LGN0232 - Genética Molecular

Identificação de Sequencias por Hibridização e Sequenciamento

5ª aula

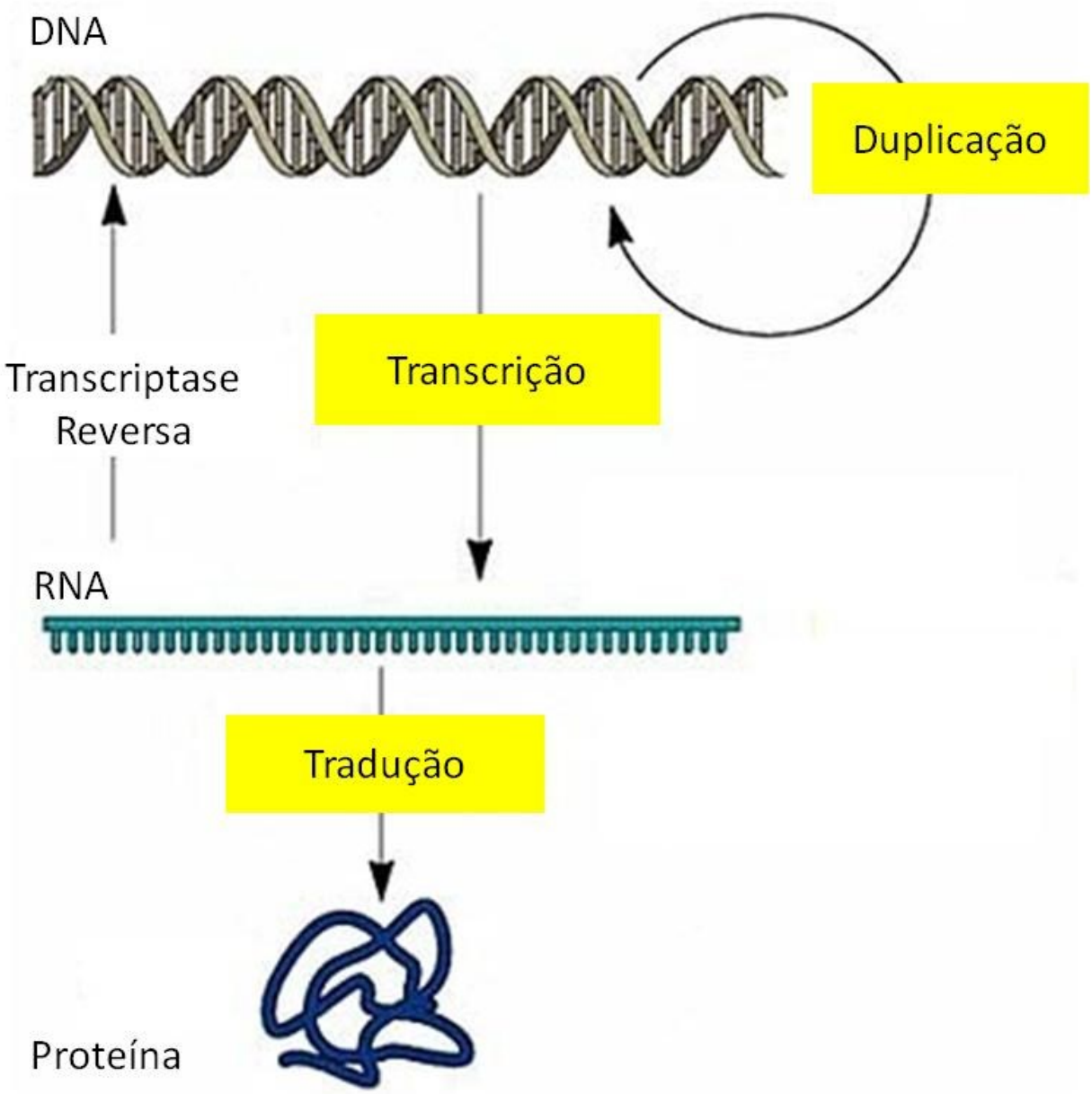
Antonio Figueira
CENA
figueira@cena.usp.br

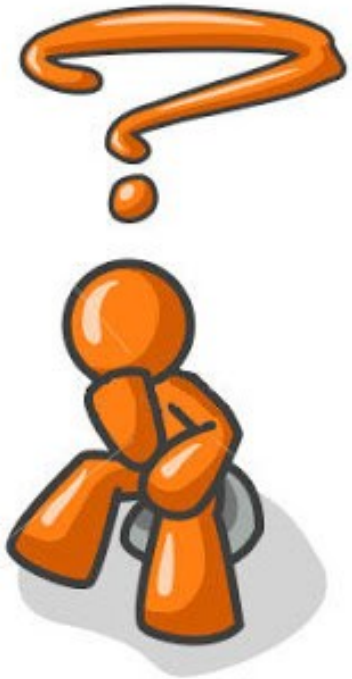
Dogma Central da Biologia Molecular

Genoma

Transcritoma

Proteoma





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

- Hibridização com sondas conhecidas
- Sequenciamento
    - Genômica, Transcritômica, Proteômica..

Técnicas de "Blotting"

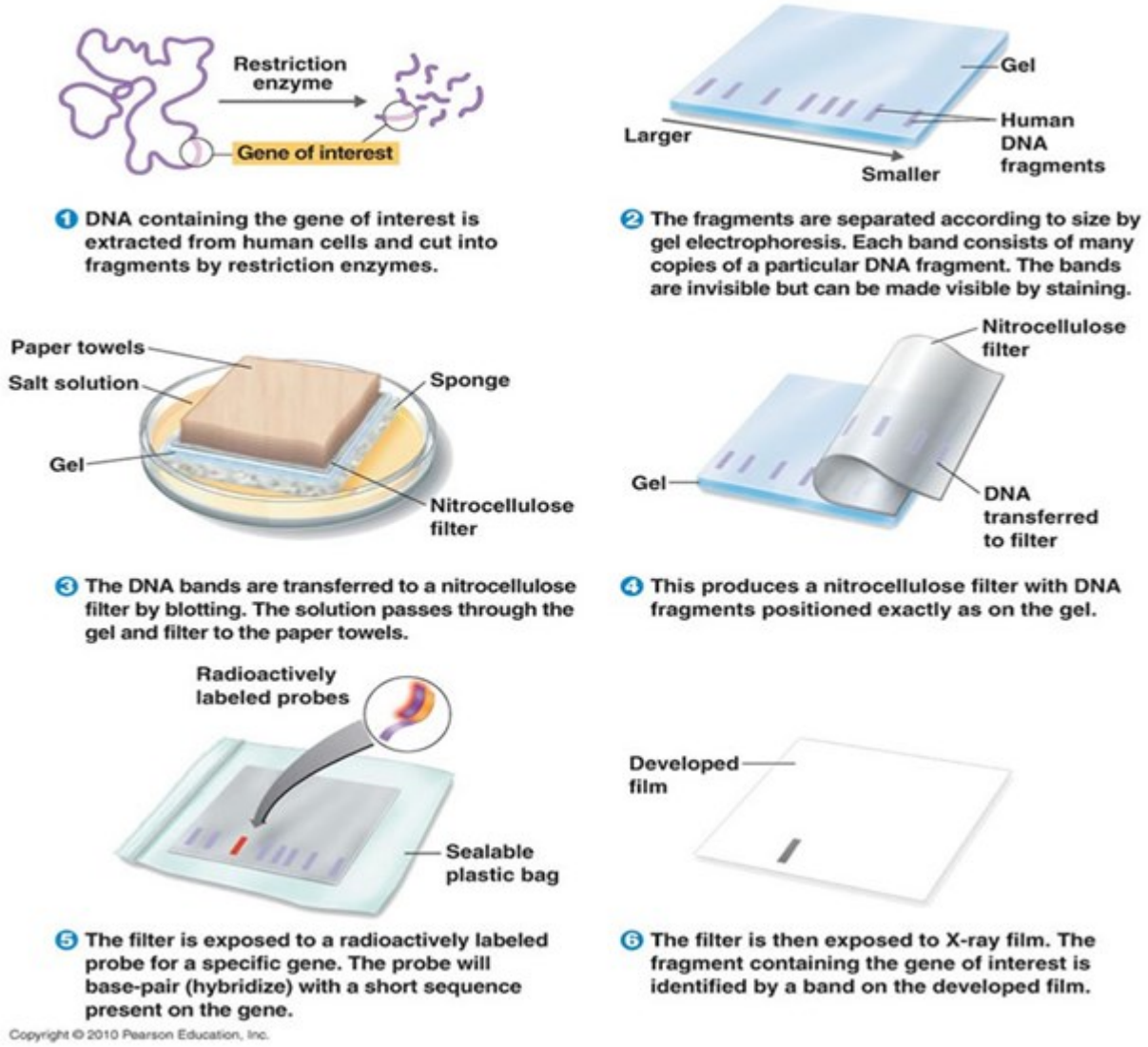
Southern blot = **técnica** utilizada que permite verificar se uma sequência de DNA específica (**gene de interesse**) está ou não presente na amostra em análise que contém uma mistura complexa = genoma inteiro de organismo digerido por enzima de restrição

northern blot = **técnica** utilizada para estudar a expressão gênica, ou seja, verificar se um determinado gene de um genoma é ou não transcrito em RNA e quantifica-lo. Essa técnica tem tal nome devido à similaridade de seu procedimento com o Southern blot (*batizada em homenagem a Edwin Southern; a diferença chave de que, em vez de DNA, a substância analisada alvo por eletroforese com uma sonda hibridizadora é RNA*)

western blot = **técnica** usada para detectar proteínas em um homogenato ou extrato de tecido biológico. Essa **técnica** usa eletroforese em gel para separar as proteínas desnaturadas por massa

Southern blot

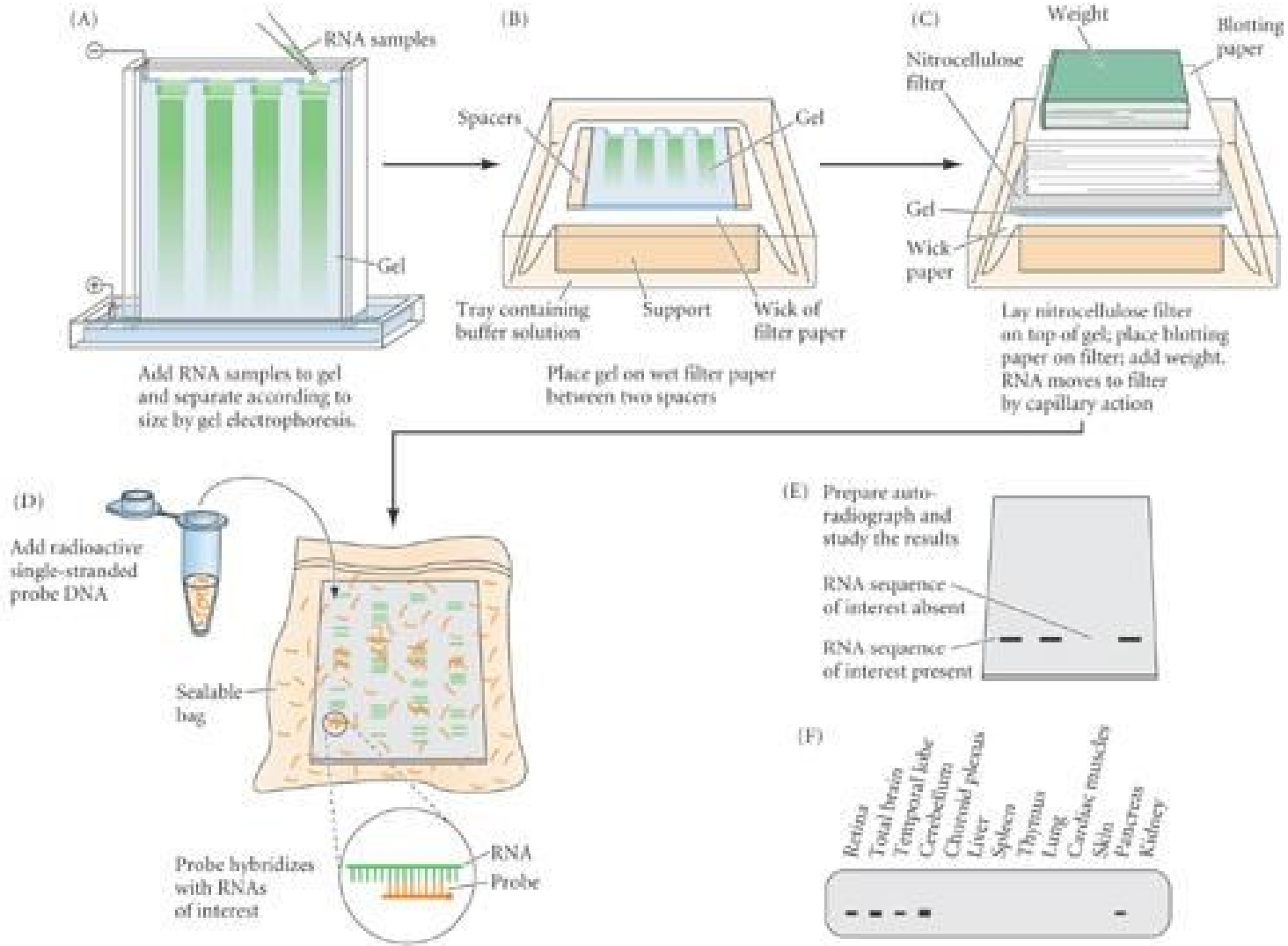
Edward **Southern**, 1975



https://www.youtube.com/watch?v=3I9wzwj0b_A

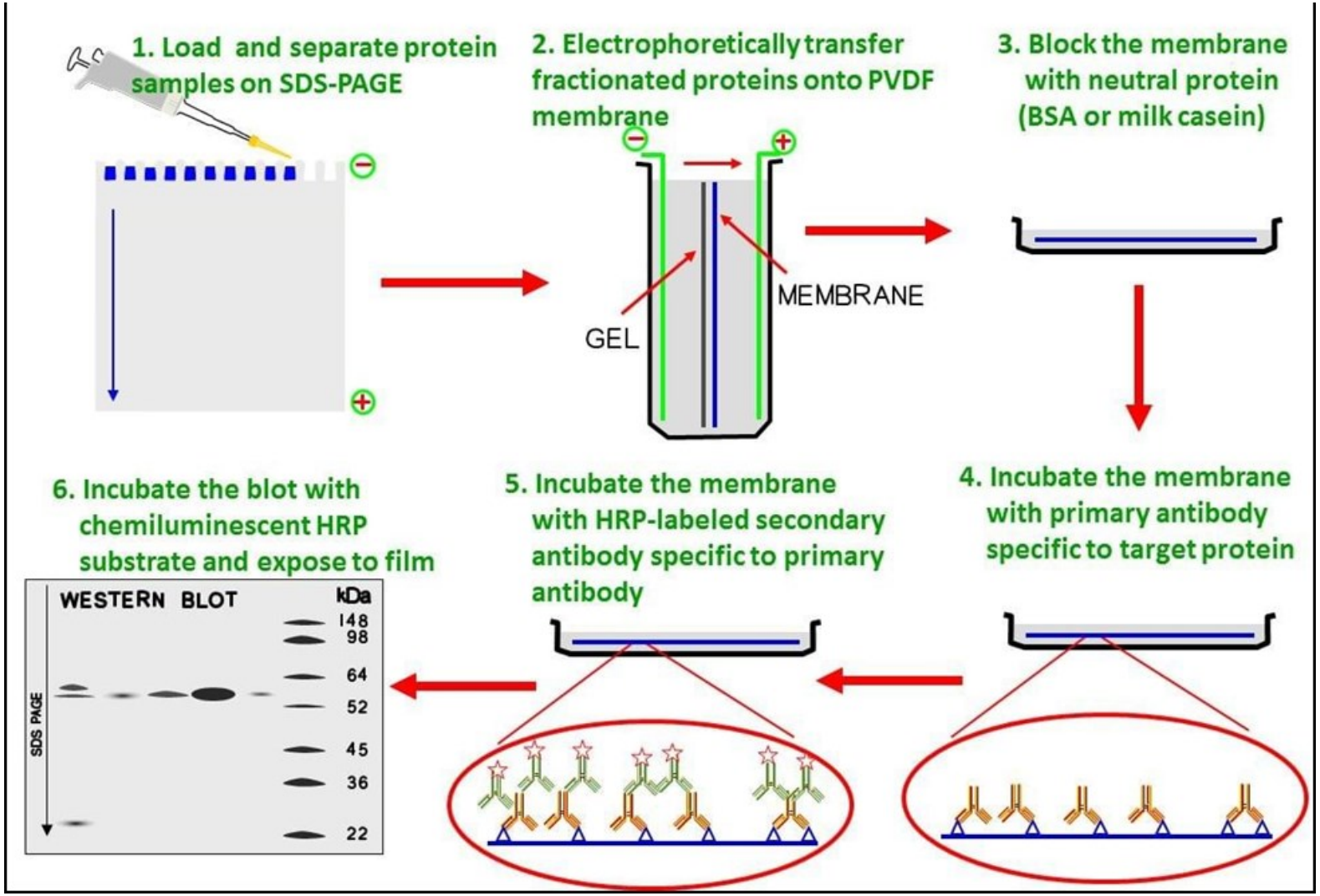
http://www.biologyexams4u.com/2013/12/southern-blotting-procedure-steps.html#.WaQd3PkrLIU

