



PCR: DA CLONAGEM AO SEQUENCIAMENTO

Aula 6

LGN0232 – Genética Molecular

Douglas Silva Domingues
Departamento de Genética
dougsd@usp.br

SUMÁRIO

- Revisando
- Clonagem
- Princípio da PCR;
- Aplicações da PCR
- Variações muito comuns: RT-PCR e qPCR
- Técnica de sequenciamento de Sanger
- Novas técnicas de sequenciamento
- Estudo dirigido.

PARA INICIAR...



Lívias 
@livasalves



Um golfinho



RECORDANDO O QUE VIMOS ATÉ AGORA

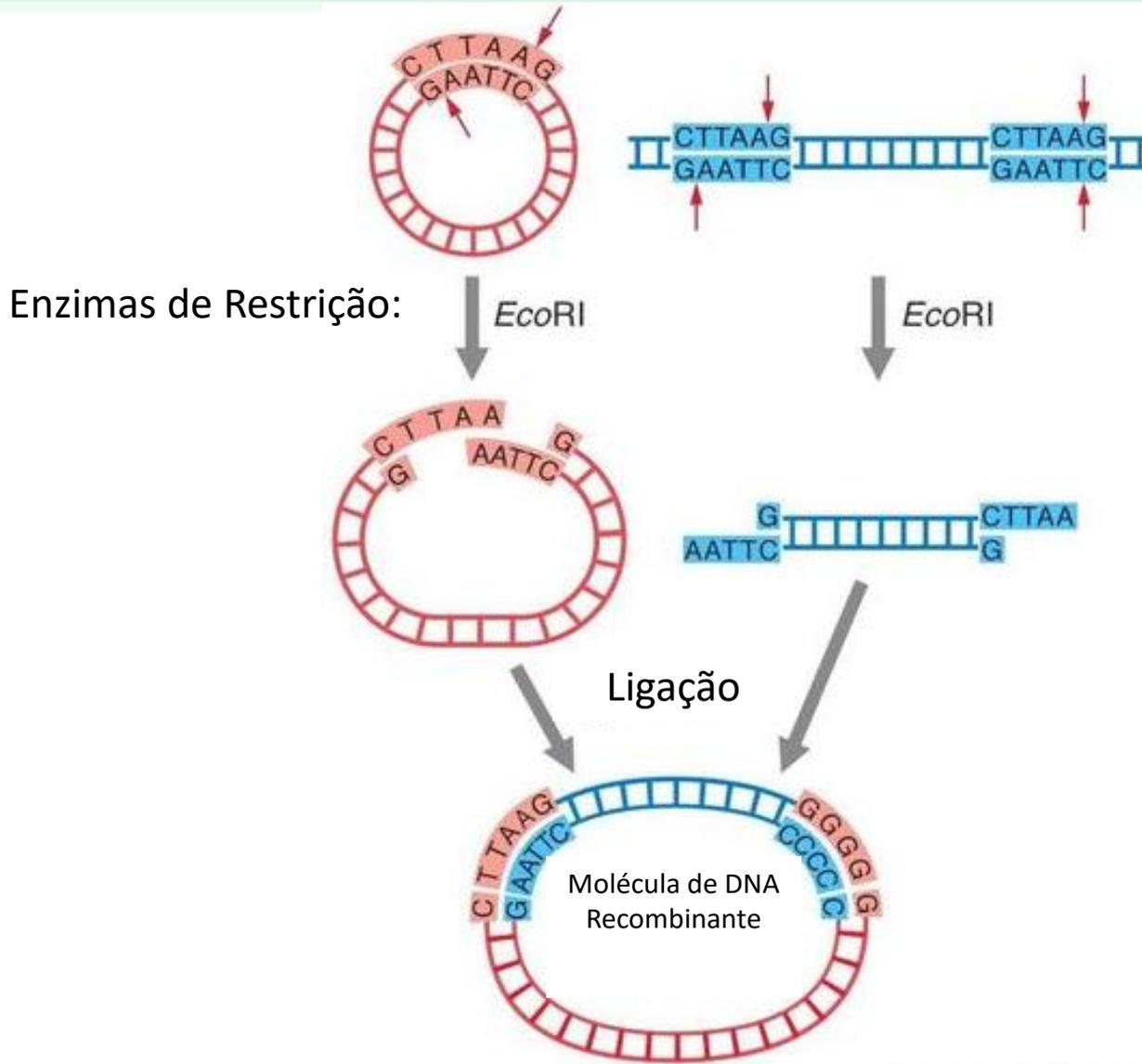
Dogma central, genes e expressão gênica

- <https://app.jove.com/embed/player?id=10798&language=Portuguese&t=1&s=1&fpv=1> (Dogma central)
