IDENTIFICAÇÃO DE SEQUÊNCIAS POR HIBRIDIZAÇÃO E SEQUENCIAMENTO



Aula 5

LGN232 – Genética Molecular

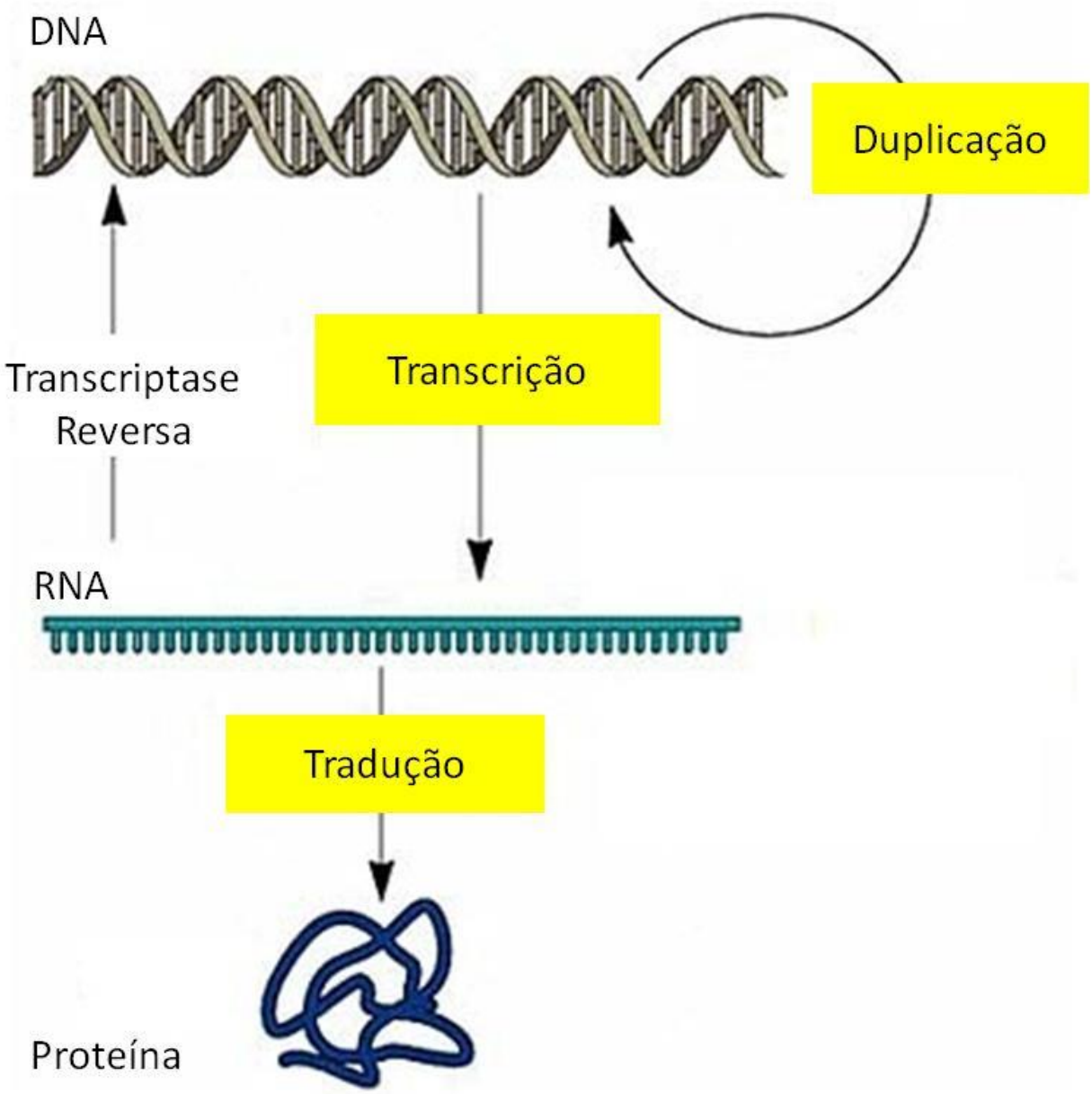
Maria Carolina Quecine
Departamento de Genética
mquecine@usp.br

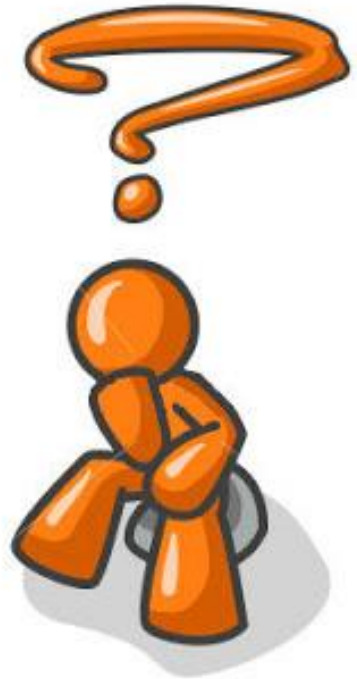
LEMBRANDO O DOGMA CENTRAL DA BIOLOGIA MOLECULAR

Genoma

Transcriptoma

Proteoma





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

- Hibridização com sondas conhecidas;
- Sequenciamento.

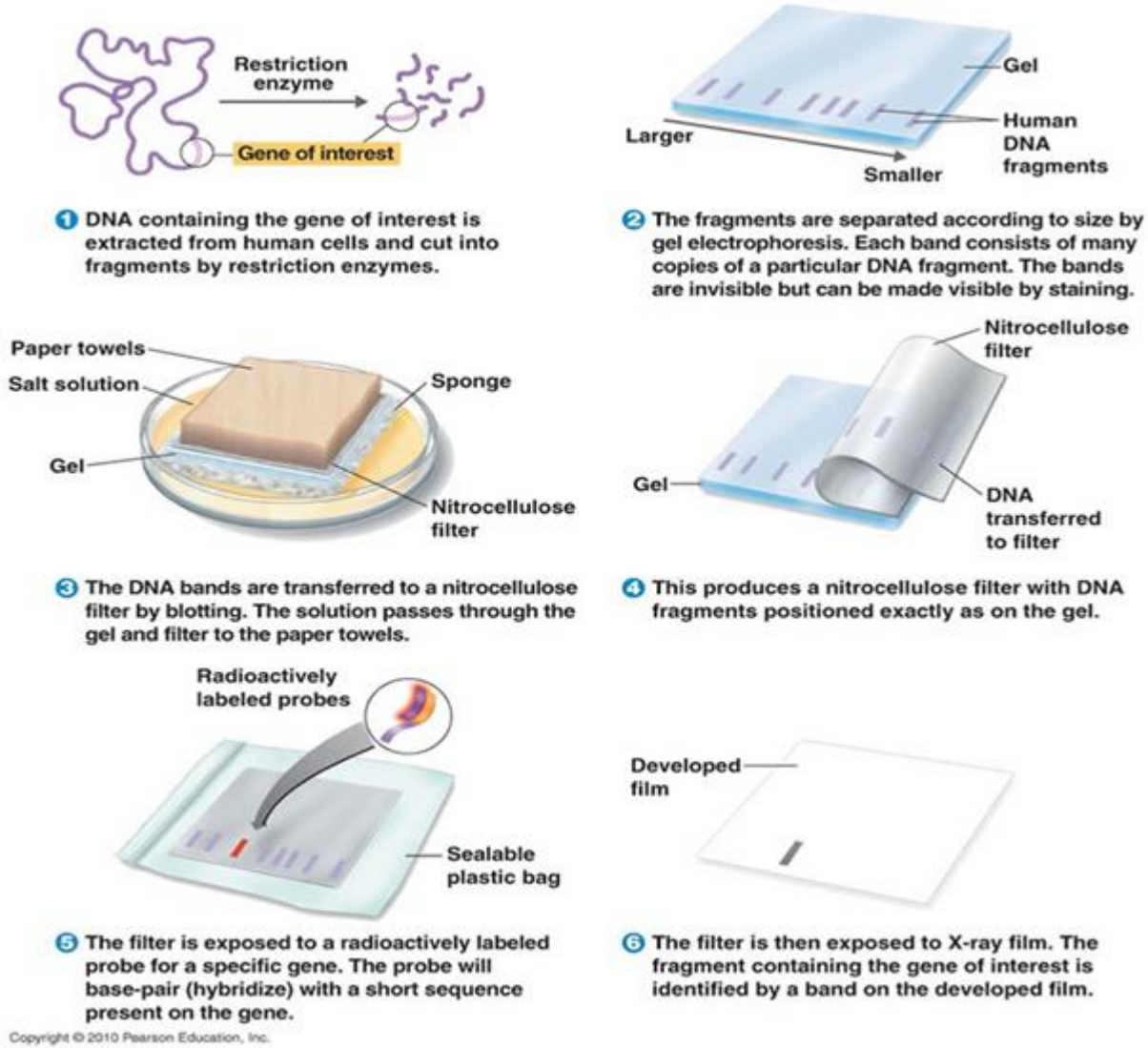
Técnicas de “Blotting”

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 contem 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).

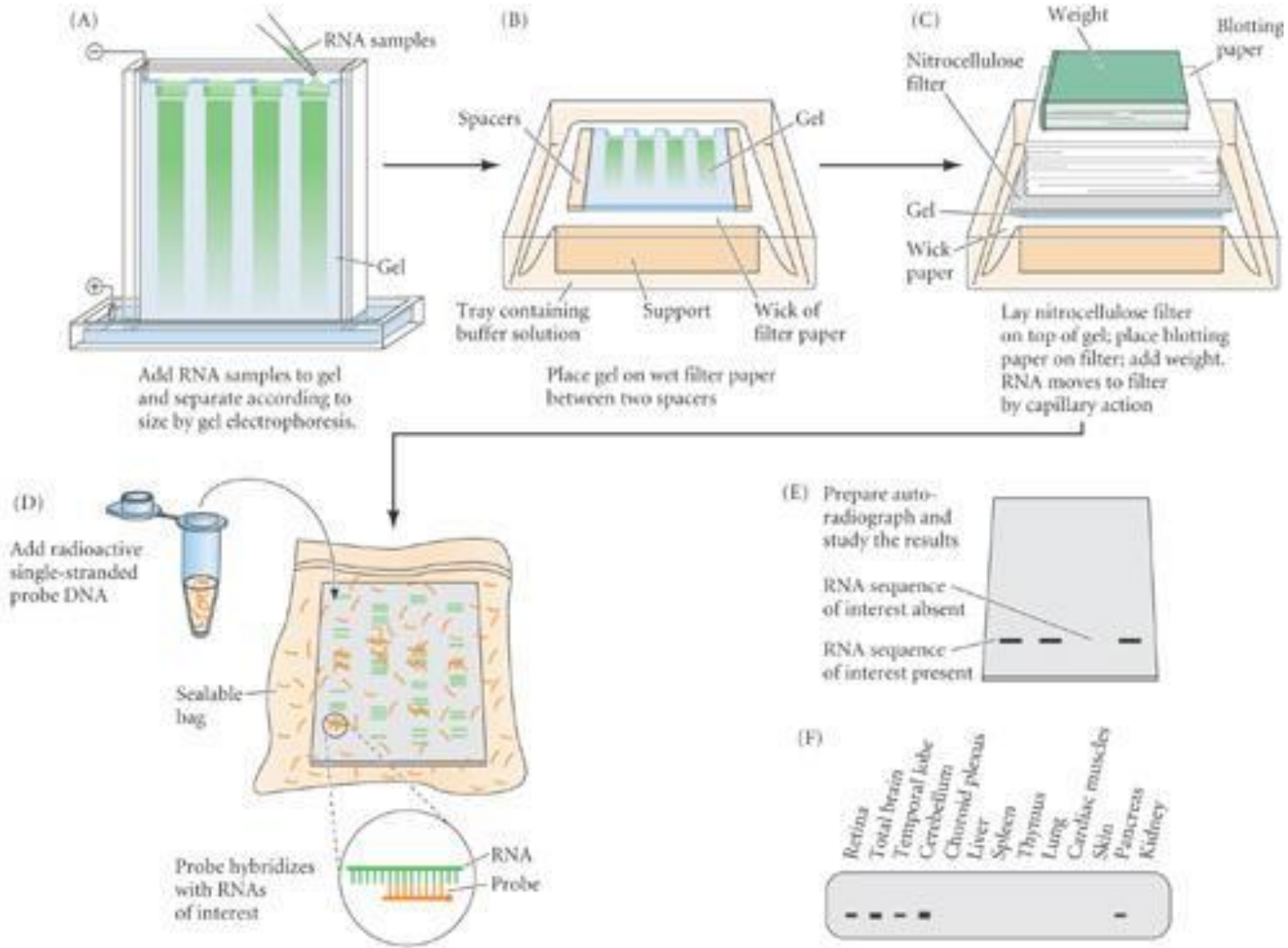
Western blot é um método em biologia molecular e bioquímica para detectar proteínas em um homogenato (células bem trituradas) ou um extrato de um tecido biológico. Essa **técnica** usa eletroforese em gel para separar as proteínas desnaturadas por massa.