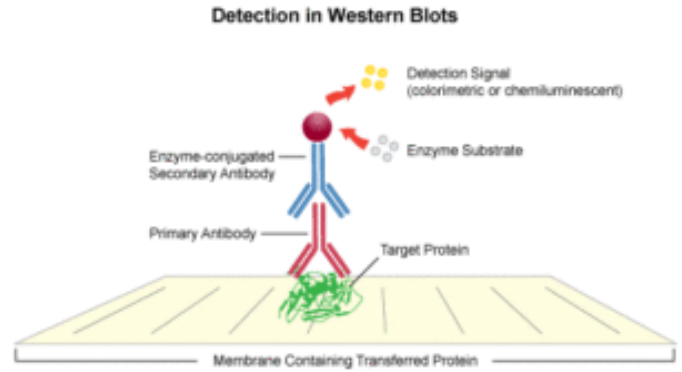
northern blot



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

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**)
- Extremamente trabalhosas!!!

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 1ª geração:

- **Método Maxam & Gilbert** (1977)*
  - Método de degradação química

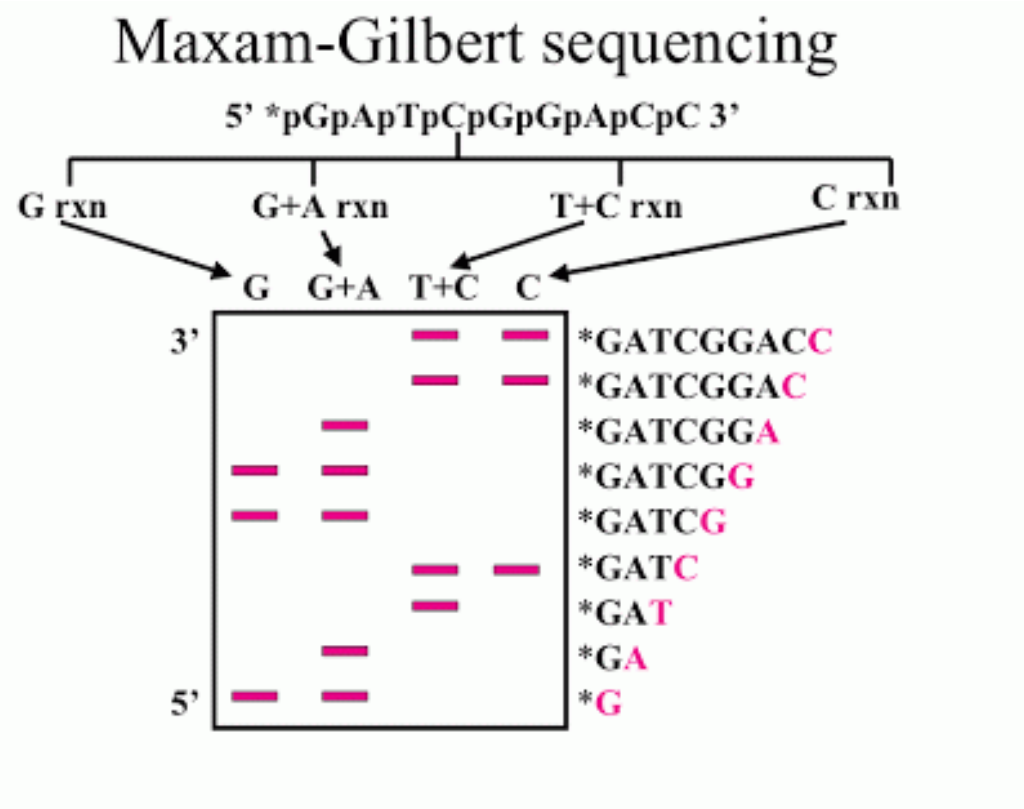
- **Método Sanger** (1977)
  - Método enzimático, dideoxi ou de término da cadeia
  - Síntese enzimática de uma fita complementar de DNA, cujo crescimento é interrompido pela adição de um dideoxinucleotídeo (ddNTP)
  - Usava marcação radioativa, depois fluorescente

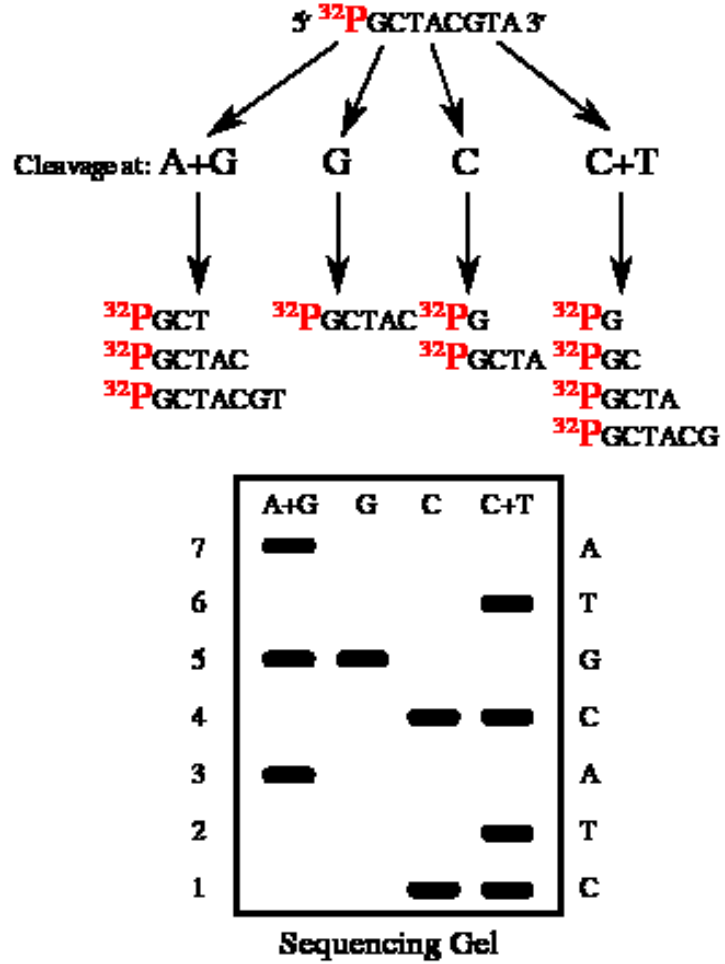
Frederick Sanger

Prêmio Nobel em Química (1980)

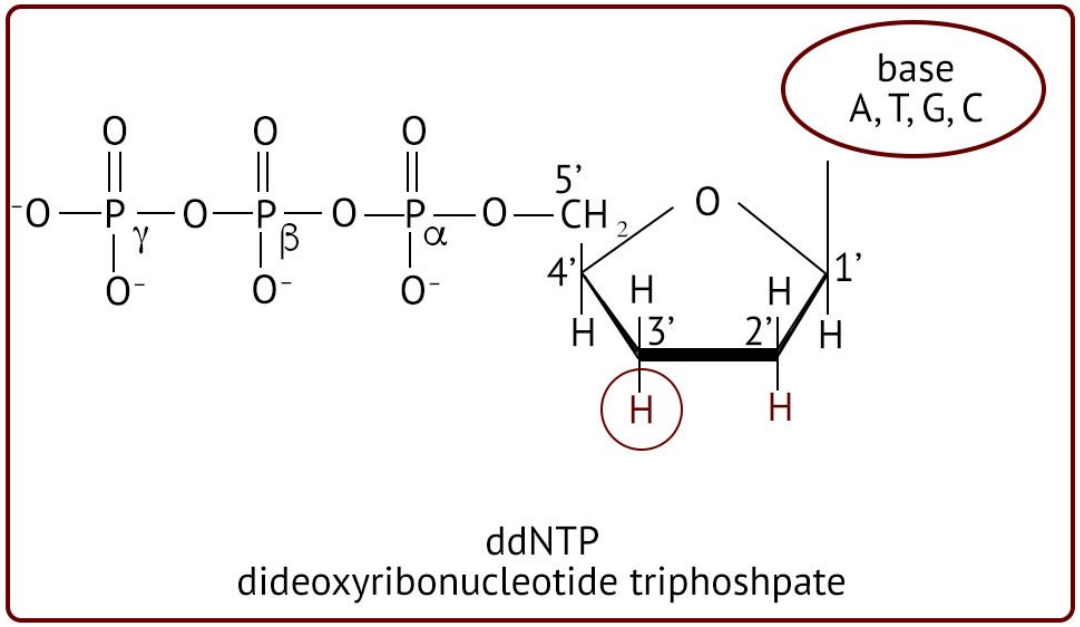
*Produtos tóxicos e perigosos à saúde, além da dificuldade de automatização, essencial para o sequenciamento de um genoma completo.