- <https://app.jove.com/embed/player?id=10984&language=Portuguese&t=1&s=1&fpv=1> (Operons)
- <https://app.jove.com/embed/player?id=10797&language=Portuguese&t=1&s=1&fpv=1> (expressão gênica em eucariotos)

DNA recombinante e enzimas de restrição

- <https://app.jove.com/embed/player?id=12009&t=1&s=1&fpv=1>
(DNA recombinante)
- <https://app.jove.com/embed/player?id=12227&t=1&s=1&fpv=1>
(Enzimas de restrição)

AULA PASSADA:



DNA RECOMBINANTE!!!!

CLONAGEM CELULAR



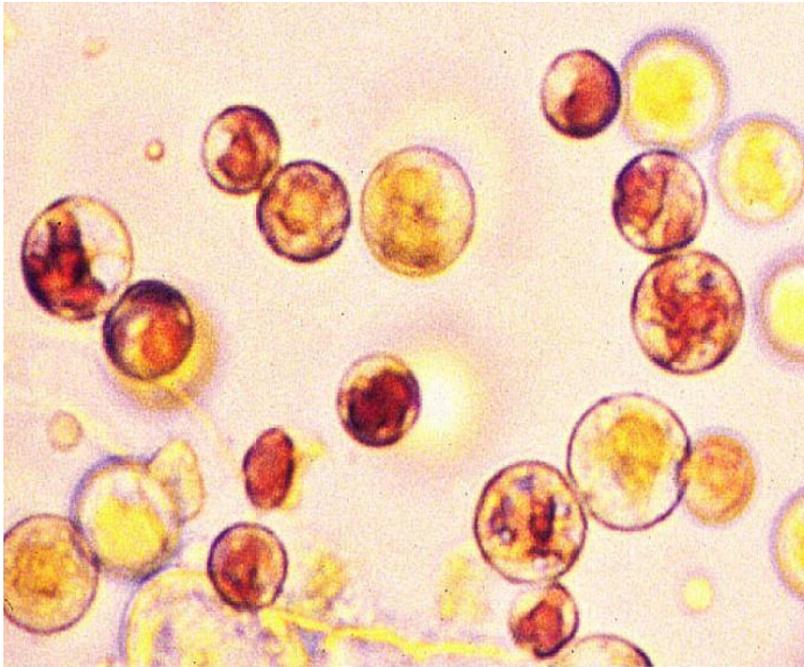
CÓPIAS IDENTICAS!!

Clone: uma coleção de moléculas ou células, todas idênticas a uma molécula ou célula original.



