

IDENTIFICAÇÃO DE SEQUÊNCIAS POR HIBRIDIZAÇÃO E SEQUENCIAMENTO

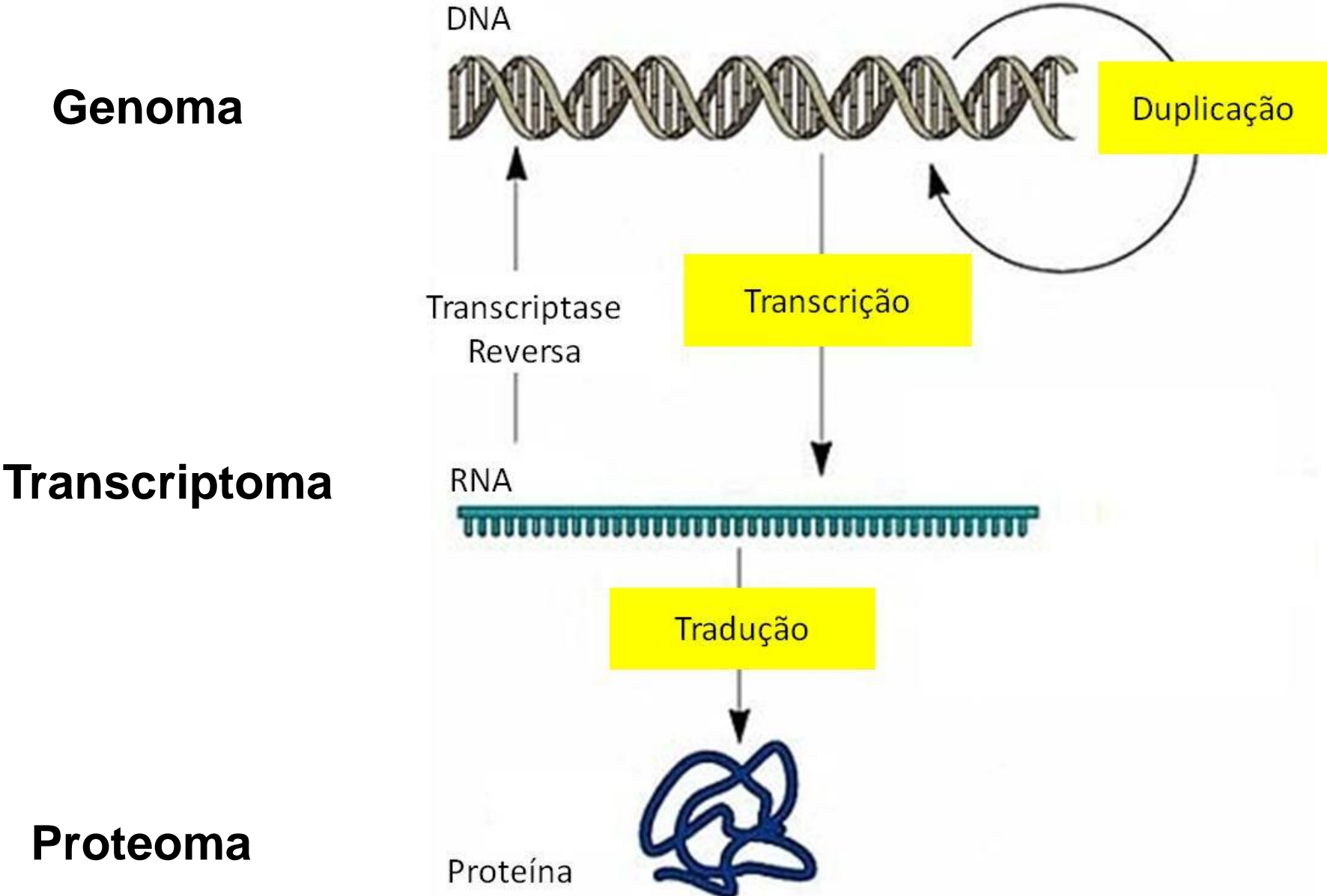
Aula 5

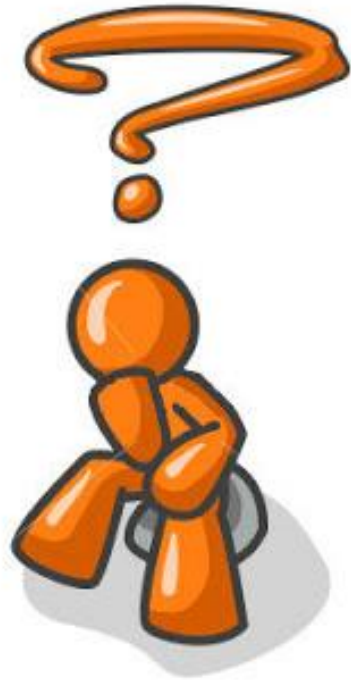
LGN232 – Genética Molecular



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mquecine@usp.br

LEMBRANDO O 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.

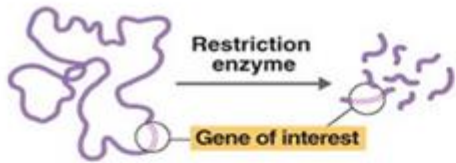
Técnicas de “Blotting”

Southern blot diz respeito a uma **técnica** utilizada na Biologia Molecular através da qual é possível verificar se uma sequência de DNA específica (gene de interesse) está ou não presente na amostra em análise que contém uma mistura complexa (genoma inteiro de um organismo).

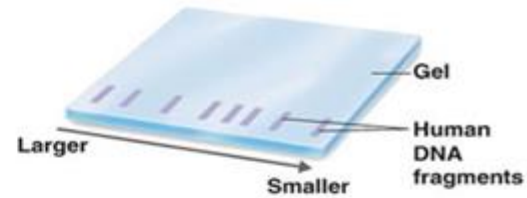
Northern Blot é uma técnica usada na pesquisa em biologia molecular para estudar a expressão gênica, ou seja, verificar se um determinado gene de um genoma é ou não transcrito em RNA e quantificar isso. Essa técnica tem tal nome devido à similaridade de seu procedimento com o Southern blot (batizada 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.

0 Southern Blot



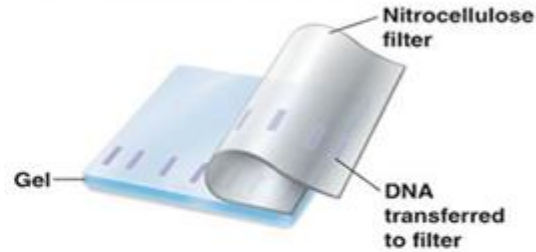
1 DNA containing the gene of interest is extracted from human cells and cut into fragments by restriction enzymes.



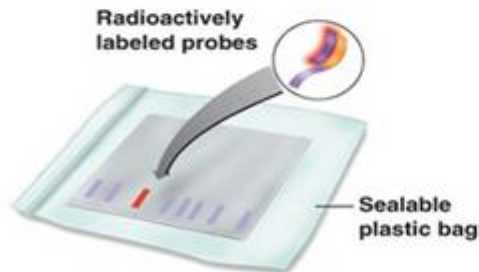
2 The fragments are separated according to size by gel electrophoresis. Each band consists of many copies of a particular DNA fragment. The bands are invisible but can be made visible by staining.



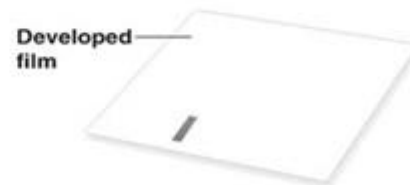
3 The DNA bands are transferred to a nitrocellulose filter by blotting. The solution passes through the gel and filter to the paper towels.



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

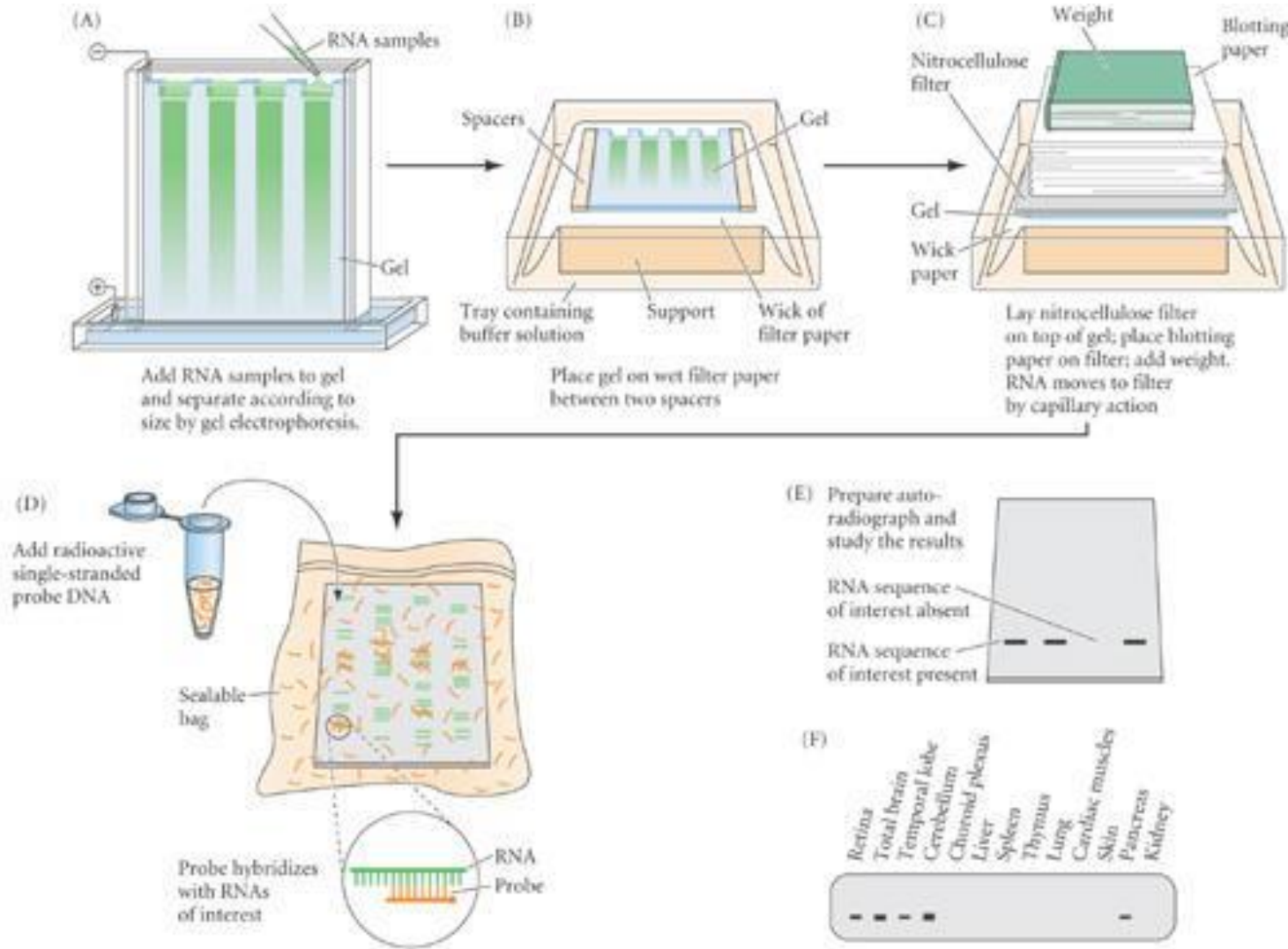


5 The filter is exposed to a radioactively labeled probe for a specific gene. The probe will base-pair (hybridize) with a short sequence present on the gene.