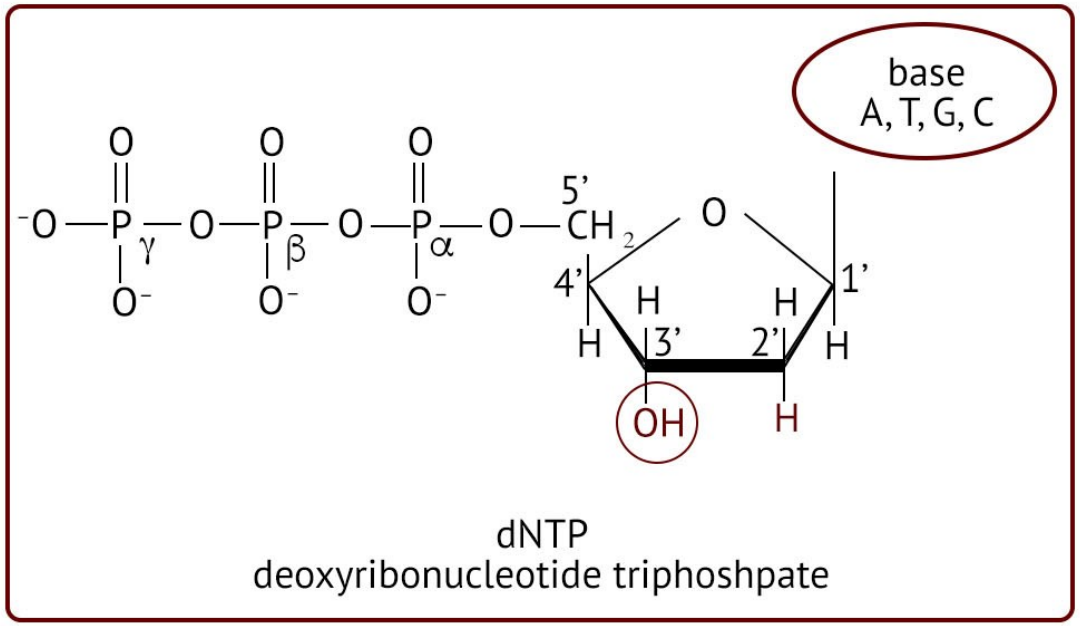
Sequenciamento por Maxam-Gilbert



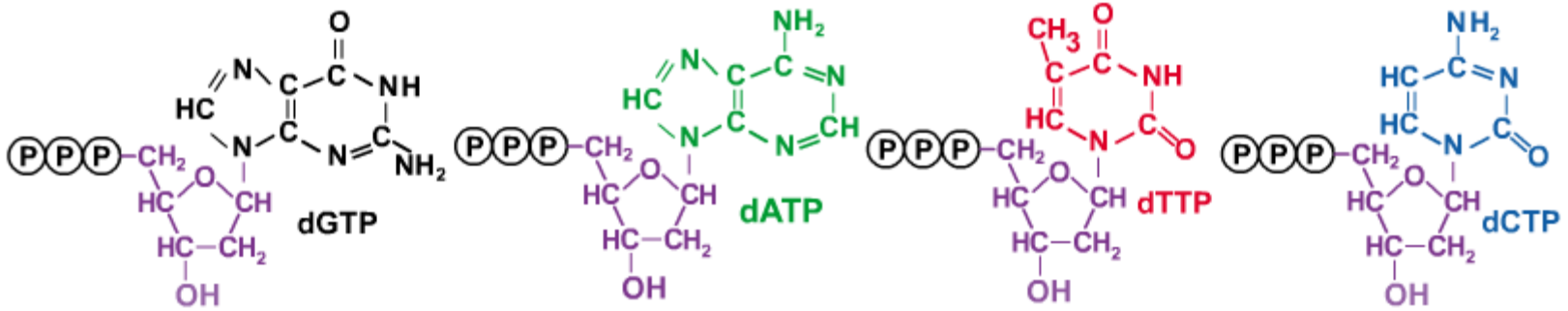


Sequenciamento por Sanger





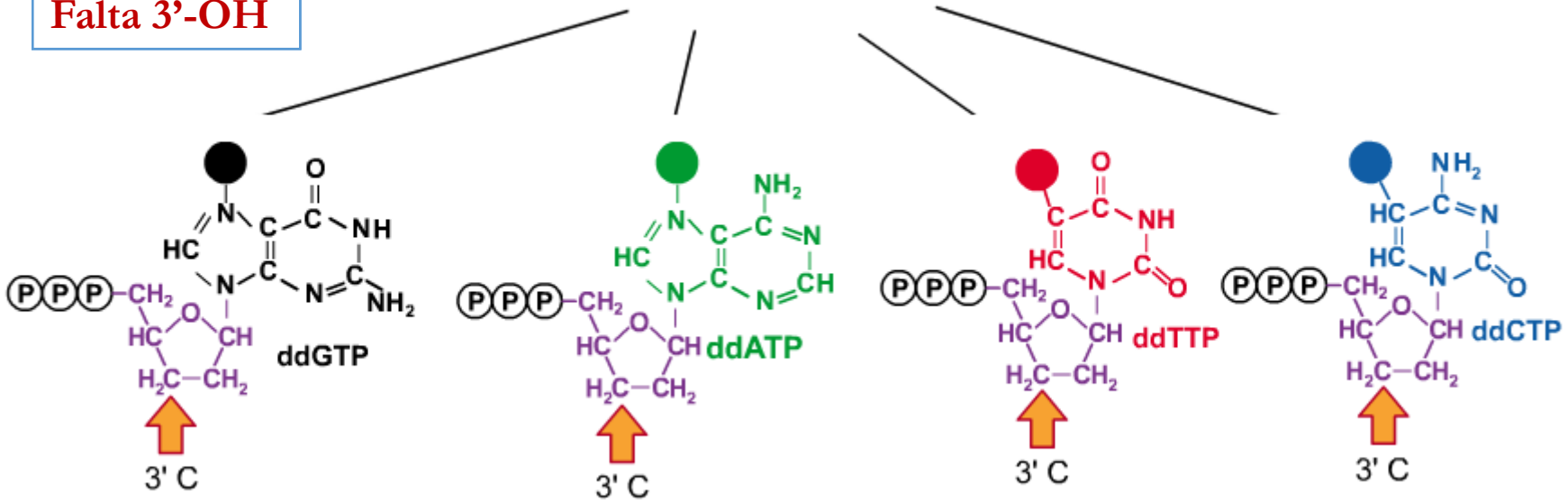
Nucleotídeos dNTPs

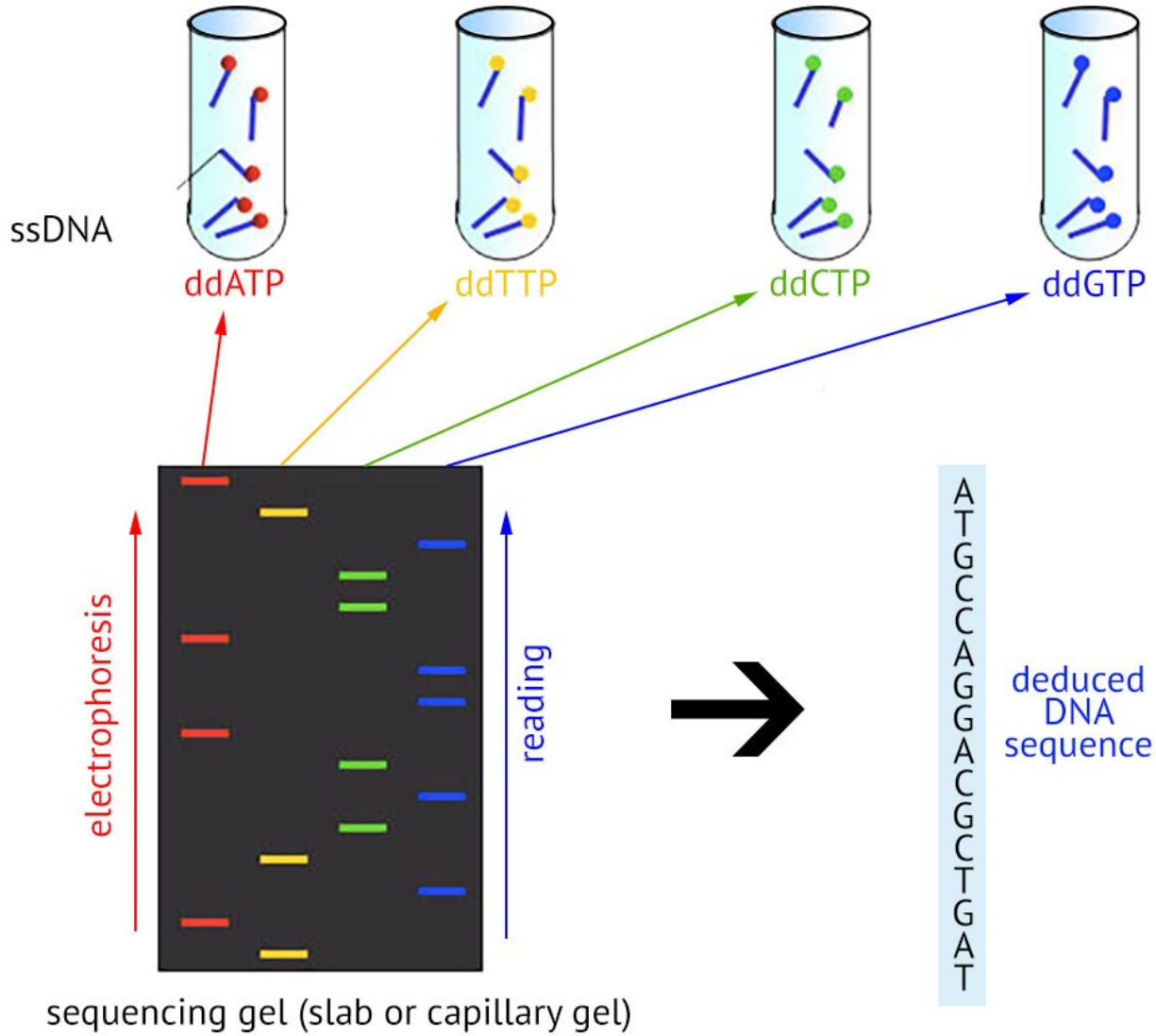


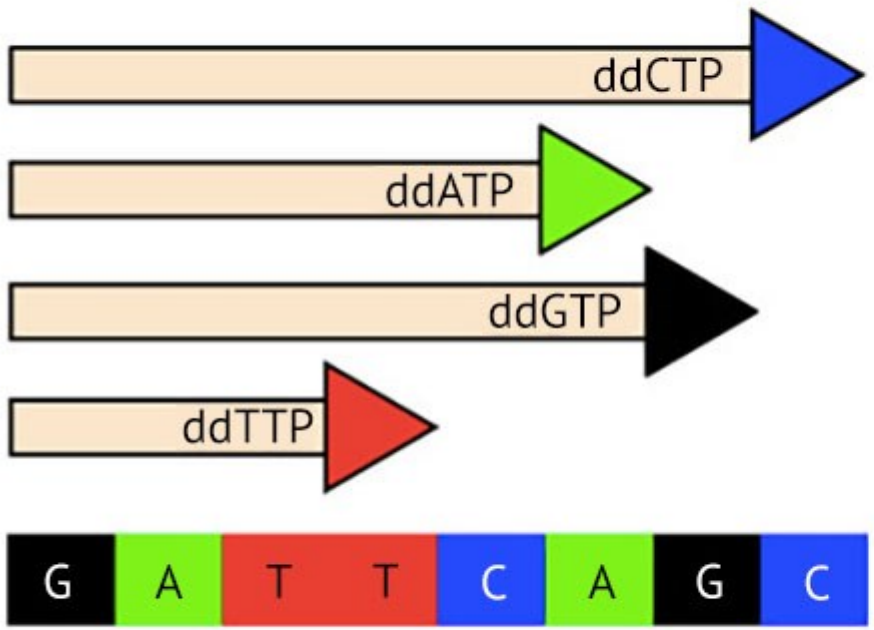
Nucleotídeos ddNTPs (Terminadores)

Corante Fluorescente

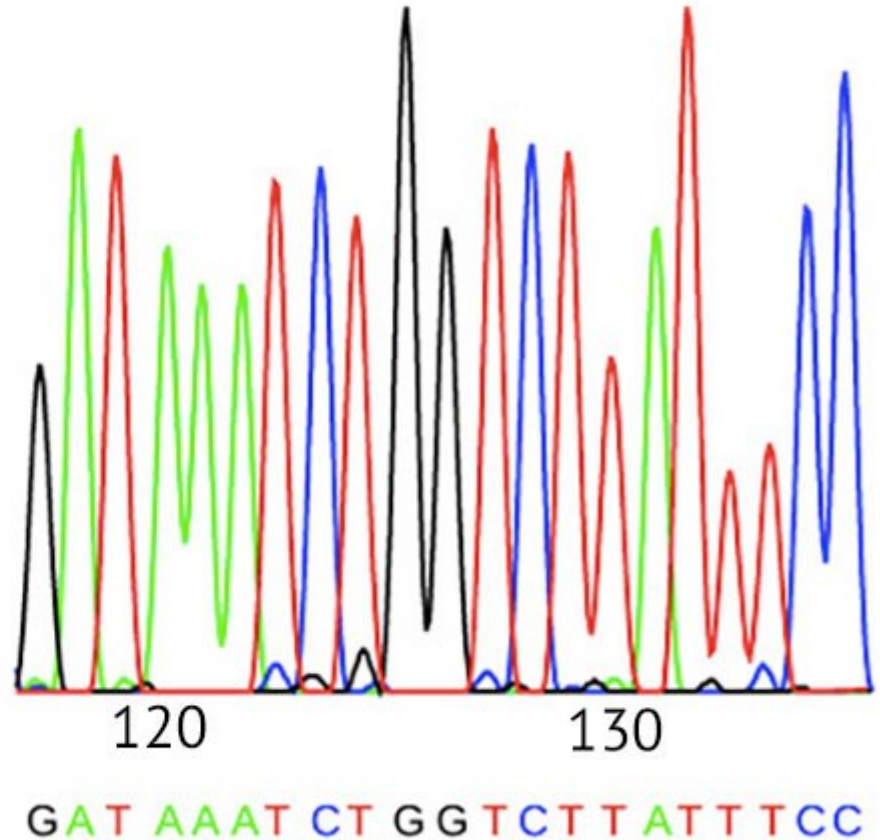
Falta 3'-OH



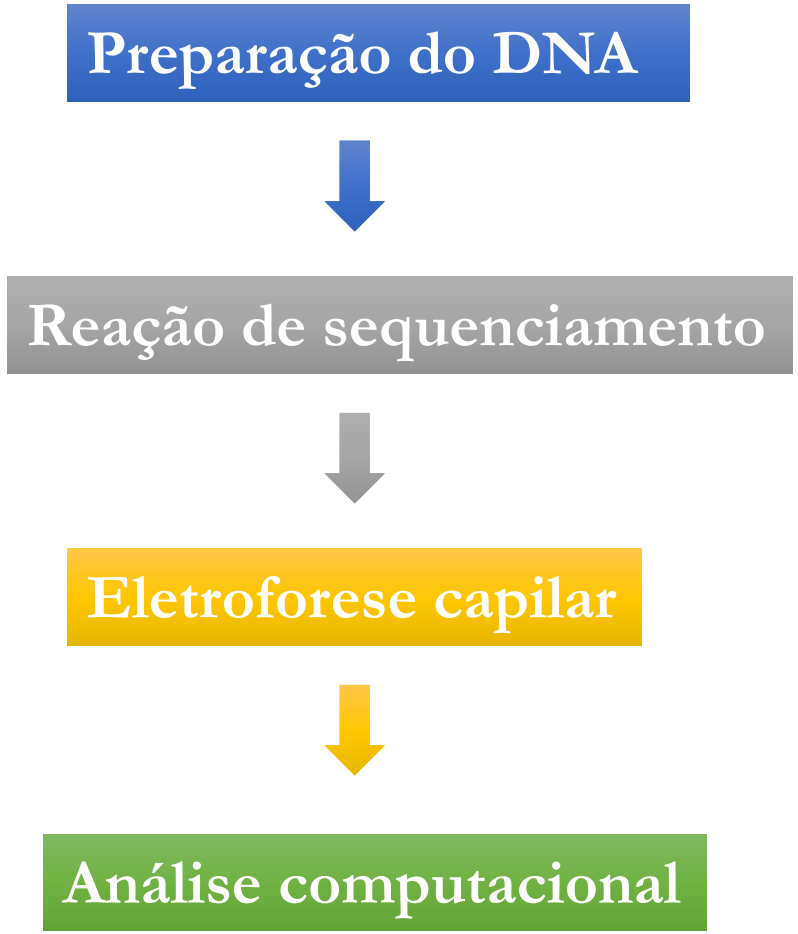

ssDNA
ddATP
ddTTP
ddCTP
ddGTP
electrophoresis
reading
sequencing gel (slab or capillary gel)
ATGCCAGGACGCTGAT
deduced DNA sequence



dye-labeled dideoxynucleotides are used to generate DNA fragments of different lengths