O Southern Blot



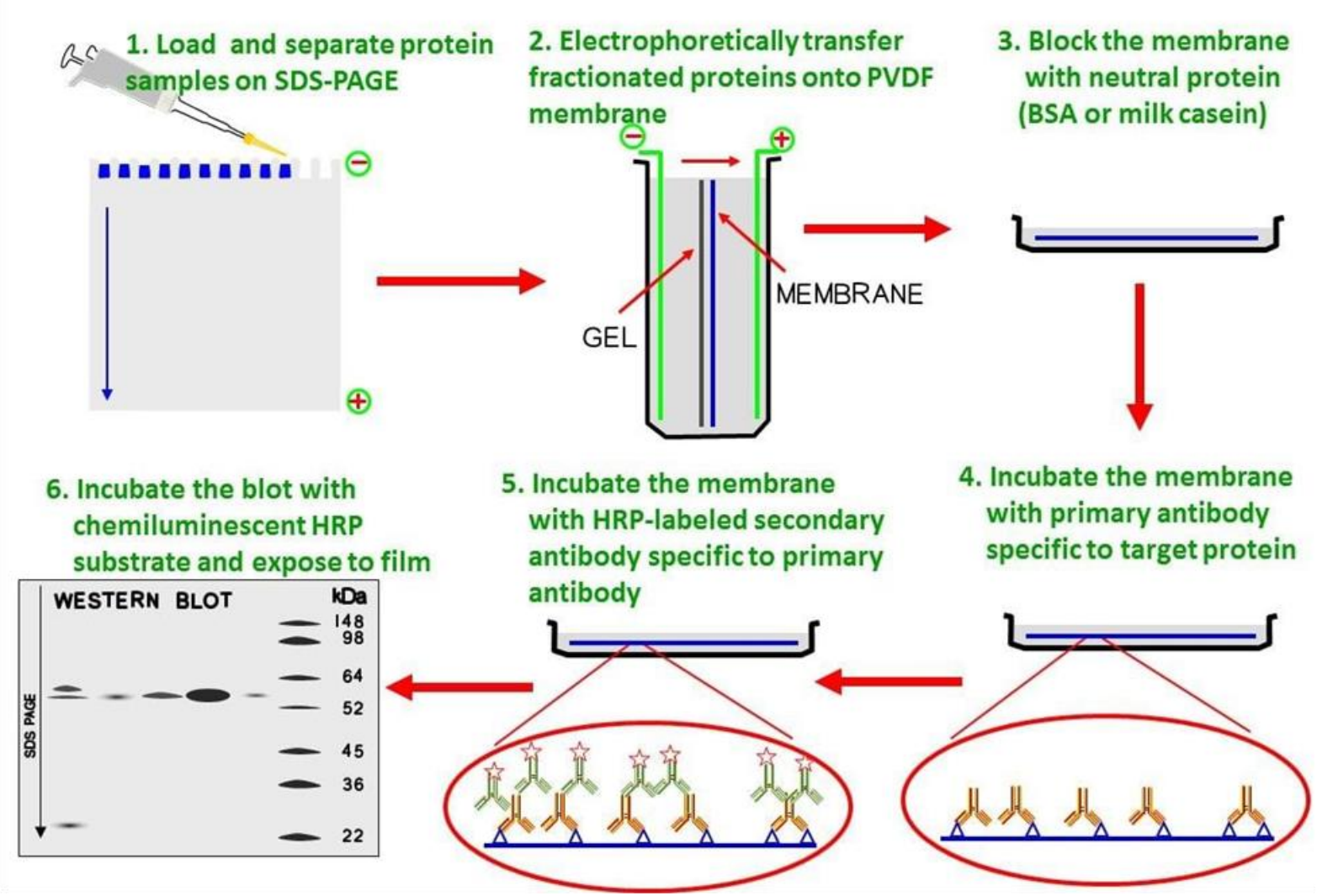
http://www.biologyexams4u.com/2013/12/southern-blotting-procedure-steps.html#.WaQd3PkrLIU

O Northern Blot



http://enfo.agt.bme.hu/drupal/en/gallery/8930

O Western Blot



http://microbeonline.com/western-blot-technique-principle-procedures-advantages-and-disadvantages/

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)

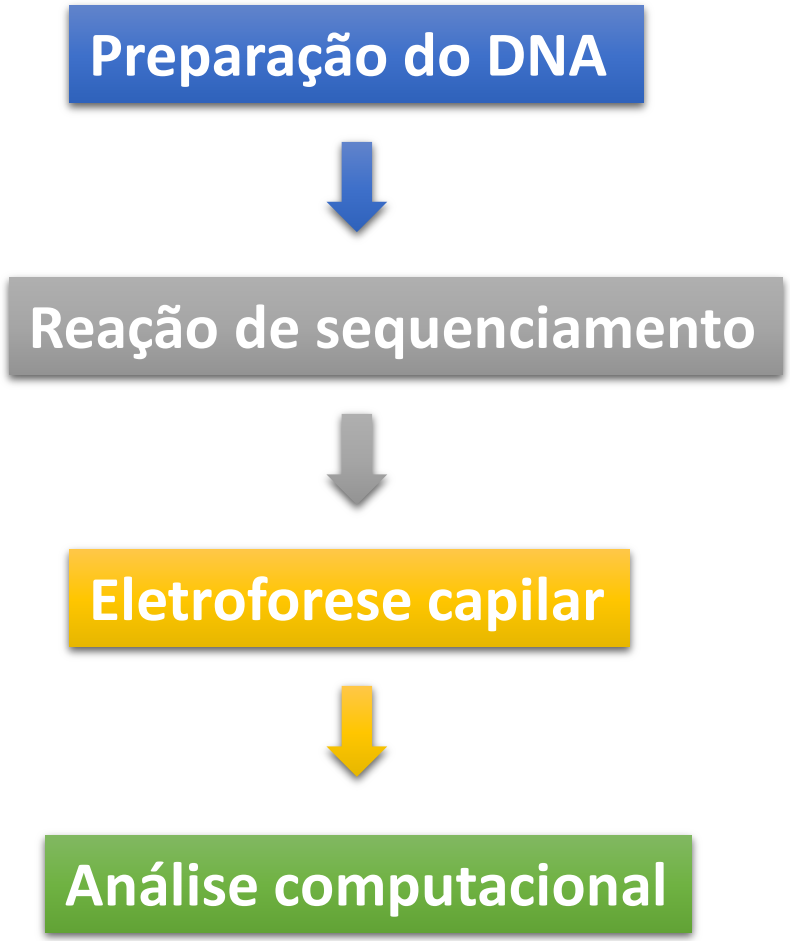


Frederick Sanger

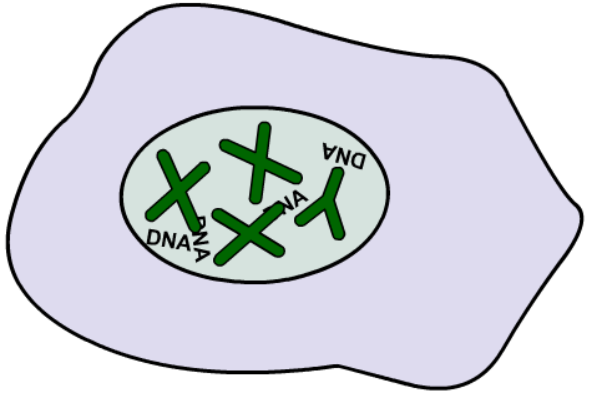
Prêmio Nobel em Química (1980)

