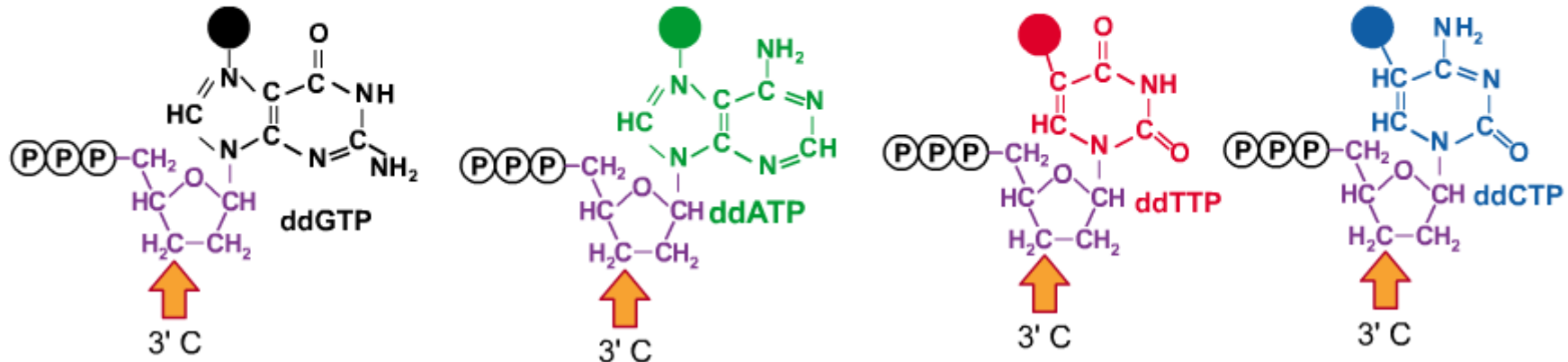
## Nucleotídeos dNTPs



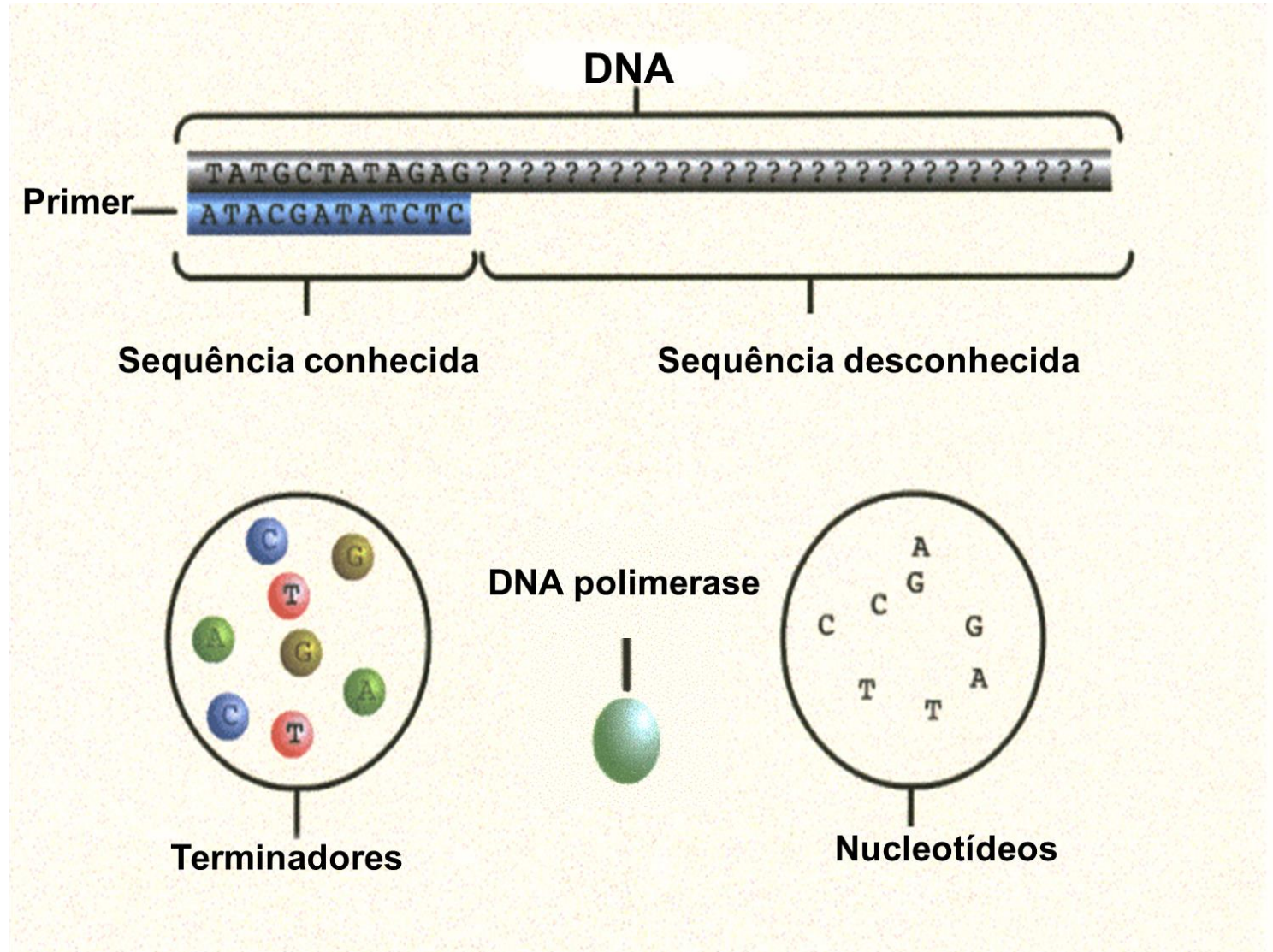
## Nucleotídeos ddNTPs (Terminadores)