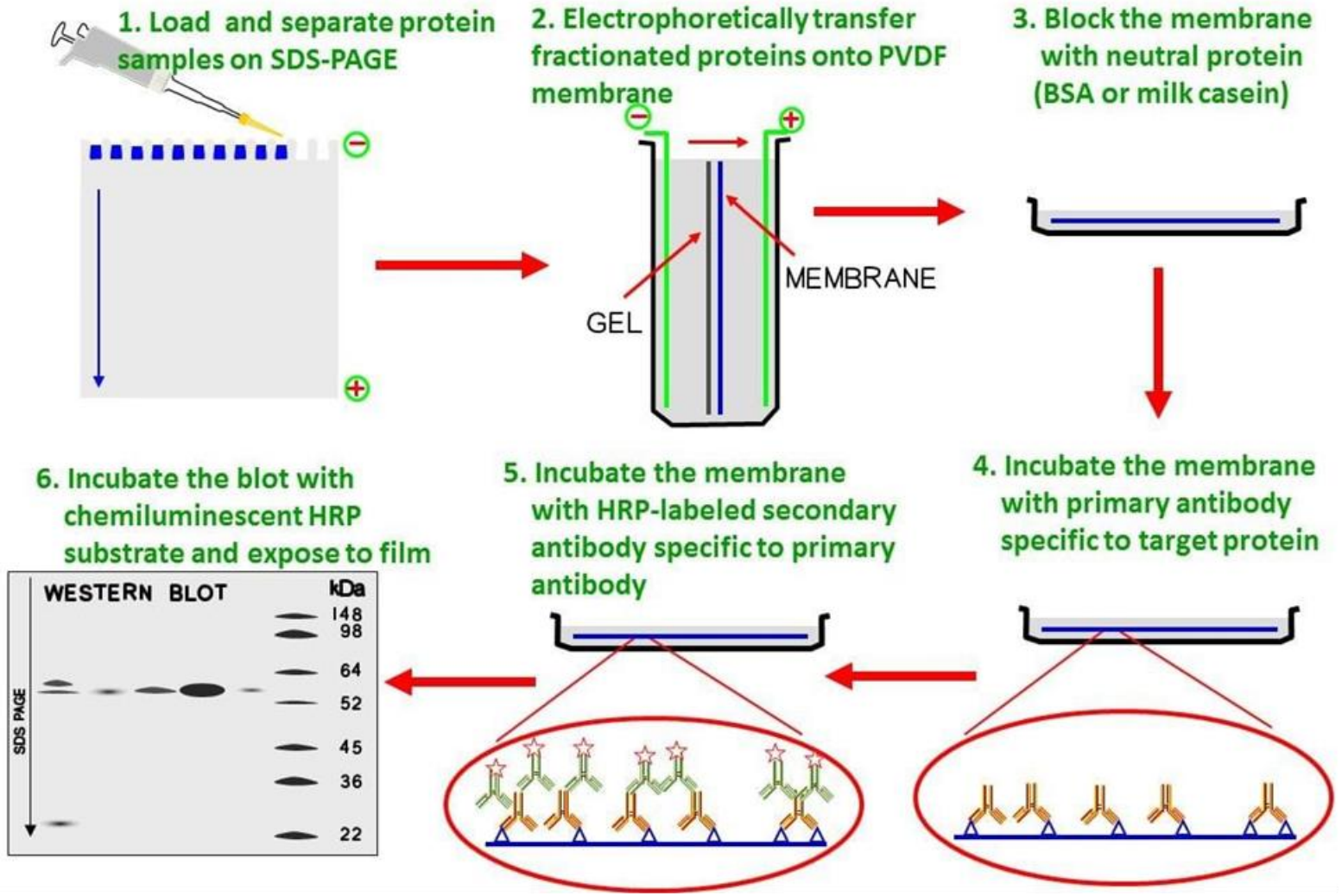


6 The filter is then exposed to X-ray film. The fragment containing the gene of interest is identified by a band on the developed film.

O Northern Blot



0 Western Blot



LIMITAÇÕES DAS TÉCNICAS

- Qualitativa ou semi-quantitativa;
- Uma sonda utilizada por vez;
- Depende de uma sequência conhecida previamente (sonda);

Ainda MUITO utilizadas para confirmação de transgênicos, validação de dados de transcriptômicas proteômicas!



- E agora, 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.

ETAPAS DO SEQUENCIAMENTO DE DNA

Preparação do DNA



Reação de sequenciamento

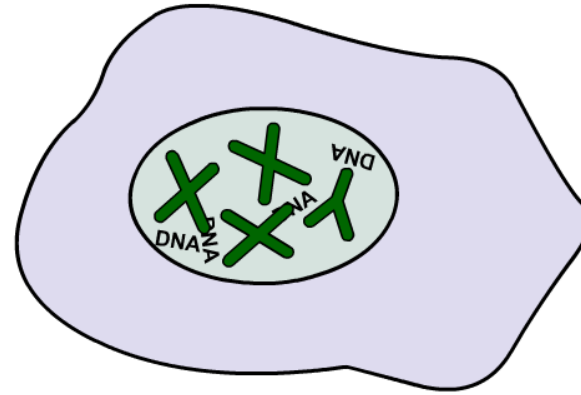


Eletroforese capilar

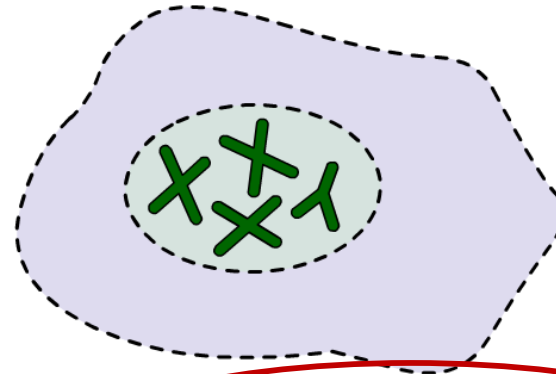


Análise computacional

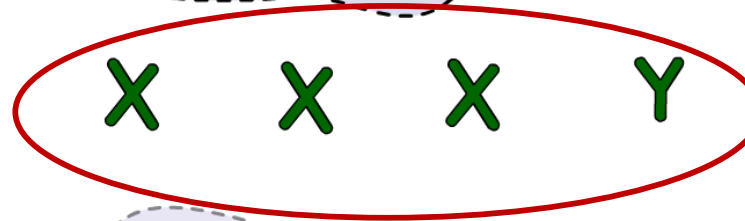
PREPARAÇÃO DO DNA



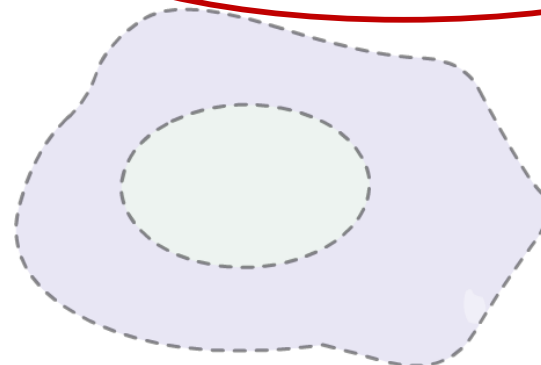
CÉLULA



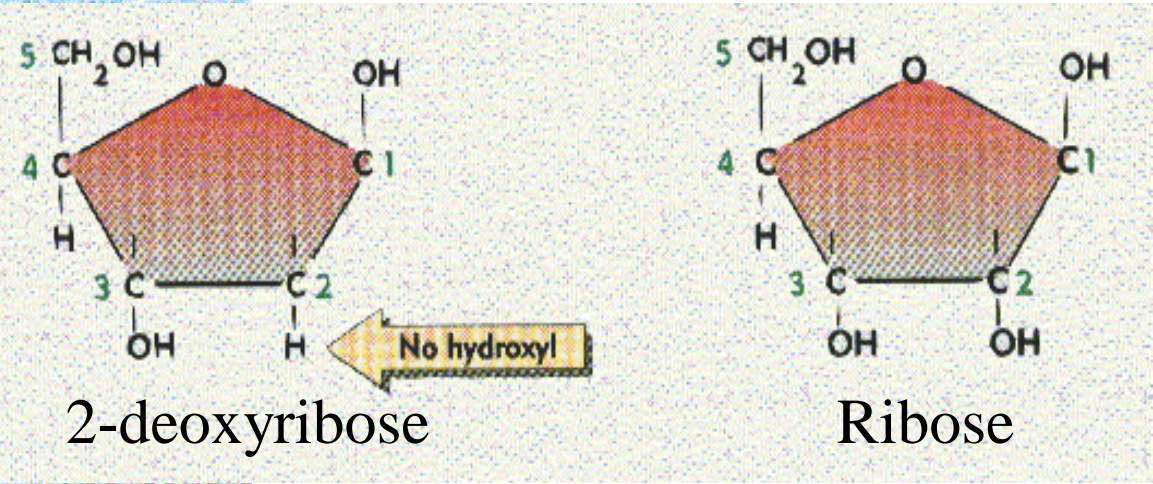
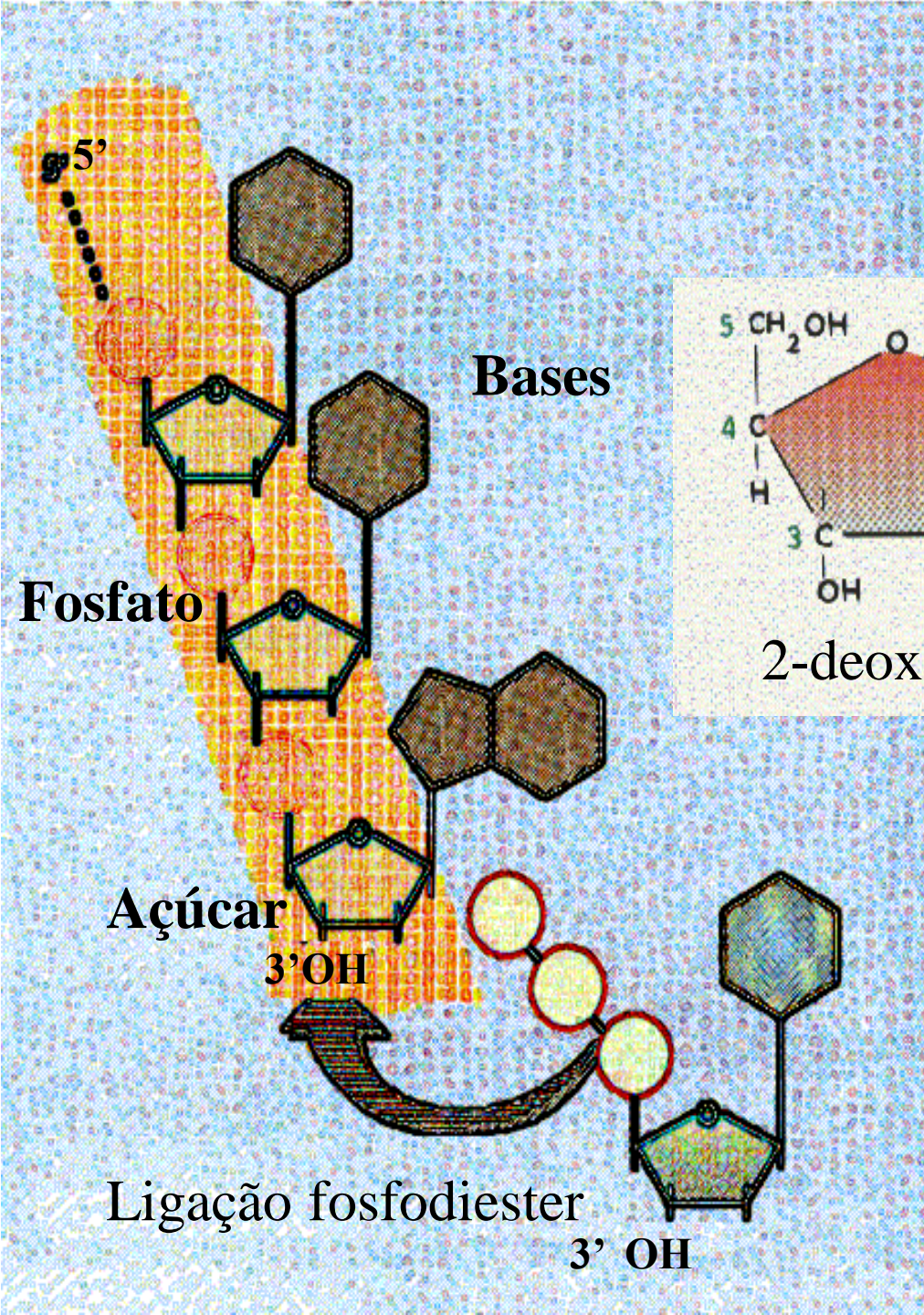
MEMBRANA
PLASMÁTICA E
PAREDE CELULAR