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

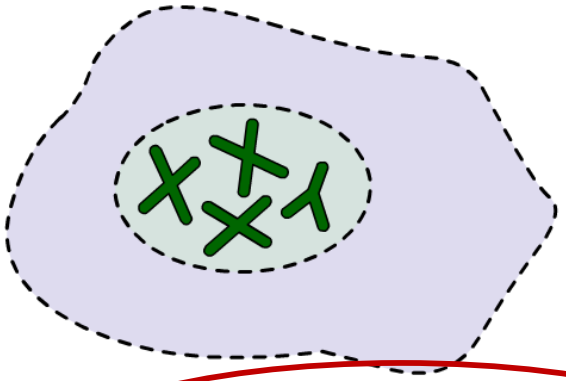
ETAPAS DO SEQUENCIAMENTO DE DNA



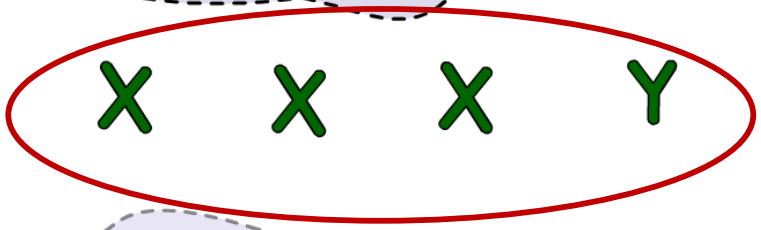
PREPARAÇÃO DO DNA



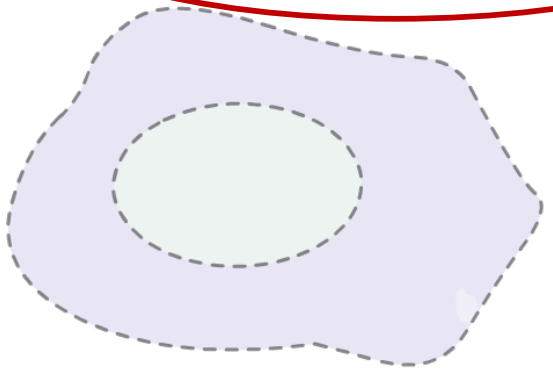
CÉLULA



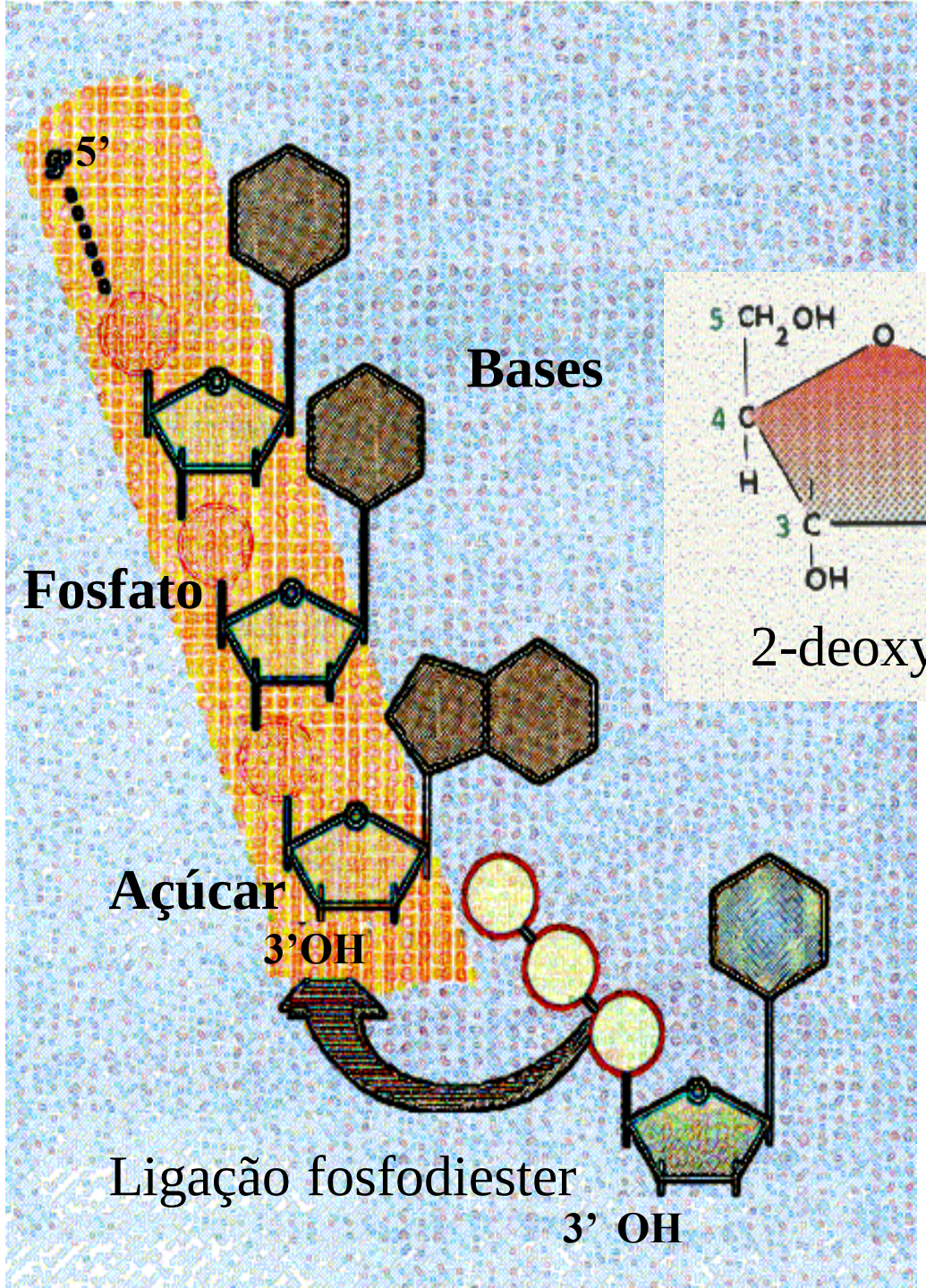
MEMBRANA
PLASMÁTICA E
PAREDE CELULAR

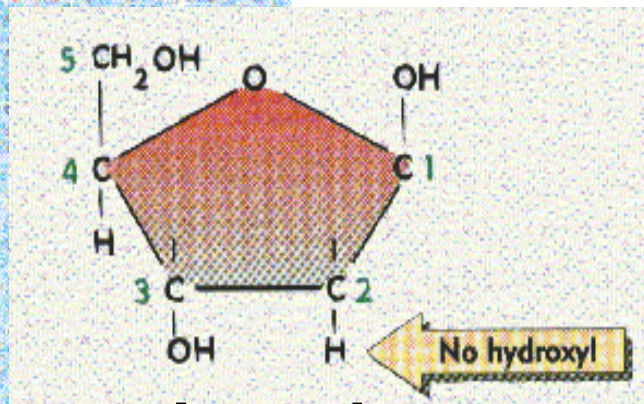


PURIFICAÇÃO DO
DNA TOTAL

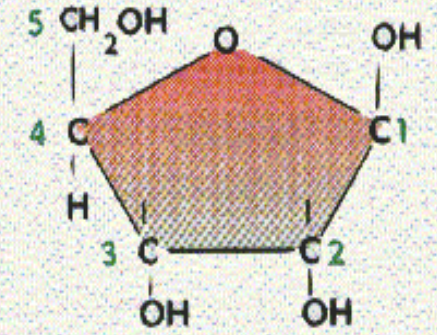


SEPARAÇÃO DOS
RESTOS CELULARES





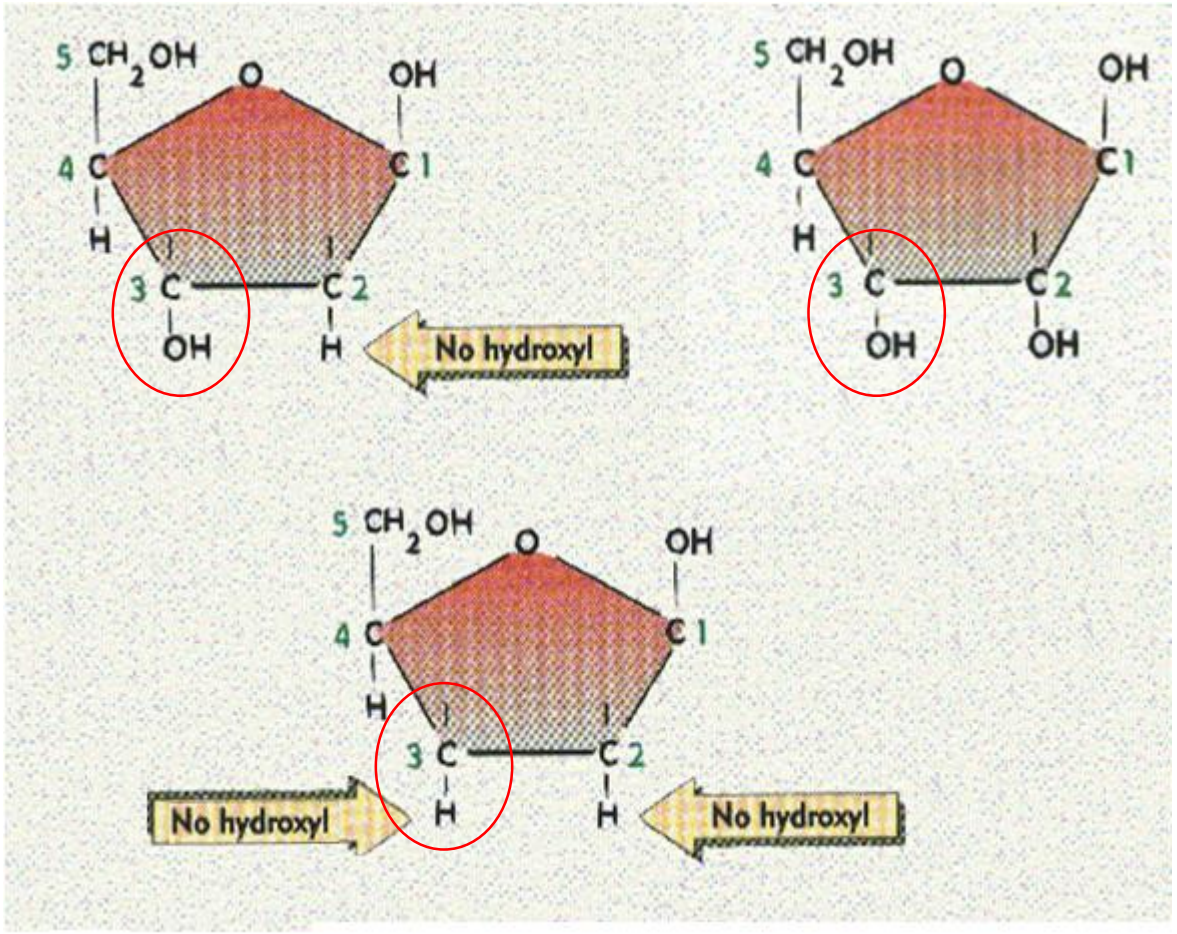
2-deoxyribose



Ribose

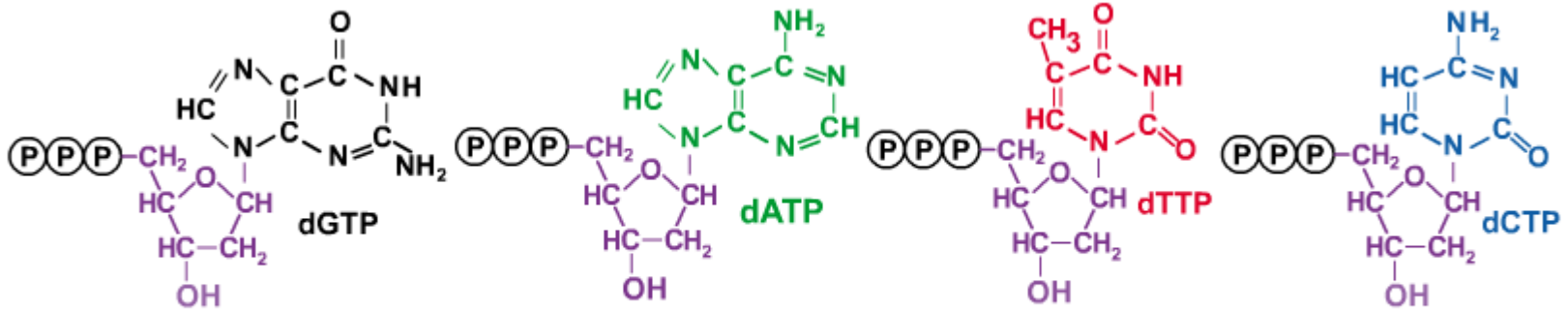
DESOXIRIBOSE

RIBOSE



DIDESOXIRIBOSE

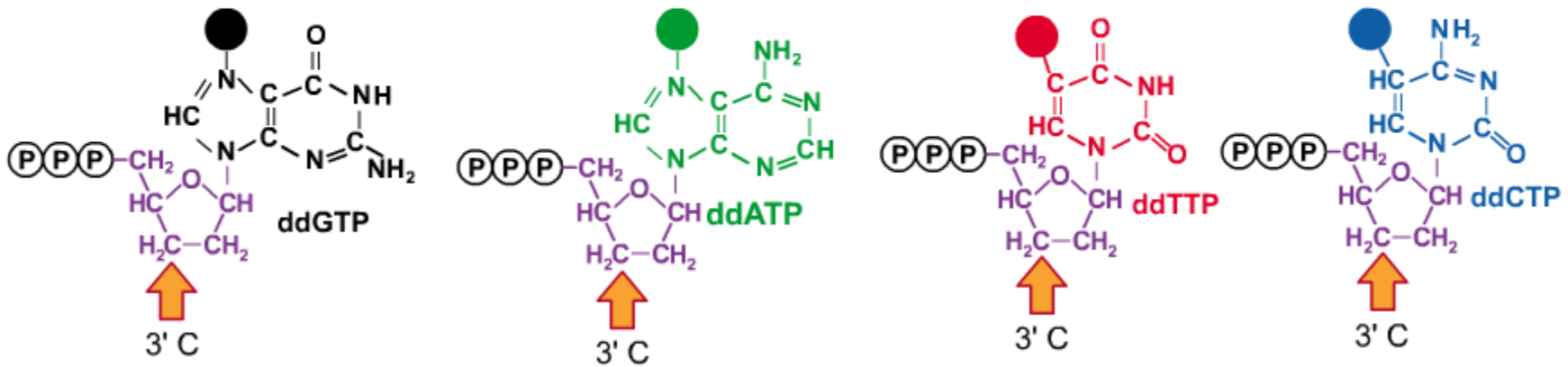
NUCLEOTÍDEOS dNTPs



NUCLEOTÍDEOS ddNTPs (TERMINADORES)

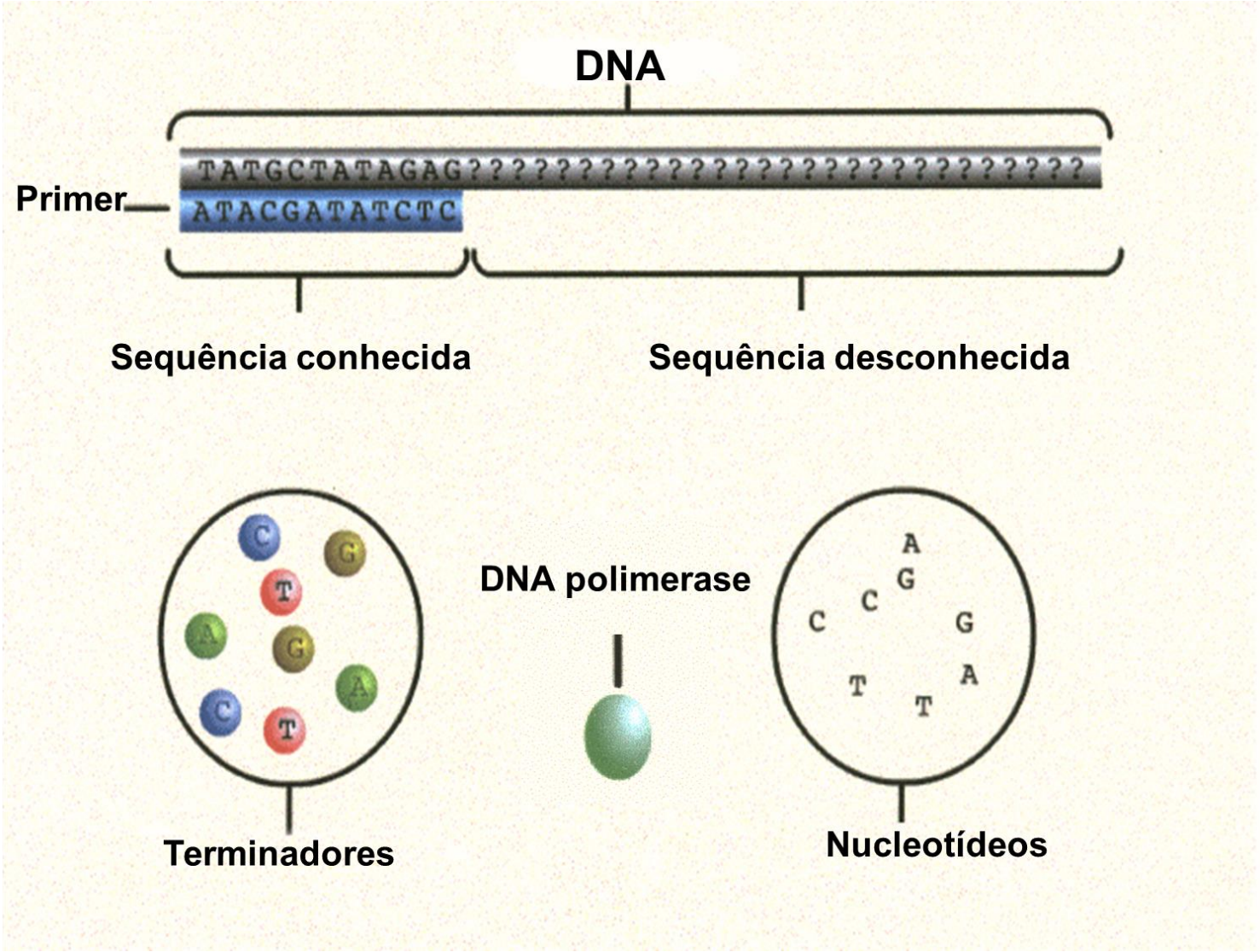
CORANTE FLUORESCENTE

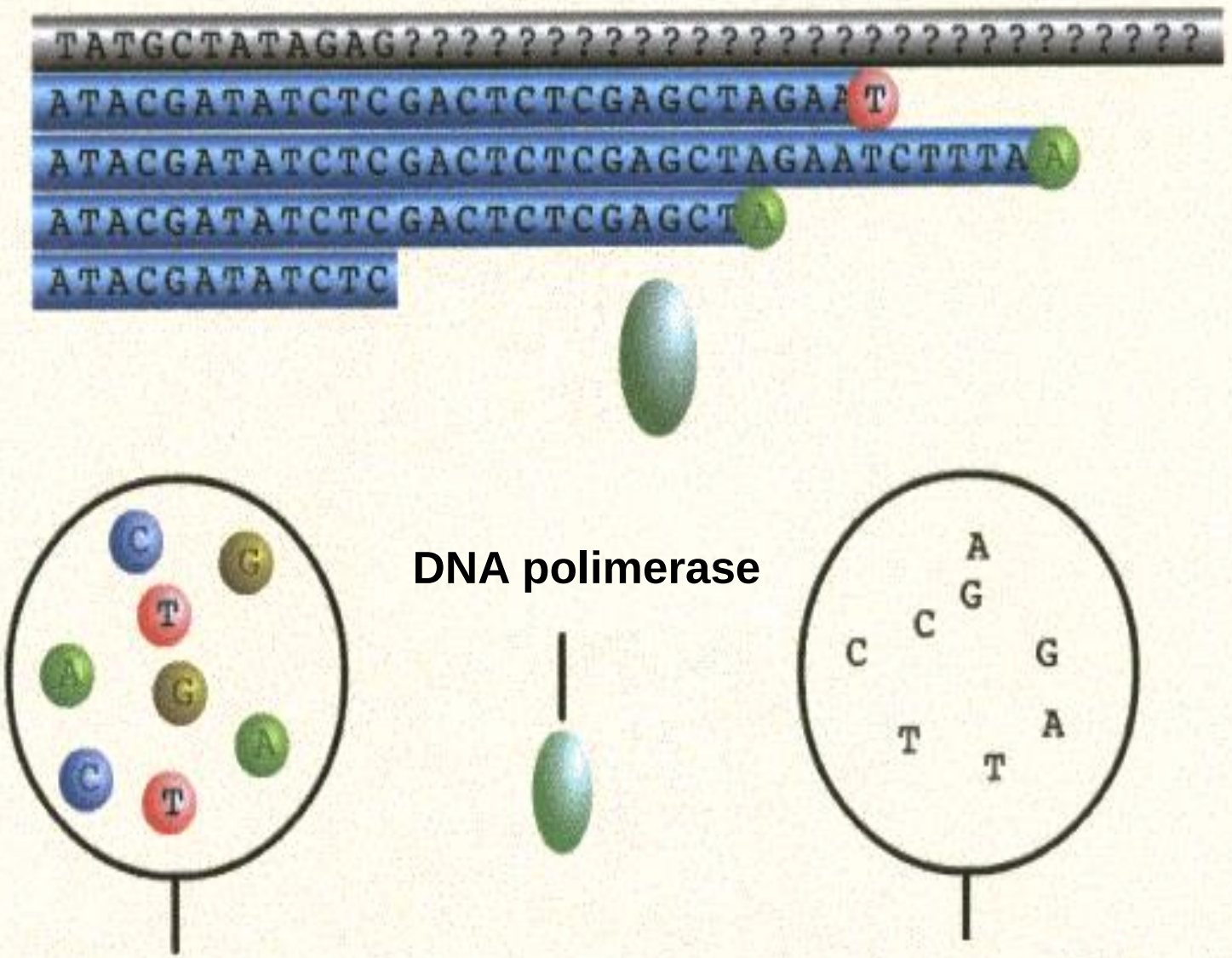
FALTA 3’ OH



REAÇÃO DE SEQUENCIAMENTO






TATGCTATAGAG??????????????????????????????
ATACGATATCTCGACTCTCGAGCTAGAAT
ATACGATATCTCGACTCTCGAGCTAGAATCTTTA
ATACGATATCTCGACTCTCGAGCT
ATACGATATCTC
DNA polimerase
Terminadores
Nucleotídeos