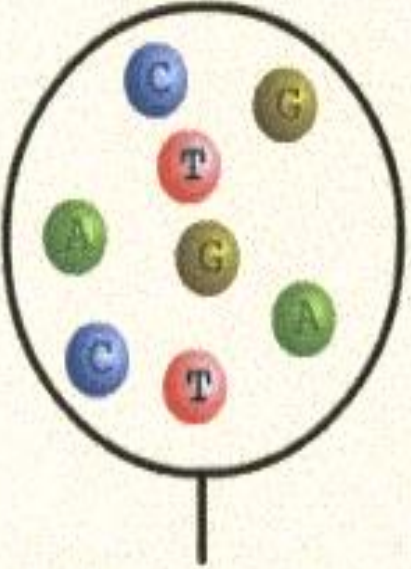
Corante Fluorescente

Falta 3'-OH



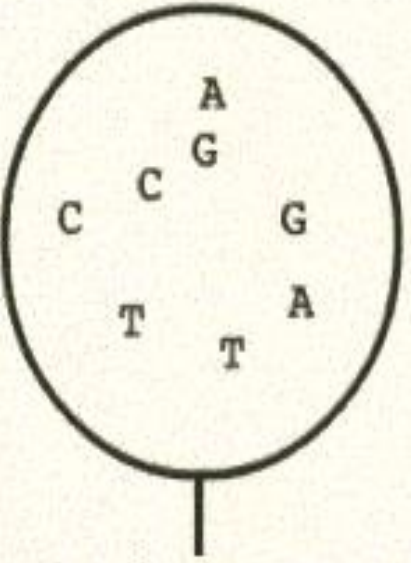
# Reação de Sequenciamento





Terminadores

DNA polimerase



Nucleotídeos

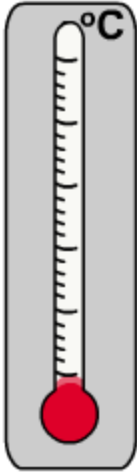
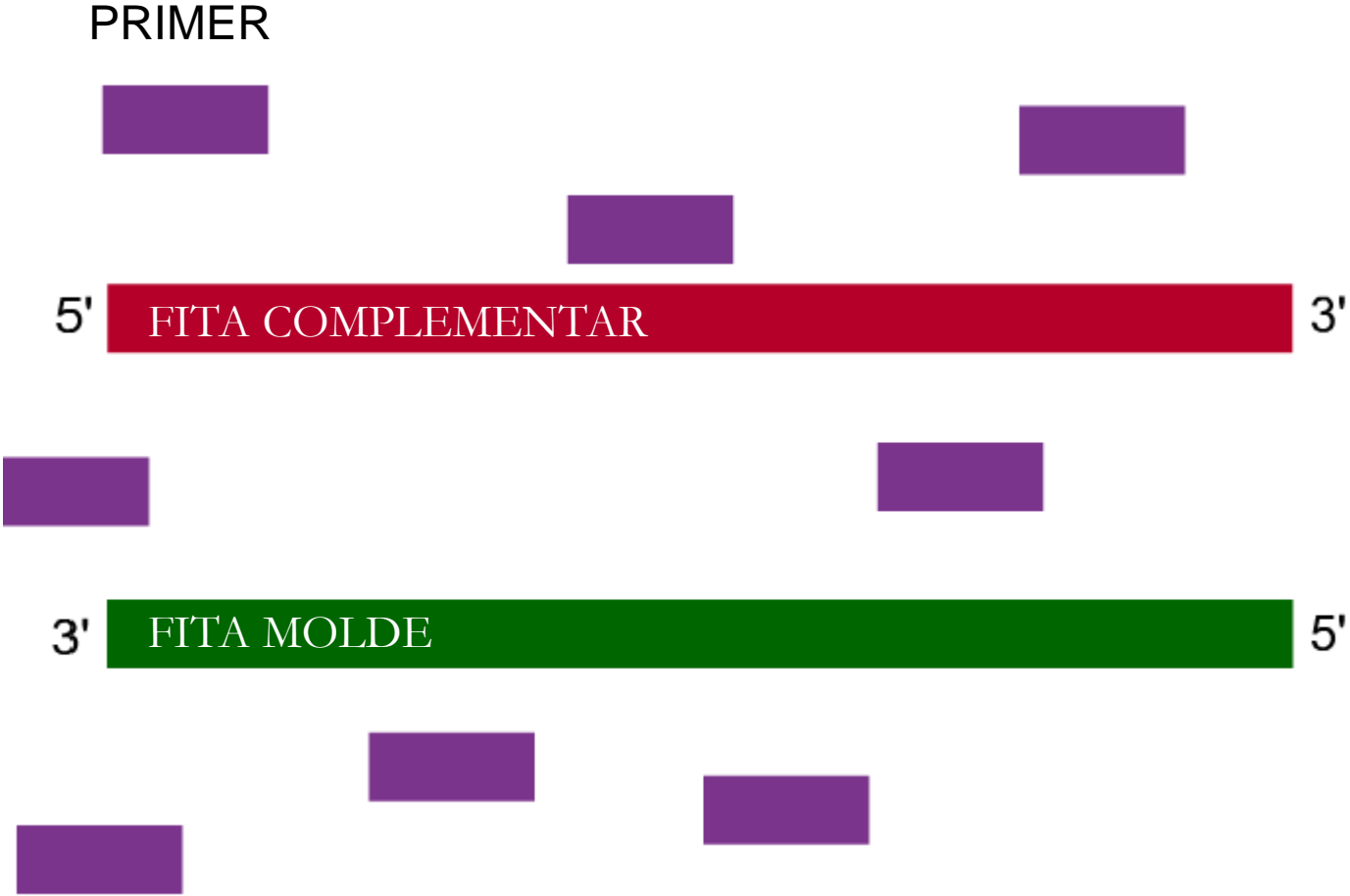
# Desnaturação do DNA



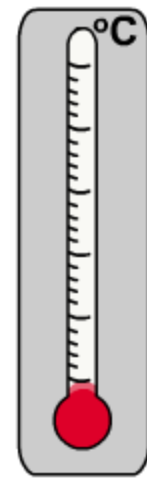
calor

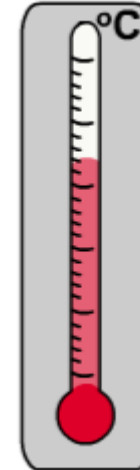
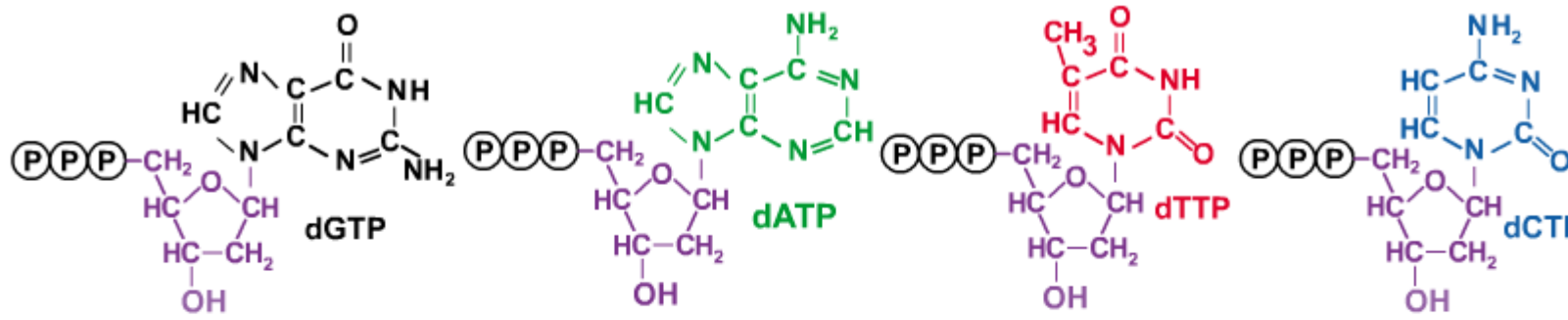


# Anelamento dos *Primers*



# Anelamento dos *Primers*

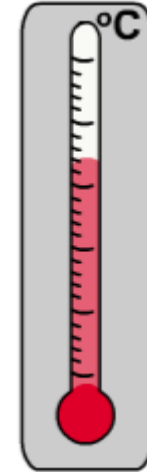




DNA POLIMERASE



# Polimerização



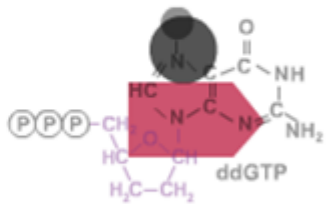
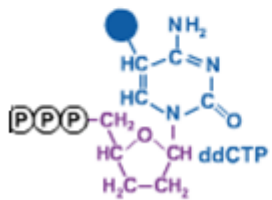
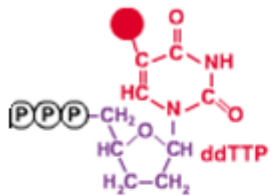
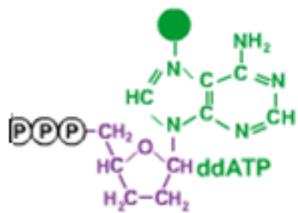
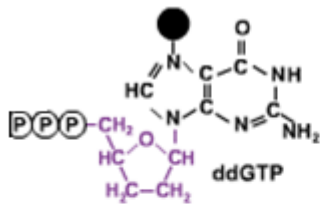
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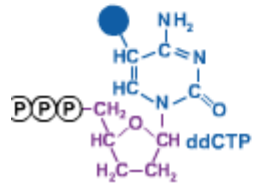
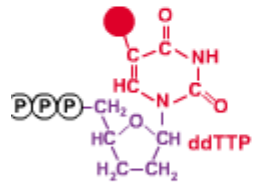
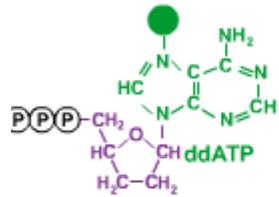
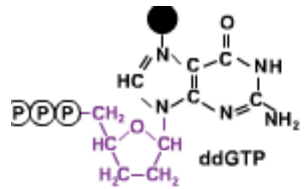


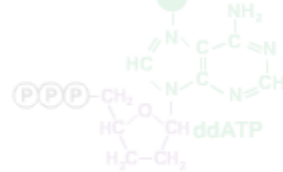
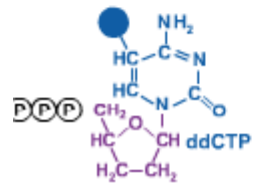
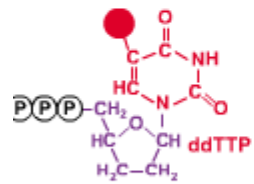
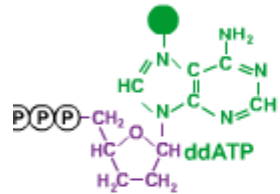
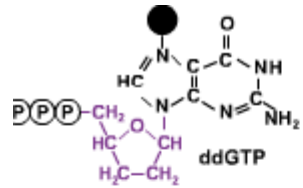
DNA POLIMERASE