PURIFICAÇÃO DO
DNA TOTAL

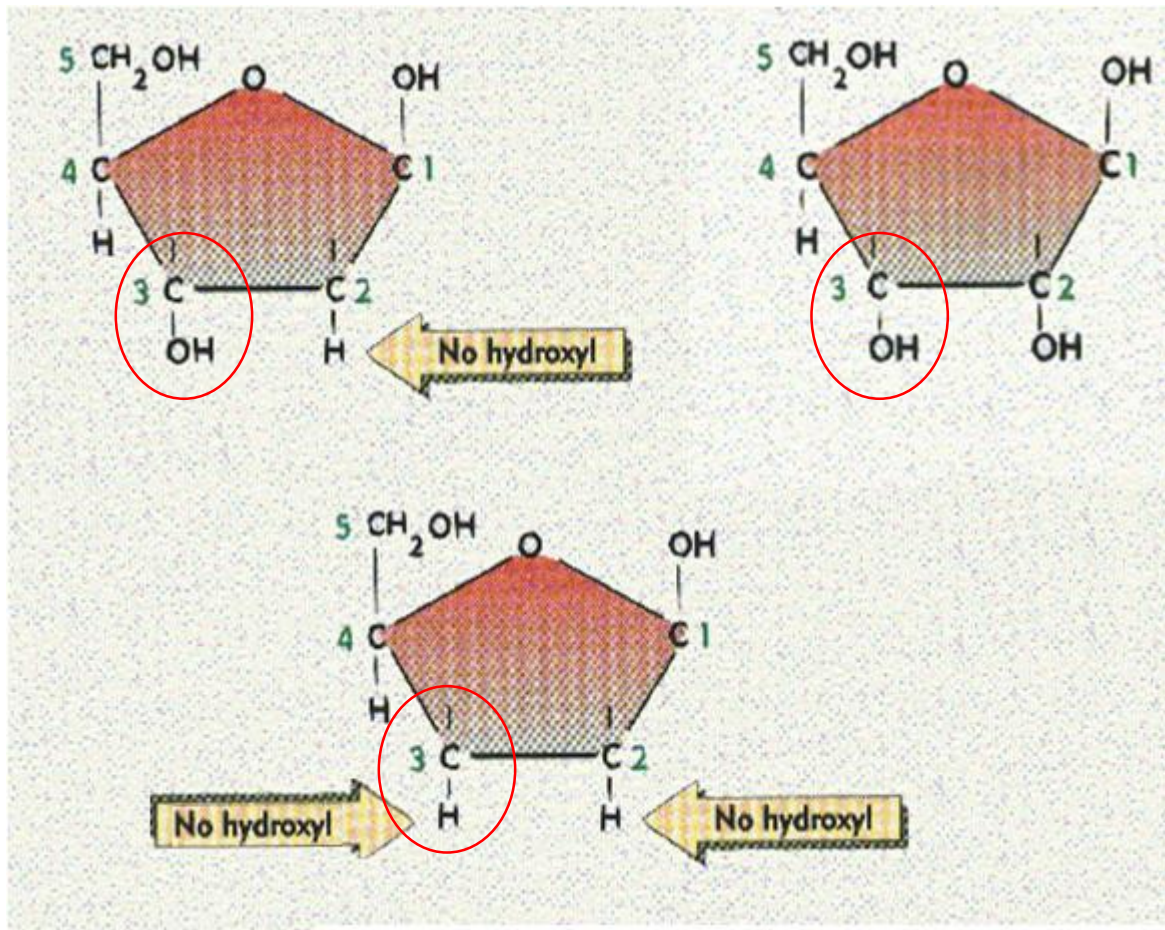


SEPARAÇÃO DOS
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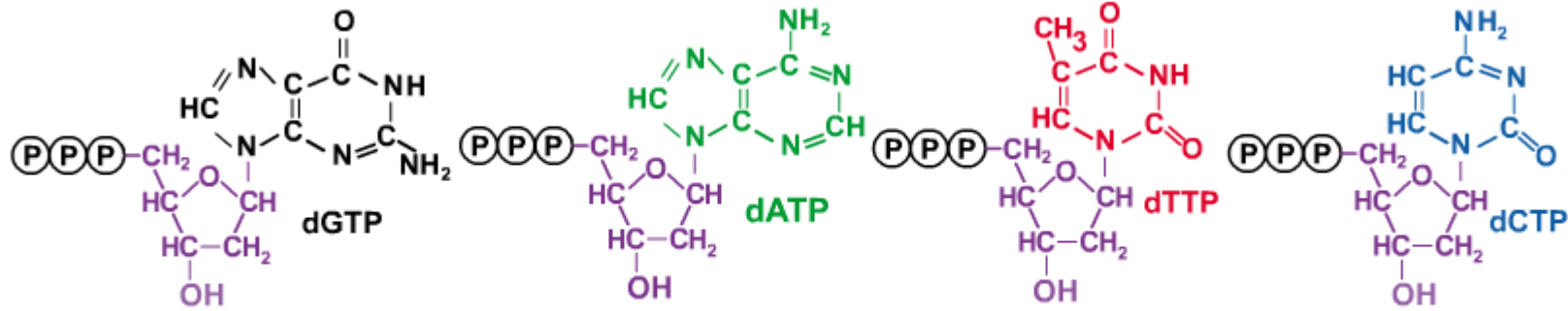
DESOXIRIBOSE

RIBOSE



DIDESOXIRIBOSE

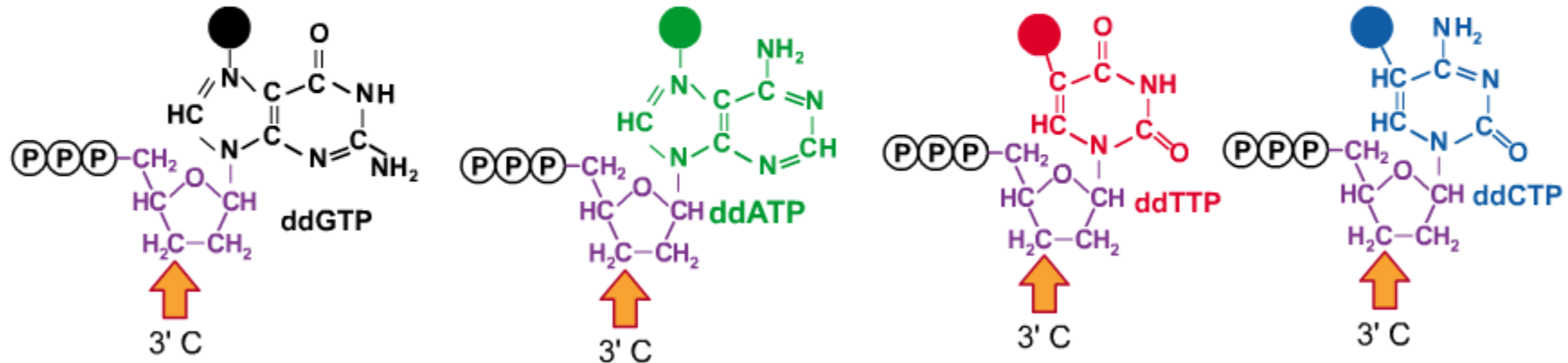
NUCLEOTÍDEOS dNTPs



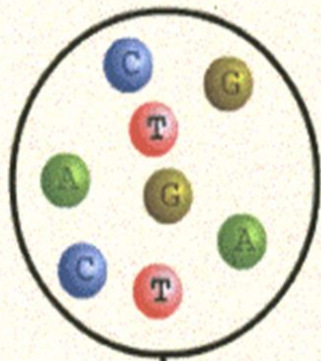
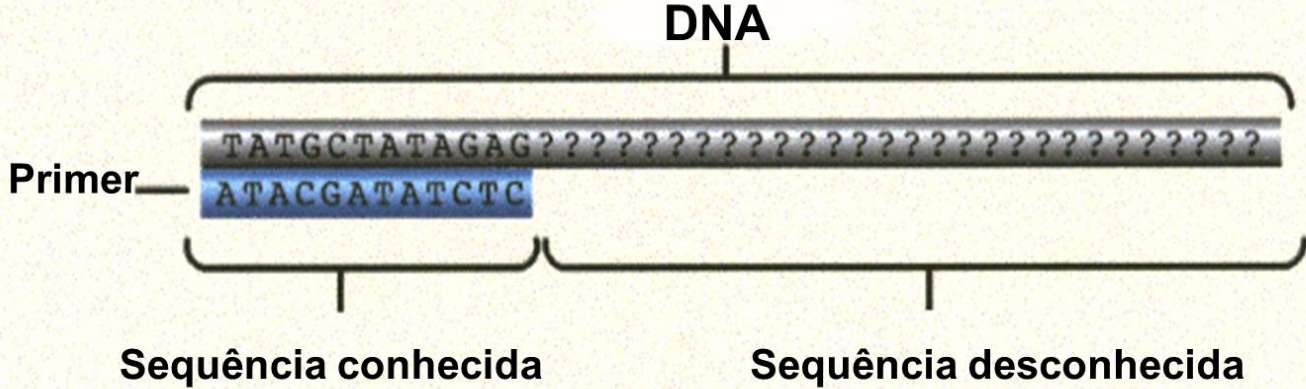
NUCLEOTÍDEOS ddNTPs (TERMINADORES)

CORANTE FLUORESCENTE

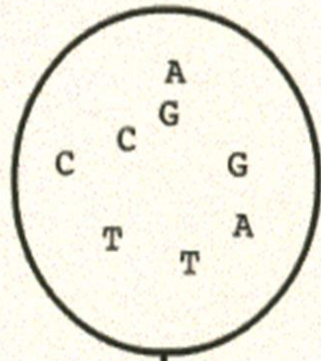
FALTA 3' OH

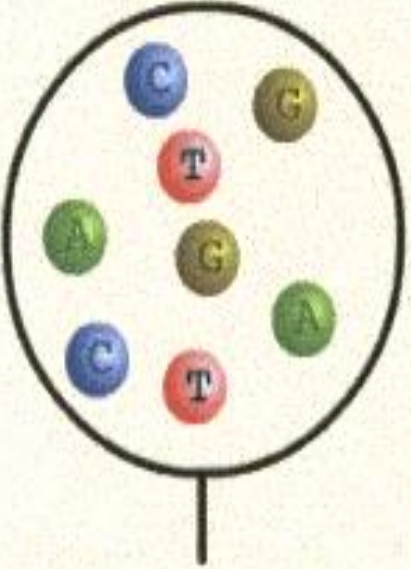


REAÇÃO DE SEQUENCIAMENTO



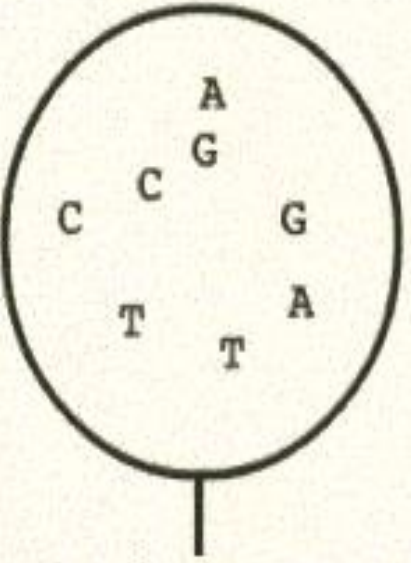
DNA polimerase





Terminadores

DNA polimerase



Nucleotídeos

DESNATURAÇÃO DO DNA

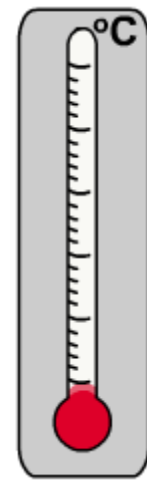
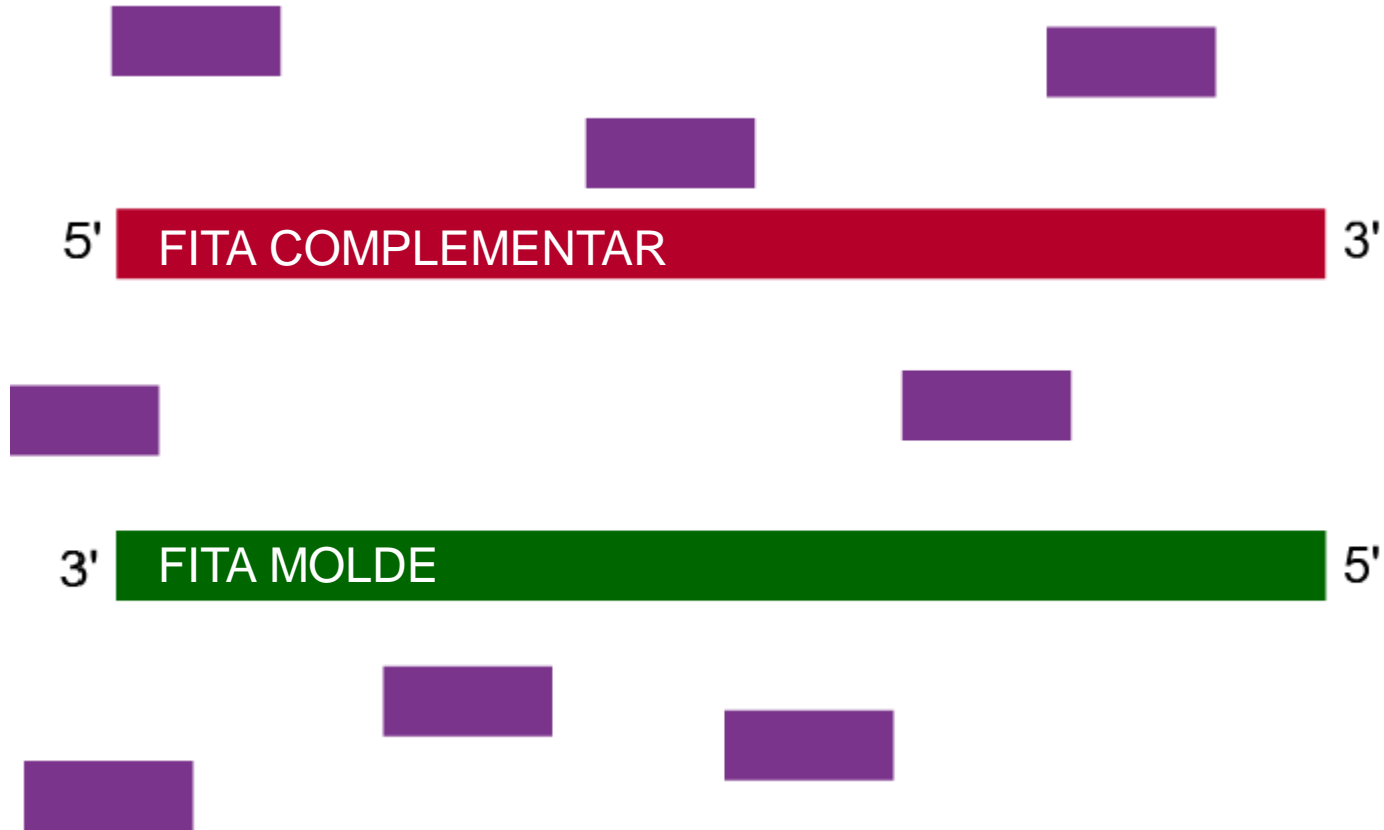