DESNATURAÇÃO DO DNA





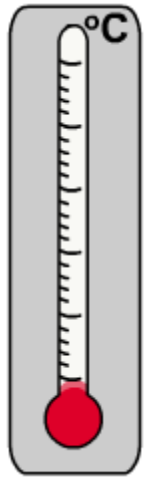
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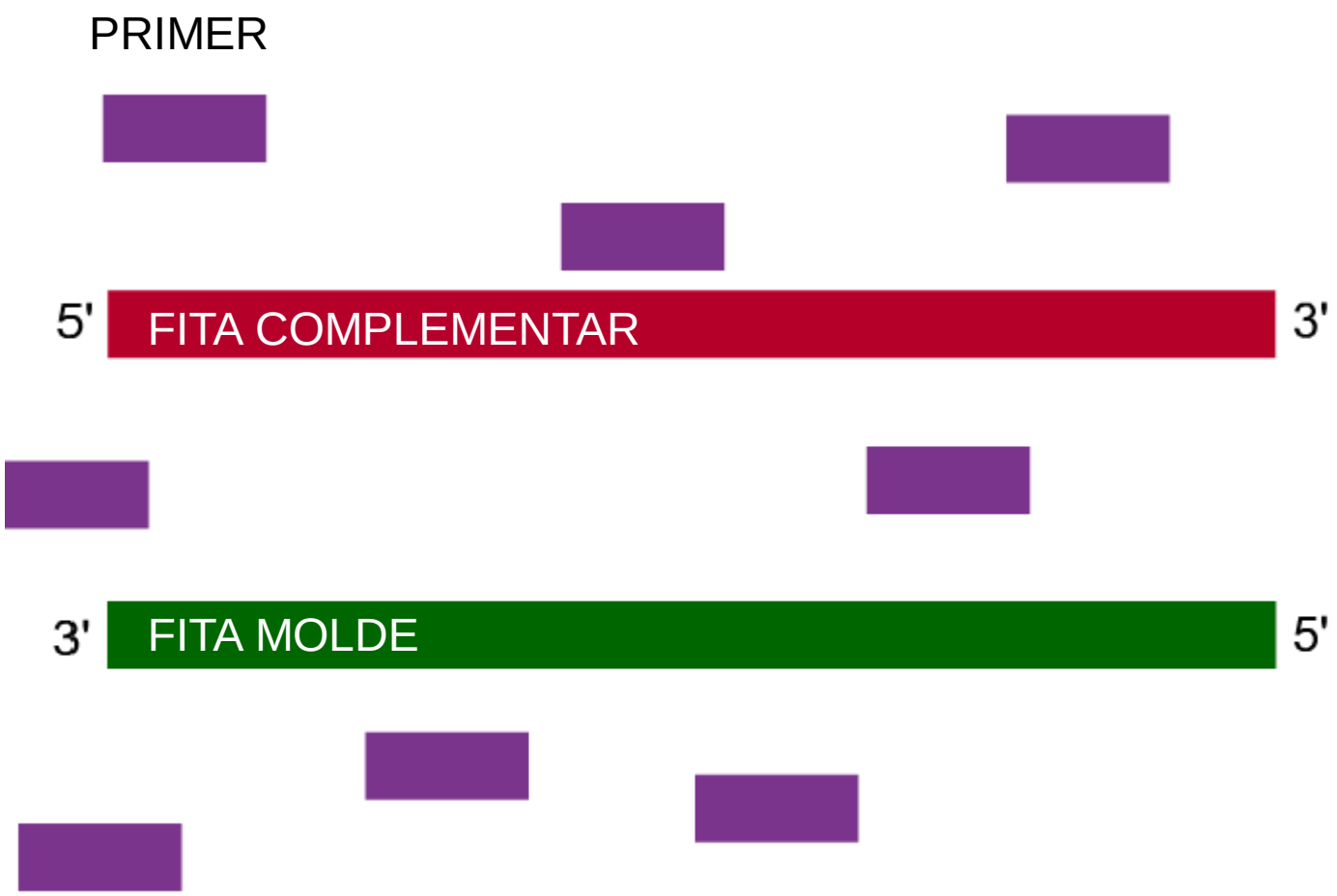




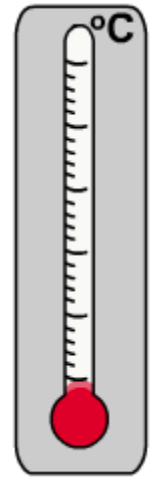


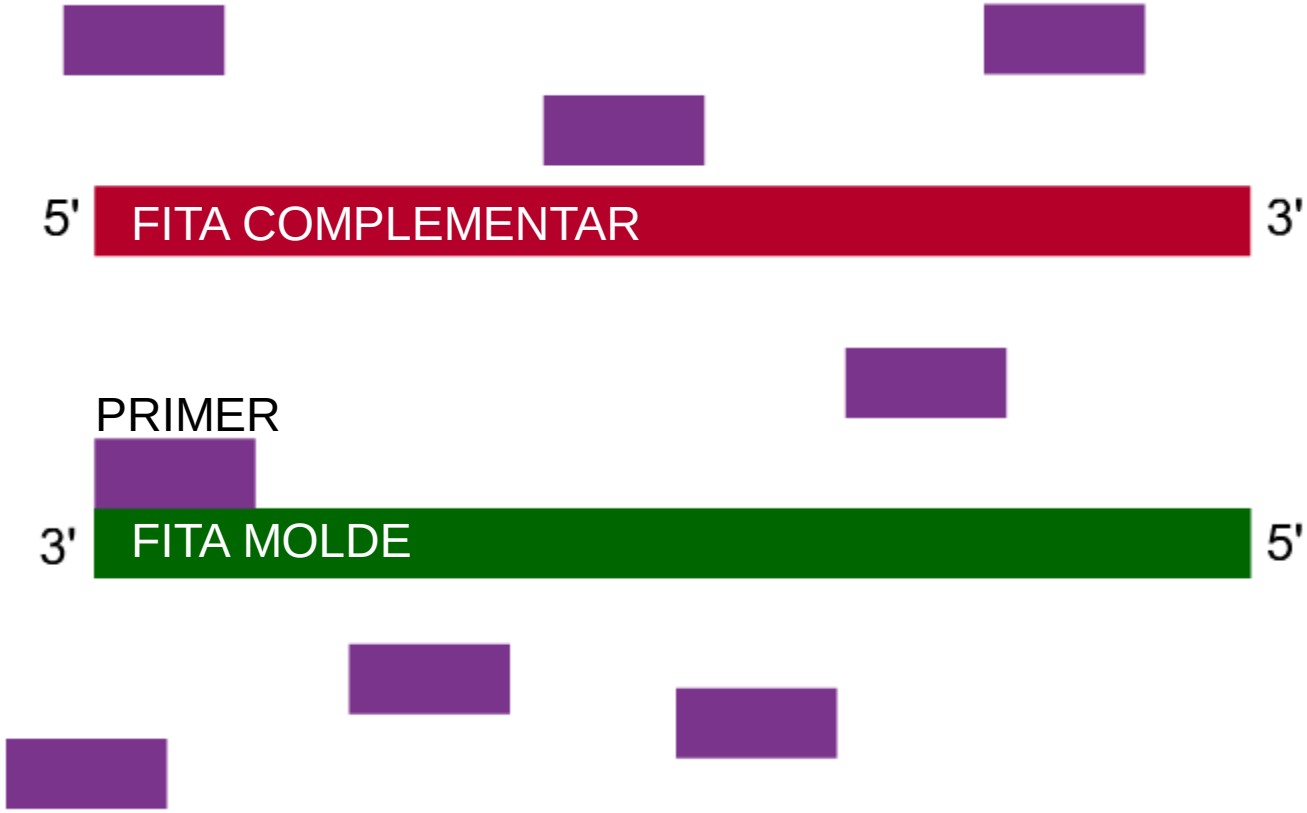
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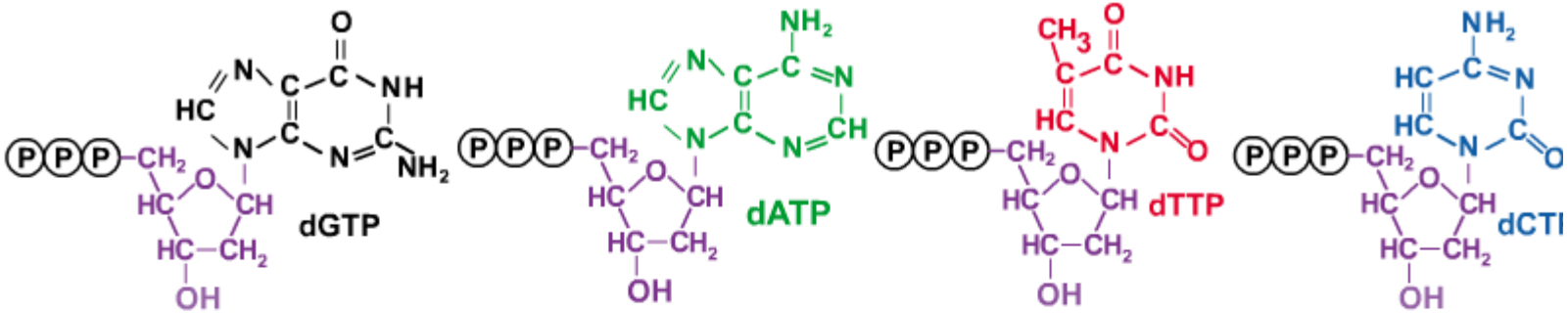


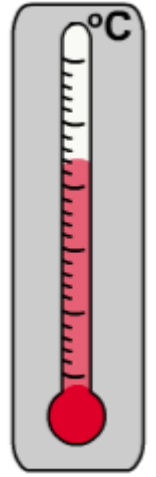


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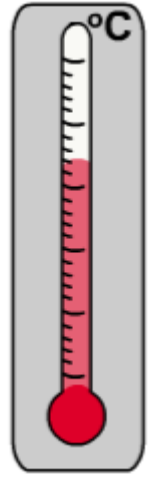


DNA POLIMERASE



POLIMERIZAÇÃO

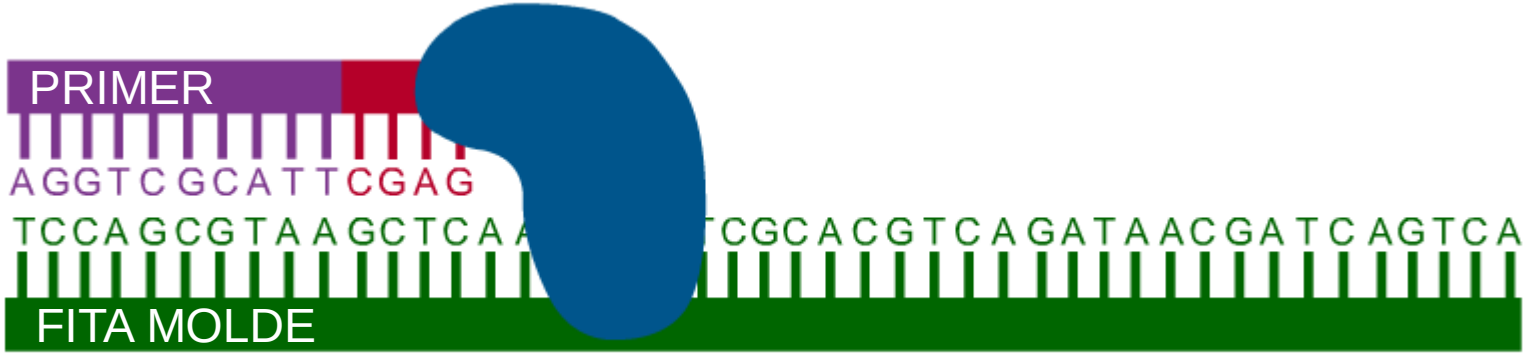


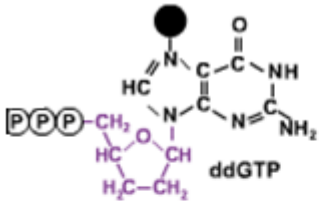


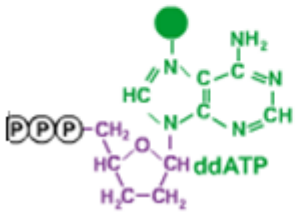
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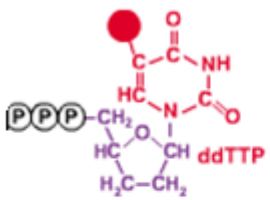