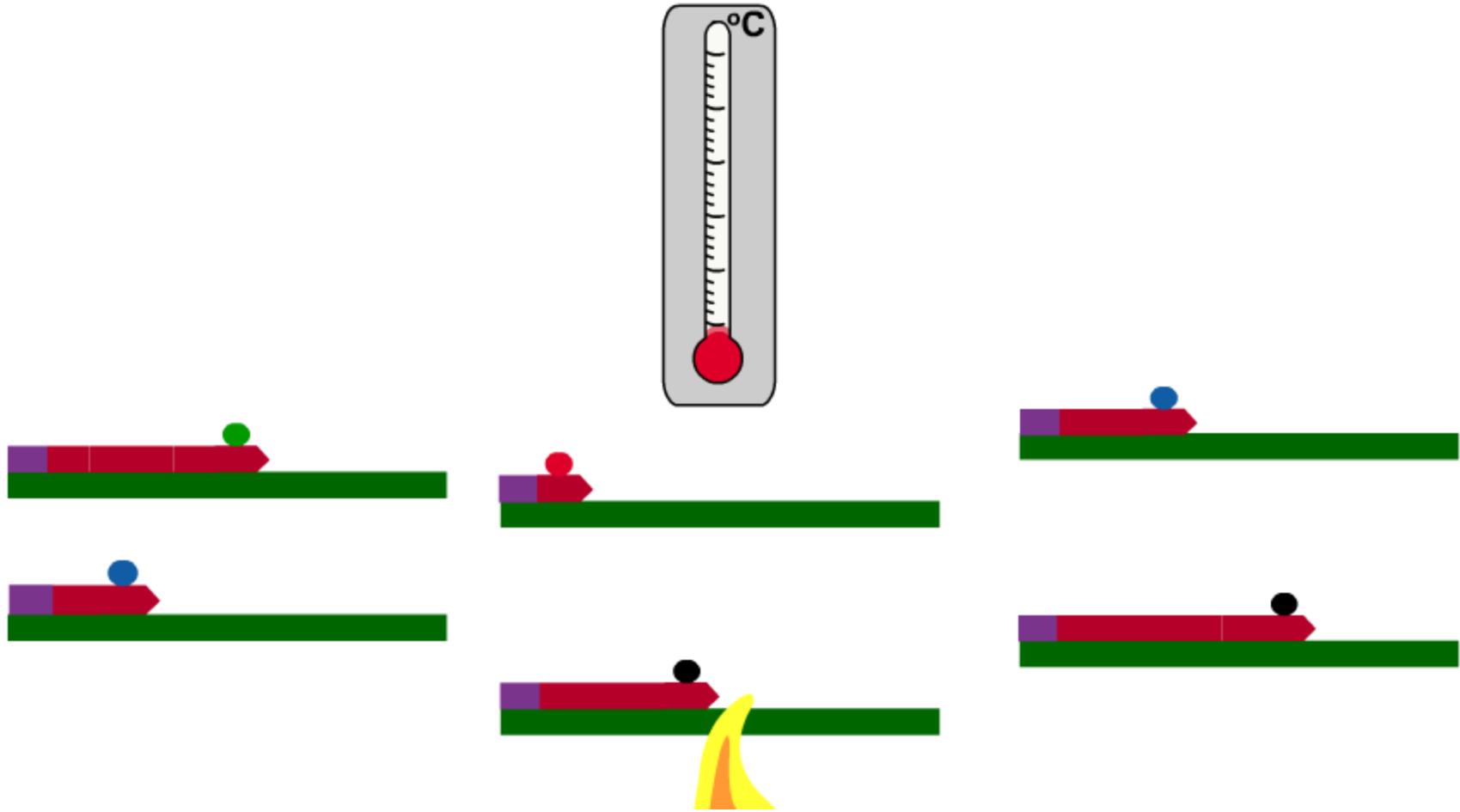


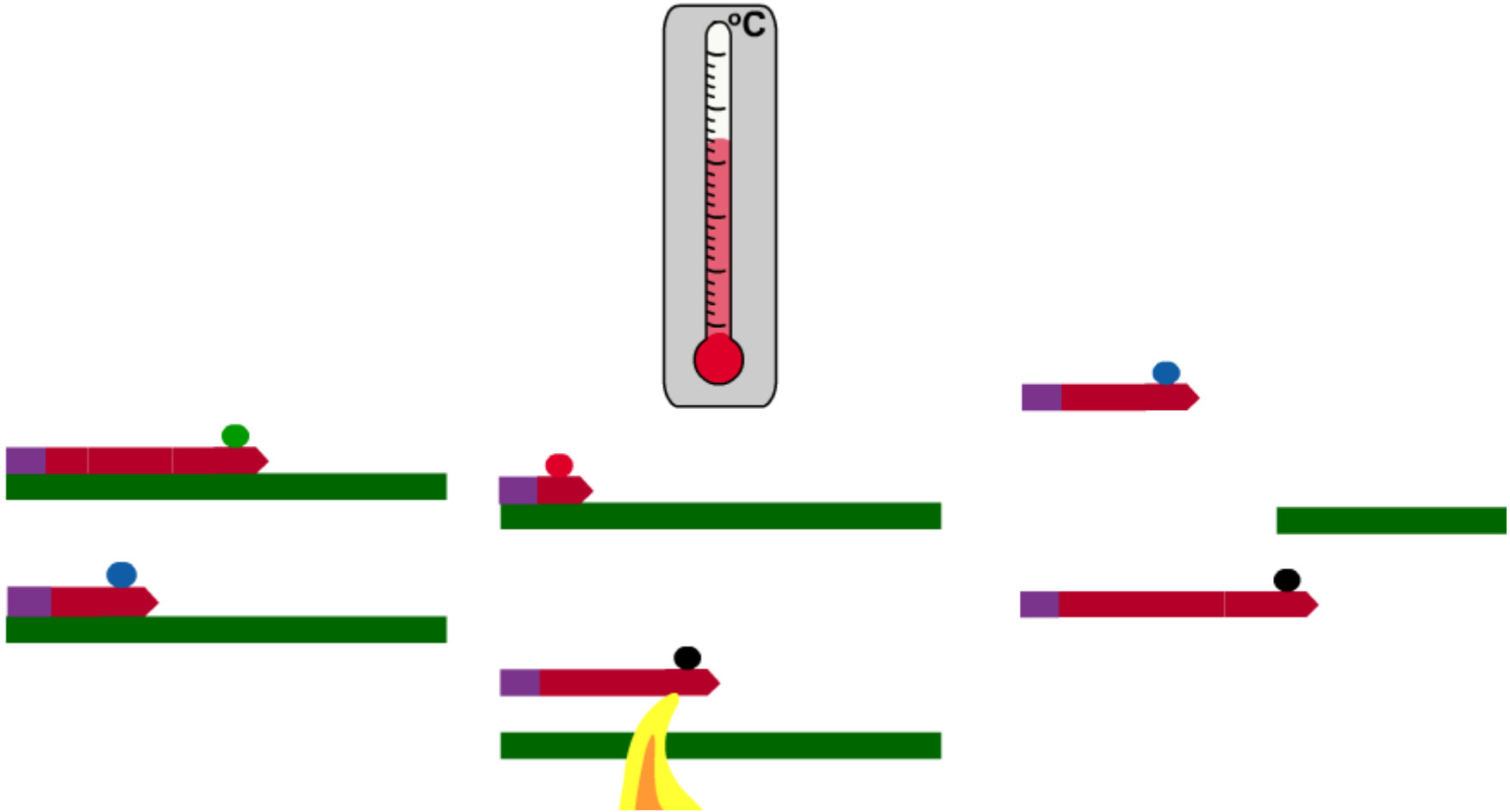


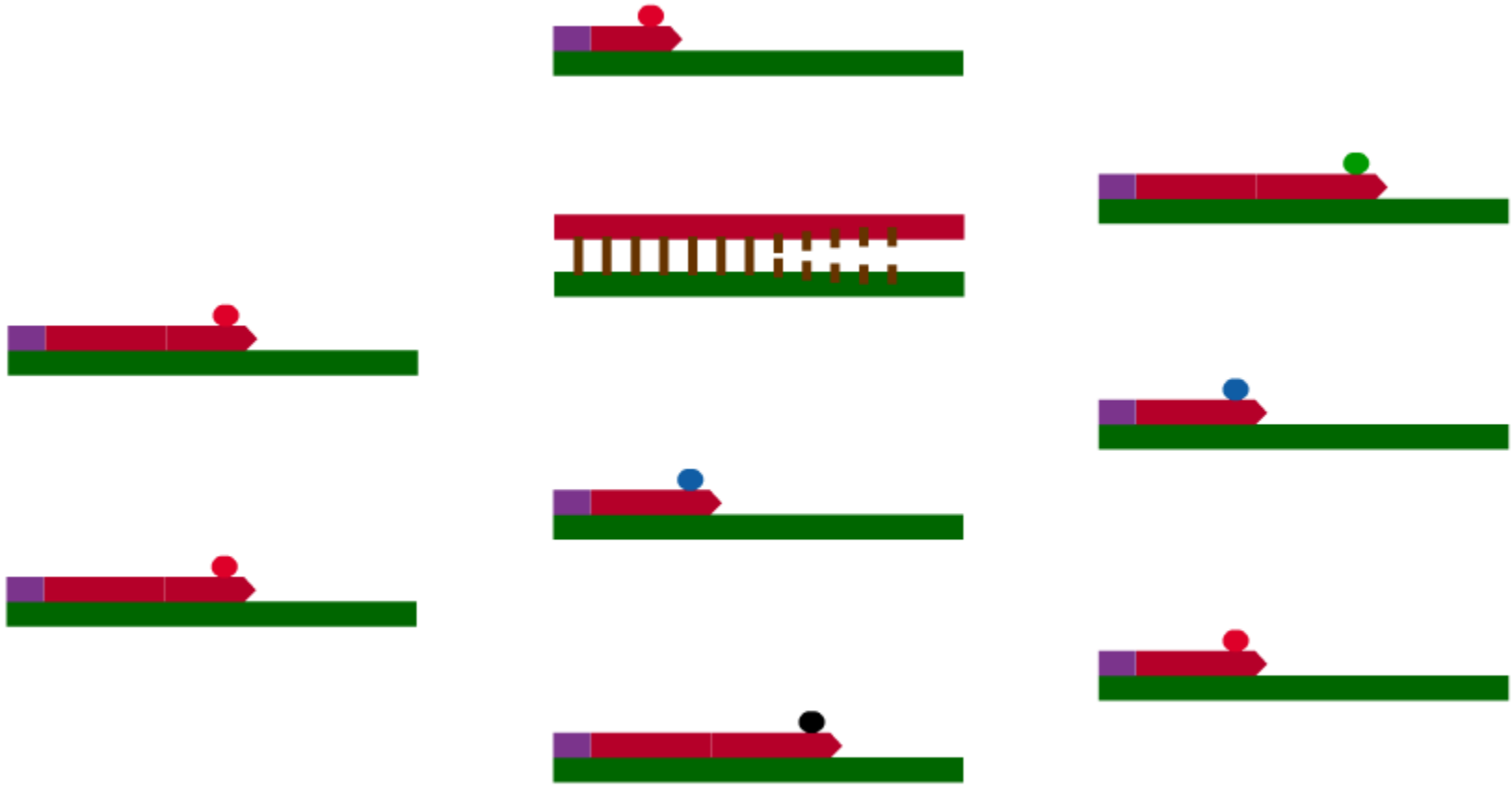




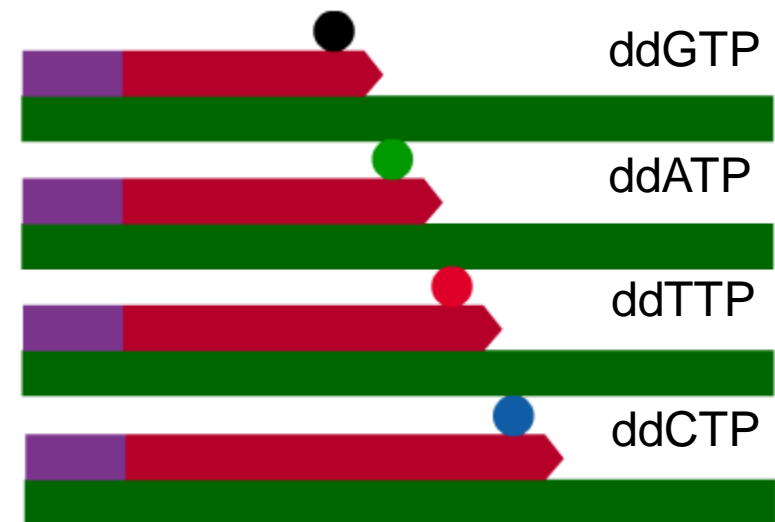








# Terminação



TATGCTATAGAG????????????????????