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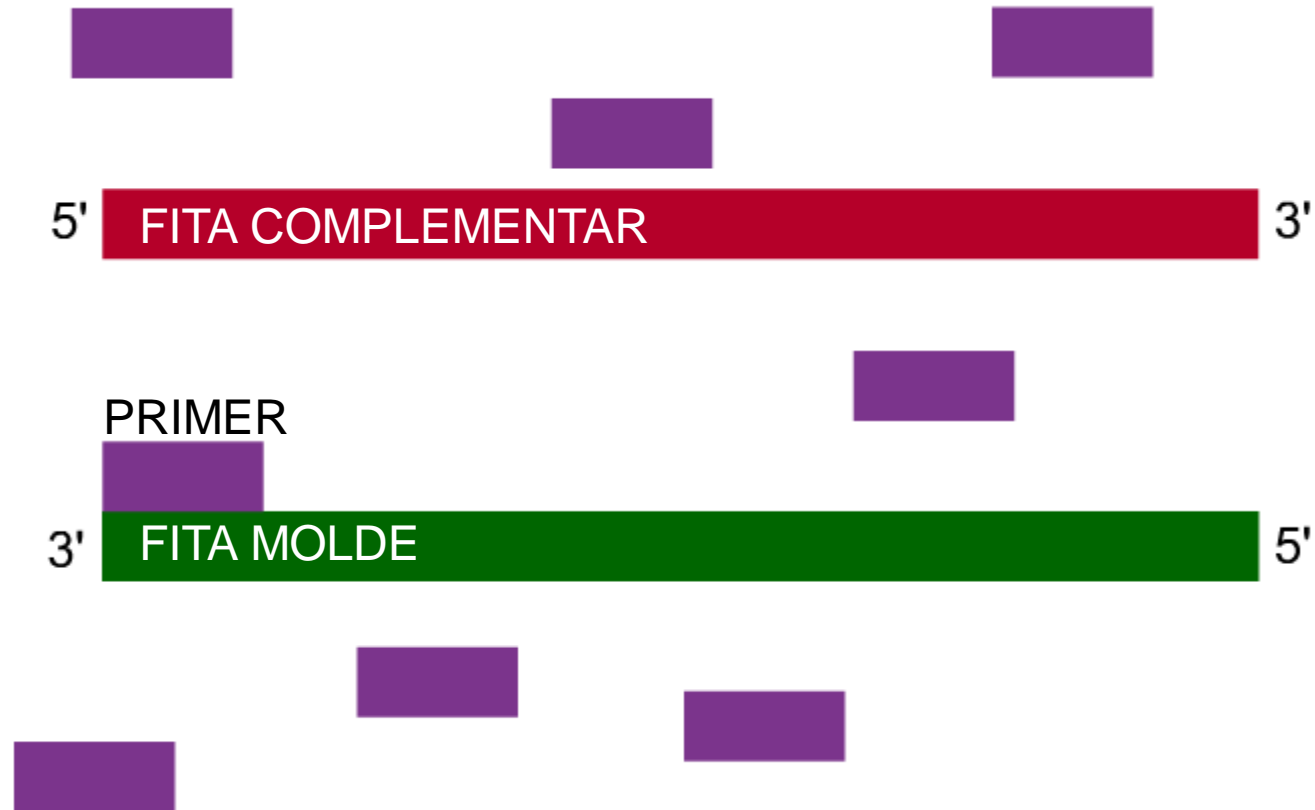
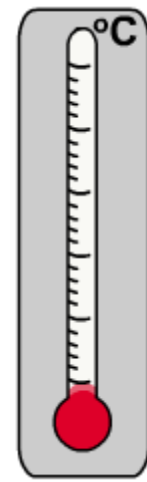


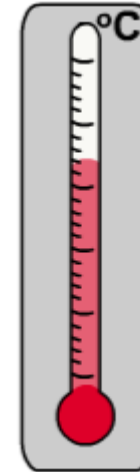
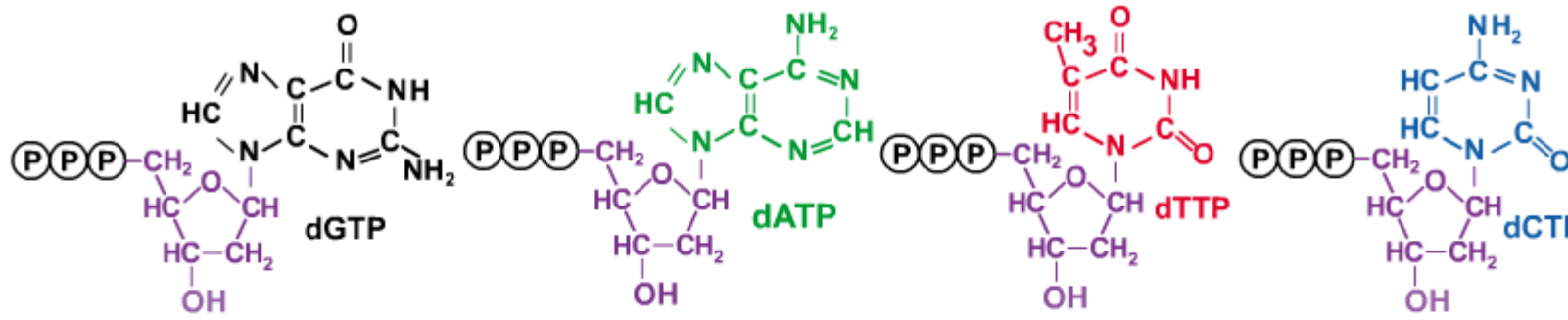
ANELAMENTO DOS PRIMERS

PRIMER



ANELAMENTO DOS PRIMERS

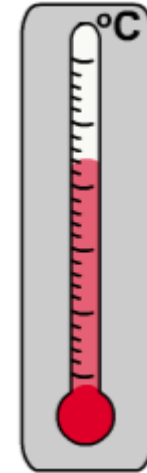




DNA POLIMERASE



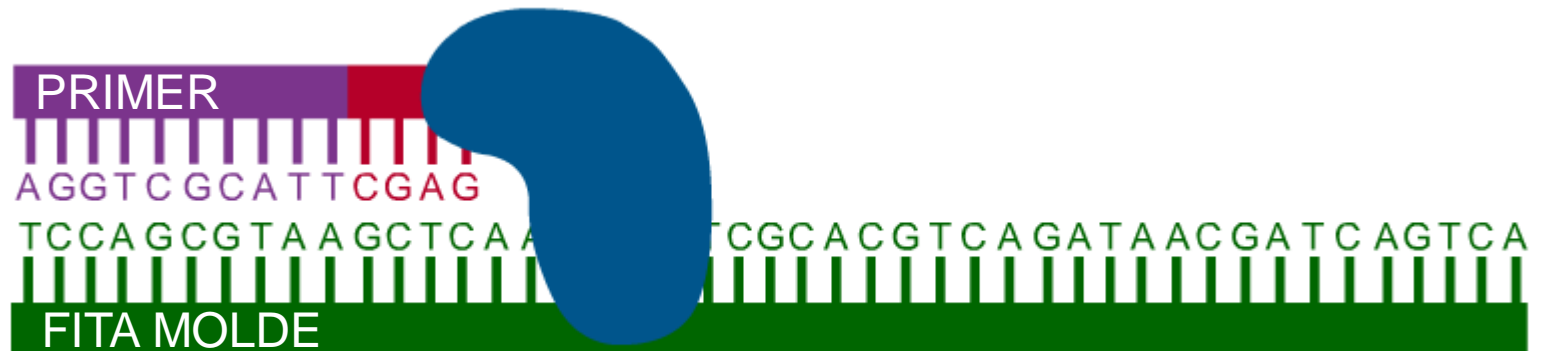
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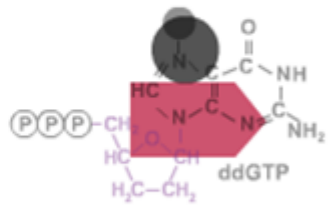
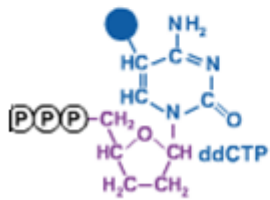
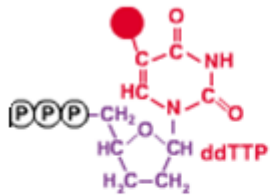
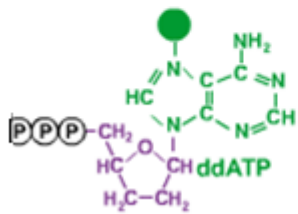
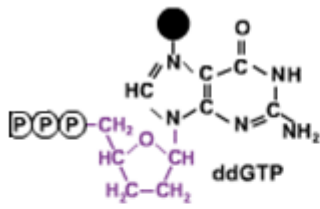


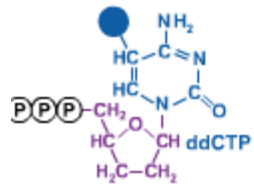
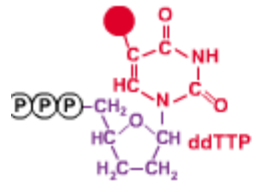
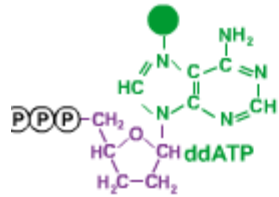
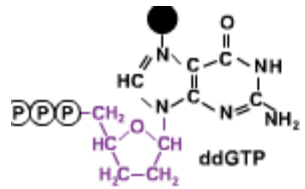
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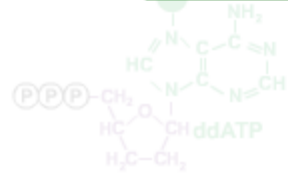
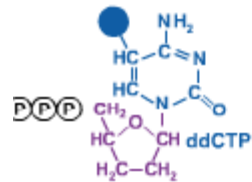
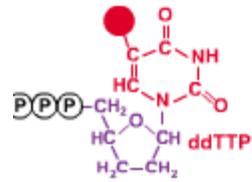
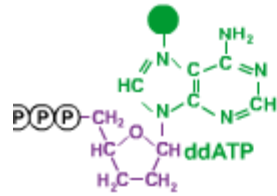
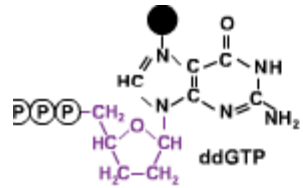