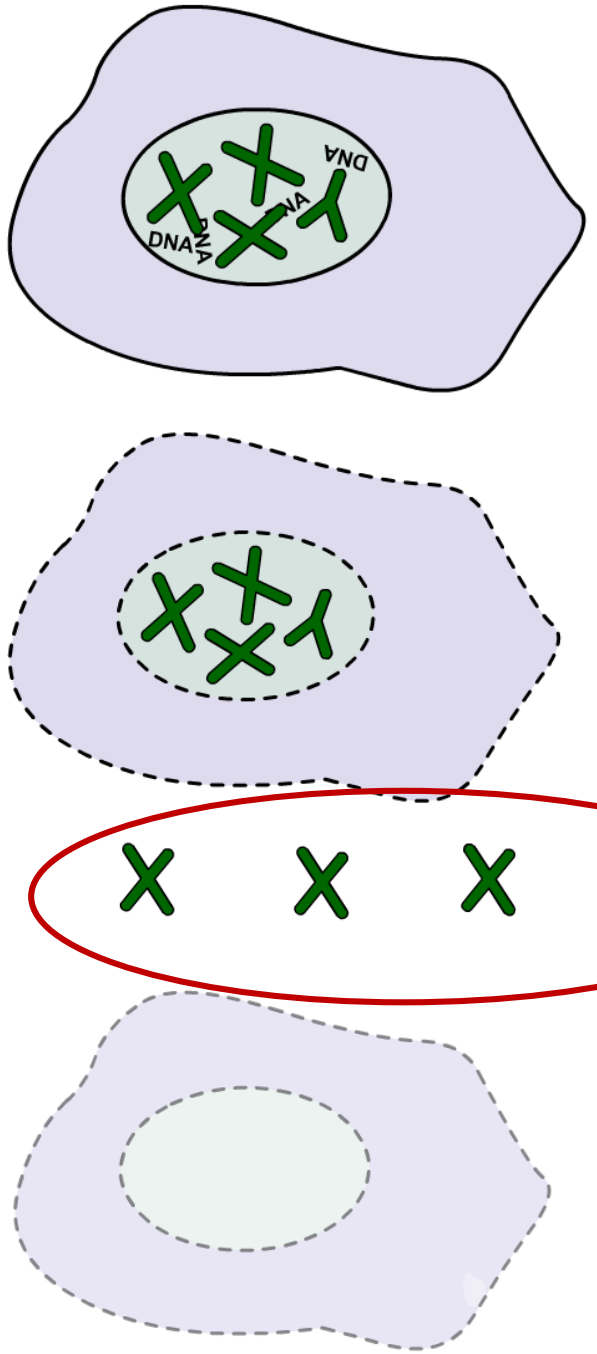


ETAPAS DO SEQUENCIAMENTO DE DNA



https://www.dnalc.org/resources/animations/cycseq.html

Preparação do DNA



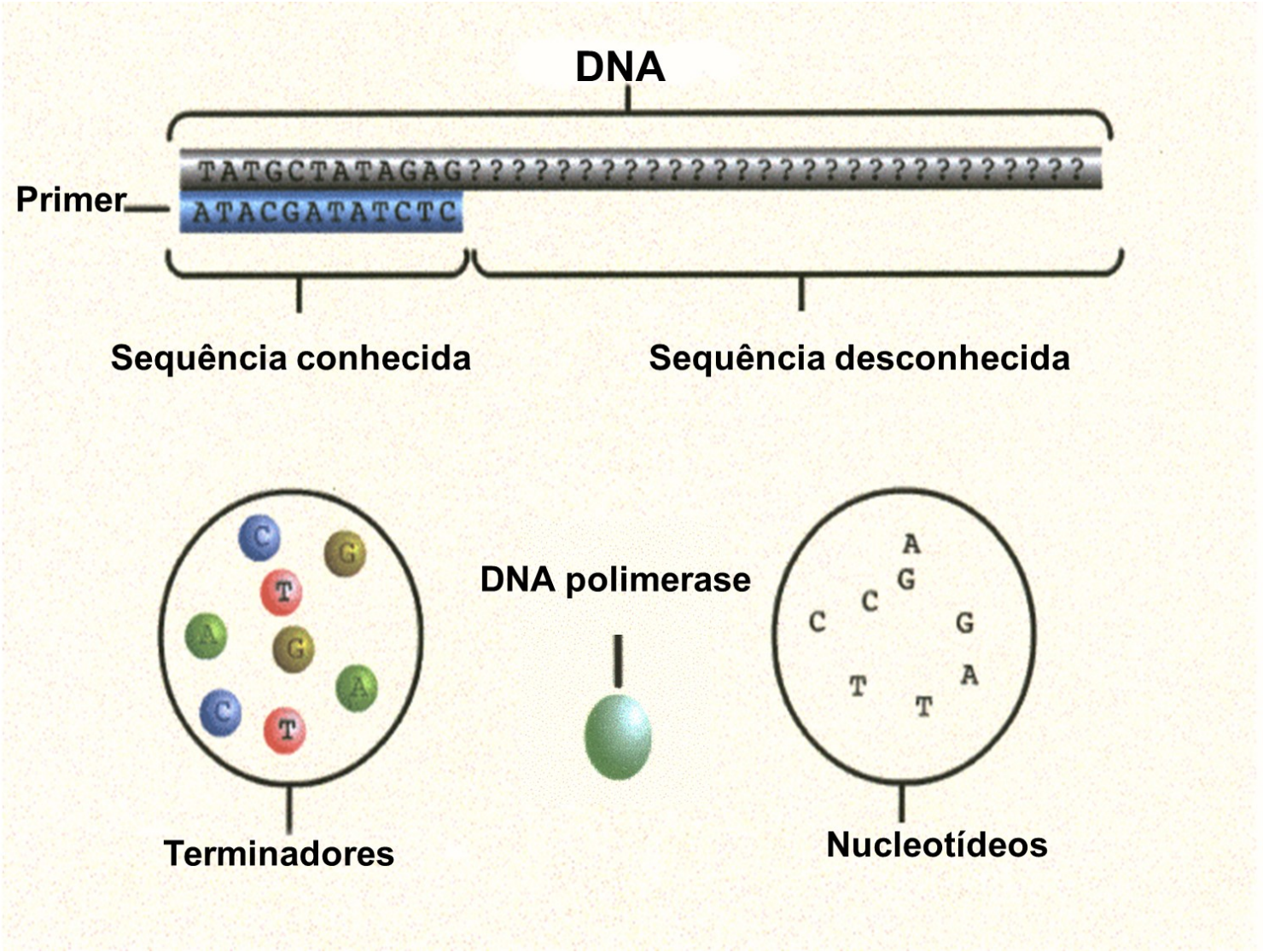
Célula

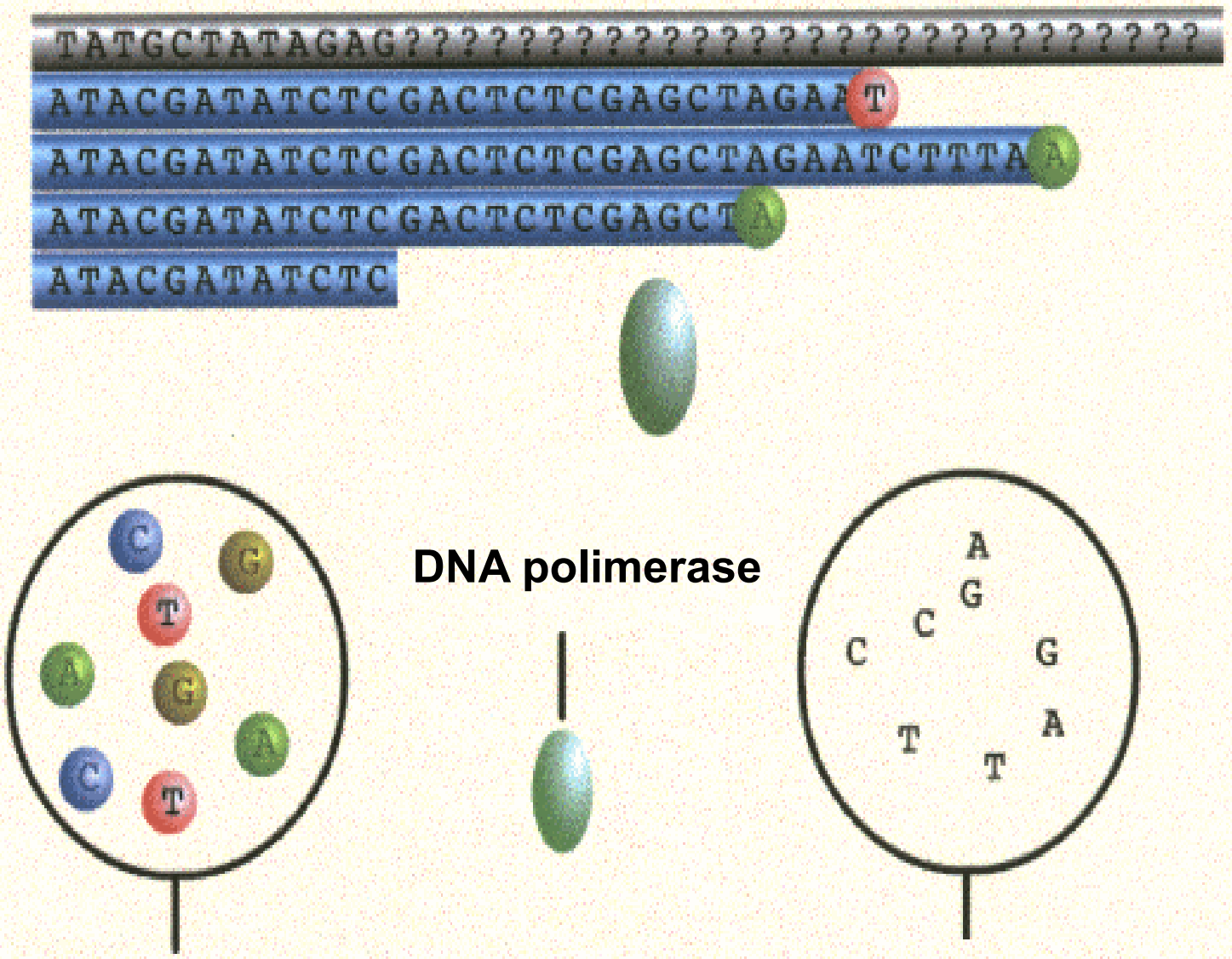
Membrana Plasmática e Parede Celular

Purificação do DNA total

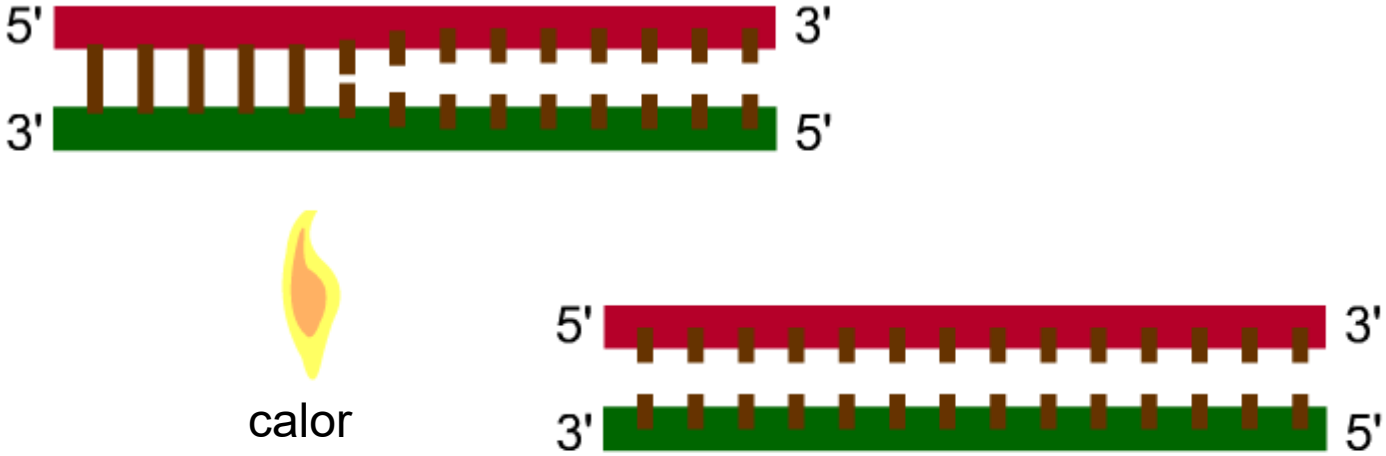
Separação dos restos celulares

Reação de Sequenciamento




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

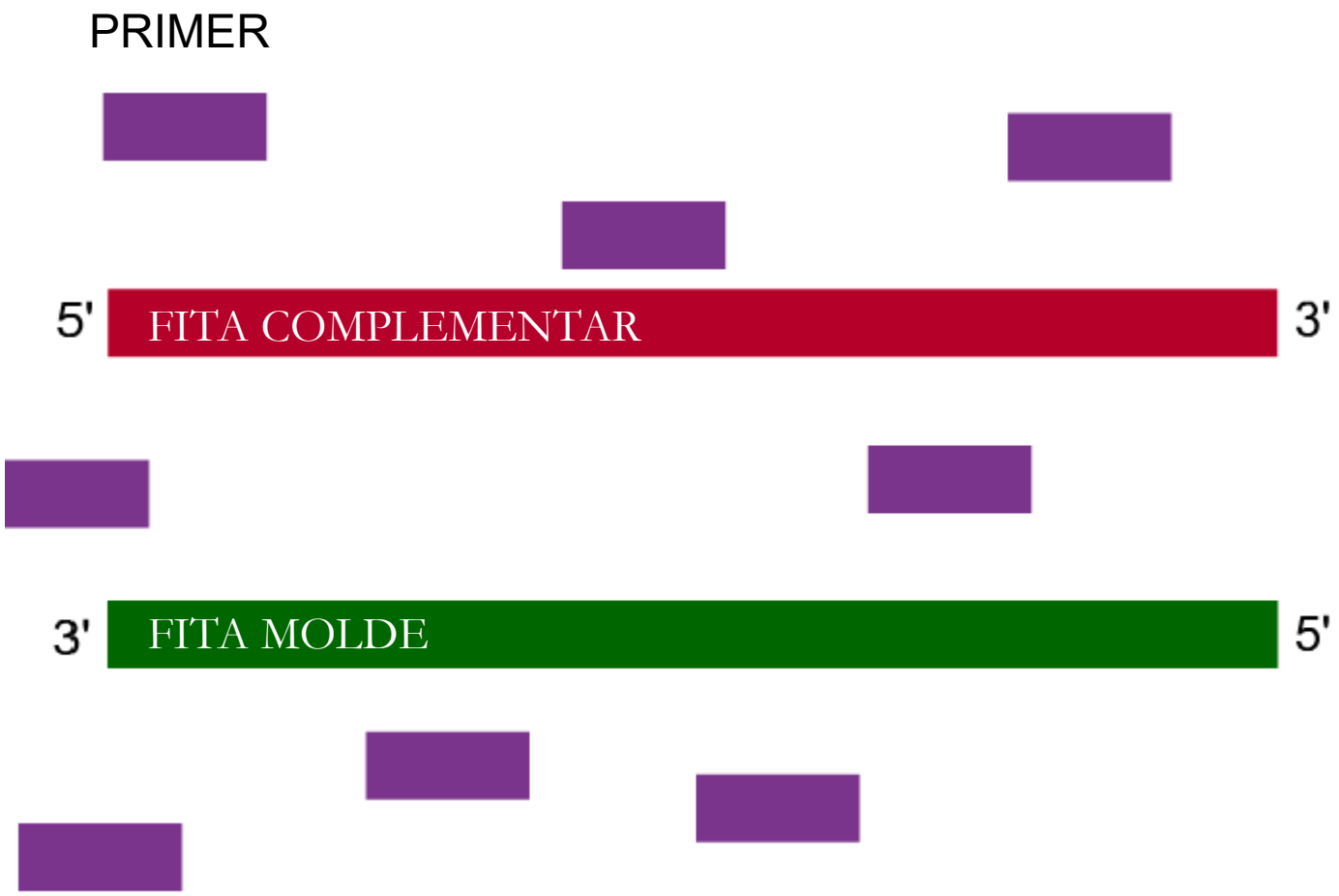
Desnaturação do DNA

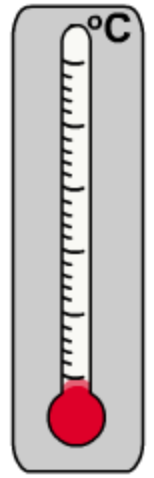


5' FITA COMPLEMENTAR 3'

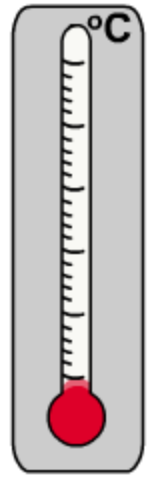
3' FITA MOLDE 5'