ATACGATATCTCGACTCTCGAGCTAGAA T

ATACGATATCTCGACTCTCGAGCTAGAATCTTTA A

ATACGATATCTCGACTCTCGAGCT A

ATACGATATCTCGACTCTCG A

ATACGATATCTCGACTCTCGAGCTAGAATCTT T

ATACGATATCTCGACTCTCGAGCTAGAATCT T

ATACGATATCTCGACTCTCGAGCTAGAATCTTTAAGGCA T

ATACGATATCTCGACTCTCGAGCTAGAATCTTTA A

ATACGATATCTCGACTCTCGAGCTAG A

ATACGATATCTCGACTCTCGAGCTAGAATCTT T

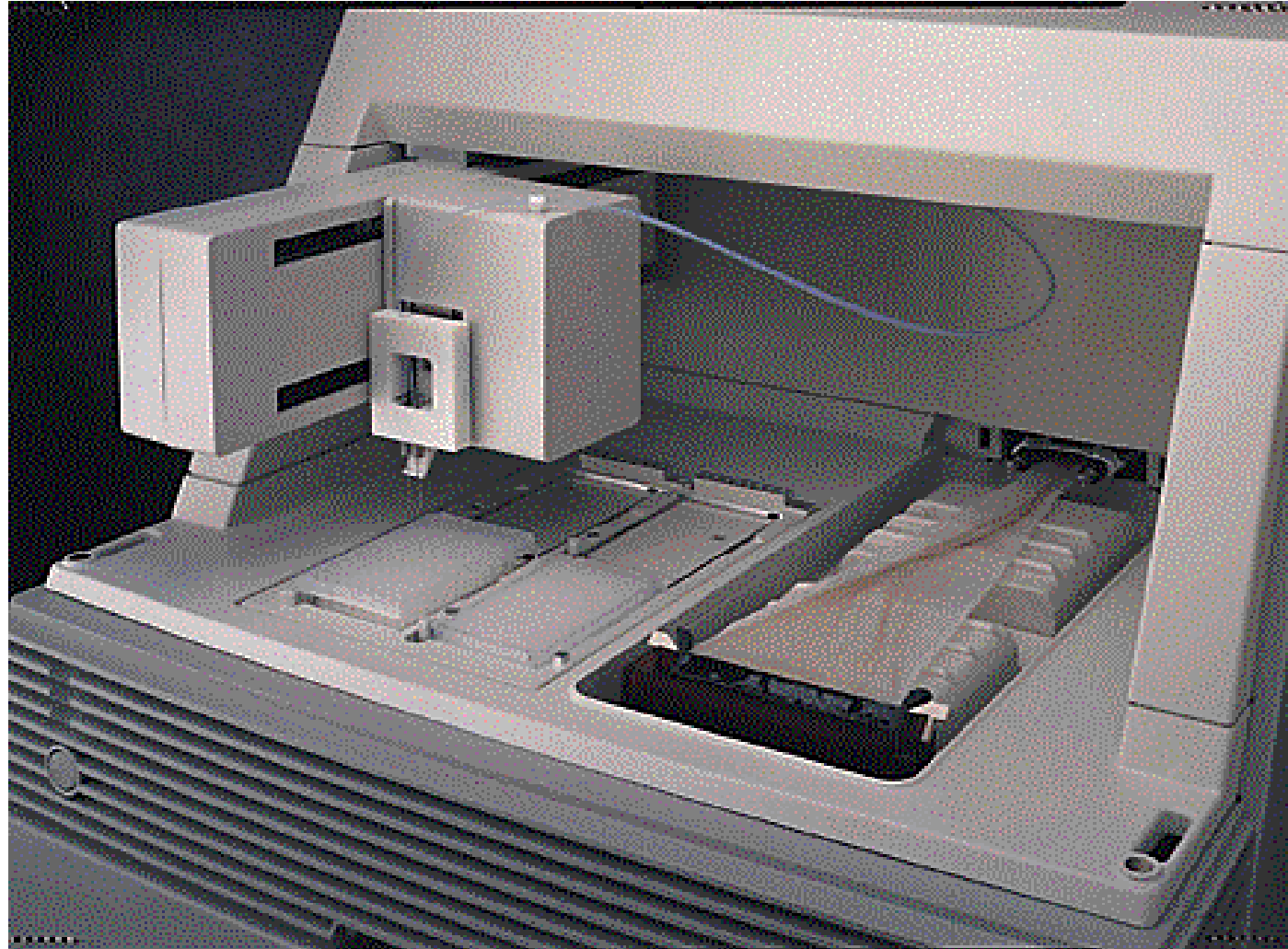