CLONAGEM MOLECULAR

Dependente de células
vivas



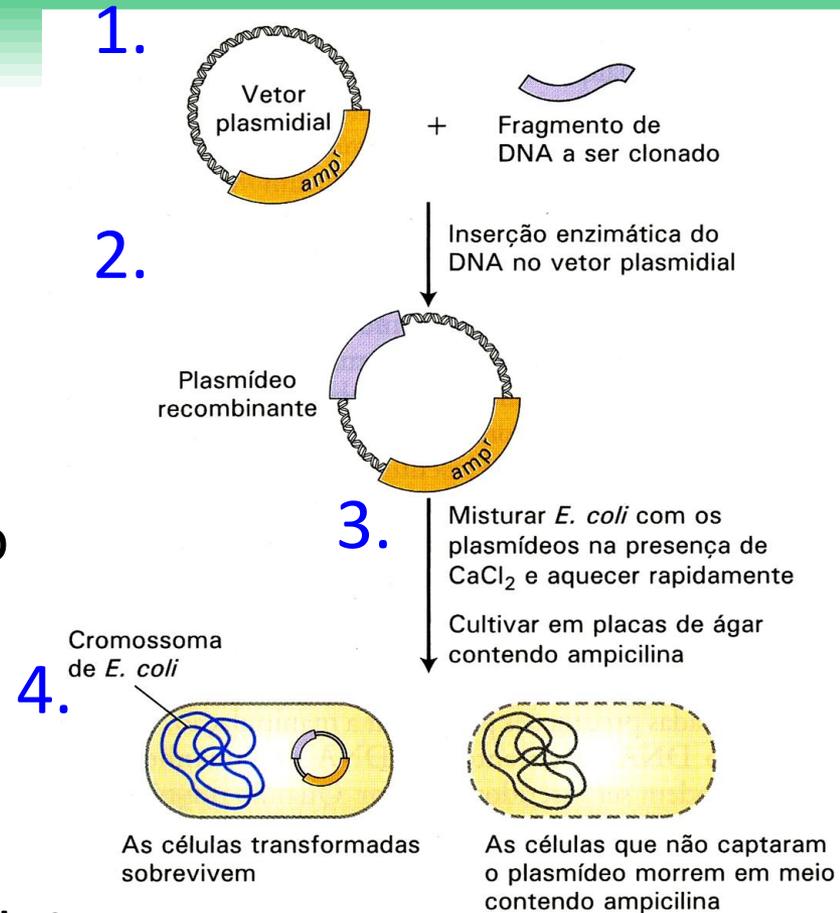
Independente de células
vivas (PCR)