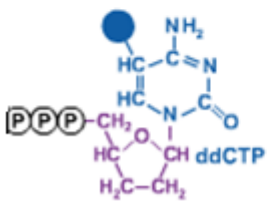
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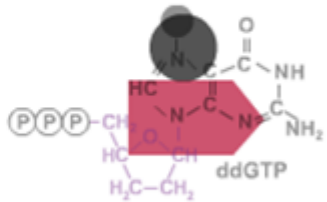



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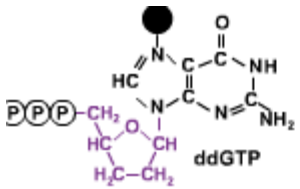

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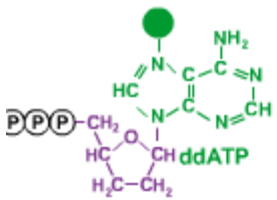

ddTTP


ddCTP


ddGTP




ddGTP

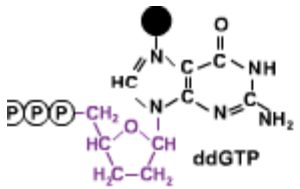

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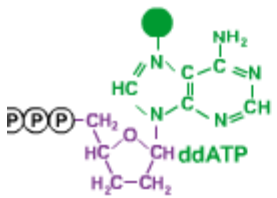

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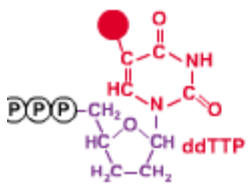

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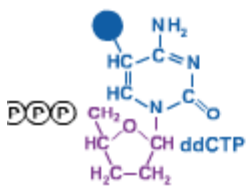


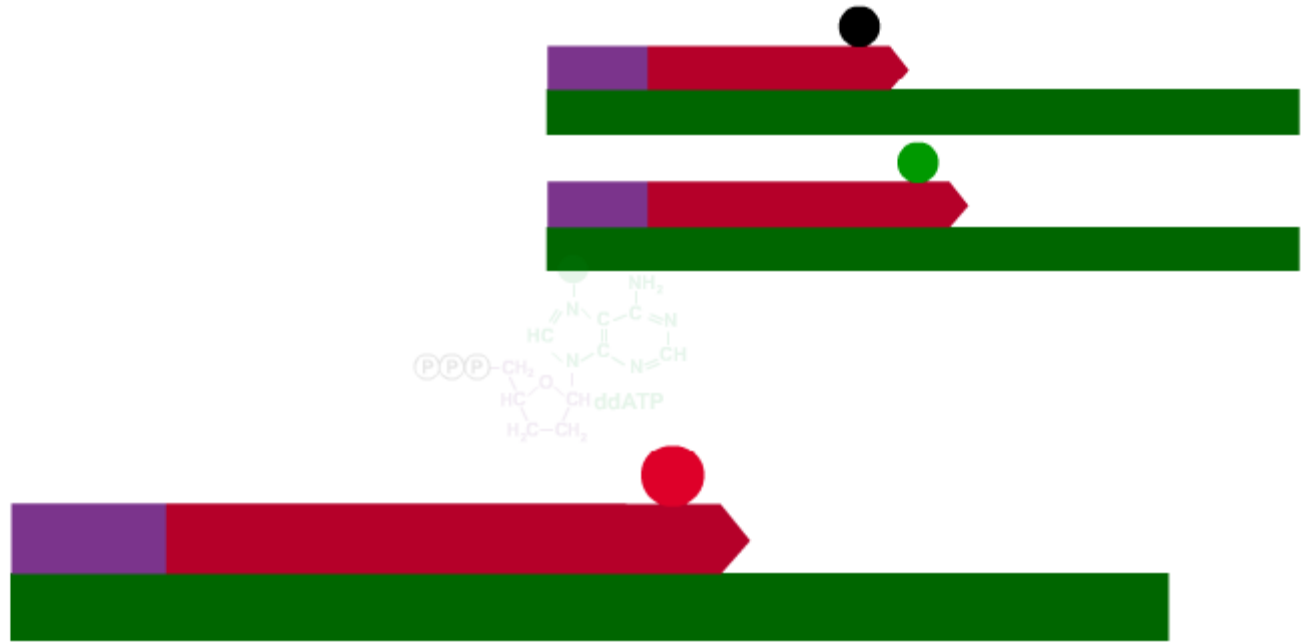


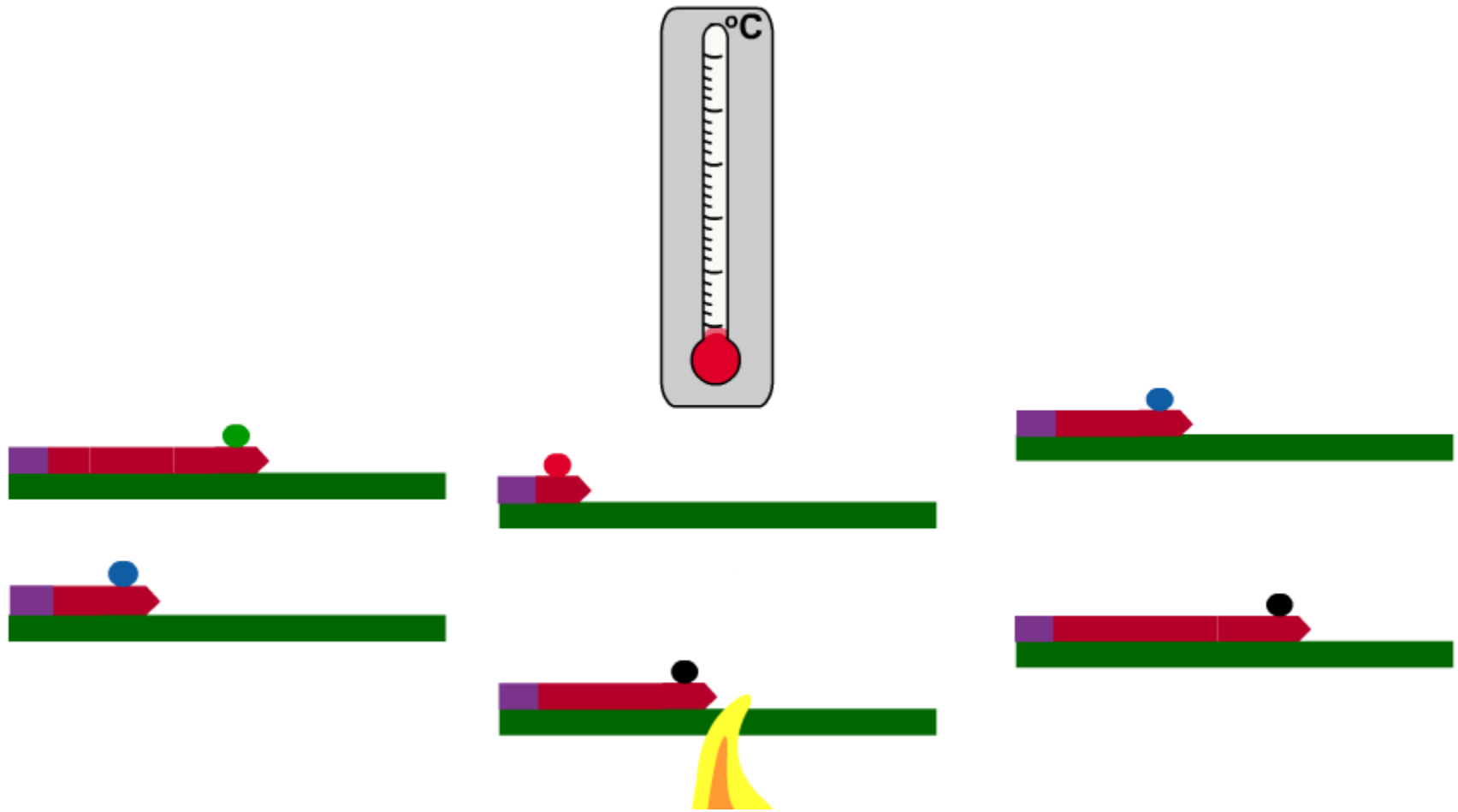

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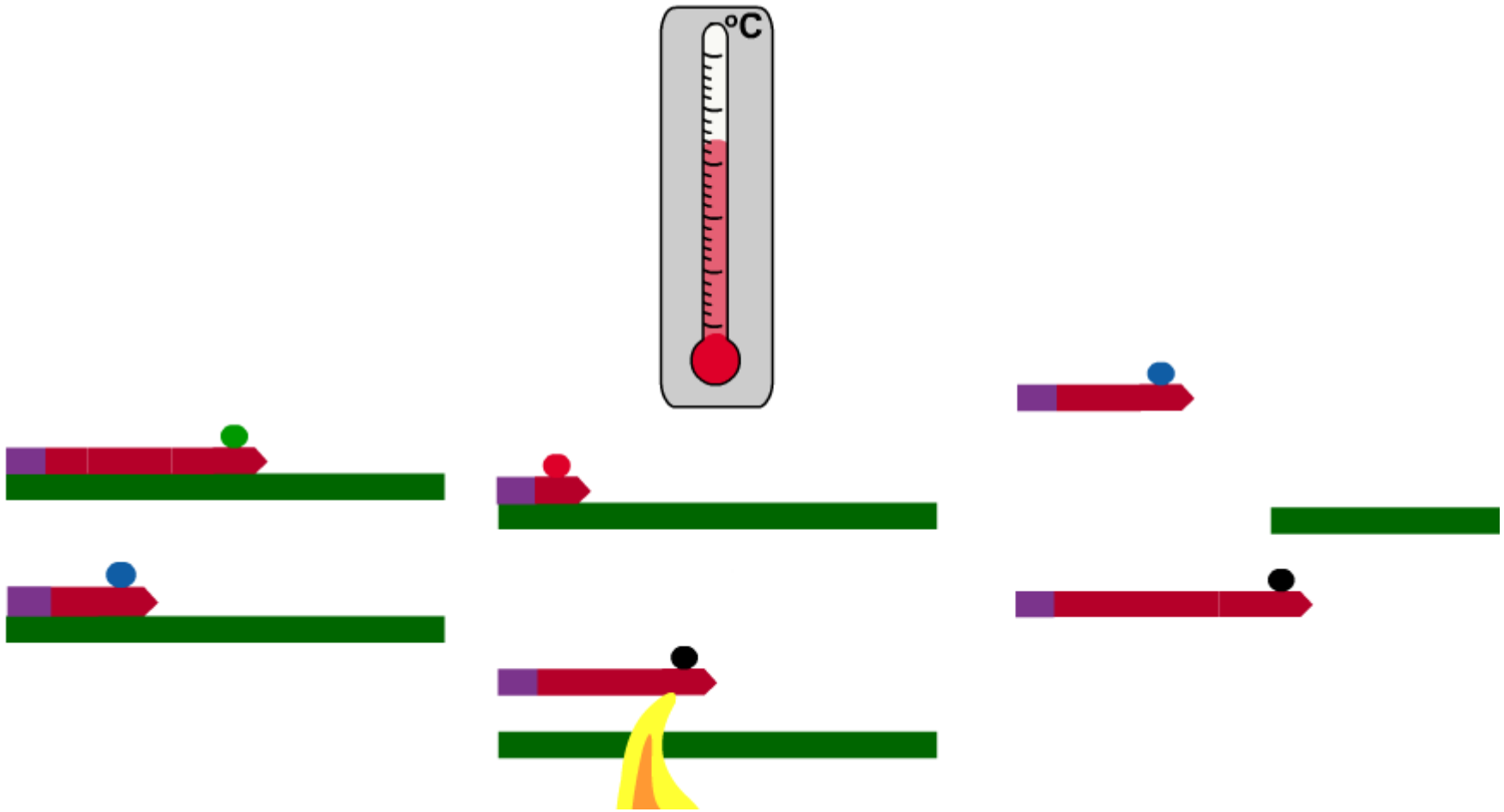