ATACGATATCTCGACTCTCGA A

ATACGATATCTCGACTCT T

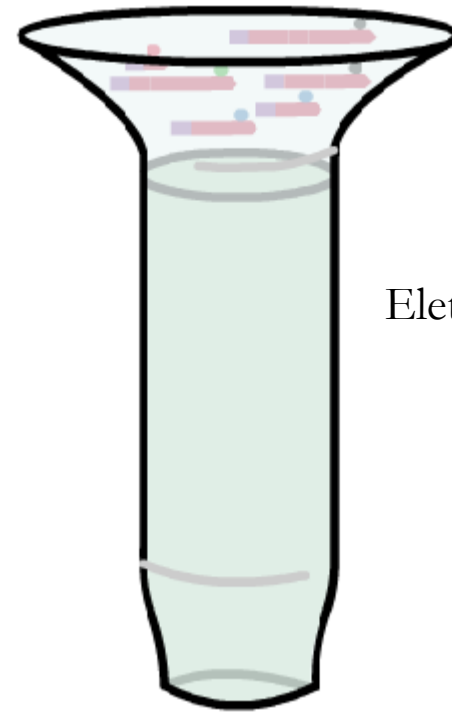
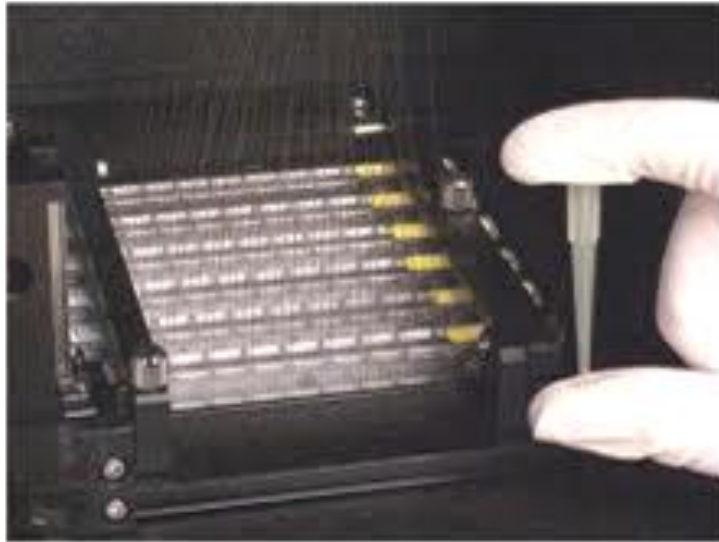
ATACGATATCTCGACTCTCGAGCTAGAATCTTTAAGGC C



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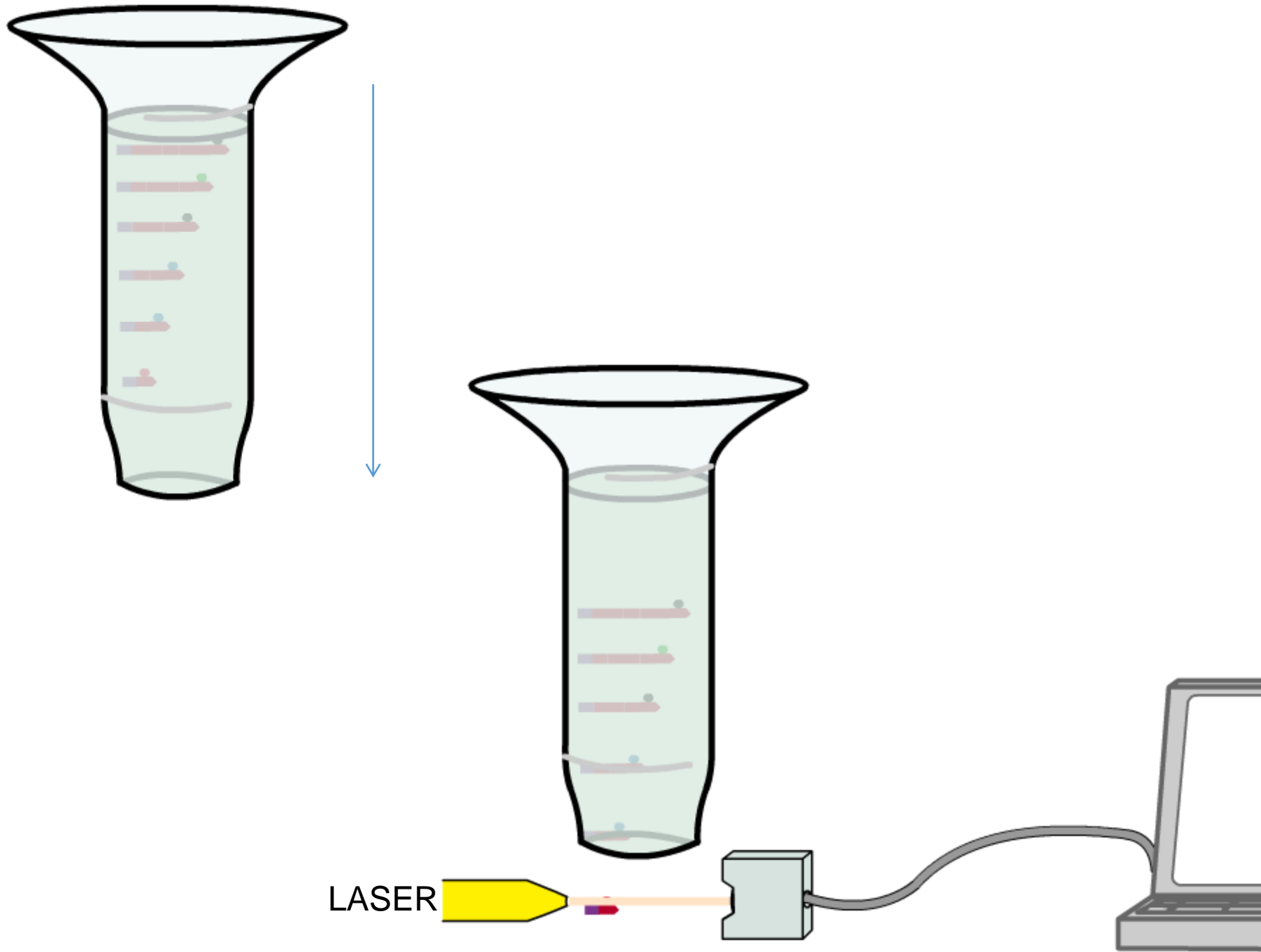


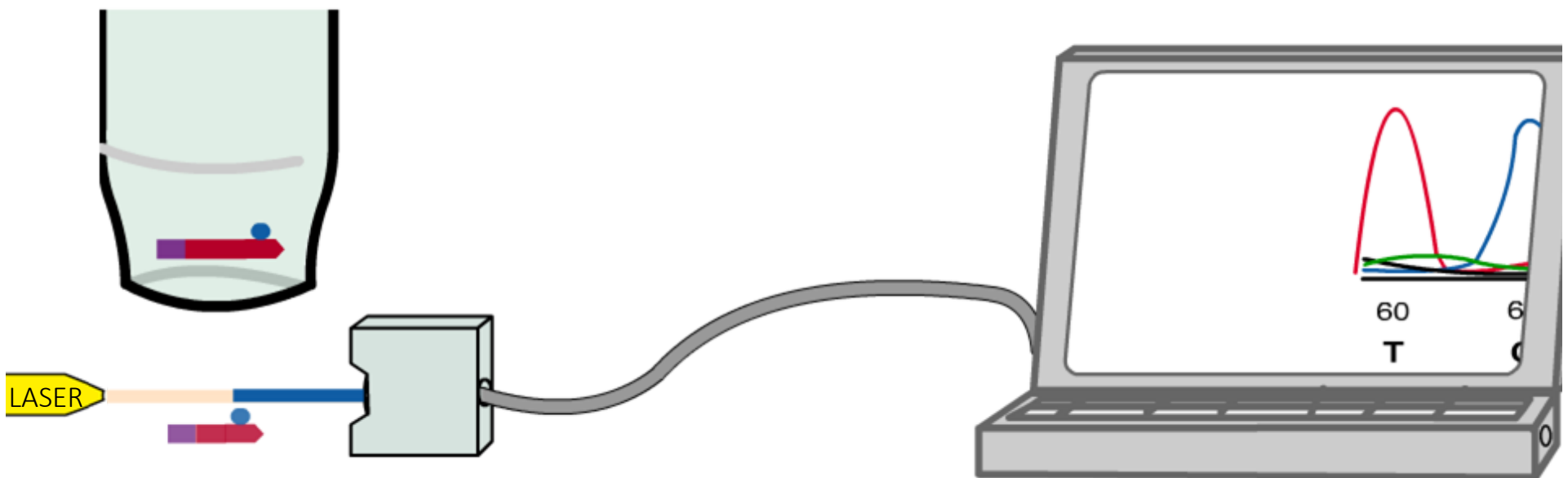
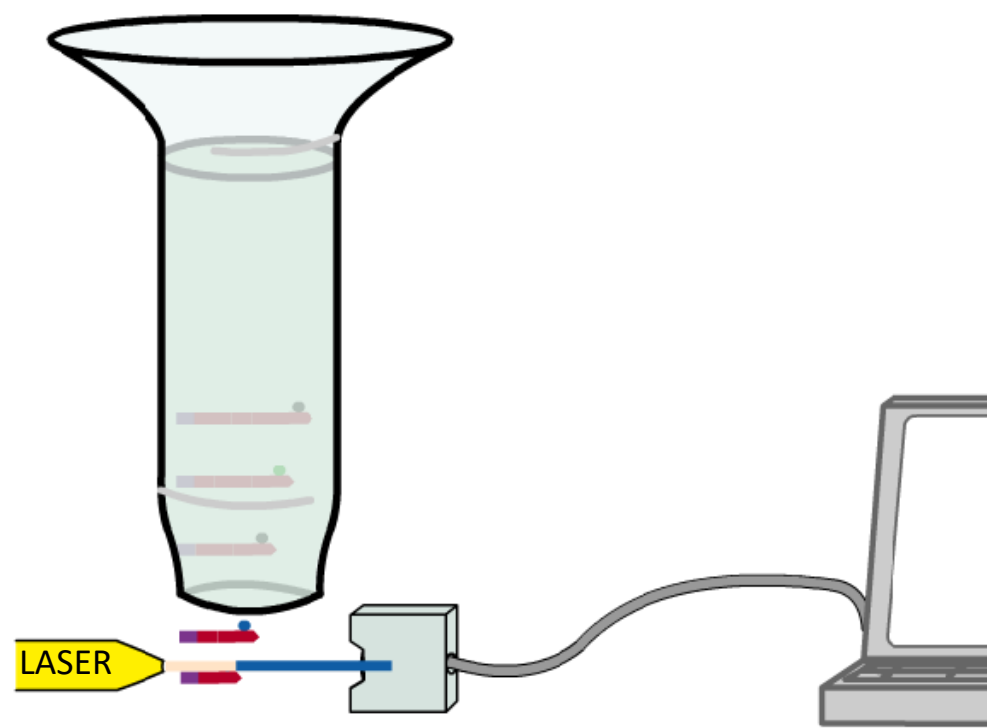
ABI 3700 Applied Biosystems