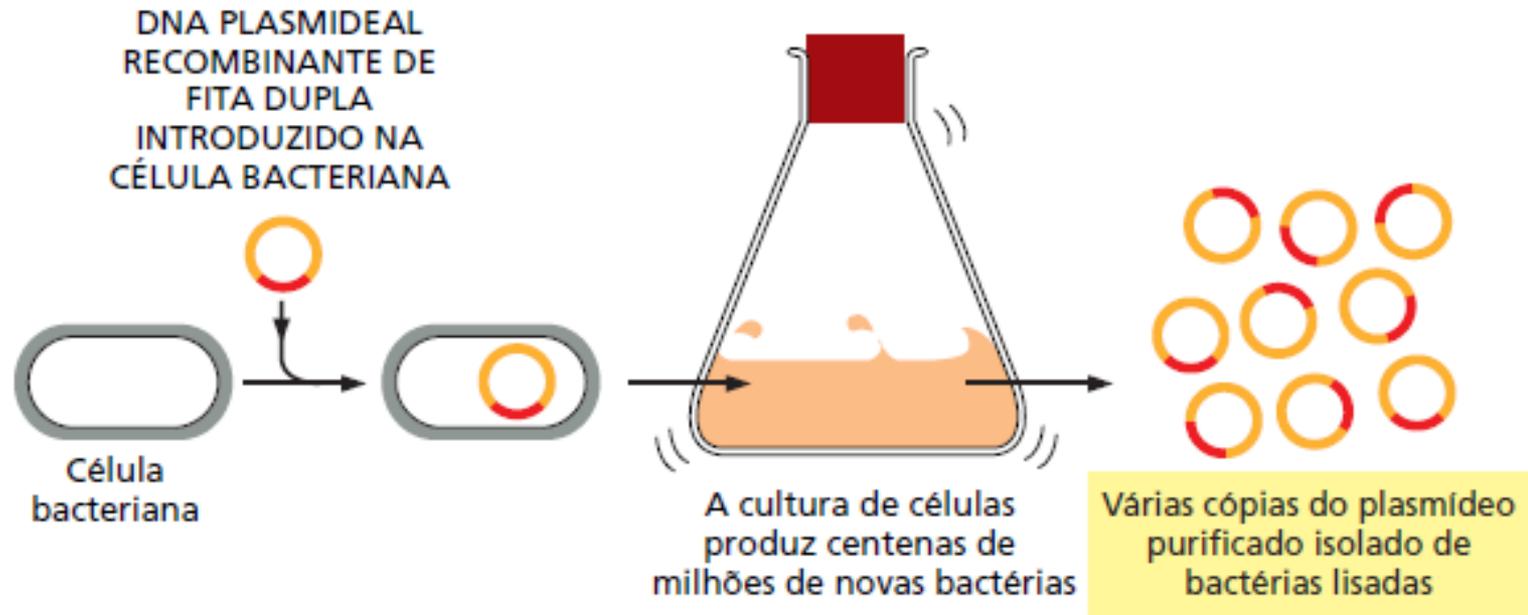
CLONAGEM MOLECULAR

RESUMO DAS ETAPAS:

1. Preparação do vetor e do inserto
2. Ligação do vetor e inserto
3. Transformação em célula hospedeira
4. Seleção de clones



CLONAGEM MOLECULAR



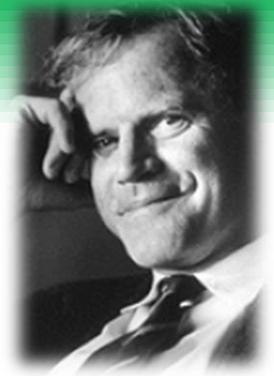
CLONAGEM MOLECULAR INDEPENDENTE DE CÉLULAS VIVAS PCR



SUMÁRIO

- Revisando
- Clonagem
- Princípio da PCR;
- Aplicações da PCR
- Variações muito comuns: RT-PCR e qPCR
- Técnica de sequenciamento de Sanger;
- Estudo dirigido.

PCR: Polymerase Chain Reaction (Reação em cadeia da polimerase)



- O processo de PCR foi **inventado** por Kary Mullis no início da década de 80 → atribuído o Premio Nobel da Química de 1993 pelo seu trabalho.
- É um método de amplificação (de criação de múltiplas cópias) de DNA **sem** o uso de um organismo vivo, por exemplo, *Escherichia coli* (bactéria) ou leveduras.

Kary Mullis, Inventor of the PCR Technique, Dies

The Nobel laureate was a proponent of LSD, a consultant for O.J. Simpson's legal defense, and the creator of a company that infused jewelry with celebrities' DNA.

Aug 12, 2019
KERRY GRENS

Kary Mullis, whose invention of the polymerase chain reaction technique earned him the Nobel Prize in Chemistry in 1993, died of pneumonia on August 7, according to [MyNewsLA.com](#). He was 74 years old.

ABOVE: FLICKR, [ERIK CHARLTON](#)

According to a 1998 profile in [The Washington Post](#), Mullis was known as a “weird” figure in science and “flamboyant” philanderer who evangelized the use of LSD, denied the evidence for both global warming and HIV as a cause of AIDS, consulted for O.J. Simpson's legal defense, and formed a company that sold jewelry embedded with celebrities' DNA. The opening paragraph of his [Nobel autobiography](#) includes a scene depicting a visit from Mullis's dying grandfather in “non-substantial form.”

“He was personally and professionally one of the more iconic personalities science has ever witnessed,” Rich Robbins, the founder and CEO of Wareham Development, a real estate developer for a number of biotech companies, tells the Emeryville, California-based paper, the [E'ville Eye](#).