ddATP


ddTTP


ddCTP




°C


°C







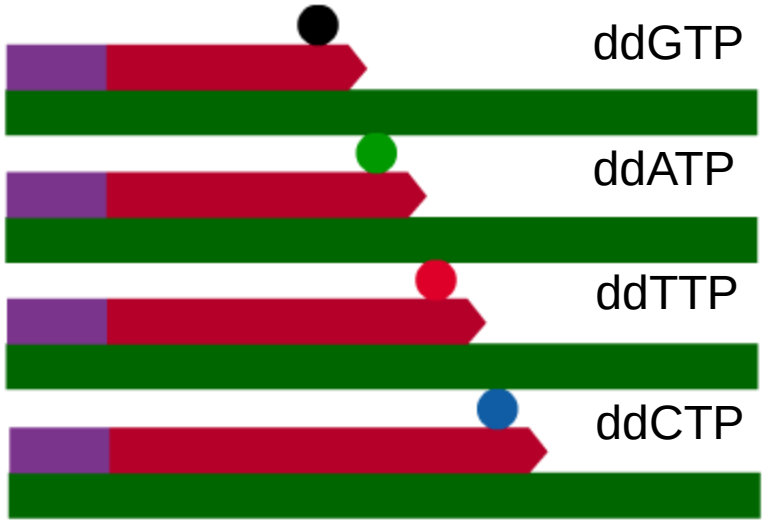


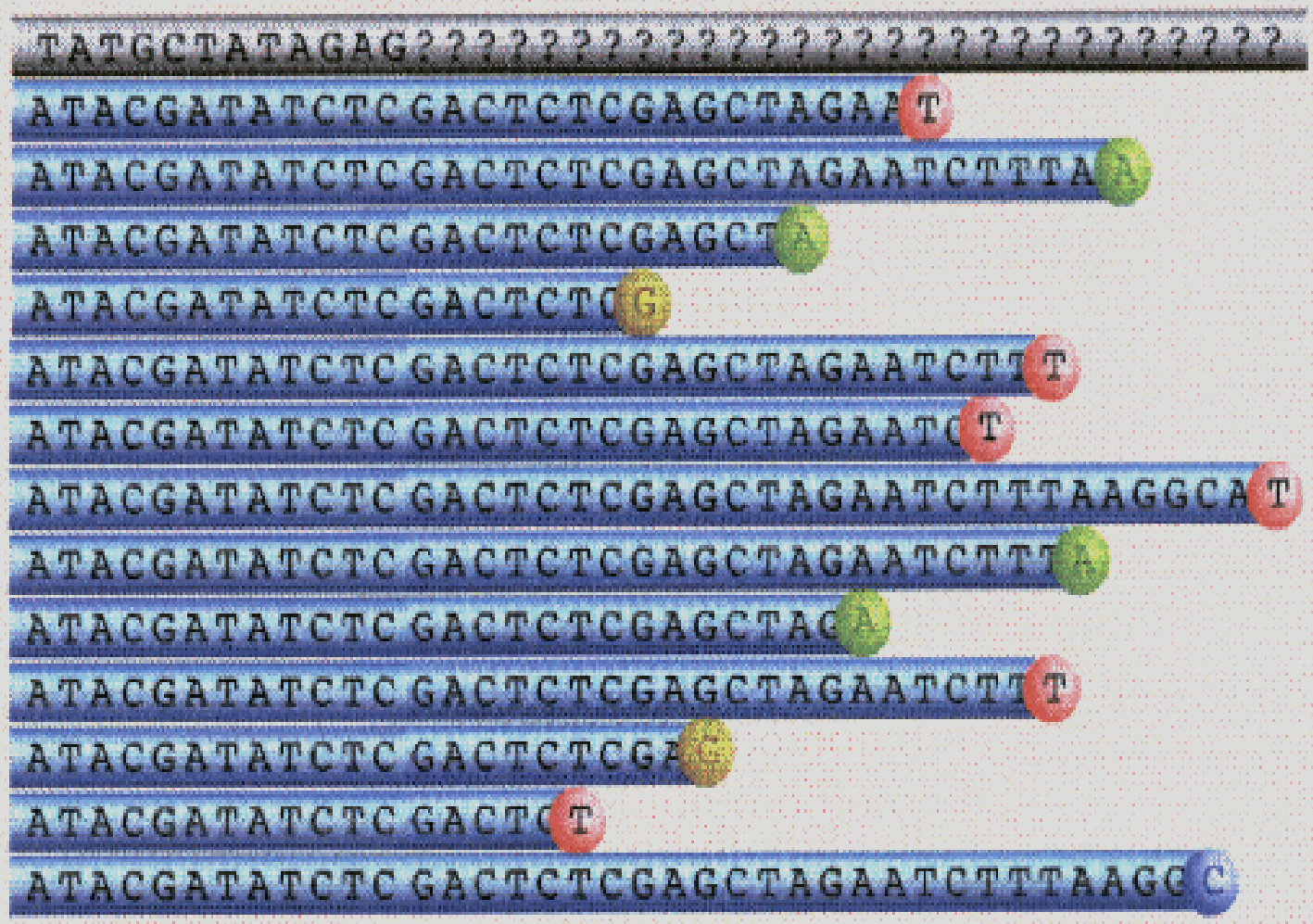


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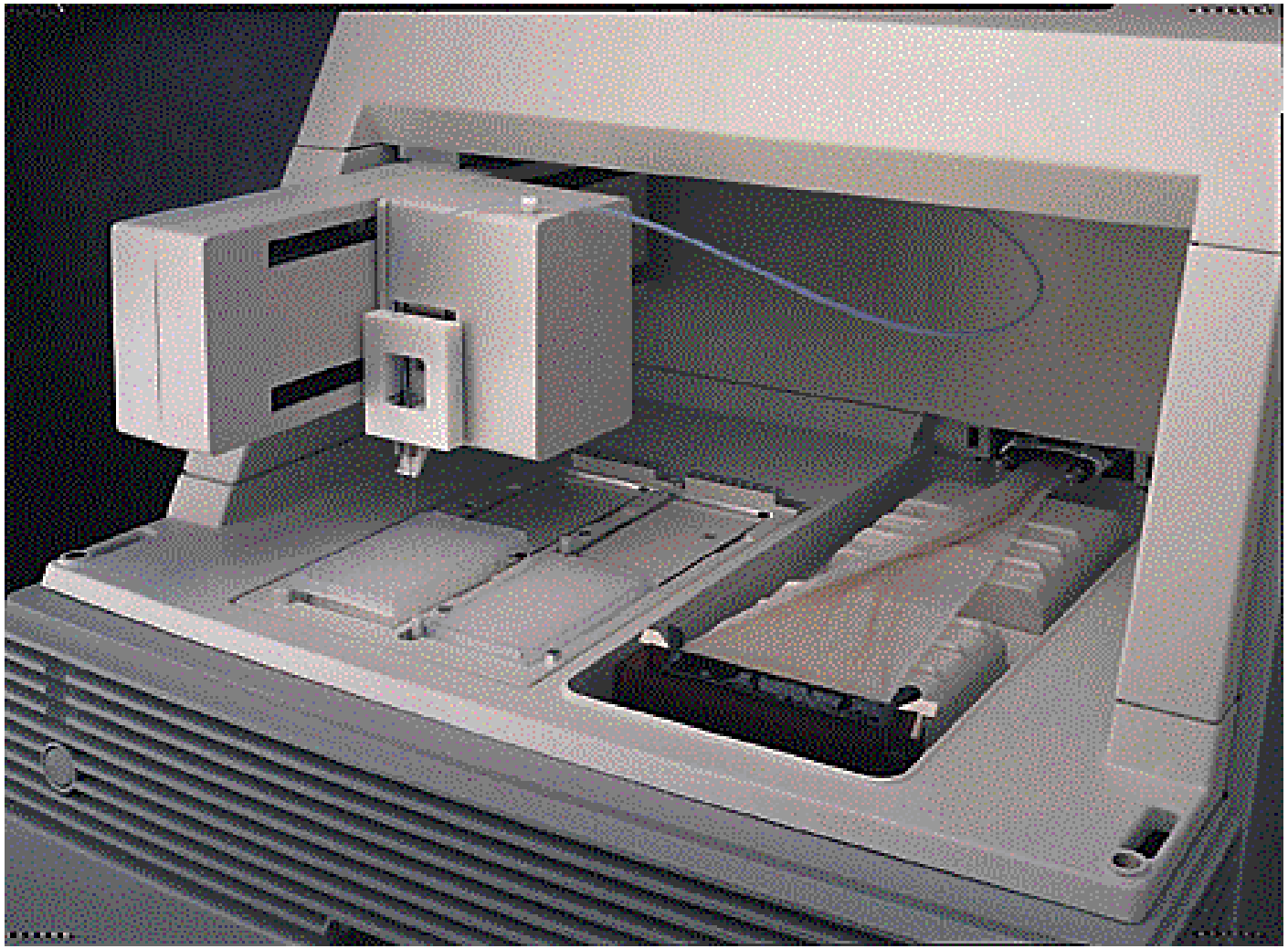

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ddATP


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PRIMER
FITA MOLDE

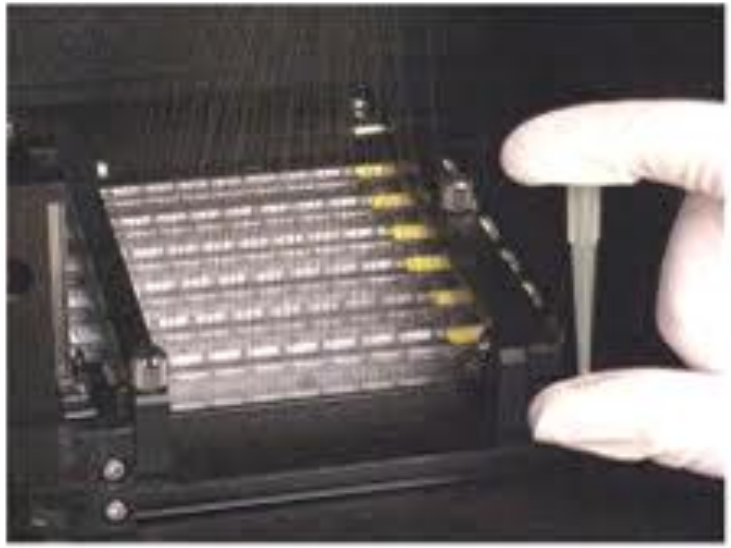

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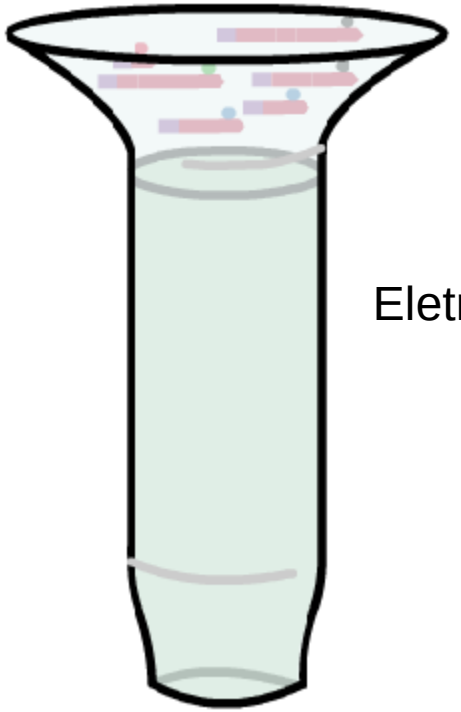