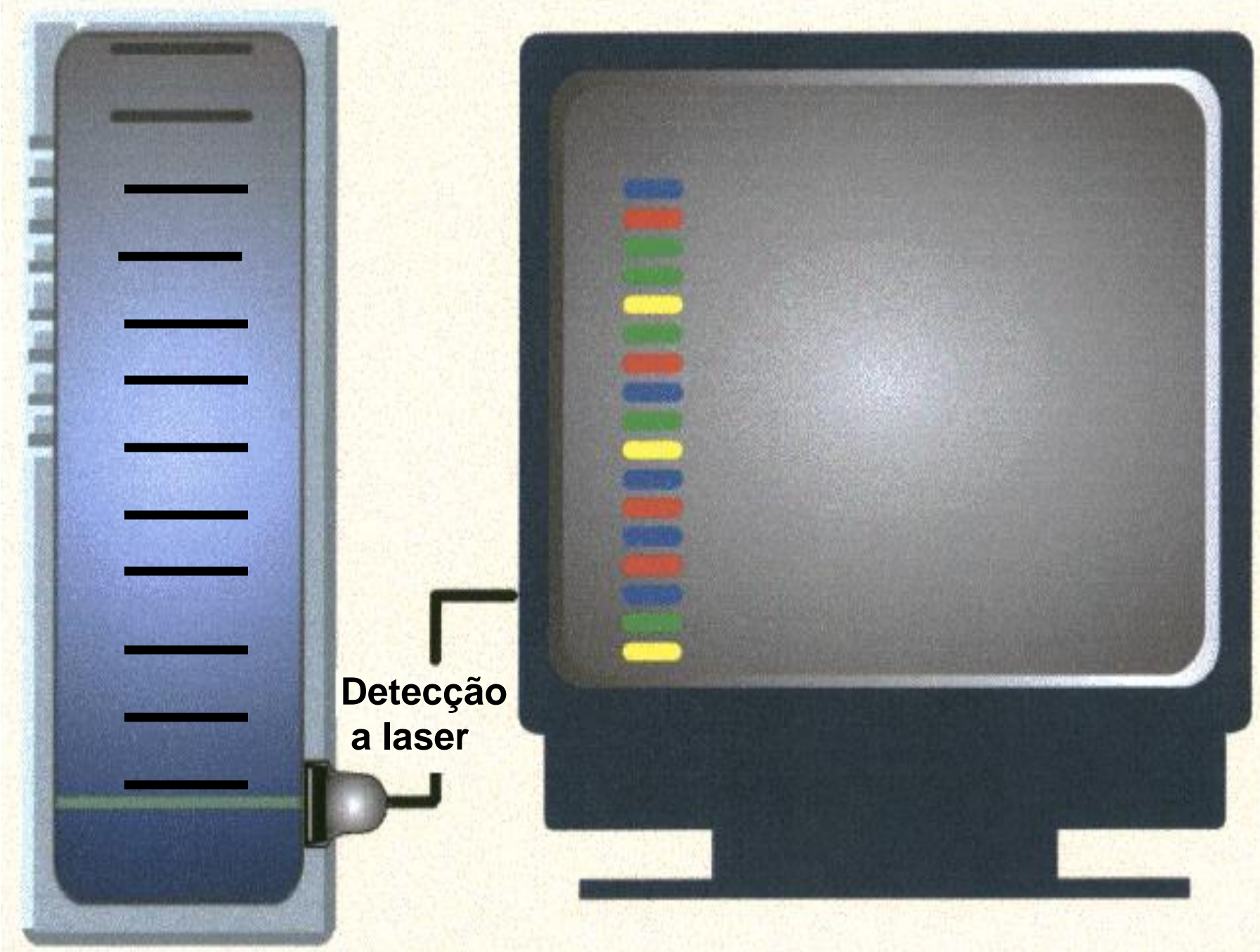


Eletroforese capilar







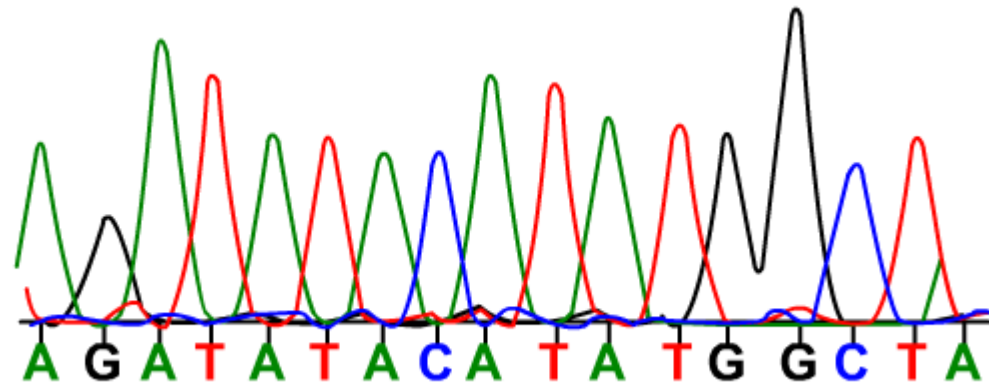
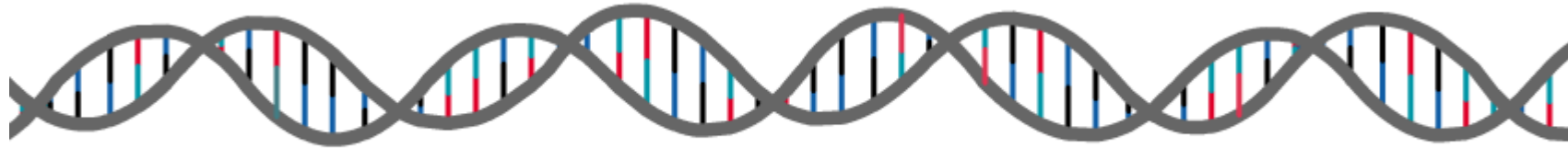


Detecção  
a laser

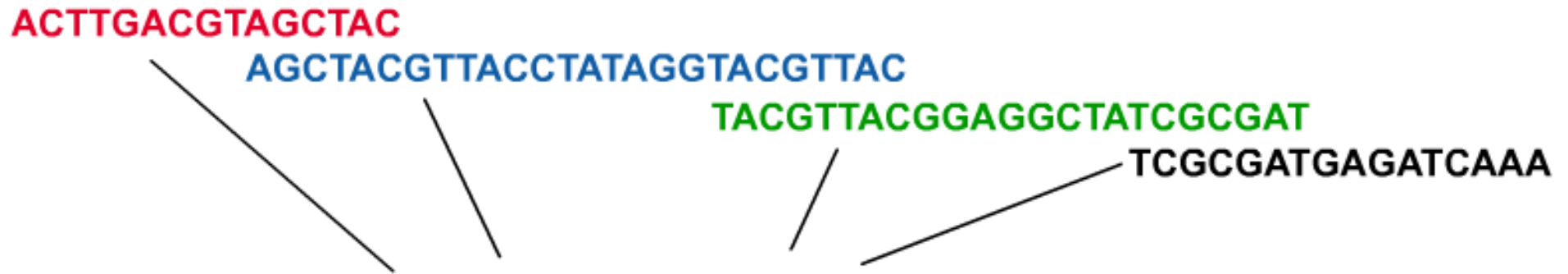


Figure 4: Support Vector Machine (SVM) Classification Results (Confusion Matrix)