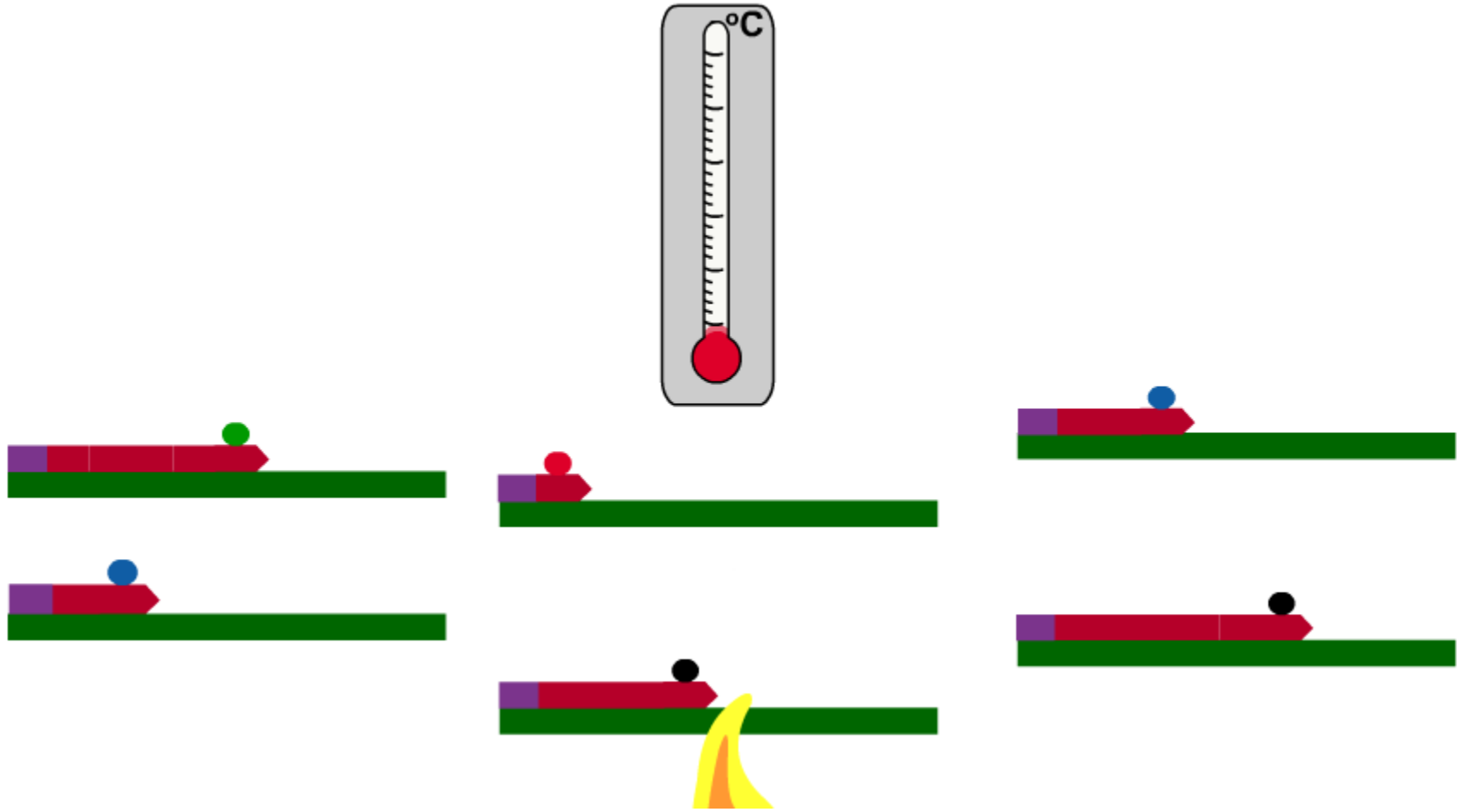
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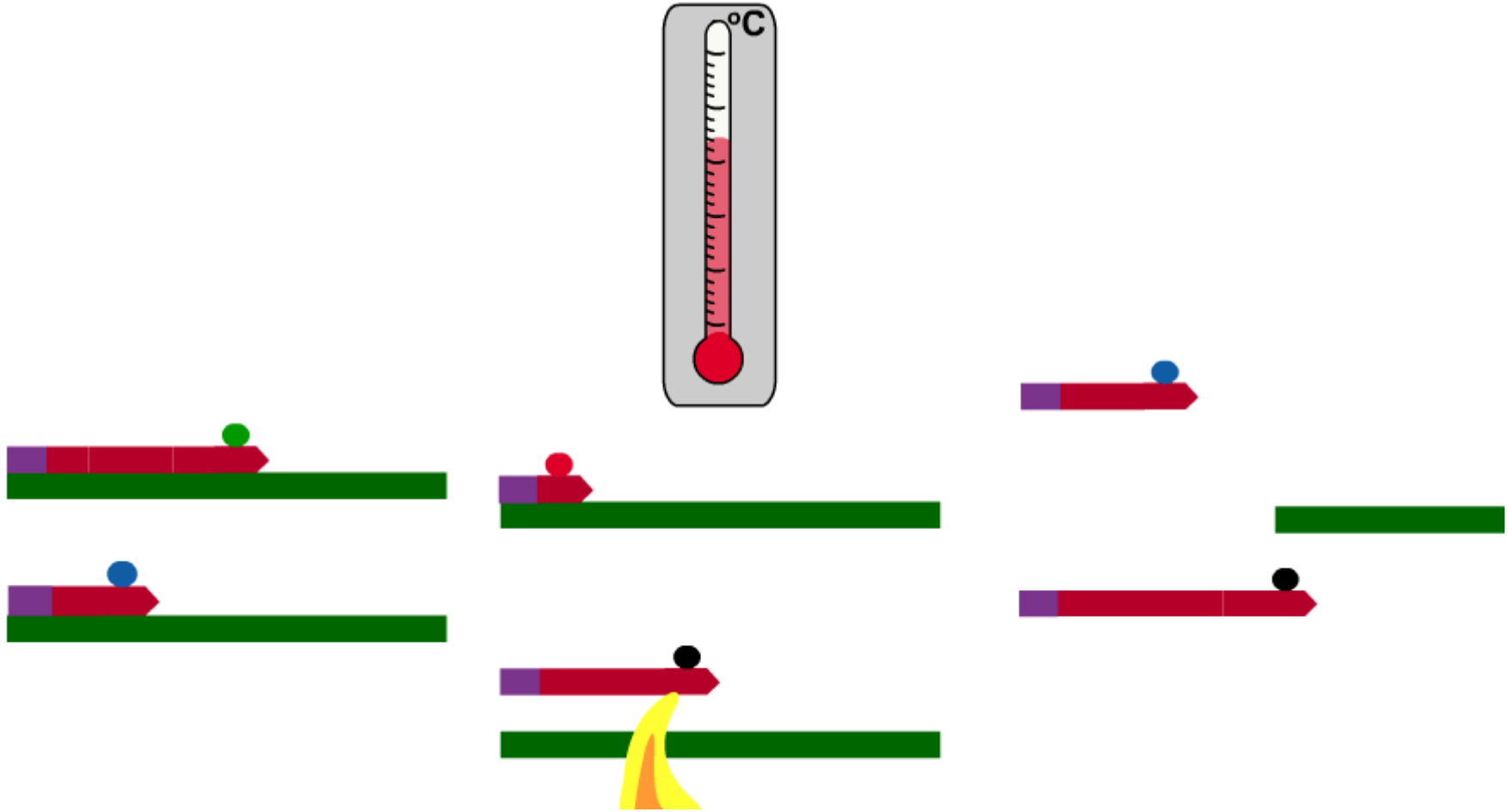


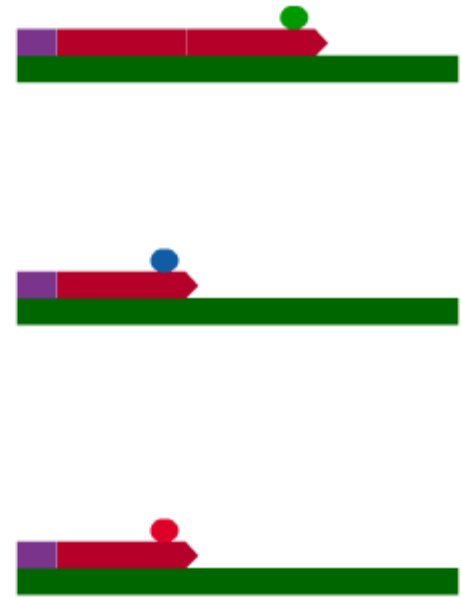
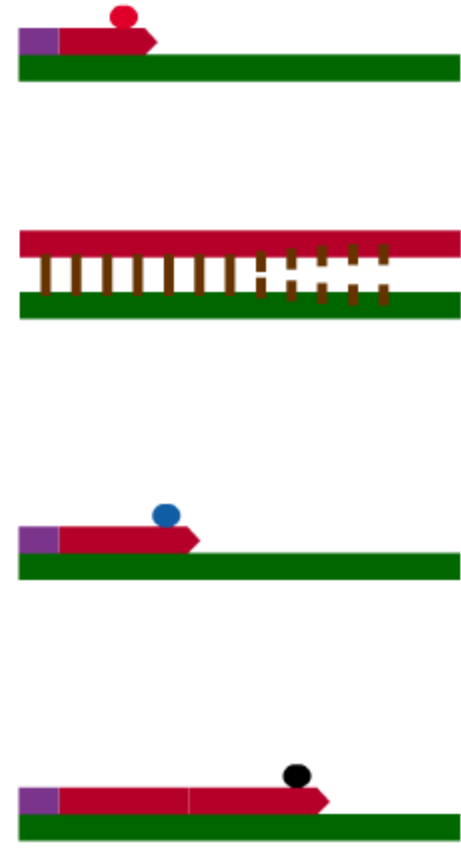
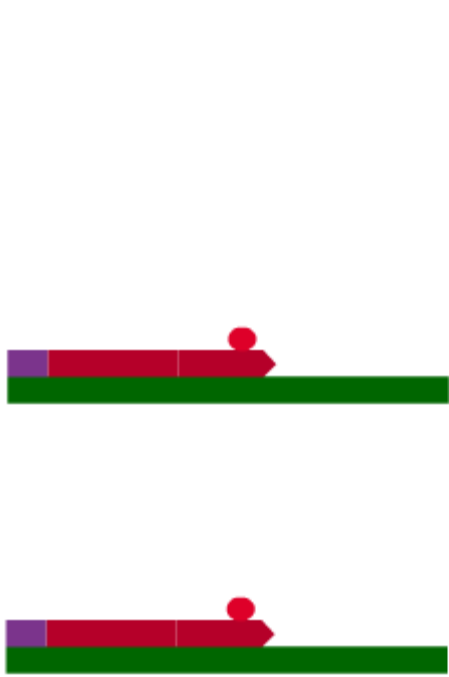