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ELETROFORESE CAPILAR



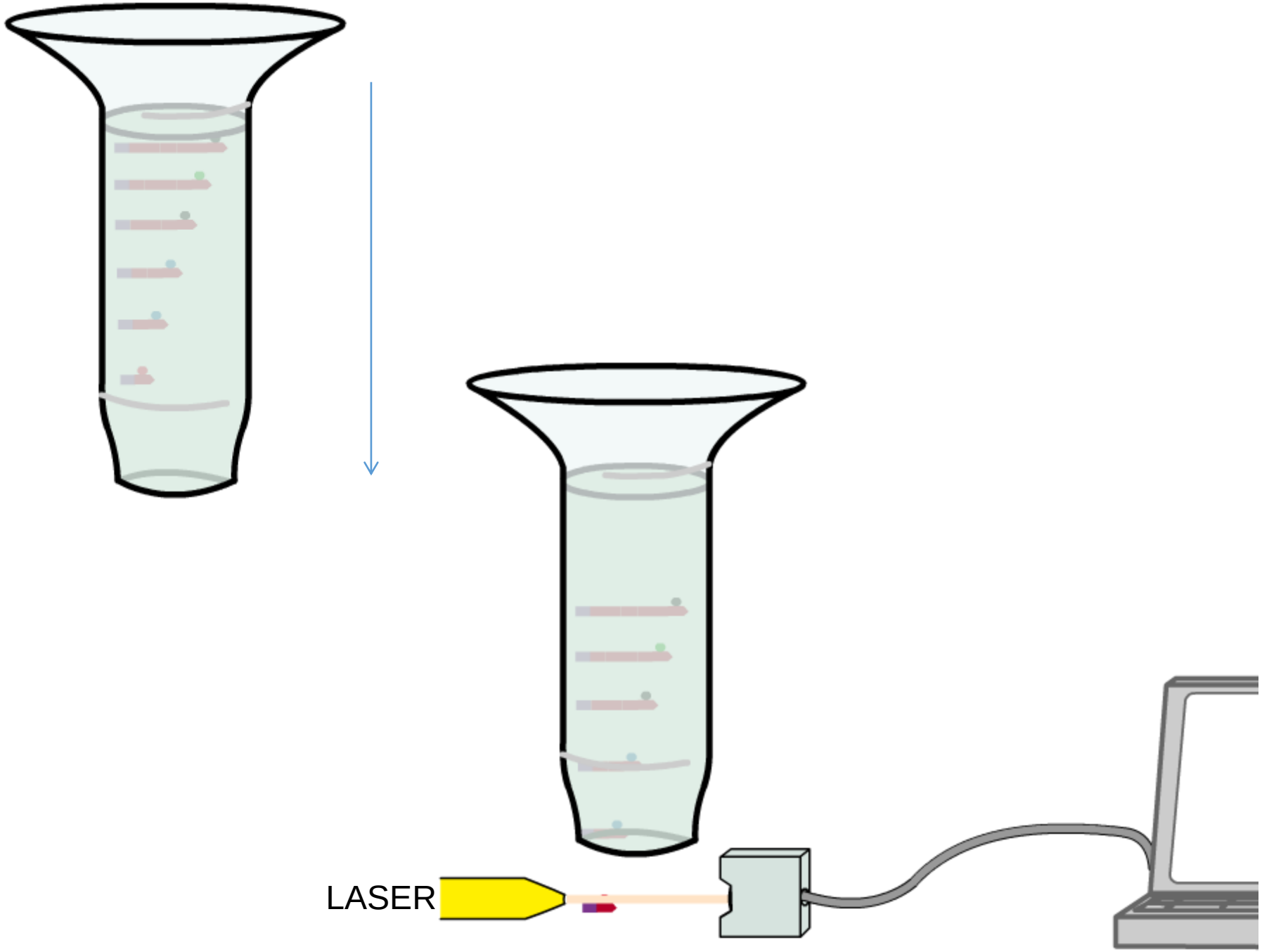
ABI 3700 Applied Biosystems

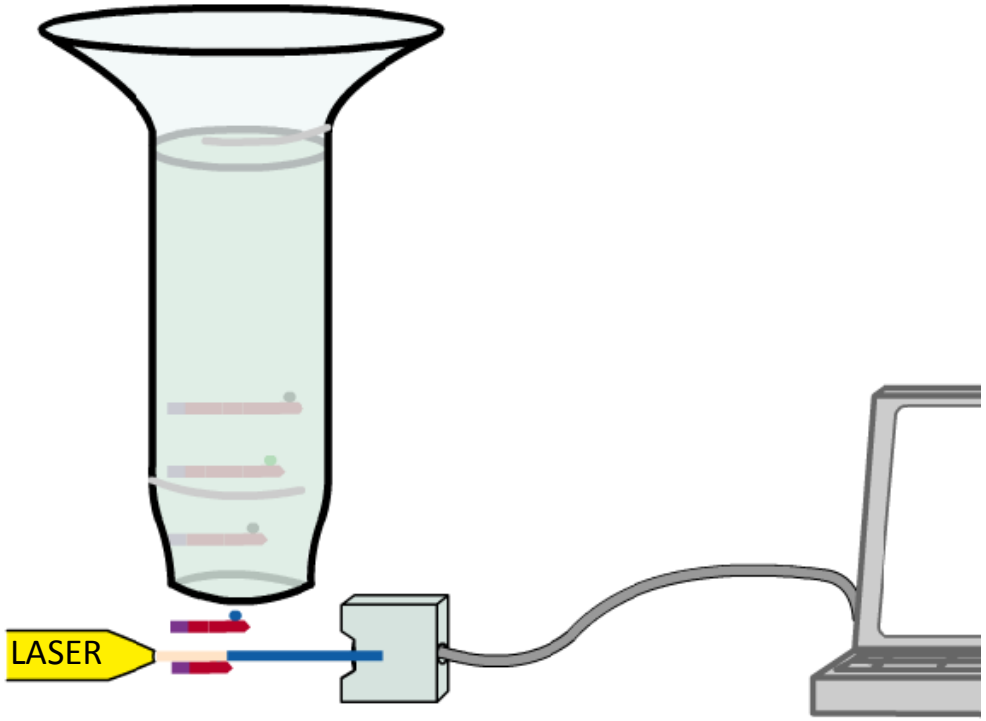


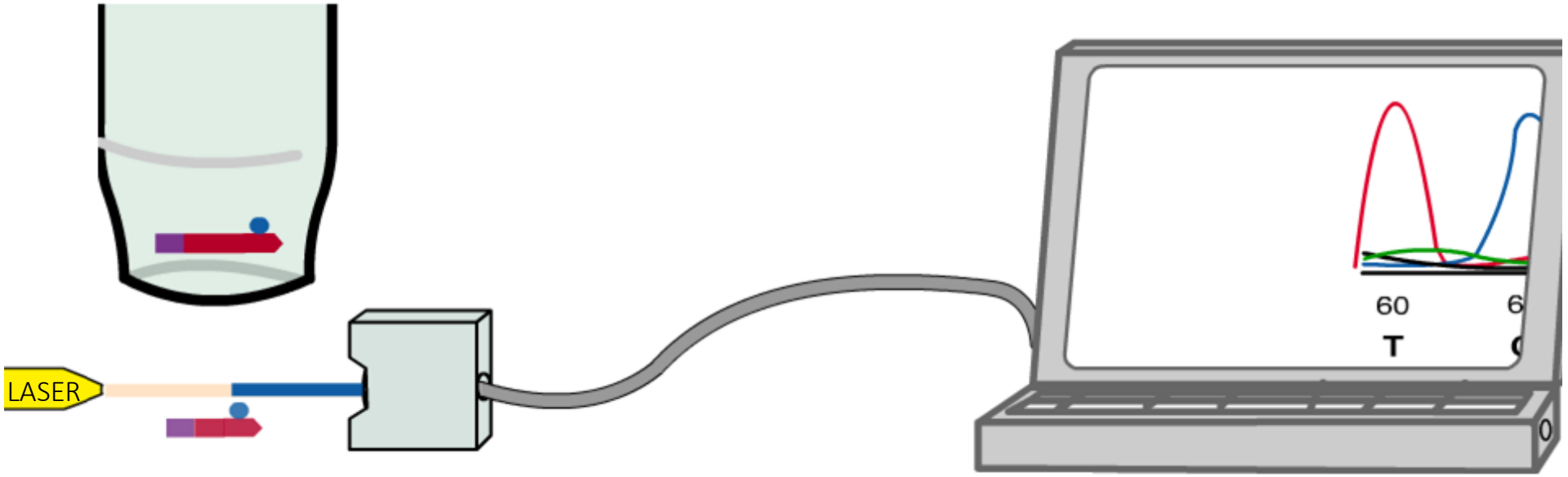


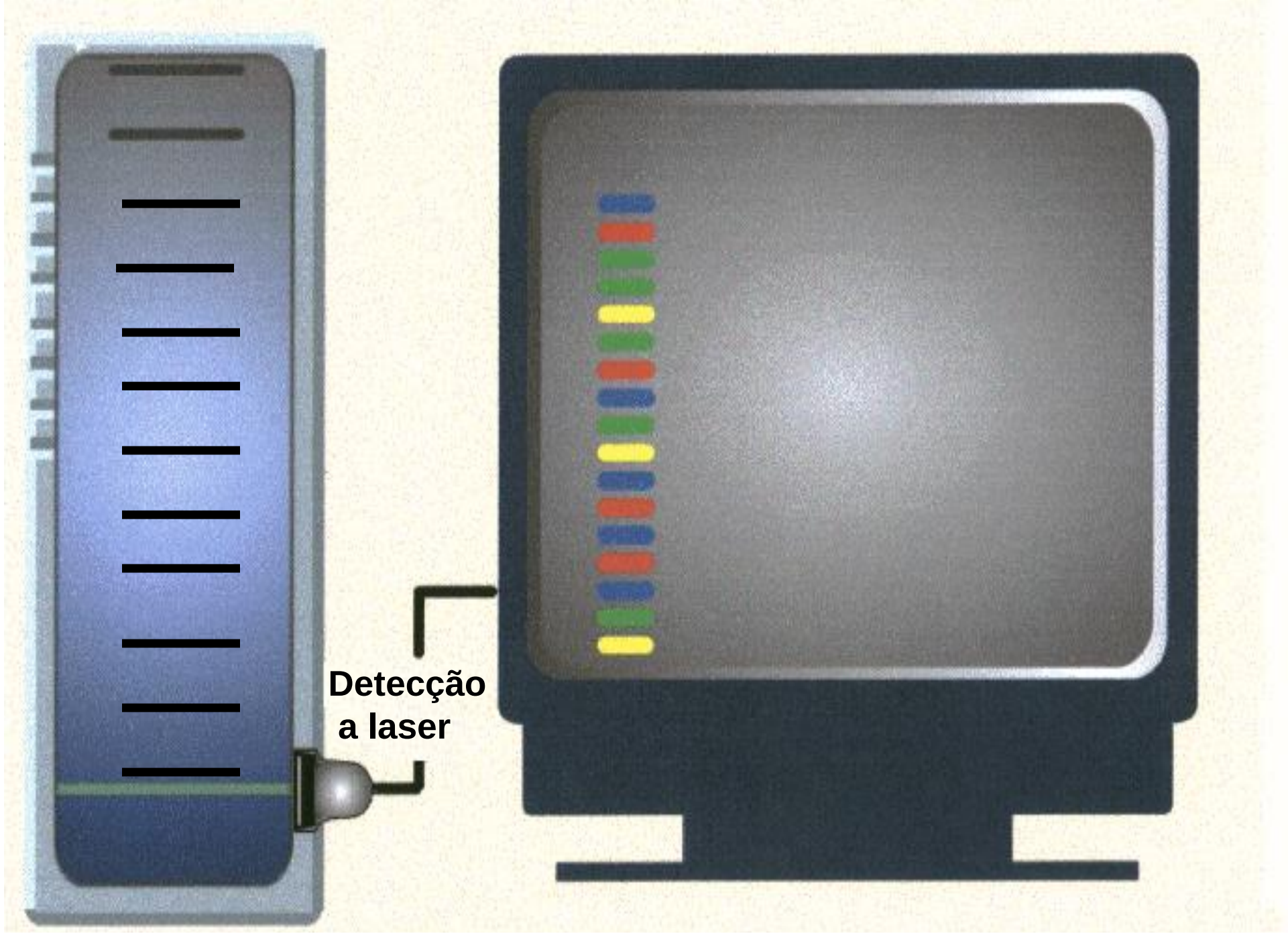
Eletroforese capilar




LASER


LASER

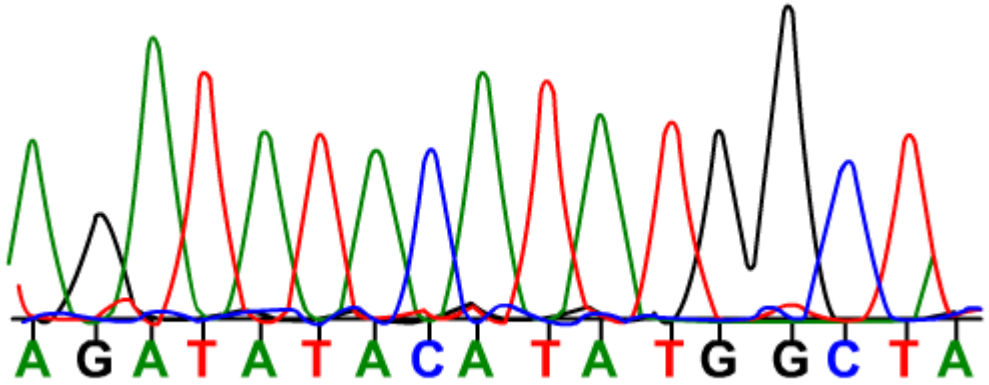

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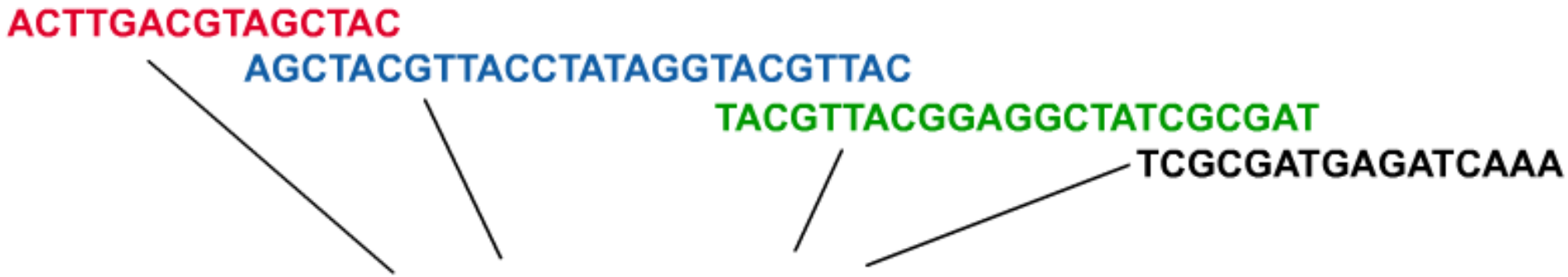