# Análise Computacional



**ACTTGACGTAGCTAC**  
**AGCTACGTTACCTATAGGTACGTTAC**  
**TACGTTACGGAGGCTATCGCGAT**  
**TCGCGATGAGATCAAA**



Fragmentos de DNA secuenciados

**ACTTGACGTAGCTAC**  
**AGCTACGTTACCTATAGGTACGTTAC**  
**TACGTTACGGAGGCTATCGCGAT**  
**TCGCGATGAGATCAAA**



**ACTTGACGTAGCTACGTTACCTATAGGTACGTTACGGAGGCTATCGCGATGAGATCAAA**

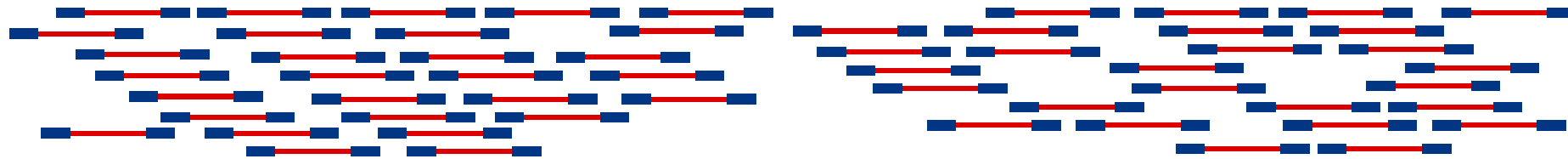
**ACTTGACGTAGCTACGTTACCTATAGGTACGTTACGGAGGCTATCGCGATGAGATCAAA**



Fragmentos Completos



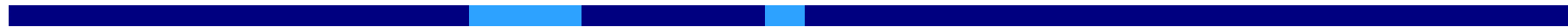
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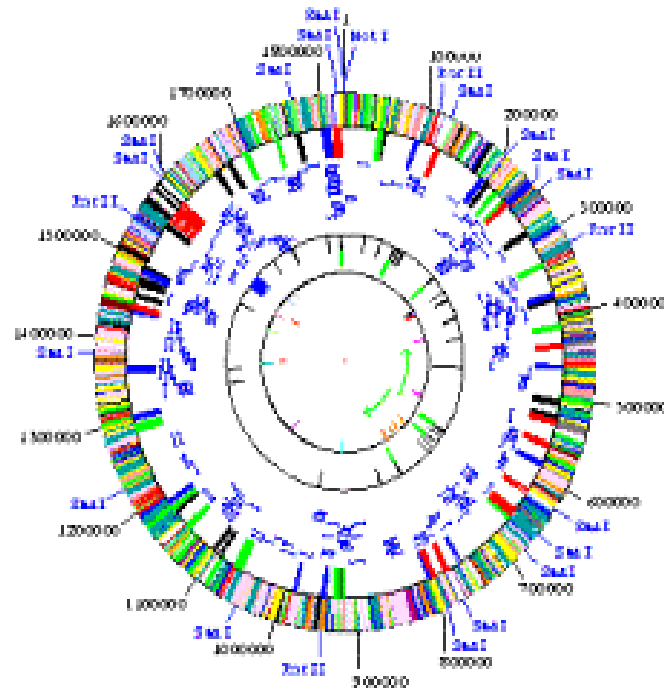
Montagem dos fragmentos