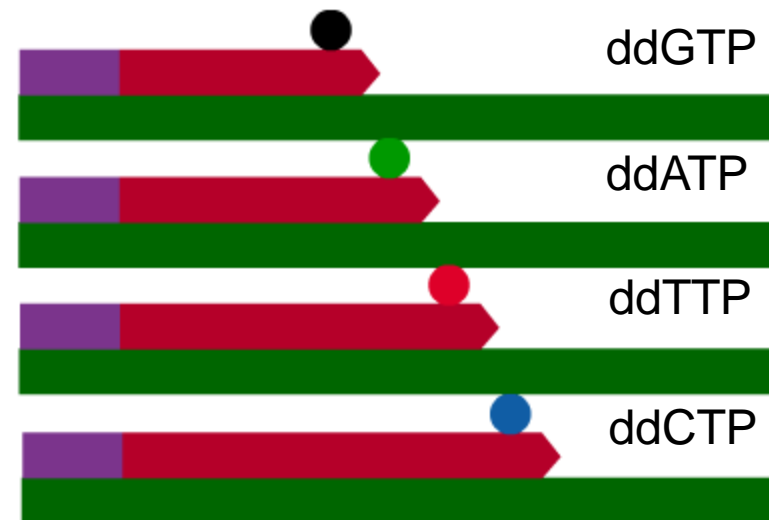








TERMINAÇÃO



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

ATACGATATCTCGACTCTCGAGCTAGAAT

ATACGATATCTCGACTCTCGAGCTAGAATCTTTA

ATACGATATCTCGACTCTCGAGCT

ATACGATATCTCGACTCTCG

ATACGATATCTCGACTCTCGAGCTAGAATCTTT

ATACGATATCTCGACTCTCGAGCTAGAATCT

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ATACGATATCTCGACTCTCGAGCTAG

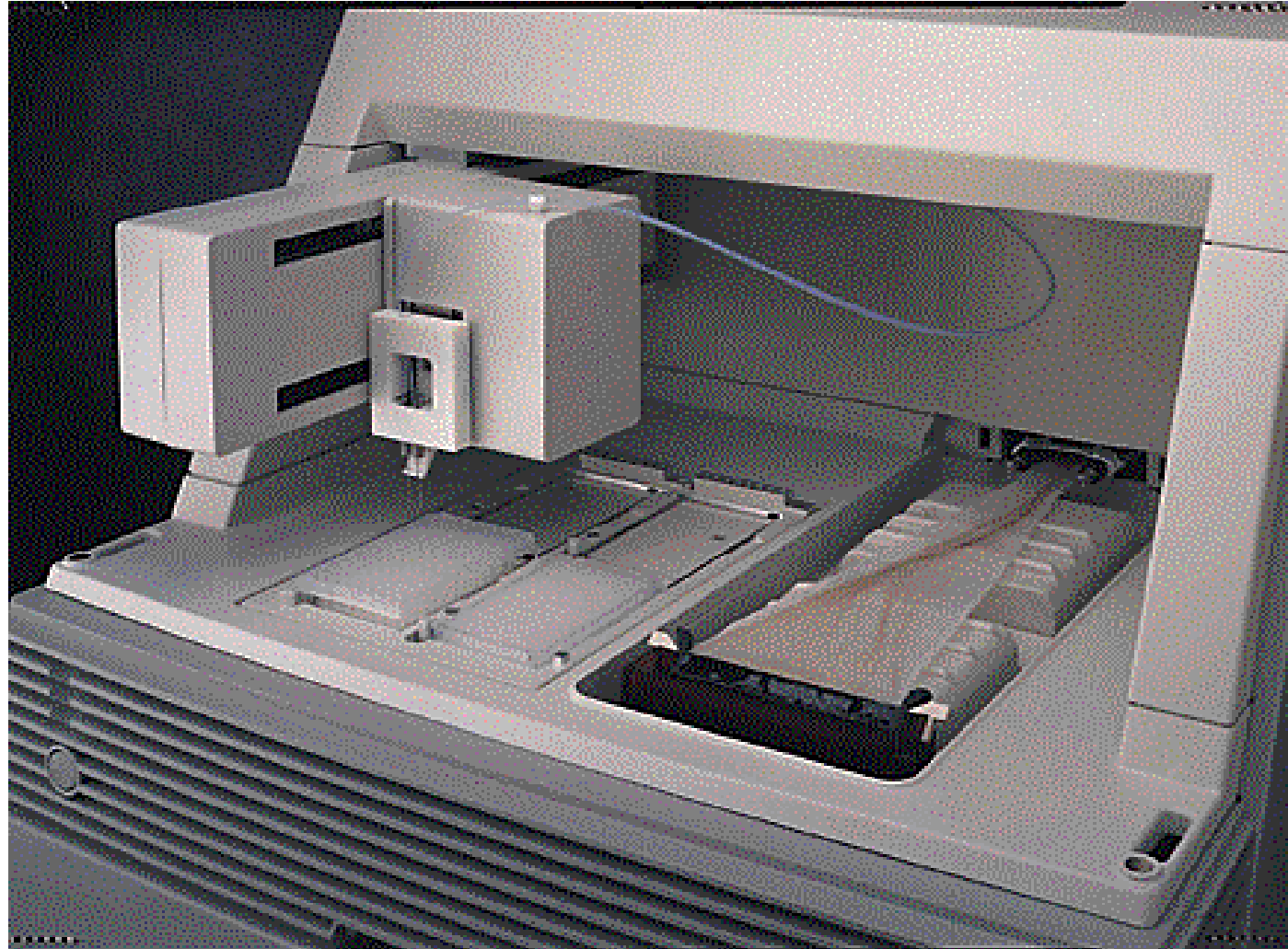
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ATACGATATCTCGACTCTCGA

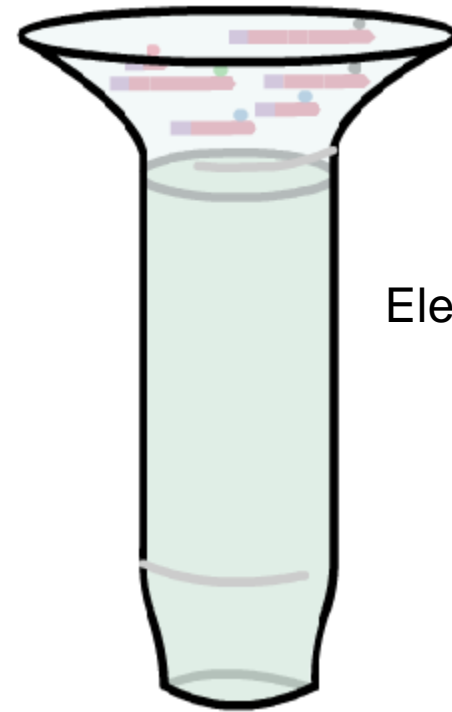
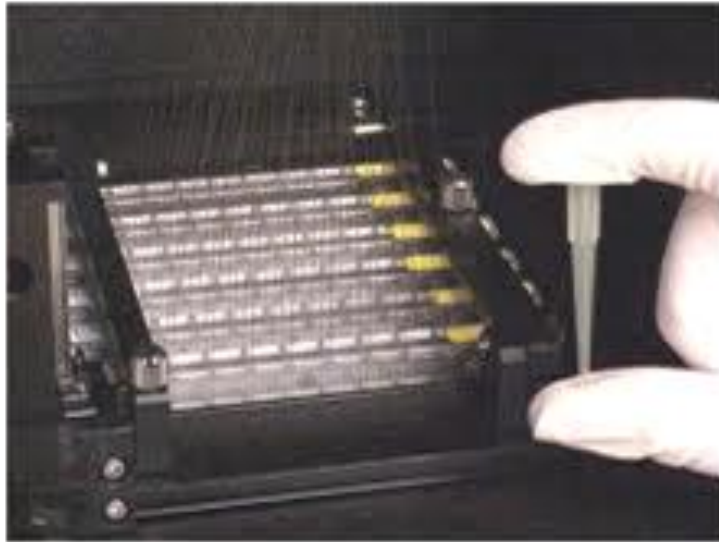
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ATACGATATCTCGACTCTCGAGCTAGAATCTTTAAGGCC

ELETROFORESE CAPILAR

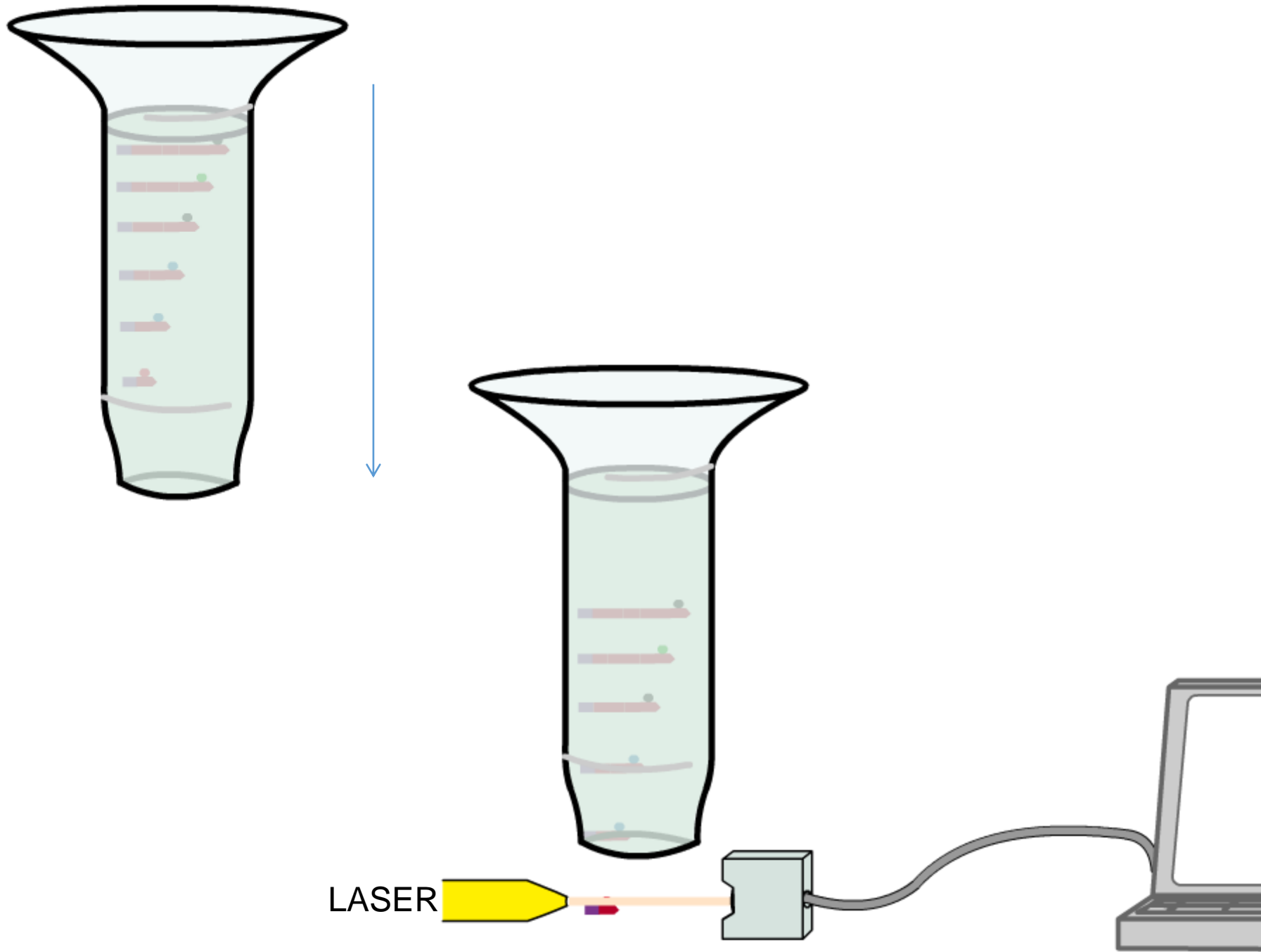


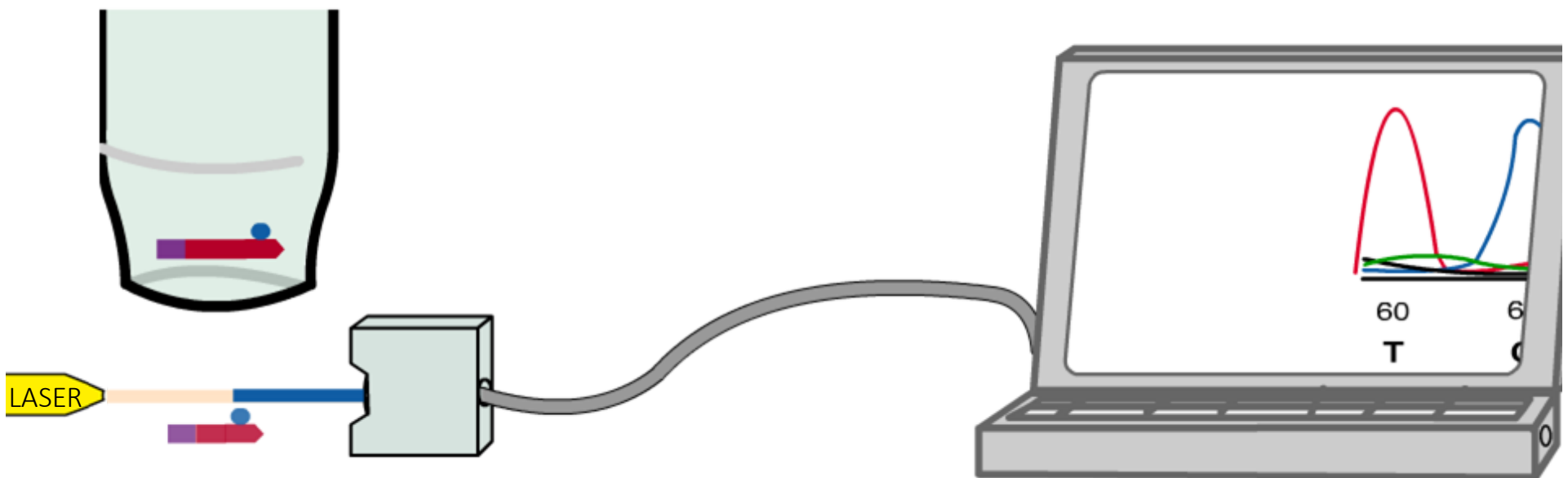
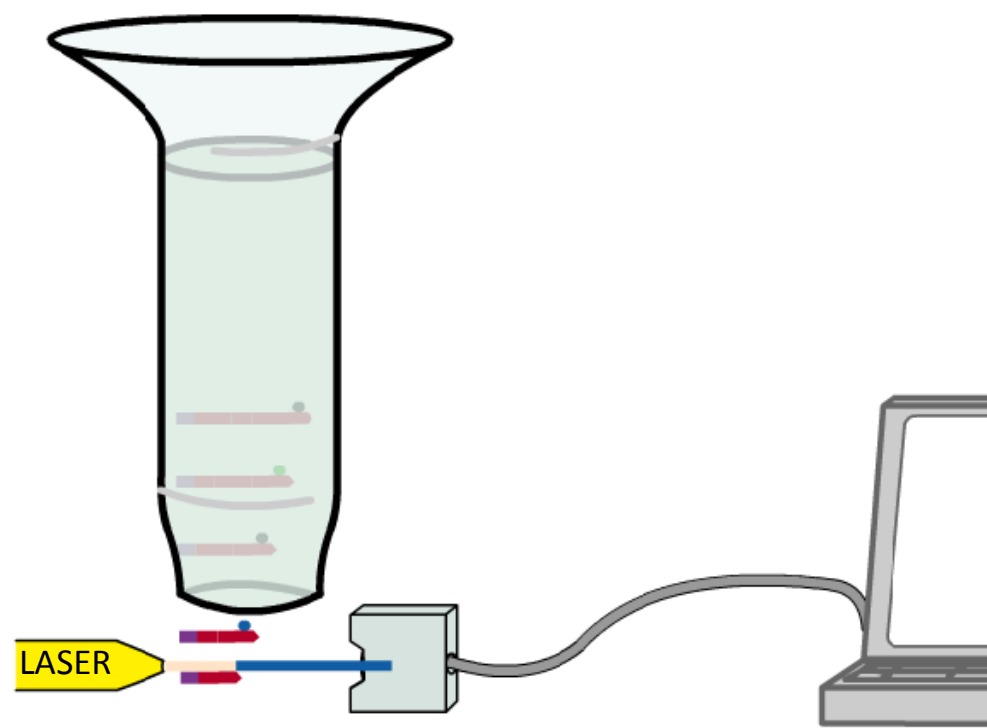
ABI 3700 Applied Biosystems