Detecção
a laser



ANÁLISE COMPUTACIONAL







FRAGMENTOS DE DNA SEQUENCIADOS

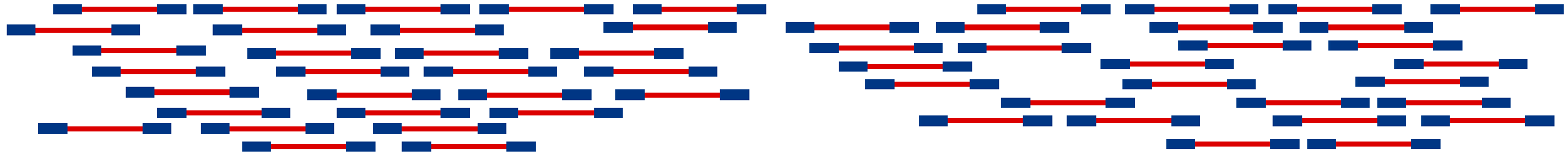






FRAGMENTOS COMPLETOS

Biblioteca de insertos pequenos



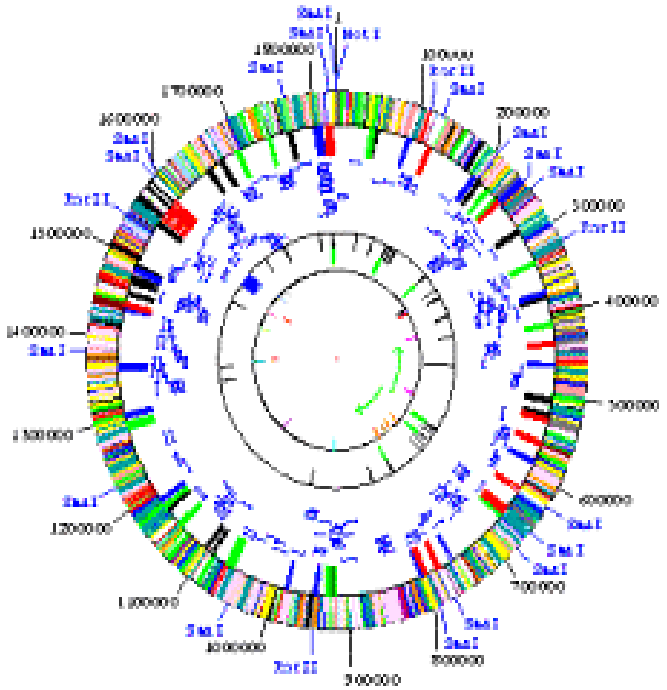
Montagem dos fragmentos

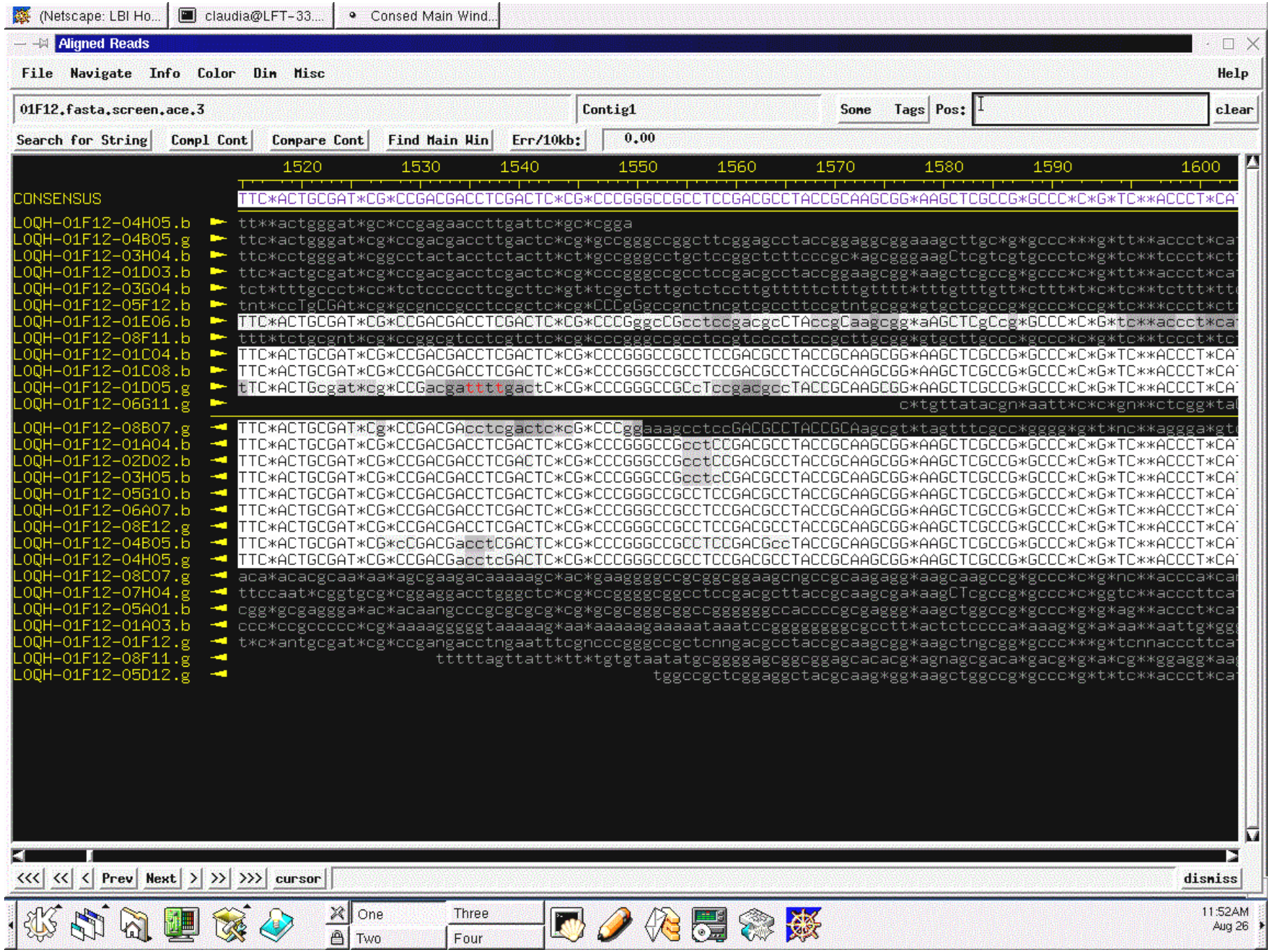


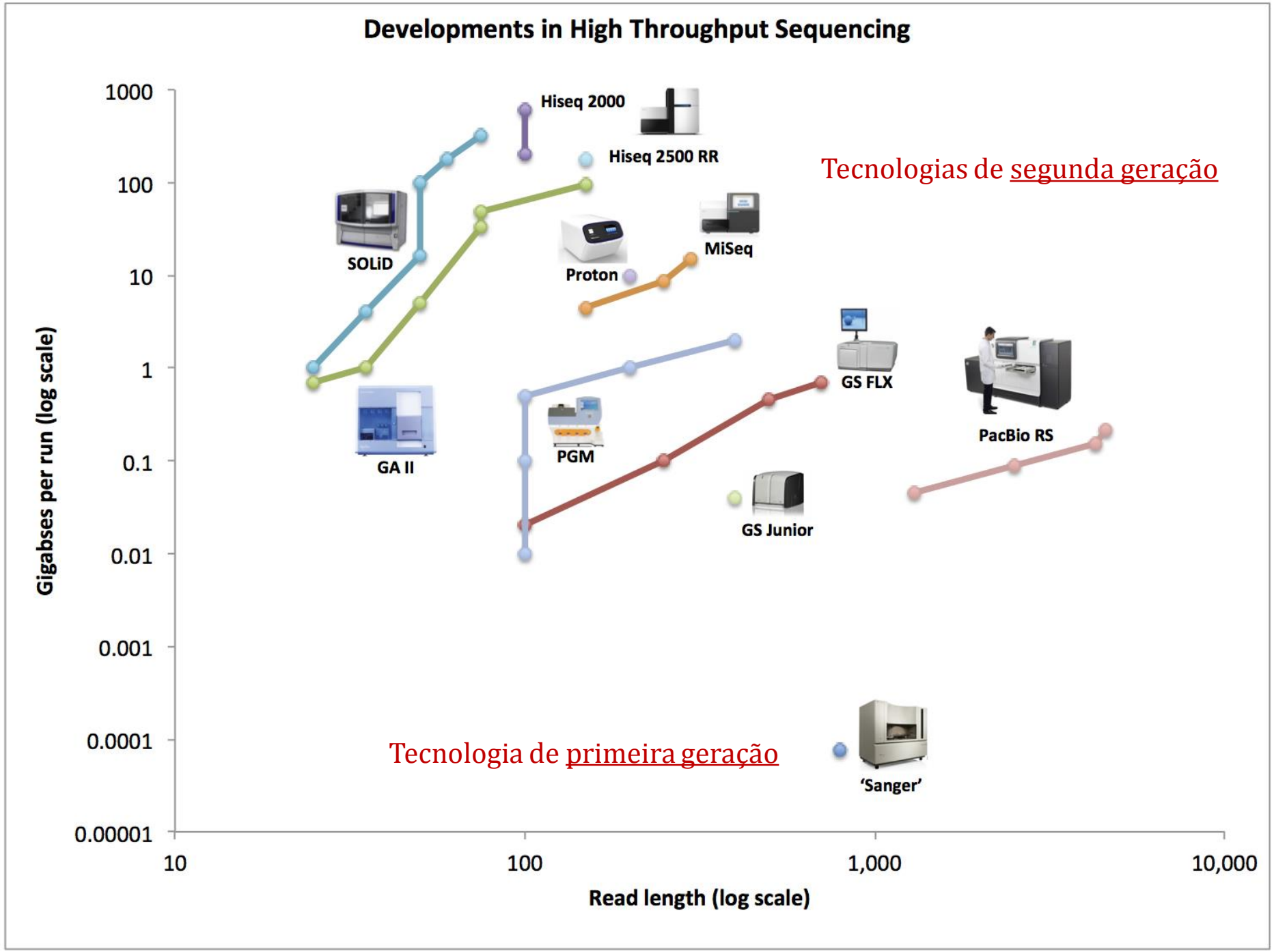
Fechamento dos gaps



Análise




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Aligned Reads
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Search for String
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Compare Cont
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Err/10kb:
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1520 1530 1540 1550 1560 1570 1580 1590 1600
CONSENSUS
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LOQH-01F12-03H04.b
LOQH-01F12-01D03.b
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Developments in High Throughput Sequencing
Gigabses per run (log scale)
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0.1
0.01
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10
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1,000
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Read length (log scale)
Hiseq 2000
Hiseq 2500 RR
SOLiD
Proton
MiSeq
GA II
PGM
GS FLX
PacBio RS
GS Junior
'Sanger'
Tecnologias de segunda geração
Tecnologia de primeira geração

Ciência Rural, Santa Maria, v.40, n.3, p.735-744, mar, 2010
ISSN 0103-8478

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

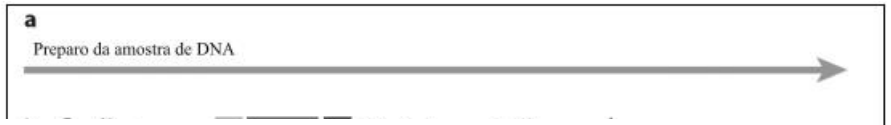
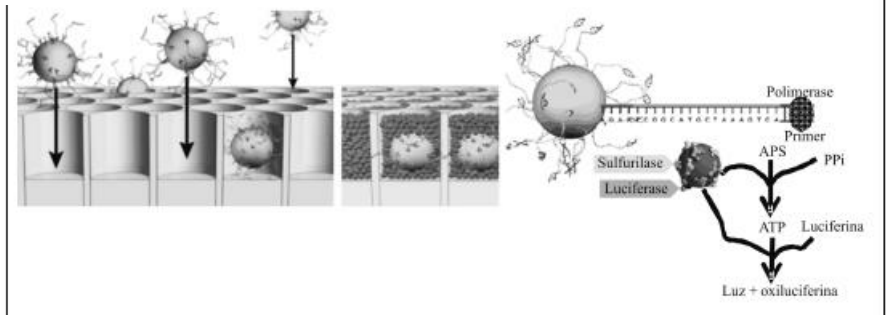
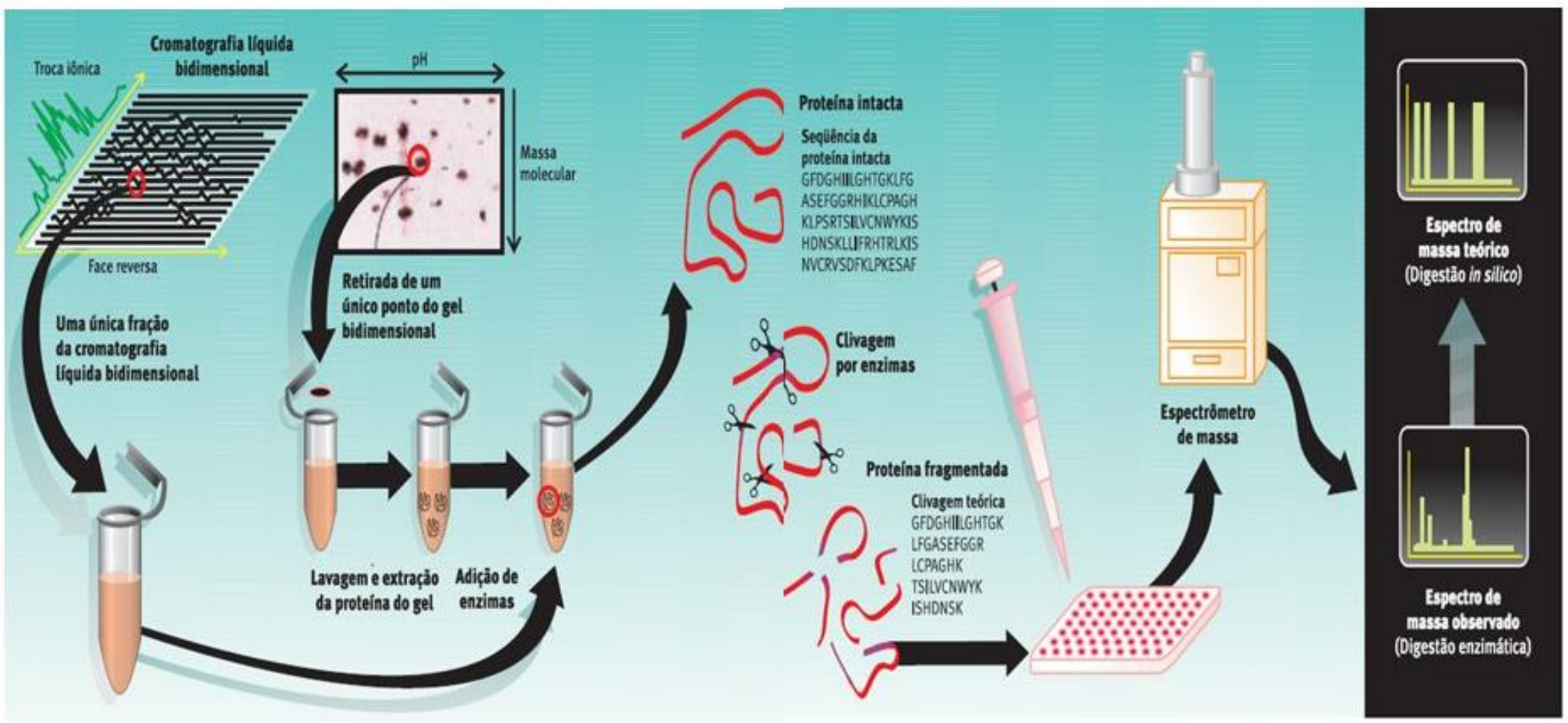


Tabela 1 - Resumo das principais características técnicas das plataformas 454 GS-FLX, Solexa e SOLiD e laboratórios no Brasil que já adquiriram essas novas plataformas. A duração da corrida inclui o tempo para o preparo, a leitura e o processamento das amostras; o custo da corrida e o valor do equipamento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

Plataforma	Corrida			Custo		Acurácia (%)	Laboratório**
	Informação (Gb)	Duração (dias)	Reads (pb)	Equipamento (U$)	Base (U$)		
GS-FLX *Titanium*	0,5	3 a 4	Até 400	531.500	10.000	99,5	- LNCC - IQ-USP
Genome analyzer (Solexa)	3	5	25-35	430.000	6.250	98,5	- Nenhum - Fiocruz
SOLiD *System*	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=3I9wzwj0b_A

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



ESTUDO DIRIGIDO

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

(LER o texto “Sequenciamento de nova geração”)

E

Capitulo 11 – Manipulando o gene /Técnicas de Biologia Molecular (páginas 197 a 241) . Menck, C.F.M.; Van Sluys, M.A. **Genética Molecular Básica: dos genes aos genomas.** Editora Guanabara Koogan, 2017.

Capítulo 5 - Técnicas de Genética Molecular (páginas 171-222). Lodish et al. Biologia Celular e Molecular. 7o Edição. Editora Artmed, 2014.