See [“PCR: Past, Present, & Future”](#)

If the K-T boundary isotopic spike is indeed the result of impact-related acid rain, the oceanic strontium isotope record may reveal other large impacts. The seawater strontium curve of Burke *et al.* (9), which spans the past 500 million years, shows at least two other prominent high spikes in the $^{87}\text{Sr}/^{86}\text{Sr}$ ratio, one in the mid-Cretaceous, at ~ 100 million years, and the other in the Pennsylvanian, at ~ 290 million years. The first appears to precede by a few million years the mass extinction event at the Cenomanian-Turonian boundary. There is also a large increase in $^{87}\text{Sr}/^{86}\text{Sr}$ across the Permian-Triassic boundary (9), the time of the most extreme mass extinction in the Phanerozoic record (17). However, the increase appears to be rather gradual, extending over 20 million to 25 million years, and is thus quite different in character from the K-T spike. Nevertheless, data are sparse for this interval, and more work will be required to determine the exact nature of the increase.

The occurrence of a spike toward higher values in the seawater $^{87}\text{Sr}/^{86}\text{Sr}$ record at the K-T boundary is tantalizing evidence for

18. I thank many colleagues at Scripps for comments on the ideas expressed in this report, in particular G. Arrhenius, S. Galer, J. Gieskes, M. Kastner, D. Lal, G. Lugmair, and H.-G. Stosch. Comments from two anonymous reviewers also improved the original manuscript. I thank P. Hey for preparation of

the manuscript. This work was supported in part by grants from the National Science Foundation and the National Aeronautics and Space Administration.

28 September 1987; accepted 7 December 1987

Primer-Directed Enzymatic Amplification of DNA with a Thermostable DNA Polymerase

RANDALL K. SAIKI, DAVID H. GELFAND, SUSANNE STOFFEL, STEPHEN J. SCHARF, RUSSELL HIGUCHI, GLENN T. HORN, KARY B. MULLIS,* HENRY A. ERLICH

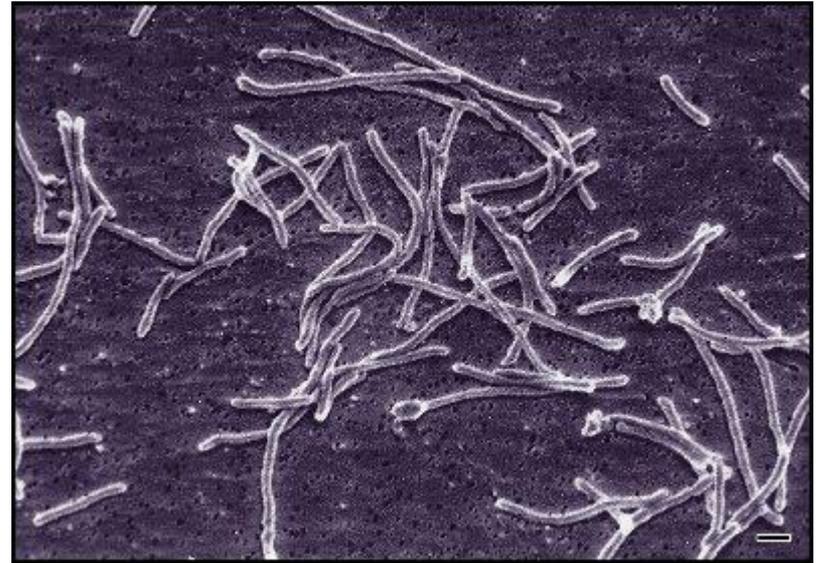
A thermostable DNA polymerase was used in an *in vitro* DNA amplification procedure, the polymerase chain reaction. The enzyme, isolated from *Thermus aquaticus*, greatly simplifies the procedure and, by enabling the amplification reaction to be performed at higher temperatures, significantly improves the specificity, yield, sensitivity, and length of products that can be amplified. Single-copy genomic sequences were amplified by a factor of more than 10 million with very high specificity, and DNA segments up to 2000 base pairs were readily amplified. In addition, the method was used to amplify and detect a target DNA molecule present only once in a sample of 10^5 cells.