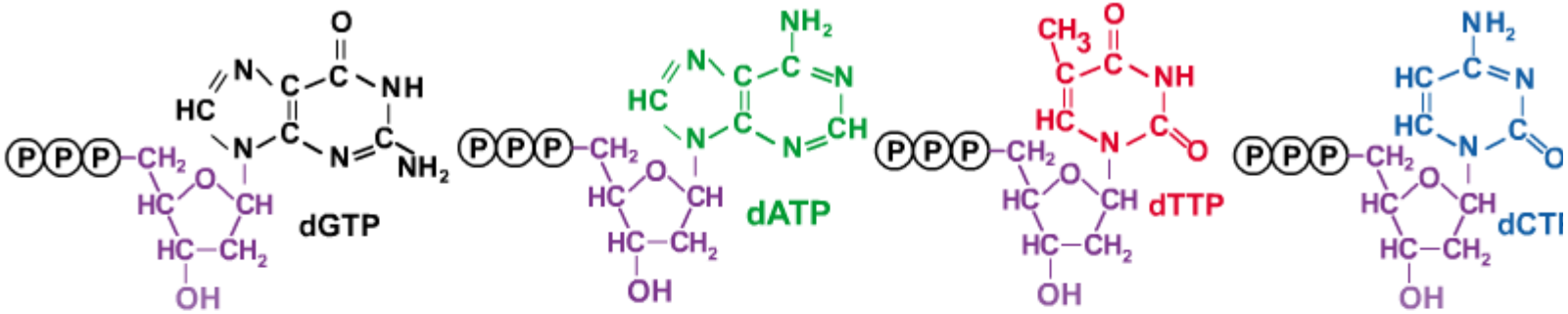
Anelamento dos *Primers*

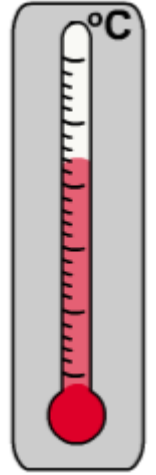




Anelamento dos *Primers*

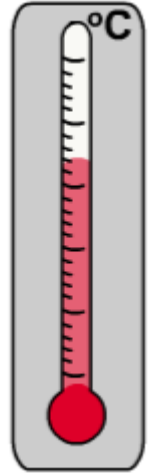






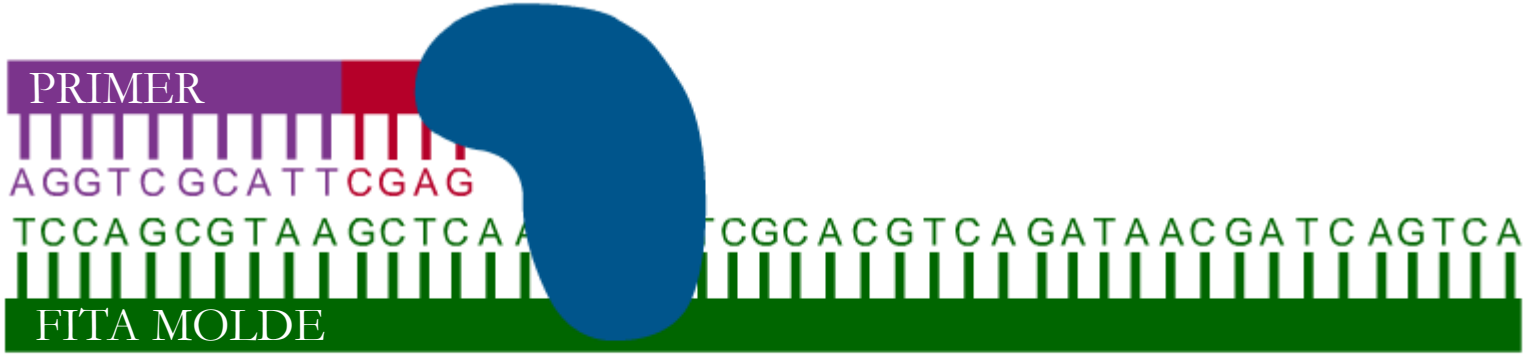
DNA POLIMERASE

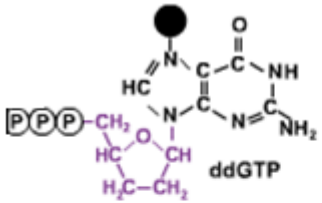
Polimerização

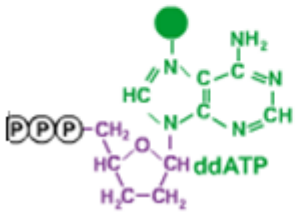


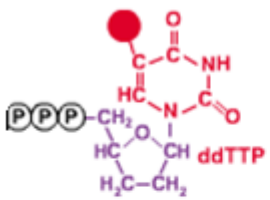
DNA POLIMERASE

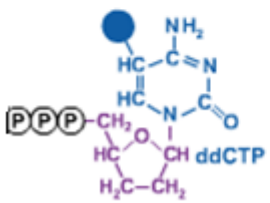
DNA POLIMERASE

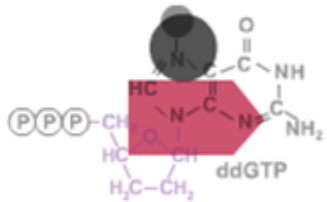


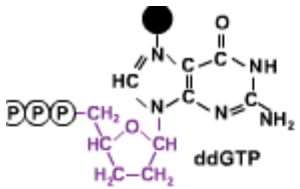

ddGTP

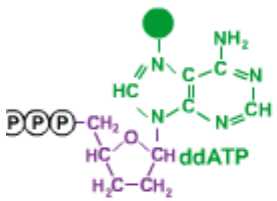

ddATP


ddTTP


ddCTP

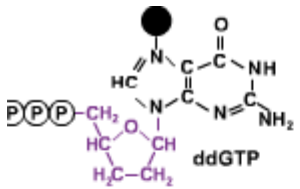

ddGTP


ddGTP


ddATP


ddTTP


ddCTP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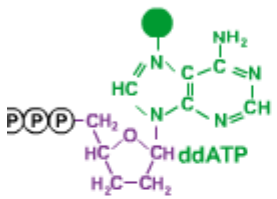
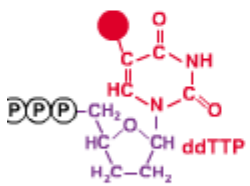

ddATP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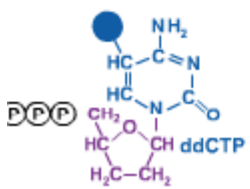
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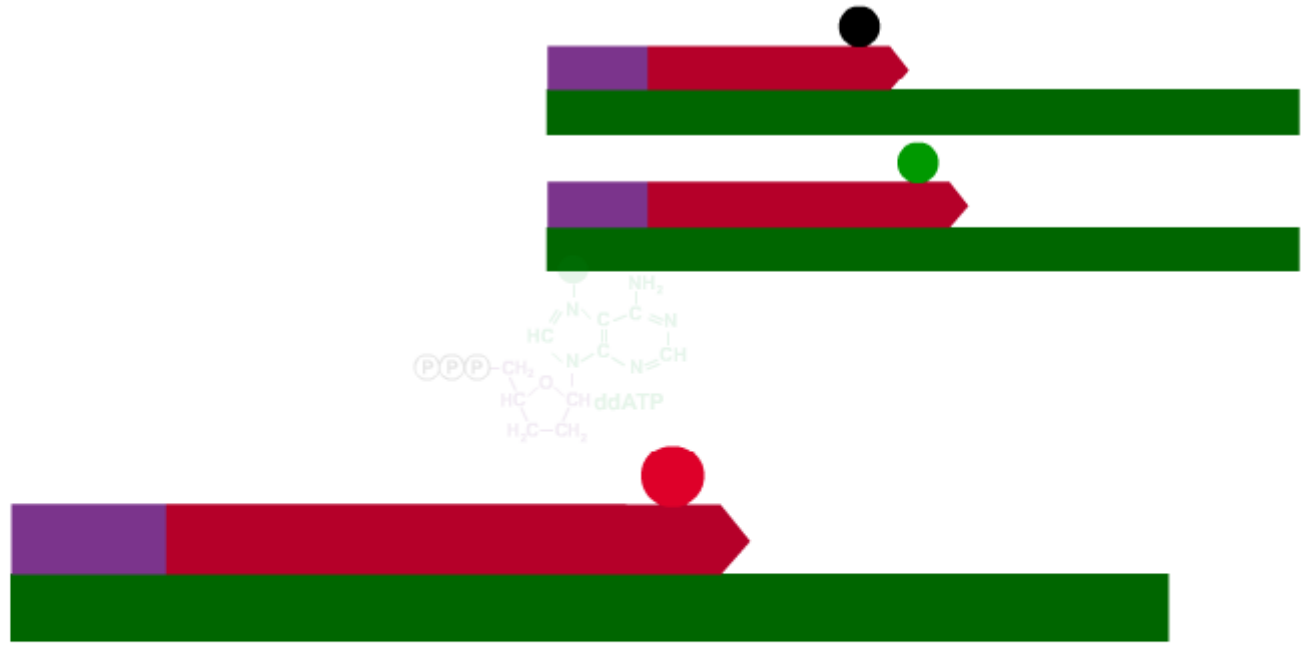

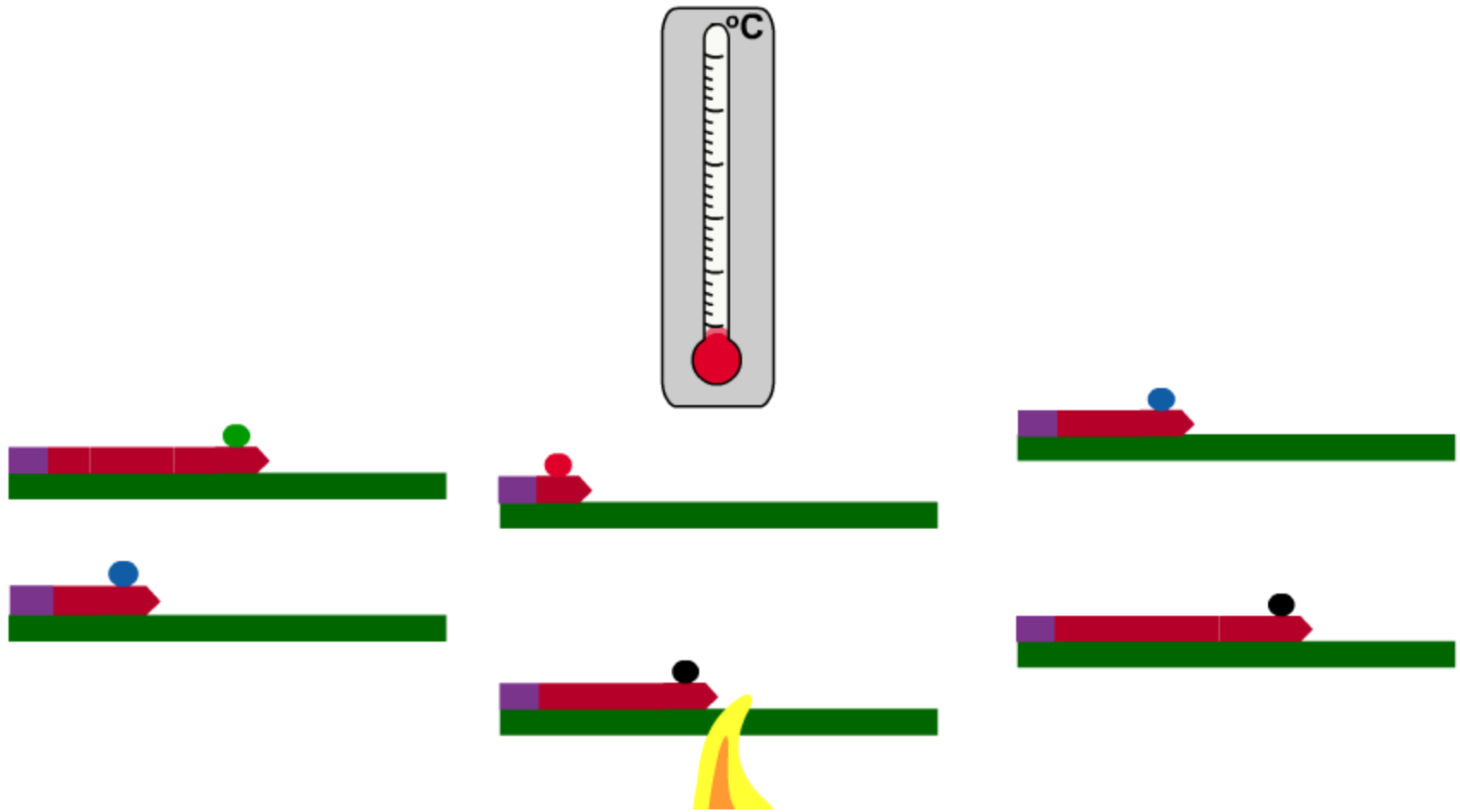
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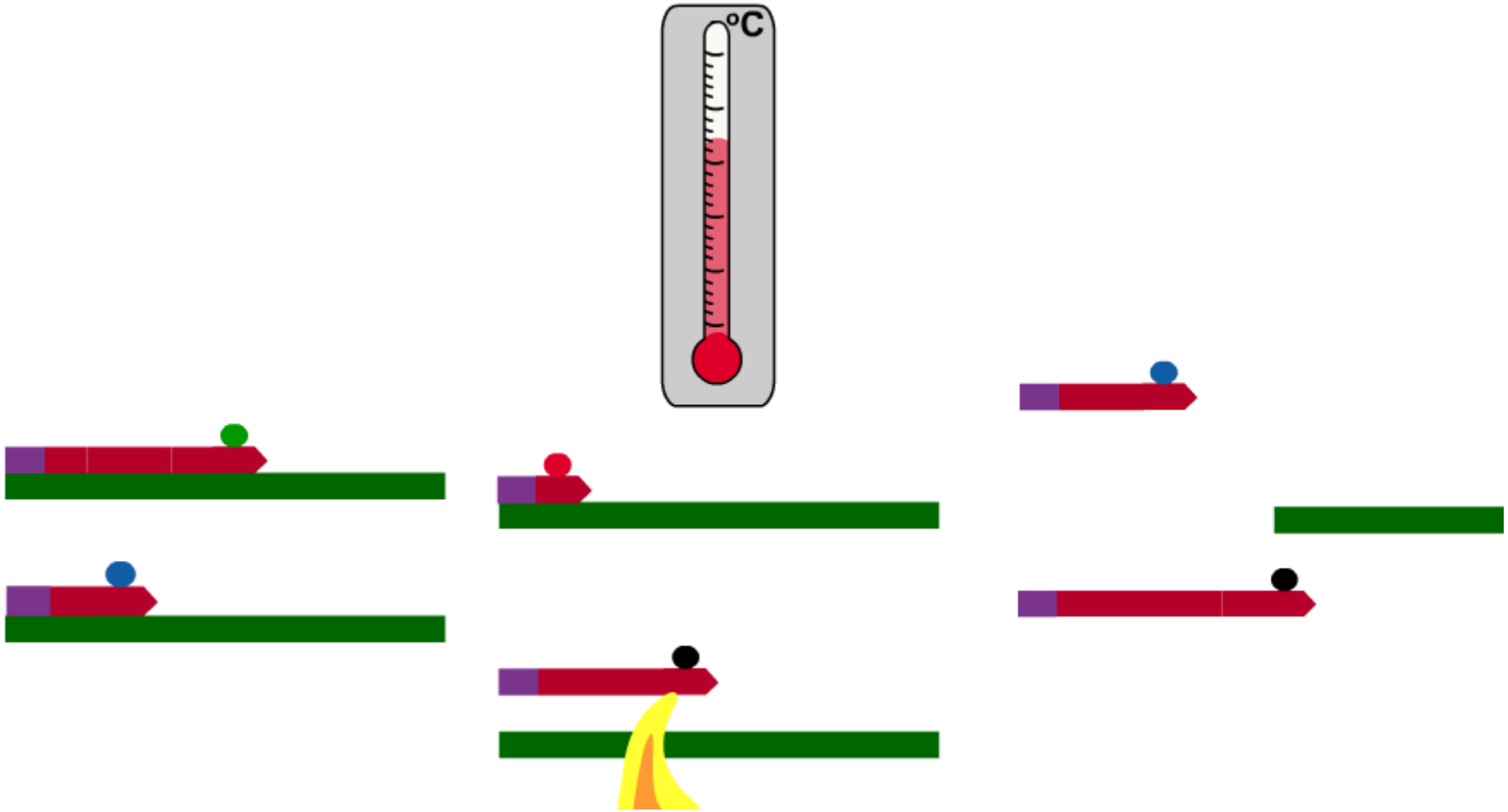