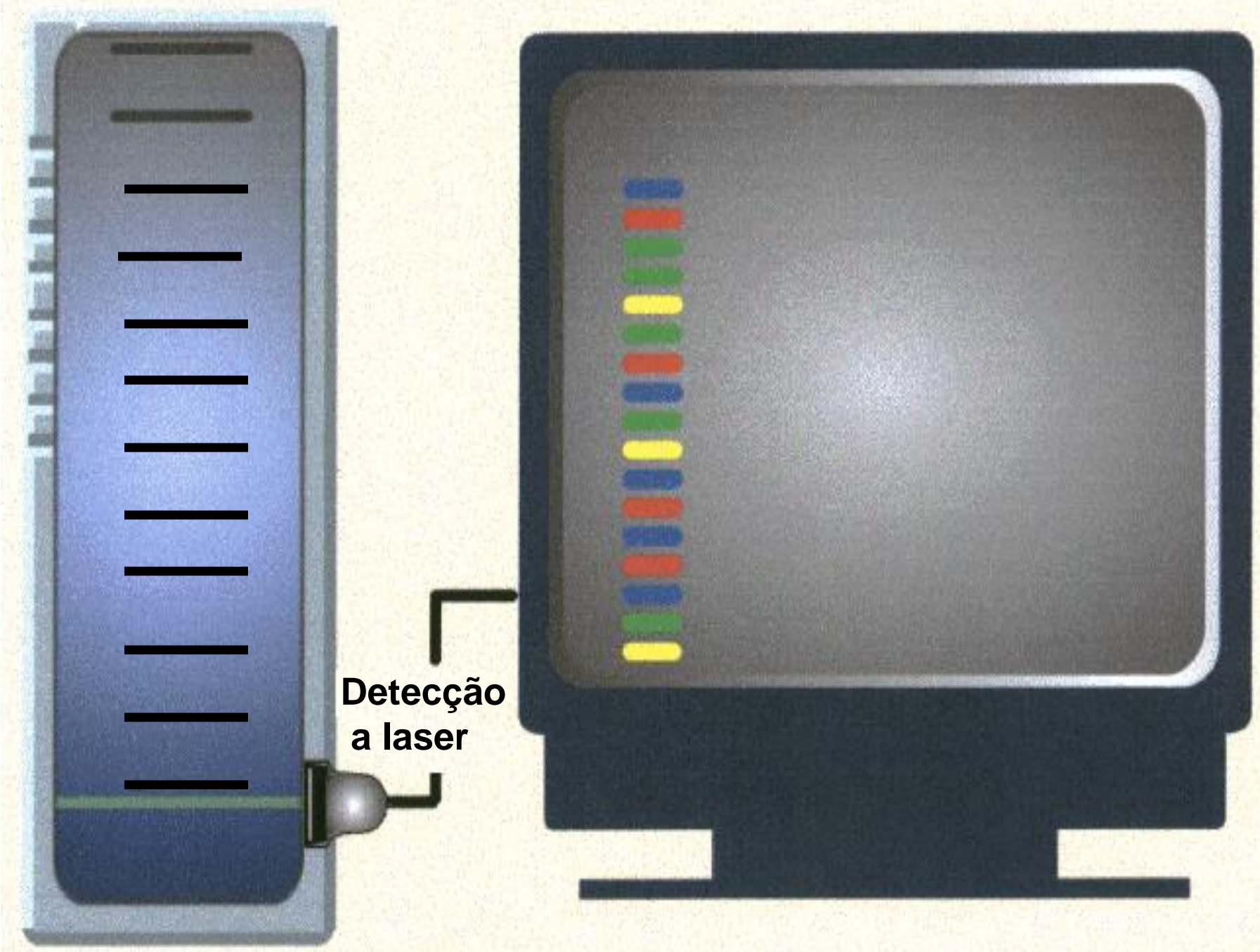


Eletroforese capilar







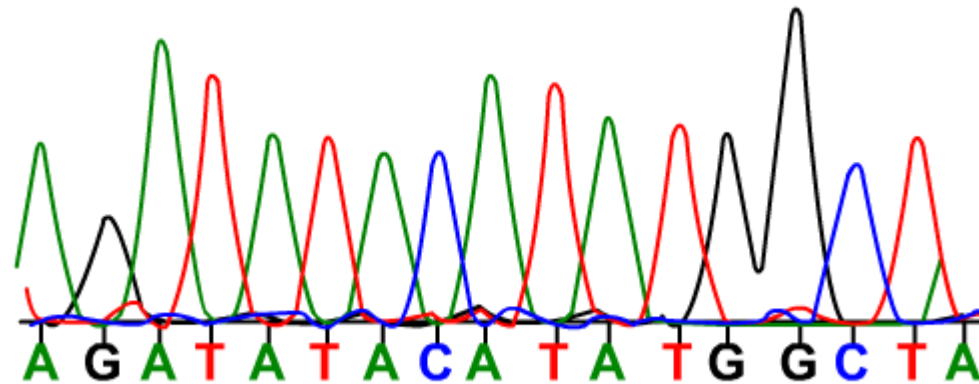
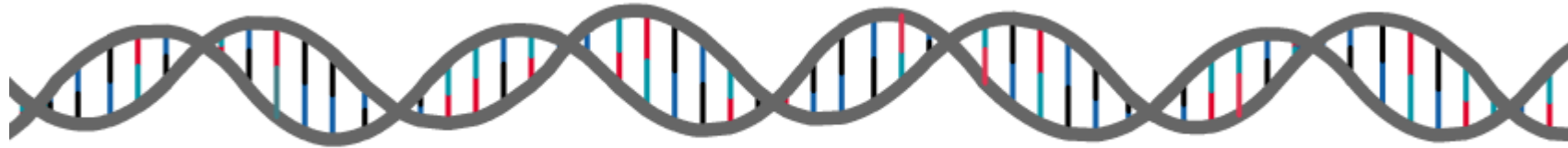


**Detecção
a laser**

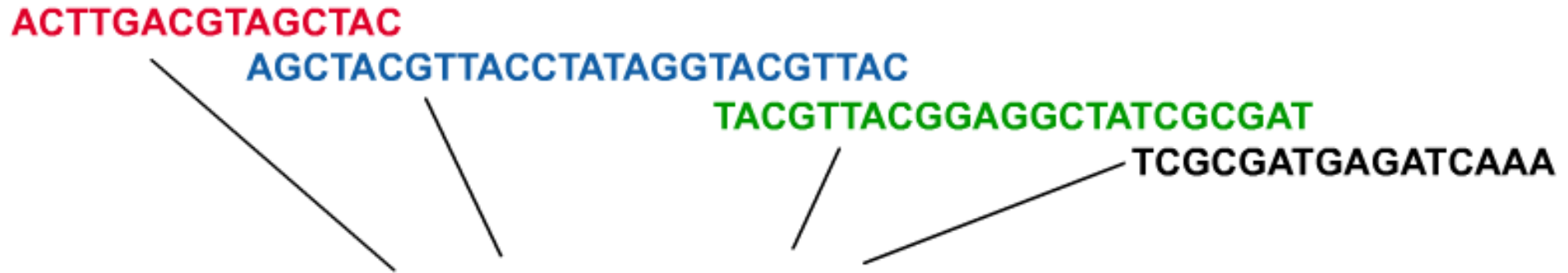


4 | Support Desk Type List & Profit - Query Tool

ANÁLISE COMPUTACIONAL



ACTTGACGTAGCTAC
AGCTACGTTACCTATAGGTACGTTAC
TACGTTACGGAGGCTATCGCGAT
TCGCGATGAGATCAAA



FRAGMENTOS DE DNA SECUENCIADOS

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AGCTACGTTACCTATAGGTACGTTAC
TACGTTACGGAGGCTATCGCGAT
TCGCGATGAGATCAAA



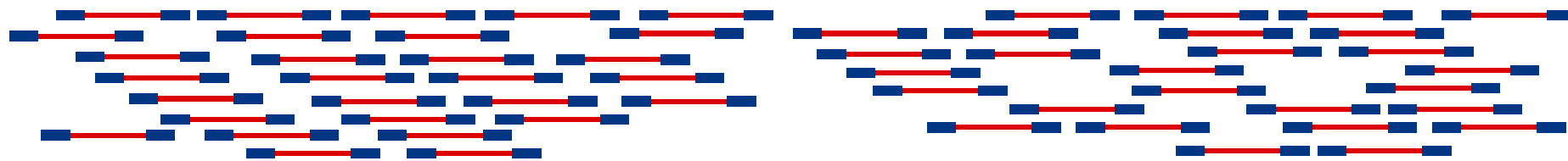
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FRAGMENTOS COMPLETOS

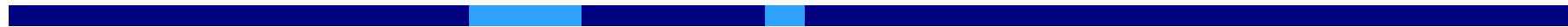
Biblioteca de insertos pequenos



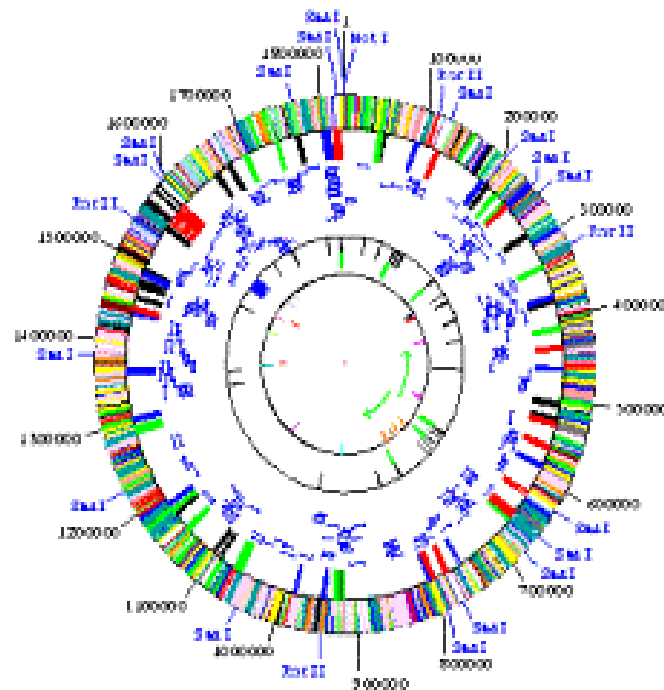
Montagem dos fragmentos



Fechamento dos gaps



Análise



Aligned Reads

File Navigate Info Color Dim Misc Help

01F12.fasta.screen.ace.3 Contig1 Sone Tags Pos: 1 clear

Search for String Compl Cont Compare Cont Find Main Min Err/10kb: 0.00

1520 1530 1540 1550 1560 1570 1580 1590 1600

CONSENSUS TTC*ACTGCGAT*CG*CCGACGACCTCGACTC*CG*CCC6GGCCGCTCCGACGCTACCGCAAGCGG*AAAGCTCGCCG*GCCC*C*G*TC**ACCCT*CA

LOQH-01F12-04H05.b ▶ tt**actgggat*gc*ccgagaaccttgattc*gc*cgga

LOQH-01F12-04B05.g ▶ ttc*actgggat*cg*ccgacgaccttgactc*cg*ggcgggcccggcttcggagcctaccggaggcggaagccttgc*ggccc**g*tt**accct*ca

LOQH-01F12-03H04.b ▶ ttc*cctgggat*cgccctactaccttactt*ct*ggcgggctgctccggctcttcccgc*agcgggaagctogtctgtgccc*c*gt**tccct*ct

LOQH-01F12-01D03.b ▶ ttc*actgcgat*cg*ccgacgacctcgactc*cg*cccgggcccgcctccgacgcctaccggaagcgg*aaagctcgccg*ggccc*c*gt**accct*ca

LOQH-01F12-03G04.b ▶ tct*tttgccct*cc*ctcccccttggcttc*gt*tcgctcttgcctccttggttttctttgtttt*ttgtttgtt*cttt*tc*tc**cttt*tt

LOQH-01F12-05F12.b ▶ tnt*ccTgCGAt*cg*ggcnccgctccgctc*cg*CCCGcGccgncctcgccttccgntgtggc*gtgctcgcg*ggccc*ccg*tc**ccct*ct

LOQH-01F12-01E06.b ▶ TTC*ACTGCGAT*CG*CCGACGACCTCGACTC*CG*CCC6ggcCGcctccgacgcCTAccgCaagcgg*aaAGCTCGcgg*GCCC*C*G*tc**accct*ca

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LOQH-01F12-01D05.g ▶ tTC*ACTGcgat*cg*CCGacgaTtTgactC*CG*CCC6GGCCGCTccgacgcctACCGCAAGCGG*AAAGCTCGCCG*GCCC*C*G*TC**ACCCT*CA