Saiki, R. K., D. H. Gelfand, S. Stoffel, S. J. Scharf, R. Higuchi, G. T. Horn, K. B. Mullis, and H. A. Erlich. "Primer-Directed Enzymatic Amplification of DNA with a Thermostable DNA Polymerase." *Science* 239 (1988): 487-491.

Thermus aquaticus

Thermus aquaticus é uma espécie de bactéria que pode suportar temperaturas elevadas, uma de várias bactérias termófilas. Esta é a fonte da enzima resistente ao calor como a Taq polimerase de DNA, um dos mais importantes enzimas na biologia molecular devido à sua utilização na reação em cadeia da polimerase (PCR), técnica de amplificação de DNA.

A bactéria foi descoberta pela primeira vez no Lower Geyser Basin do Parque Nacional de Yellowstone



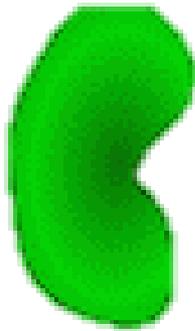
Thermus aquaticus



REAÇÃO DE PCR



DNA molde



DNA polimerase

H_2O + tampão



primers

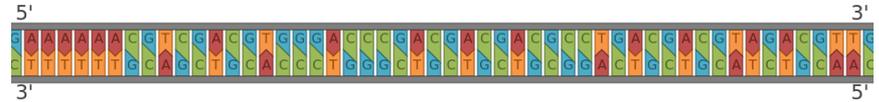
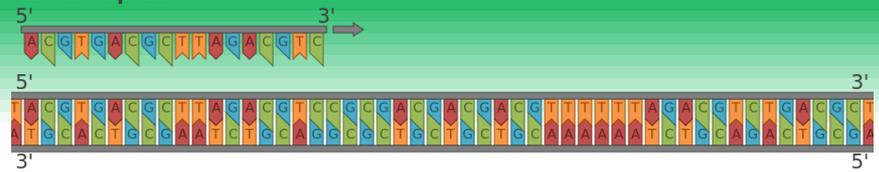
A T C G

Nucleotídeos

(dNTPs) – dATP, dTTP,
dCTP, dTTP

DESENHO DE PRIMERS:

Forward primer



molbiol-tools.ca/PCR.htm

DESIGN PCR PRIMERS

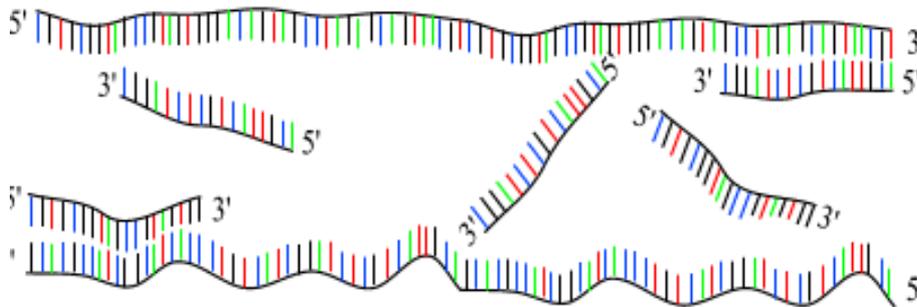
BACKGROUND INFORMATION: For sites describing PCR theory, as well as companies marketing PCR products you might want to begin by visiting [Highveld](#). For PCR techniques see [PCRlink.com](#).