°C





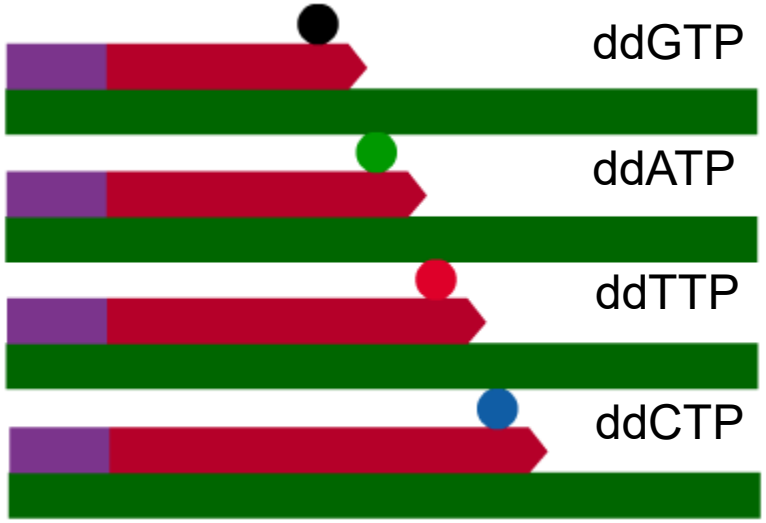


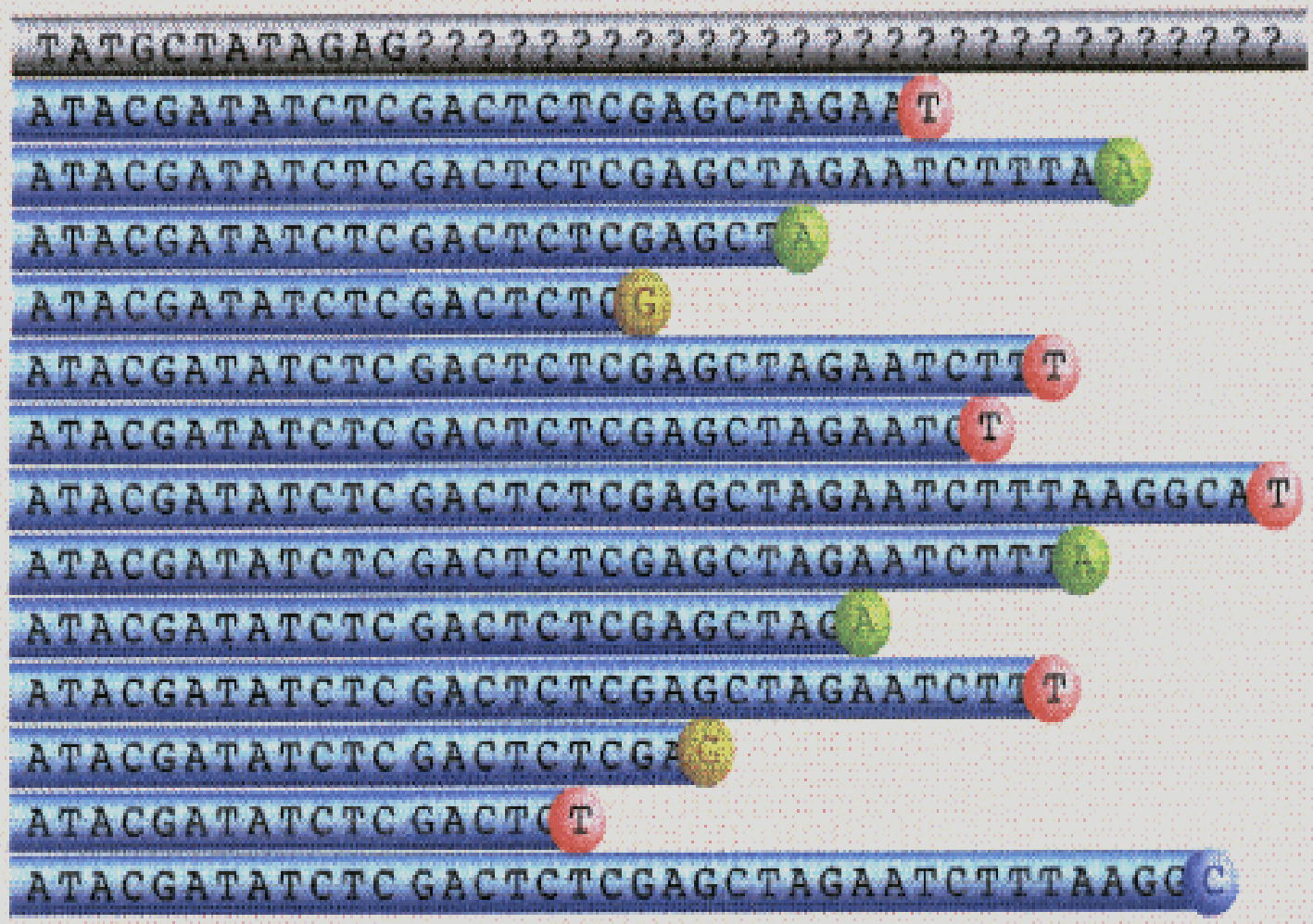


Terminação


ddGTP
ddATP


ddTTP
PRIMER
FITA MOLDE


ddGTP
ddATP
ddTTP
ddCTP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ATACGATATCTCGACTCTCGAGCT
ATACGATATCTCGACTCTC
ATACGATATCTCGACTCTCGAGCTAGAATCTT
ATACGATATCTCGACTCTCGAGCTAGAATC
ATACGATATCTCGACTCTCGAGCTAGAATCTTTAAGGCA
ATACGATATCTCGACTCTCGAGCTAGAATCTT
ATACGATATCTCGACTCTCGAGCTAC
ATACGATATCTCGACTCTCGAGCTAGAATCTT
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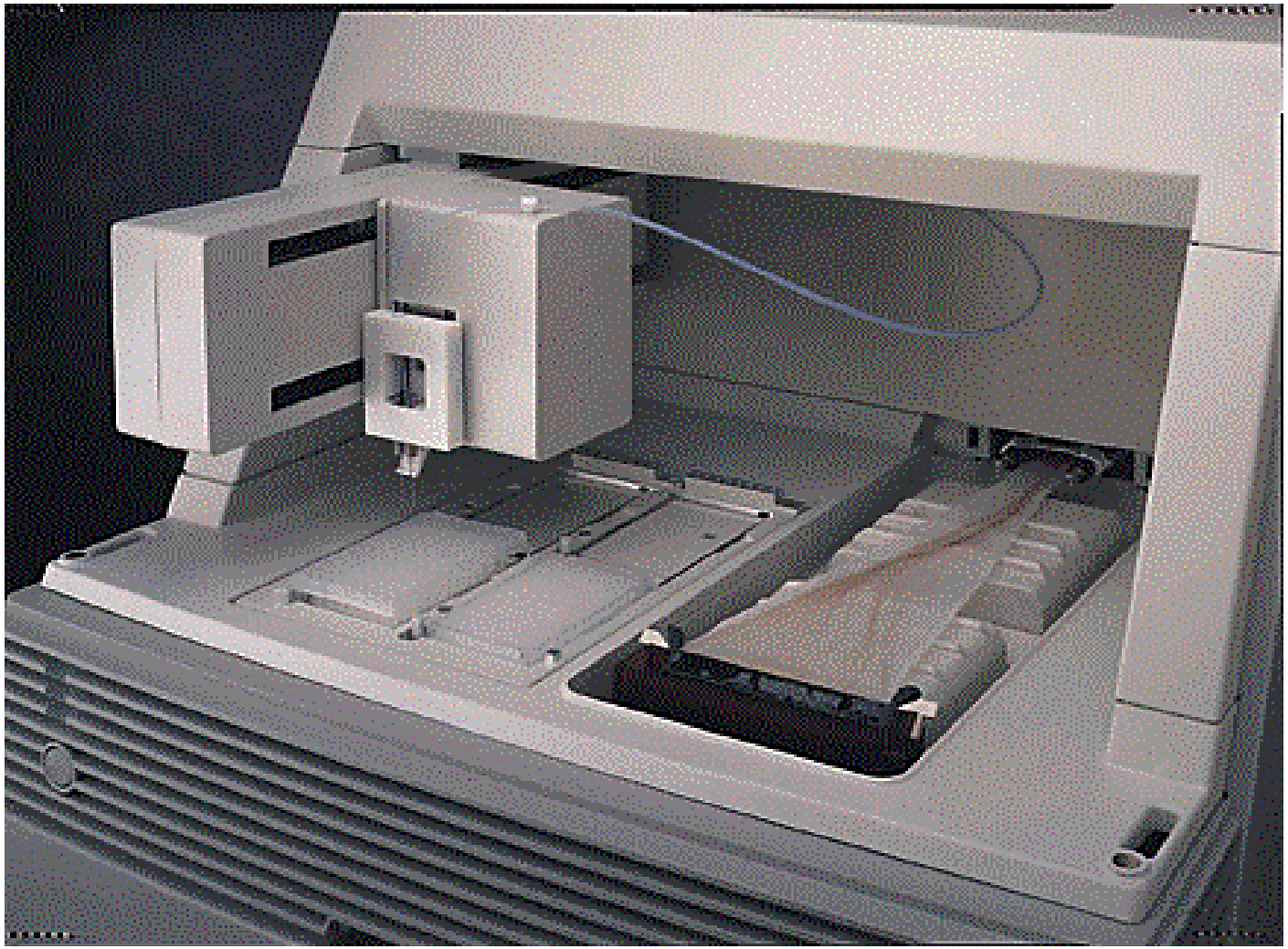
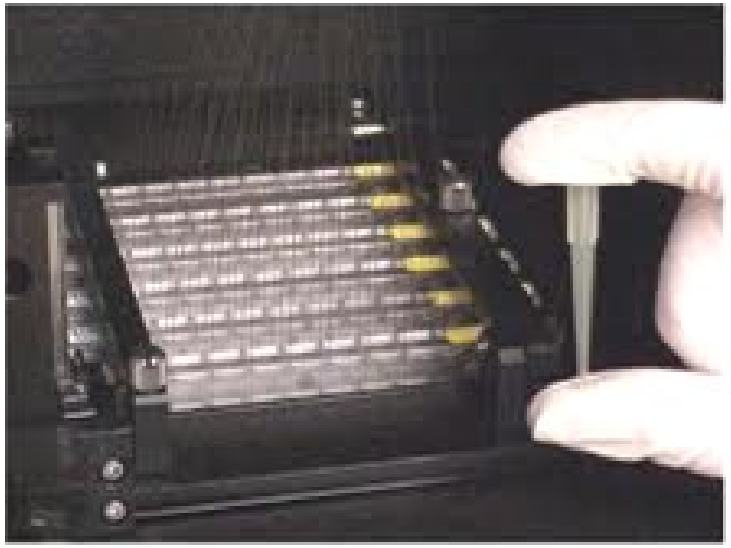
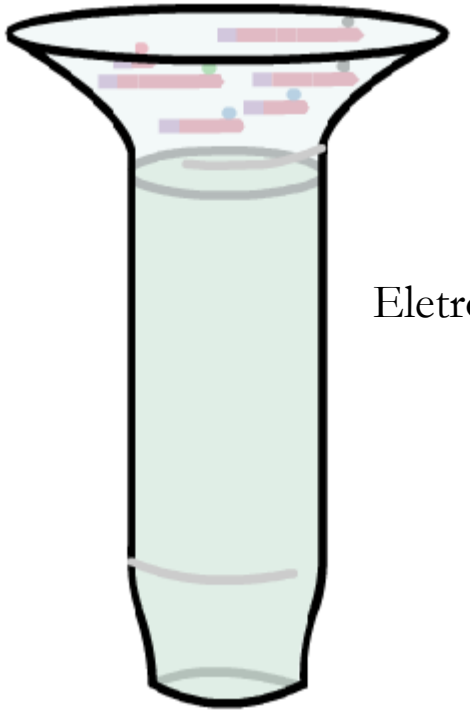
Eletroforese Capilar