Fechamento dos gaps

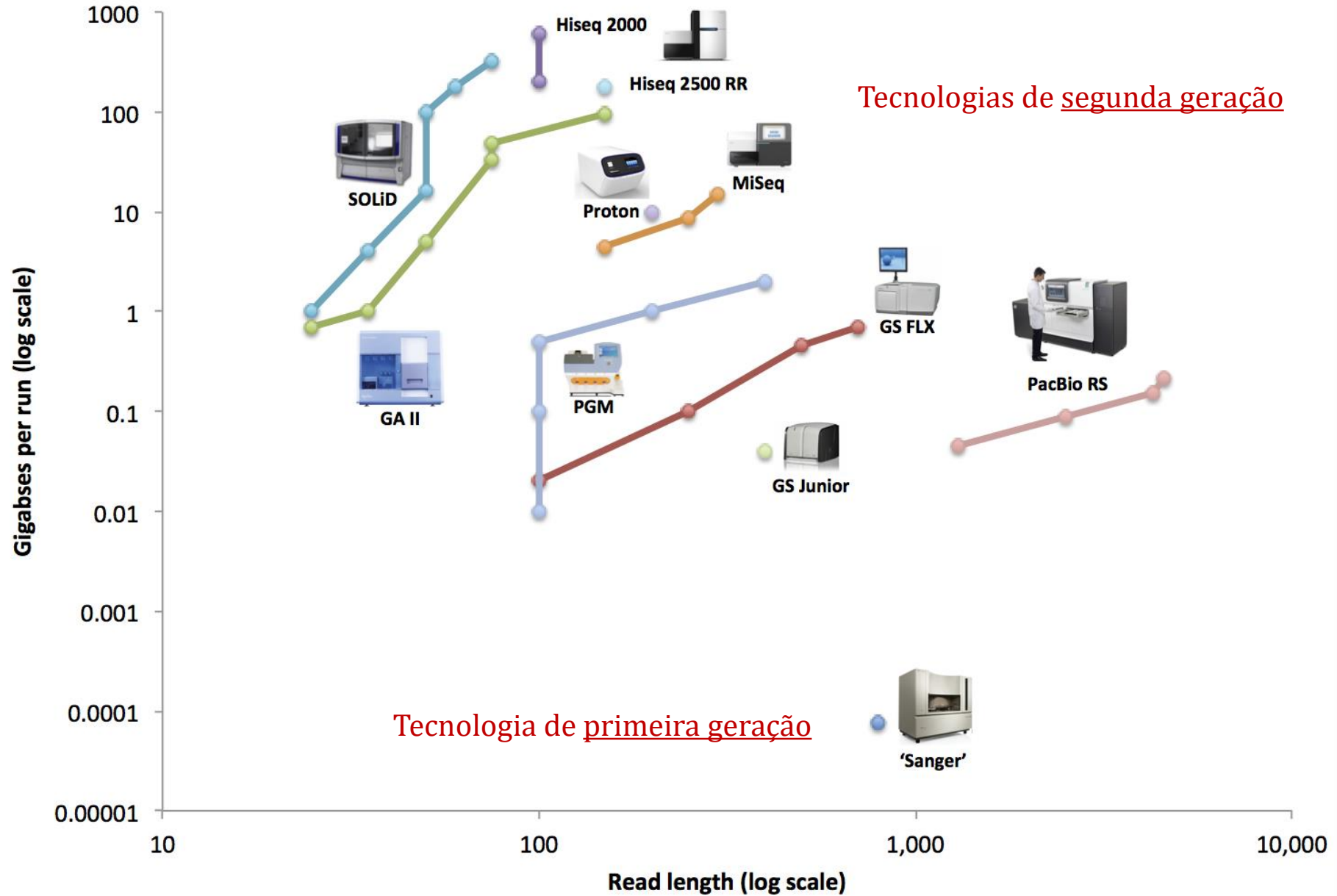


Análise



	1520	1530	1540	1550	1560	1570	1580	1590	1600
CONSENSUS	TTC*ACTGCGAT*CG*CCGACGACCTCGACTC*CG*CCC								
LOQH-01F12-04H05.b	tt**actgggat*gc*ccgagaaccttgattc*gc*cgga								
LOQH-01F12-04B05.g	ttc*actgggat*cg*ccgacgaccttgactc*cg*gccggg								
LOQH-01F12-03H04.b	ttc*cctgggat*cg*ccgactactactctactt*ct*gccggg								
LOQH-01F12-01D03.b	ttc*actgcgat*cg*ccgacgacctcgactc*cg*cccggg								
LOQH-01F12-03G04.b	tct*tttgccct*cc*ctcccccttggcttc*gt*tcgctt								
LOQH-01F12-05F12.b	tnt*ccTgCGAT*cg*ggcnccgacctcgctc*cg*CCGg								
LOQH-01F12-01E06.b	TTC*ACTGCGAT*CG*CCGACGACCTCGACTC*CG*CCC								
LOQH-01F12-08F11.b	ttt*tctgcnt*cg*ccggcgctctgctctc*cg*cccggg								
LOQH-01F12-01C04.b	TTC*ACTGCGAT*CG*CCGACGACCTCGACTC*CG*CCC								
LOQH-01F12-01C08.b	TTC*ACTGCGAT*CG*CCGACGACCTCGACTC*CG*CCC								
LOQH-01F12-01D05.g	tTC*ACTGcgat*cg*CCGacgaTttgactC*CG*CCC								
LOQH-01F12-06G11.g	c*tgttatacgn*aatt*c*c*gn**ctcgg*ta								
LOQH-01F12-08B07.g	TTC*ACTGCGAT*CG*CCGACGACctcgactc*CG*CCC								
LOQH-01F12-01A04.b	TTC*ACTGCGAT*CG*CCGACGACCTCGACTC*CG*CCC								
LOQH-01F12-02D02.b	TTC*ACTGCGAT*CG*CCGACGACCTCGACTC*CG*CCC								
LOQH-01F12-03H05.b	TTC*ACTGCGAT*CG*CCGACGACCTCGACTC*CG*CCC								
LOQH-01F12-05G10.b	TTC*ACTGCGAT*CG*CCGACGACCTCGACTC*CG*CCC								
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LOQH-01F12-04H05.g	TTC*ACTGCGAT*CG*CCGACGacctcGACTC*CG*CCC								
LOQH-01F12-08C07.g	aca*acacgcaa*aa*agcgaagacaaaaagc*ac*ga								
LOQH-01F12-07H04.g	ttccaat*cggtgcg*ccgaggacctgggctc*cg*ccc								
LOQH-01F12-05A01.b	cgg*gcgaggga*ac*acaangcccgcgcgc*cg*gcg								
LOQH-01F12-01A03.b	ccc*ccgcccc*cg*aaaaggggtaaaaag*aa*aaa								
LOQH-01F12-01F12.g	t*c*antgcgat*cg*ccgangacctngaatttognccc								
LOQH-01F12-08F11.g	tttttagttatt*tt*tgtgtaatatgcggggagcgg								
LOQH-01F12-05D12.g	tggcgcctcggaggctacgcaag*gg*aagctggccg								

# Developments in High Throughput Sequencing

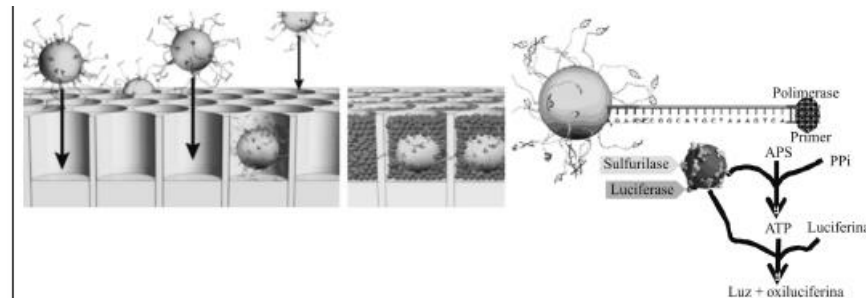


## Sequenciamento de DNA de nova geração e suas aplicações na genômica de plantas

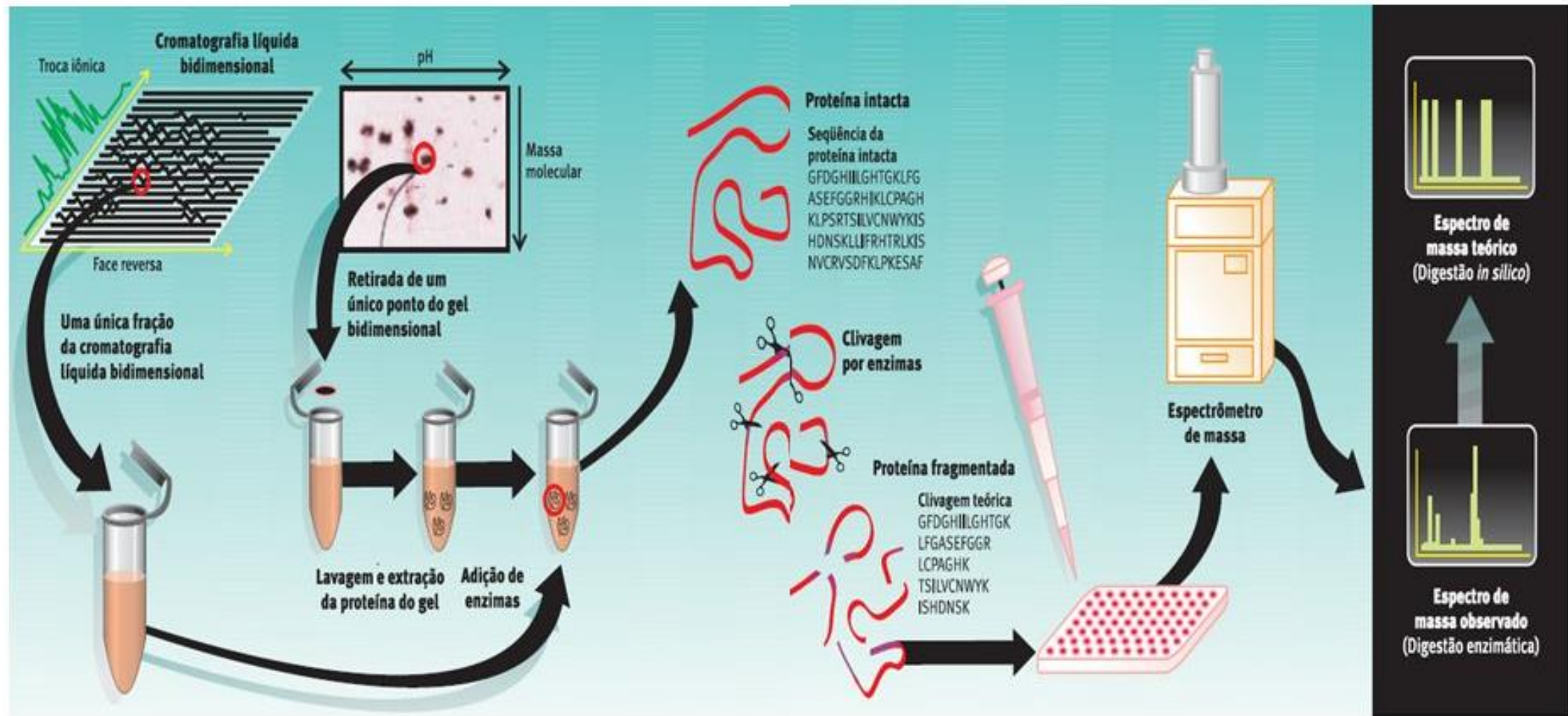


Tabela 1 - Resumo das principais características técnicas das plataformas 454 GS-FLX, Solexa e SOLiD e laboratórios no Brasil que já adquiriram essas novas plataformas. A duração da corrida inclui o tempo para o preparo, a leitura e o processamento das amostras; o custo da corrida e o valor do equipamento são fornecidos na capacidade máxima do equipamento.

Plataforma	-----Corrida-----			-----Custo-----		Acurácia (%)	Laboratório**
	Informação (Gb)	Duração (dias)	Reads (pb)	Equipamento (U\$)	Base (U\$)		
GS-FLX <i>Titanium</i>	0,5	3 a 4	Até 400	531.500	10.000	99,5	- LNCC - IQ-USP
<i>Genome analyzer</i> (Solexa)	3	5	25-35	430.000	6.250	98,5	- Nenhum - Fiocruz
SOLiD <i>System</i>	25	4-12	35-50	599.000	10.000	99	- Instituto Ludwig - UFPA



# O sequenciamento de proteínas é bem mais complexo!!!



# Sequenciamento de Nova Geração NGS

- **Illumina – sequenciamento por síntese**

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

- **PacBio - Single Molecule Real Time Sequencing - Pacific Biosciences**

<https://www.youtube.com/watch?v=v8p4ph2MAvI>

- **Nanopore DNA sequencing**

<https://www.youtube.com/watch?v=E9-Rm5AoZGw>

# Animações

<http://www.dnalc.org/resources/animations/cycseq.html>

<http://www.dnalc.org/ddnalc/resources/sangerseq.html>

<https://www.youtube.com/watch?v=iTBTHmhNNbE>

[https://www.youtube.com/watch?v=3I9wzwj0b\\_A](https://www.youtube.com/watch?v=3I9wzwj0b_A)

<https://www.youtube.com/watch?v=vK-HlMaitnE>



# ESTUDO DIRIGIDO

1. Conceito dos diferentes “blottings”;
2. Sequenciamento por Sanger;
3. Novas técnicas de sequenciamento

(LER o texto “Sequenciamento de nova geração”)