LOQH-01F12-06G11.g ▶ c*tggtatacgn*aaatt*c*c*gn**ctcgg*ta

LOQH-01F12-08B07.g ▶ TTC*ACTGCGAT*CG*CCGACGAcctcgactc*CG*CCCggaaagcctccGACGCTACCGCAagcgt*tagtttcgcc*gggg*gt*nc**aggga*gt

LOQH-01F12-01A04.b ▶ TTC*ACTGCGAT*CG*CCGACGACCTCGACTC*CG*CCC6GGCCGcctCCGACGCTACCGCAAGCGG*AAAGCTCGCCG*GCCC*C*G*TC**ACCCT*CA

LOQH-01F12-02D02.b ▶ TTC*ACTGCGAT*CG*CCGACGACCTCGACTC*CG*CCC6GGCCGcctCCGACGCTACCGCAAGCGG*AAAGCTCGCCG*GCCC*C*G*TC**ACCCT*CA

LOQH-01F12-03H05.b ▶ TTC*ACTGCGAT*CG*CCGACGACCTCGACTC*CG*CCC6GGCCGcctcCGACGCTACCGCAAGCGG*AAAGCTCGCCG*GCCC*C*G*TC**ACCCT*CA

LOQH-01F12-05G10.b ▶ TTC*ACTGCGAT*CG*CCGACGACCTCGACTC*CG*CCC6GGCCGCTCCGACGCTACCGCAAGCGG*AAAGCTCGCCG*GCCC*C*G*TC**ACCCT*CA

LOQH-01F12-06A07.b ▶ TTC*ACTGCGAT*CG*CCGACGACCTCGACTC*CG*CCC6GGCCGCTCCGACGCTACCGCAAGCGG*AAAGCTCGCCG*GCCC*C*G*TC**ACCCT*CA

LOQH-01F12-08E12.g ▶ TTC*ACTGCGAT*CG*CCGACGACCTCGACTC*CG*CCC6GGCCGCTCCGACGCTACCGCAAGCGG*AAAGCTCGCCG*GCCC*C*G*TC**ACCCT*CA

LOQH-01F12-04B05.b ▶ TTC*ACTGCGAT*CG*cCGACGacctCGACTC*CG*CCC6GGCCGCTCCGACGcctACCGCAAGCGG*AAAGCTCGCCG*GCCC*C*G*TC**ACCCT*CA

LOQH-01F12-04H05.g ▶ TTC*ACTGCGAT*CG*CCGACGacctcGACTC*CG*CCC6GGCCGCTCCGACGCTACCGCAAGCGG*AAAGCTCGCCG*GCCC*C*G*TC**ACCCT*CA

LOQH-01F12-08C07.g ▶ aca*acacgcaa*aa*agcgaagacaaaaagc*ac*gaaggggccgcccgggaagcngccgcaagagg*aaagcaagccg*ggccc*c*gt*nc**accct*ca

LOQH-01F12-07H04.g ▶ ttccaat*cggtgcg*ccgaggacctgggctc*cg*cccgggcccgcctccgacgcctaccgcaagcgg*aaagCTcgccg*ggccc*c*ggtc**accct*ca

LOQH-01F12-05A01.b ▶ cgg*gcgaggga*ac*acaangcccgcgcgc*cg*gcgcccggccggggggccaccocgcgaggg*aaagctggccg*ggccc*gg*ag**accct*ca

LOQH-01F12-01A03.b ▶ ccc*ccgcccc*cg*aaaaggggtaaaaag*aa*aaaaagaaaaataaatccggggggggcgcctt*actctcccc*aaag*ga*aa*aat*gg*gg

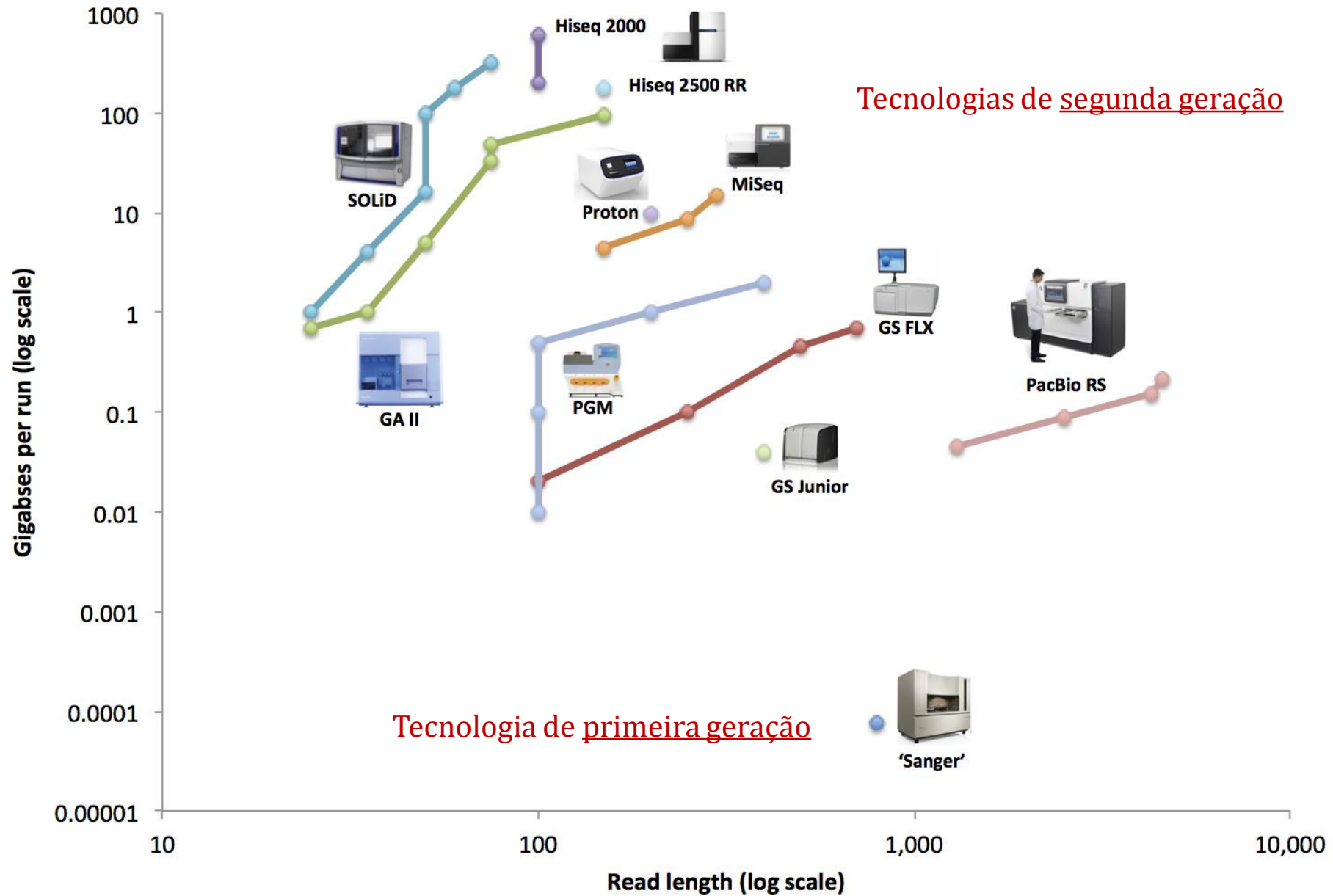
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LOQH-01F12-08F11.g ▶ tttttagttatt*tt*gtgtaatatgcggggagcggcggagcacacg*agnagcgaaca*ggacg*ga*cg**ggagg*aa

LOQH-01F12-05D12.g ▶ tggcgcctcggaggctacgcaag*gg*aaagctggccg*ggccc*gt*tc**accct*ca

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Developments in High Throughput Sequencing



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

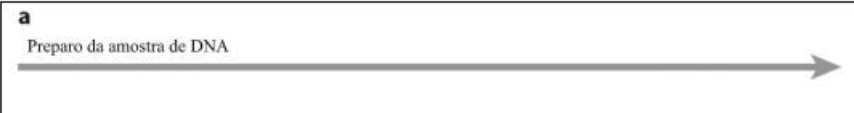
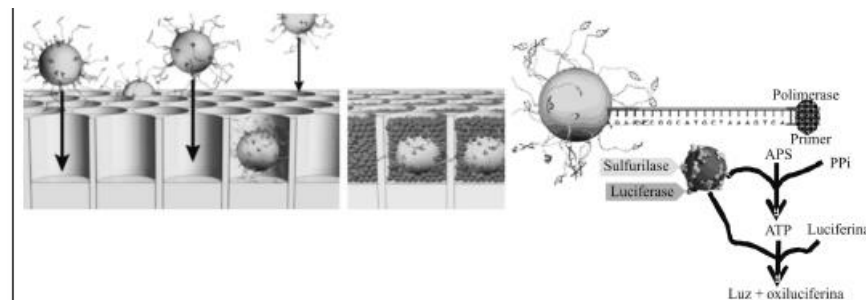
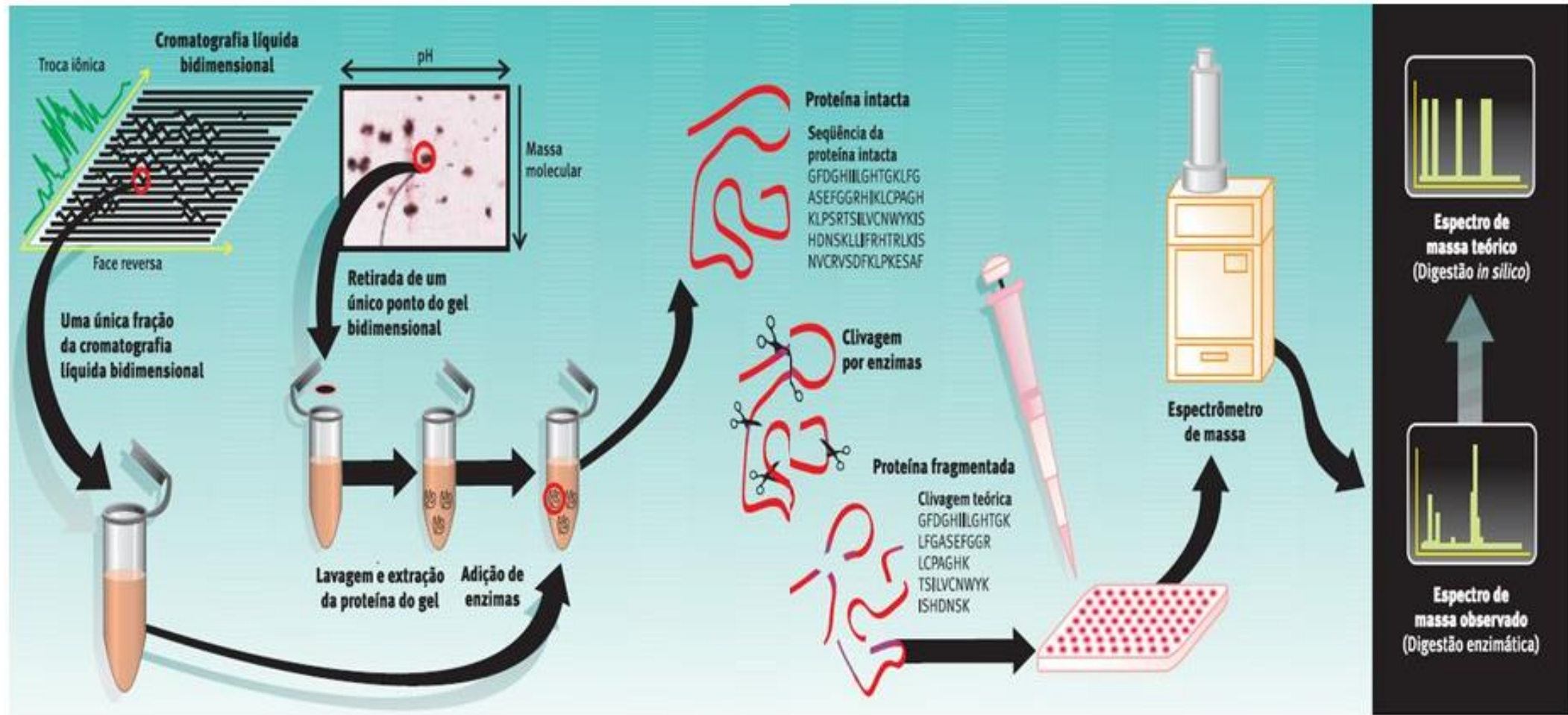


Tabela 1 - Resumo das principais características técnicas das plataformas 454 GS-FLX, Solexa e SOLiD e laboratórios no Brasil que já adquiriram essas novas plataformas. A duração da corrida inclui o tempo para o preparo, a leitura e o processamento das amostras; o custo da corrida e o valor do equipamento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



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=3l9wzWj0b_A

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



ESTUDO DIRIGIDO

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

(LER o texto “Sequenciamento de nova geração”)