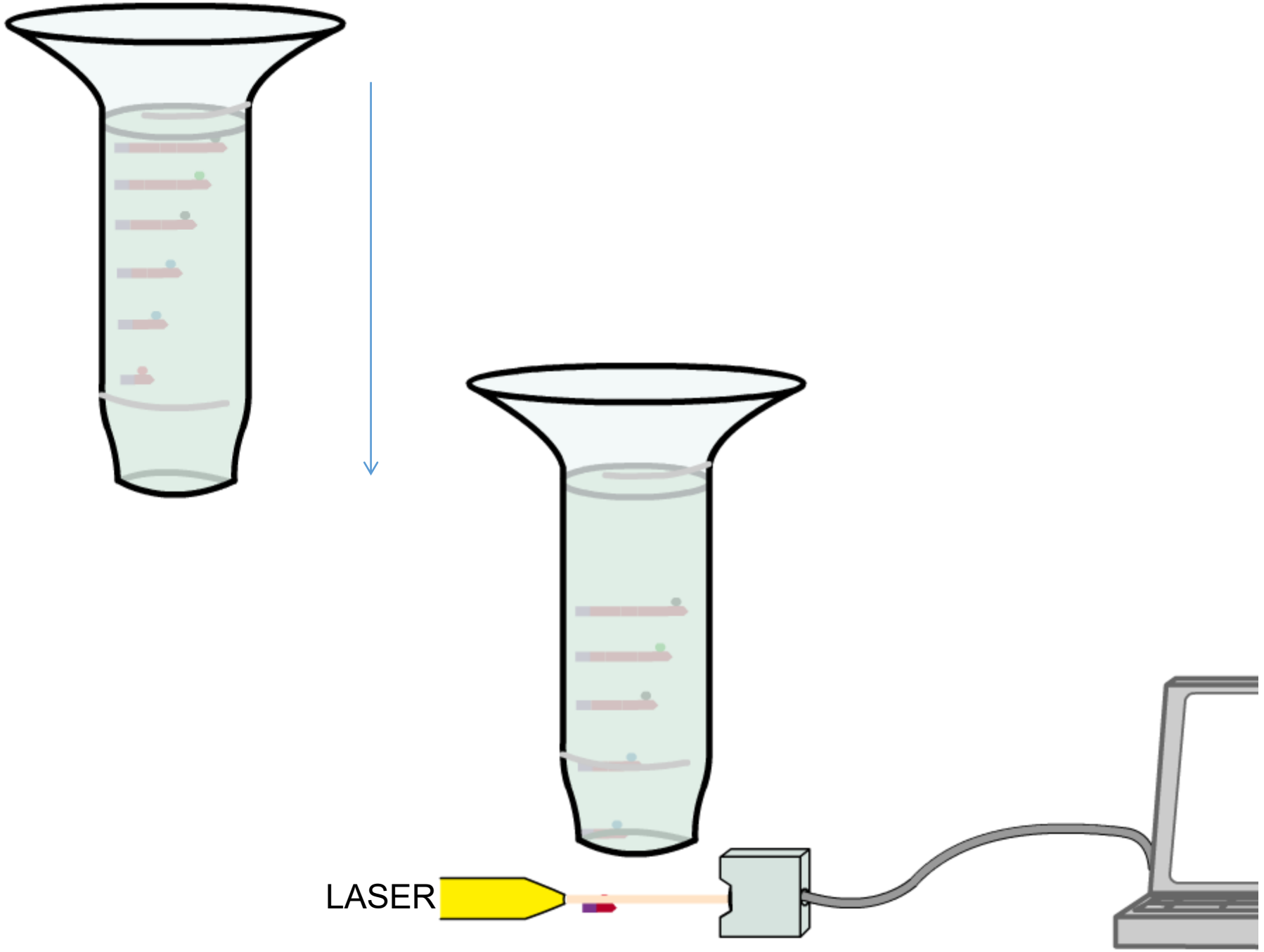


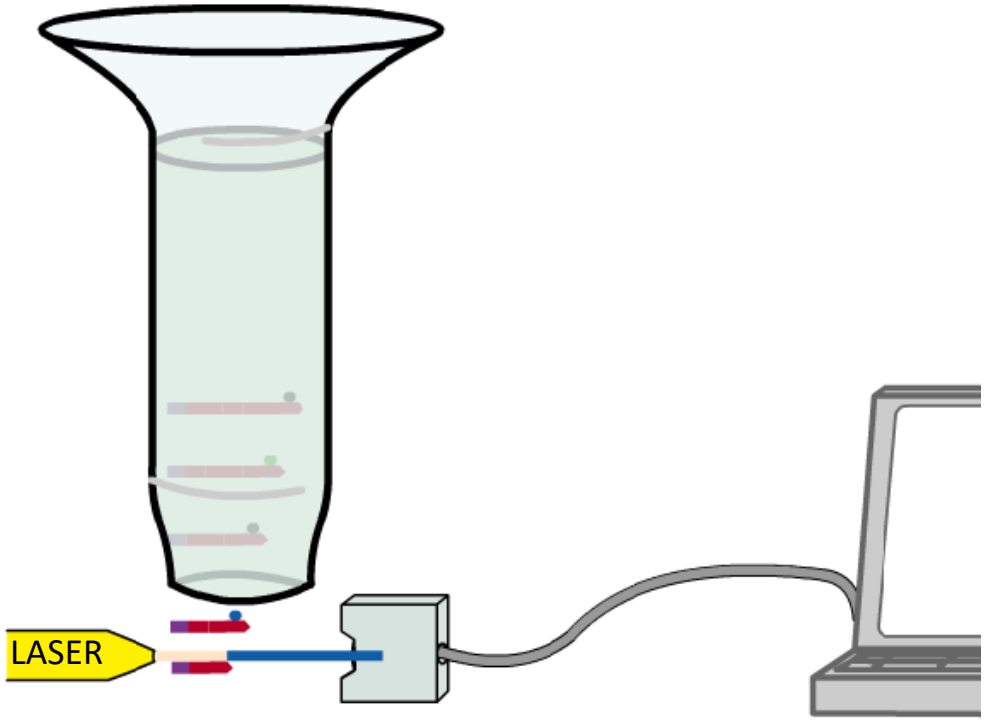
ABI 3700 Applied Biosystems

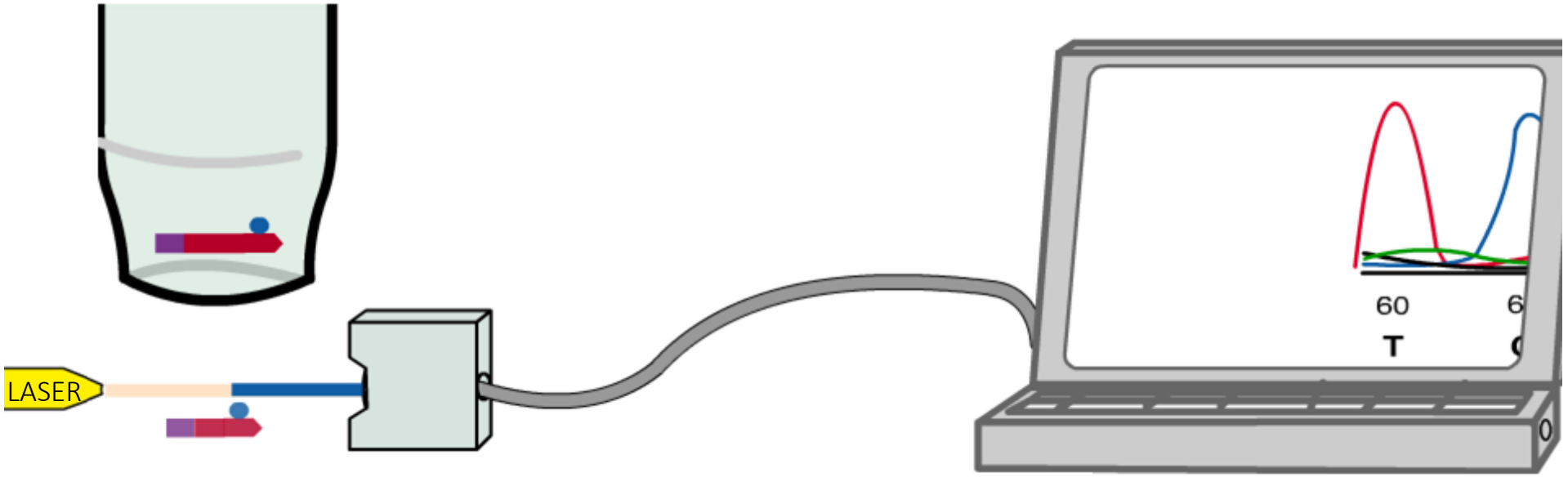




Eletroforese capilar


LASER


LASER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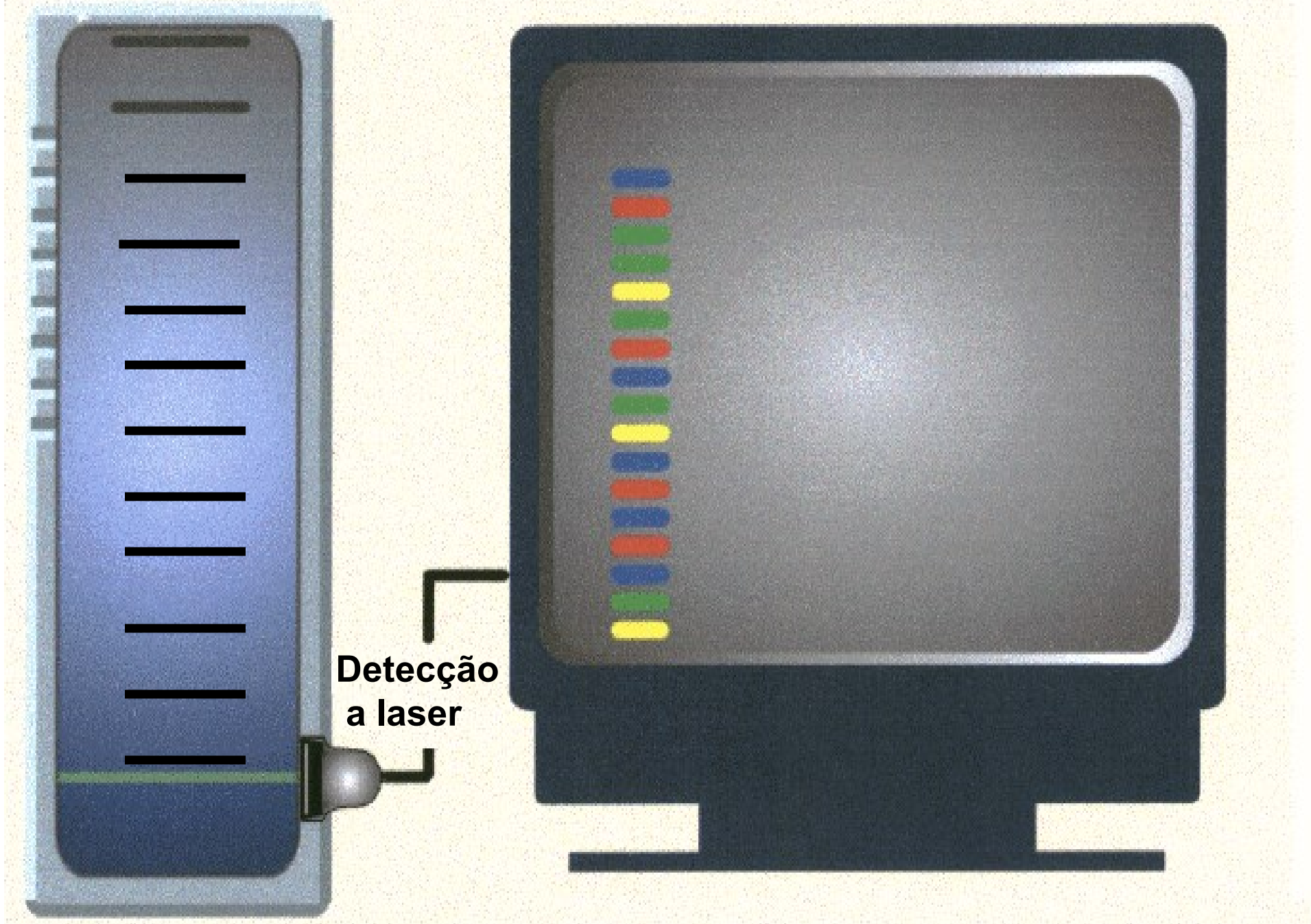
Detecção
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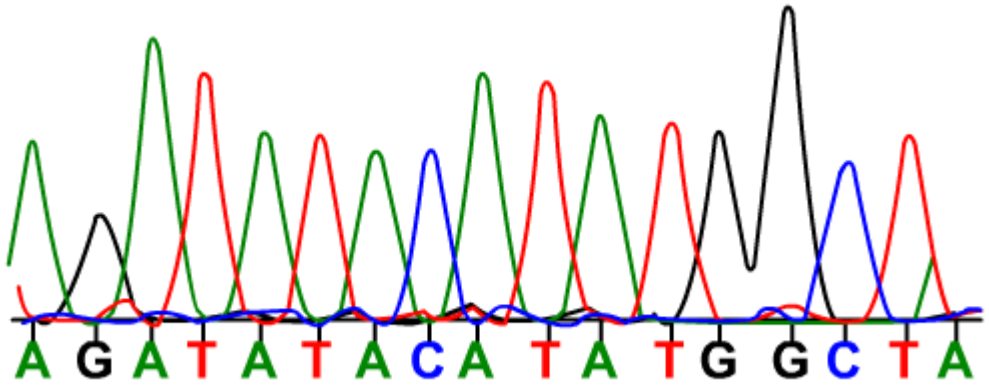
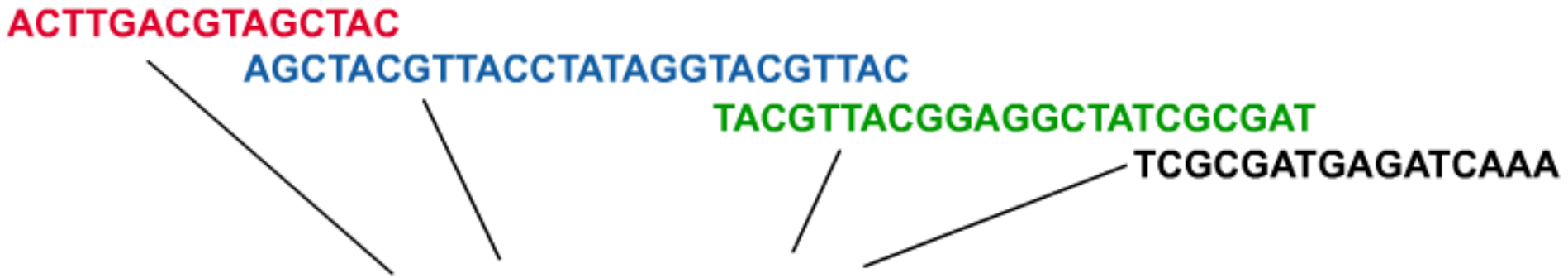
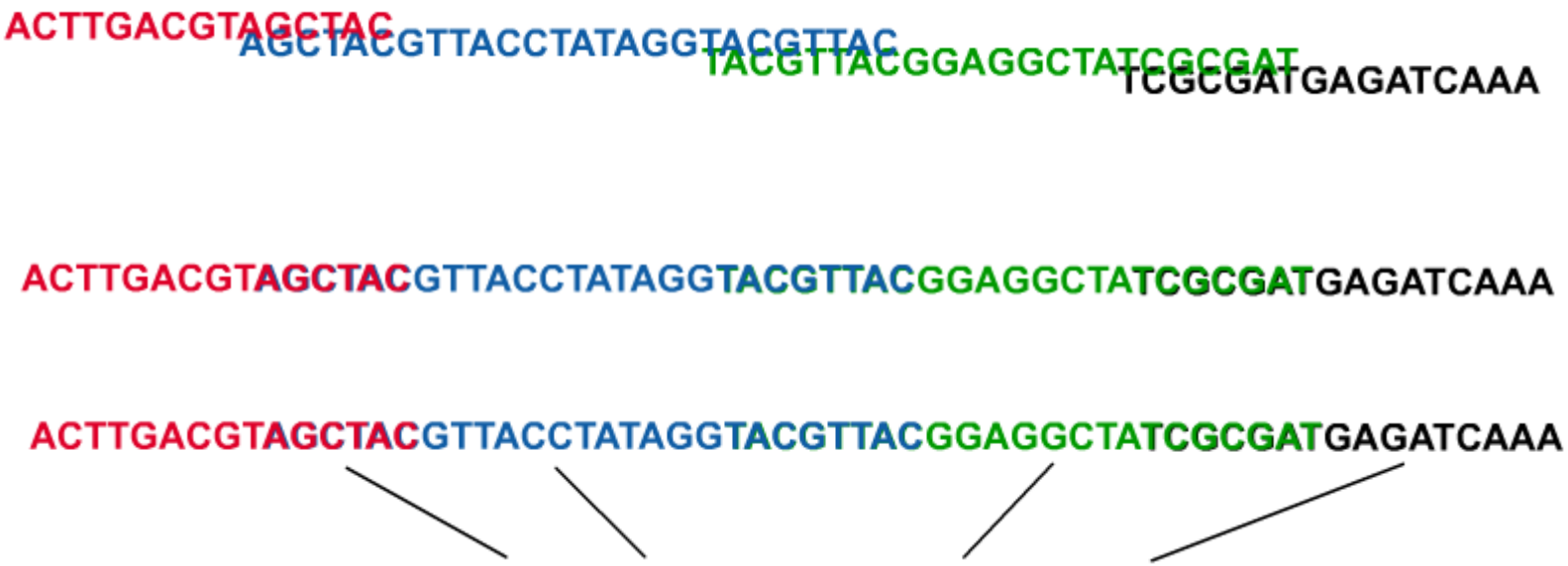
Análise Computacional



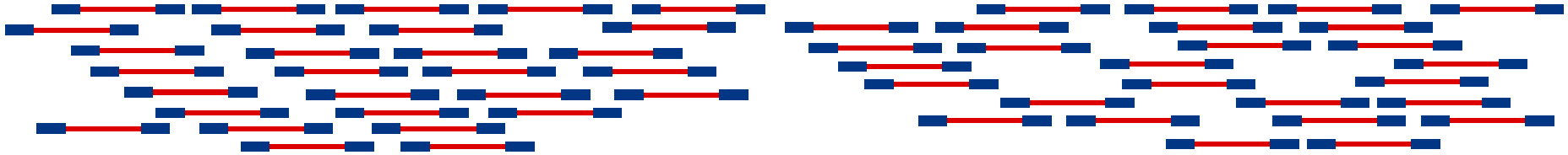


Fragmentos de DNA sequenciados



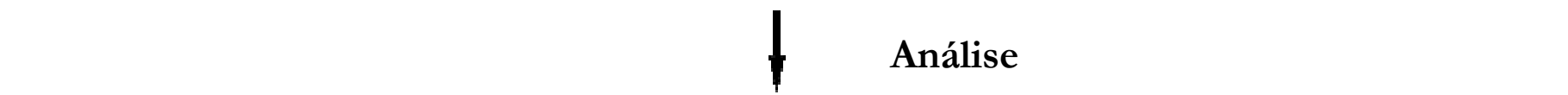
Fragmentos Completos

Biblioteca de insertos pequenos

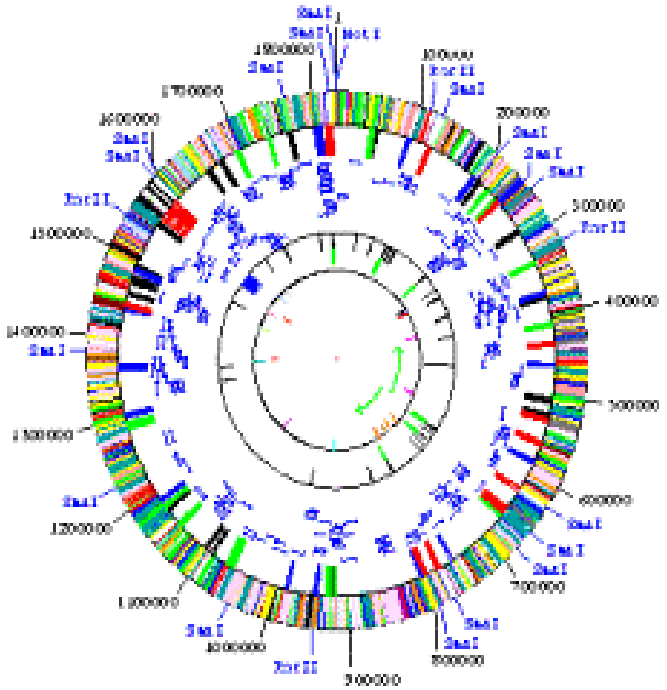


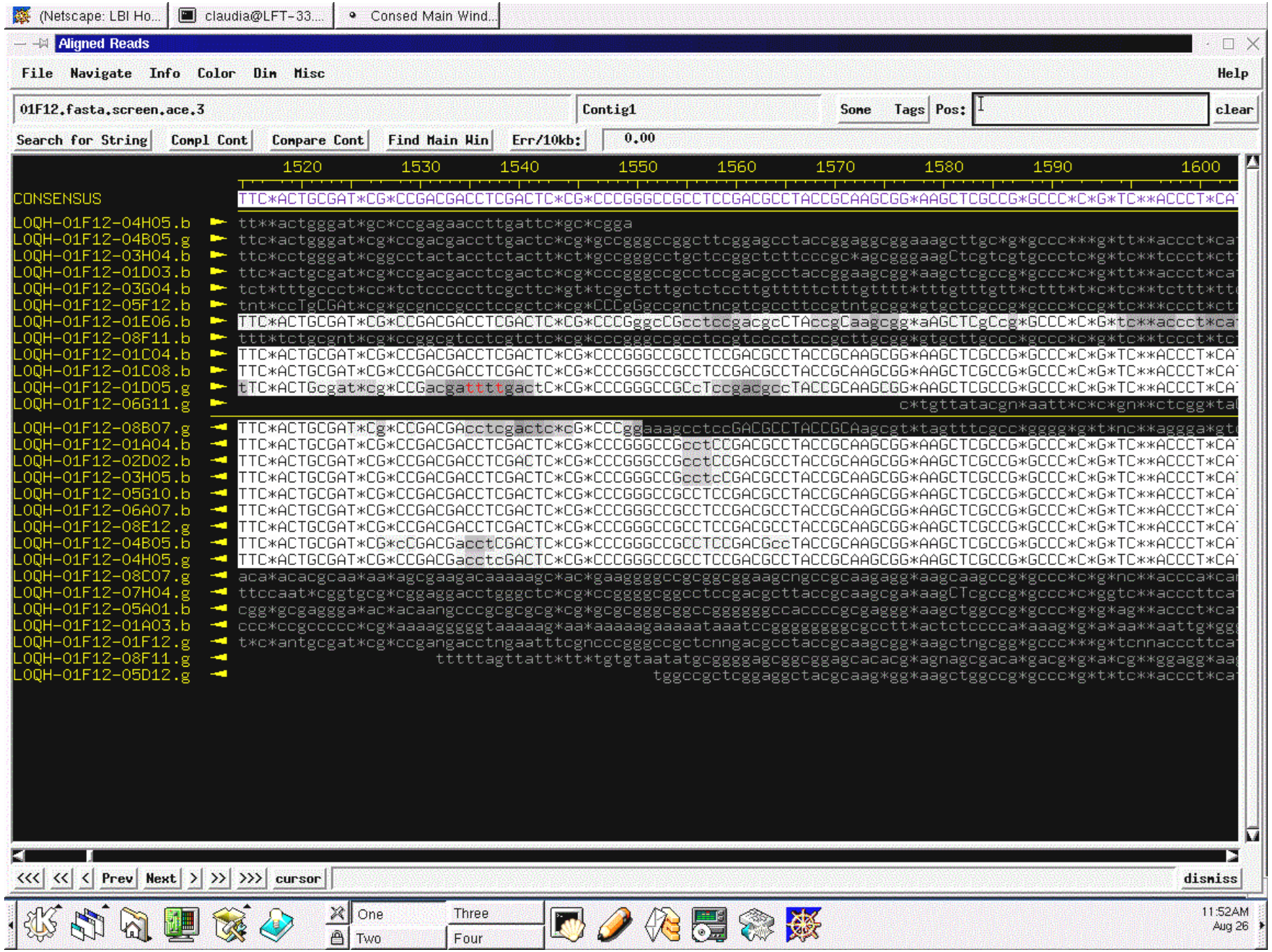
Montagem dos fragmentos

Fechamento dos gaps



Análise

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CONSENSUS
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LOQH-01F12-04B05.g
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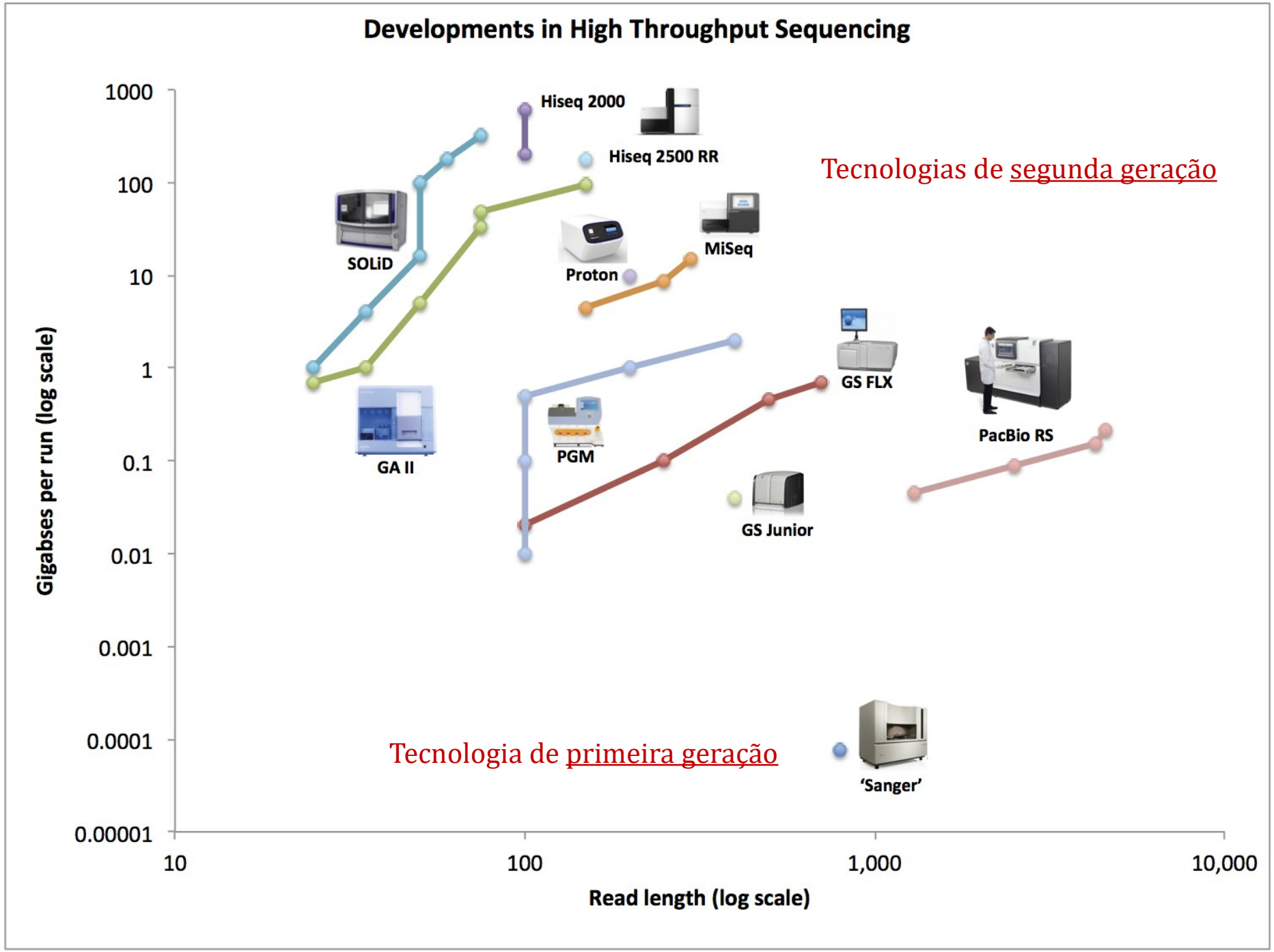
Developments in High Throughput Sequencing
Hiseq 2000
Hiseq 2500 RR
SOLiD
Proton
MiSeq
GS FLX
PacBio RS
GA II
PGM
GS Junior
'Sanger'
Tecnologias de segunda geração
Tecnologia de primeira geração
Gigabses per run (log scale)
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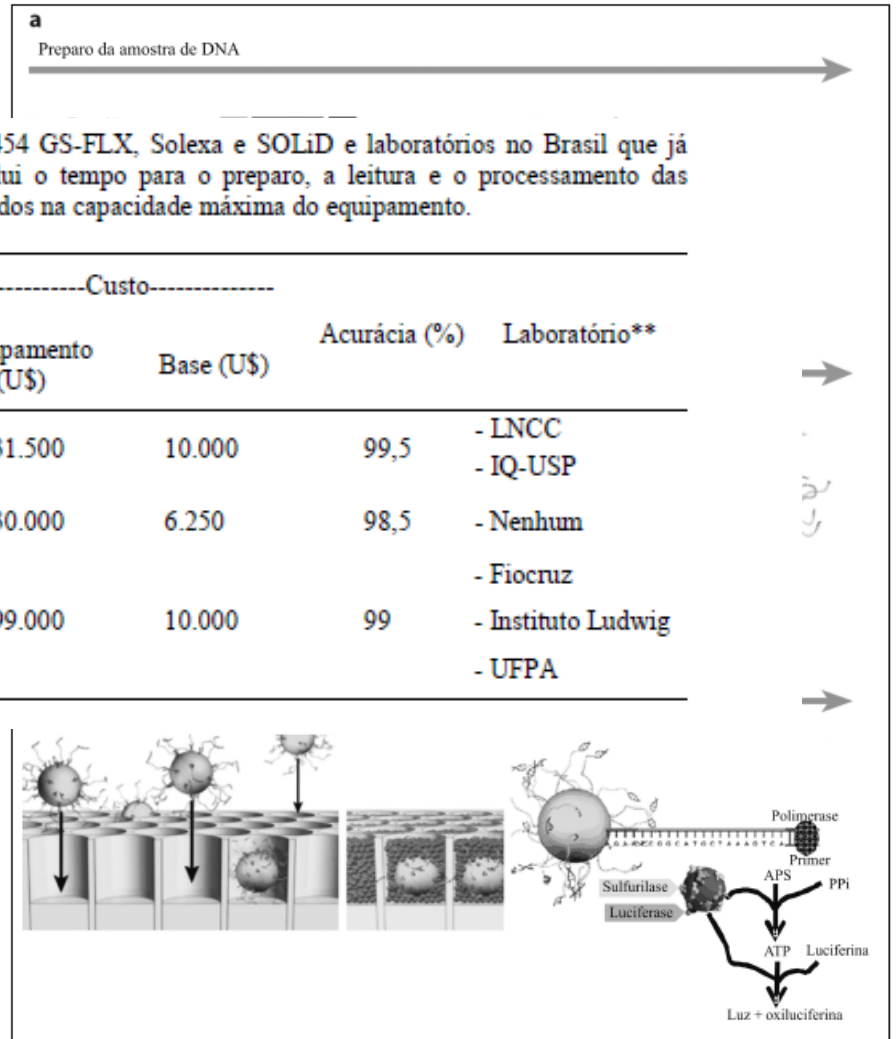
1
0.1
0.01
0.001
0.0001
0.00001
10
100
1,000
10,000
Read length (log scale)

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

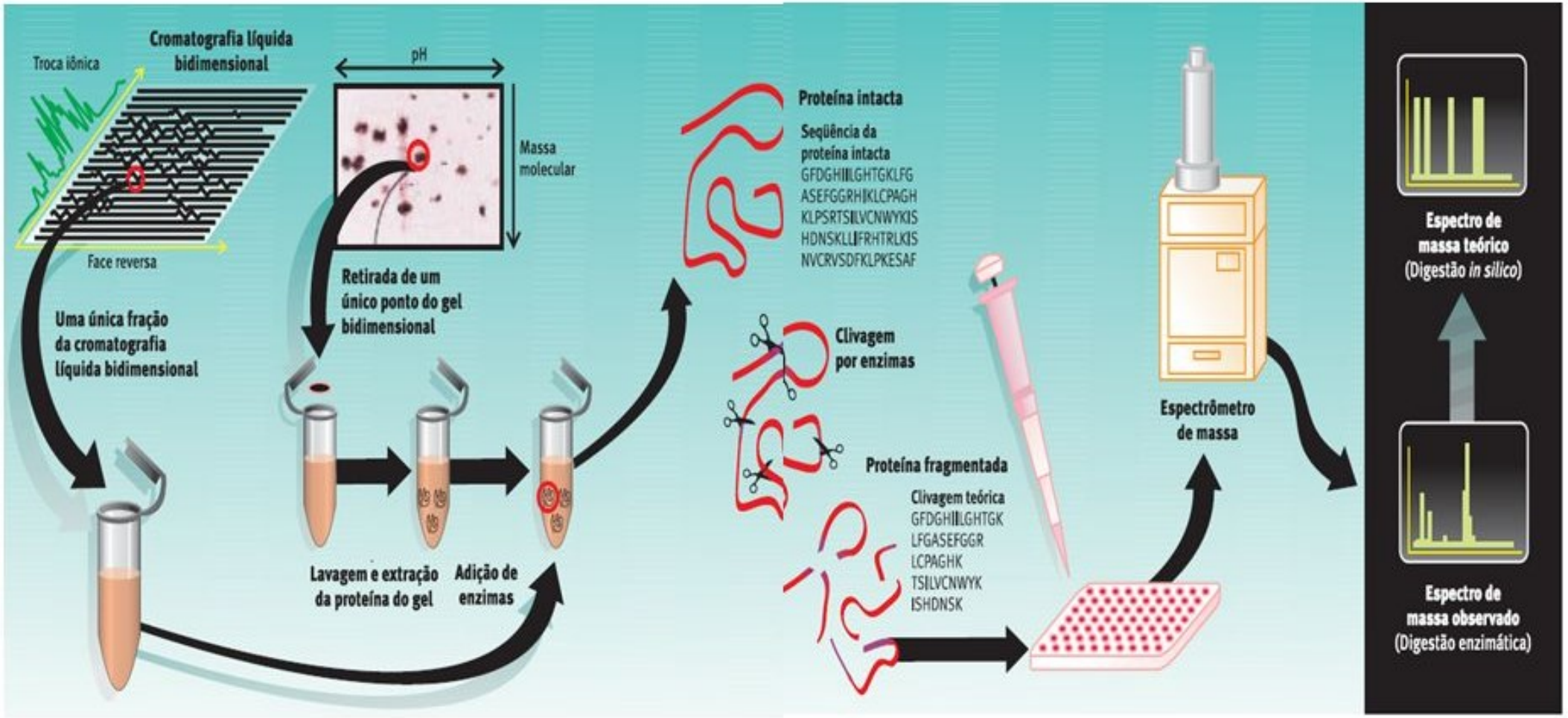
Sequenciamento de DNA de nova geração e suas aplicações na genômica de plantas

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!!!



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=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”