There are several excellent sites for designing PCR primers:

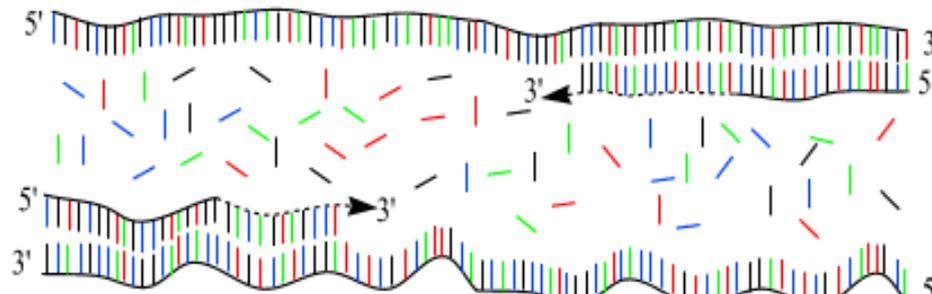
- [Primer3: WWW primer tool](#) (*University of Massachusetts Medical School, U.S.A.*) – This site has a very powerful PCR primer design program permitting one considerable control over the nature of the primers, including size of product desired, primer size and T_m range, and presence/absence of a 3'-GC clamp.
- [GeneFisher - Interactive PCR Primer Design](#) (*Universitat Bielefeld, Germany*) - a very good site allowing great control over primer design.
- [PCR Now](#) (*Computational Biology Group, PathoGene, Southwestern Medical Center, U.S.A.*) - created to design Real-Time Polymerase Chain Reaction (RT-PCR) primers for any number of user-defined coding sequences. Great control over primer properties. If you are interested in designing primers specific to published organismal or viral genes see the related site [PathoGene](#).
- [Primer3Plus](#) - a new improved web interface to the popular Primer3 primer design program (**Reference:** A. Untergasser et al. 2007. Nucl. Acids Res. **35**(Web Server issue):W71-W74)
- [BiSearch](#) Primer Design and Search Tool - this is a useful tool for primer-design for any DNA template and especially for bisulfite-treated genomes. The ePCR tool provides fast detection of mispriming sites and alternative PCR products in cDNA libraries and native or bisulfite-treated genomes. (**Reference:** Arányi T et al. 2006. BMC Bioinformatics **7**: 431).
- [Primer-BLAST](#) was developed at NCBI to help users make primers that are specific to the input PCR template. It uses [Primer3](#) to design PCR primers and then submits them to BLAST search against user-selected database. The blast results are then automatically analyzed to avoid primer pairs that can cause amplification of targets other than the input template.
- [MFEprimer-2.0](#) allows users to check primer specificity against genomic DNA and messenger RNA/complementary DNA sequence databases quickly and easily. This server uses a k-mer index algorithm to accelerate the search process for primer binding sites and uses thermodynamics to evaluate binding stability between each primer and its DNA template. Several important characteristics, such as the sequence, melting temperature and size of each amplicon, either specific or non-specific, are reported. (**Reference:** Qu W et al. 2012. Nucl. Acids Res. **40** (Web Server issue): W205-W208)
- [Primer Design and Search Tool](#)
- [PrimerDesign-M](#) - includes several options for multiple-primer design, allowing researchers to efficiently design walking primers that cover long DNA targets, such as entire HIV-1 genomes, and that optimizes primers simultaneously informed by genetic diversity in multiple alignments and experimental design constraints given by the user. PrimerDesign-M can also design primers that include DNA barcodes and minimize primer dimerization. PrimerDesign-M finds optimal primers for highly variable DNA targets and facilitates design flexibility by suggesting alternative designs to adapt to experimental conditions. (**Reference:** Yoon H & Leitner T. 2015. Bioinformatics **31**:1472-1474).



Passo 1 – desnaturação
1 minuto – 94°C



Passo 2 – anelamento
45 segundos – 55°C



Passo 3 – extensão
45 segundos – 72°C

PCR: *Polymerase Chain Reaction*

- **1. Desnaturação** (94-96°C, 30-600 segundos).

Durante a desnaturação, a cadeia dupla do DNA é separada em duas cadeias simples.

A DNA polimerase é estável a altas temperaturas pois é obtida de organismos que vivem em ambientes extremos (extremófilos). A DNA polimerase mais usada é a *Taq* polimerase (obtida de *Thermus aquaticus*).

- **2. Annealing** (emparelhamento, pareamento, hibridização) (45-80° C, 30-120 segundos).

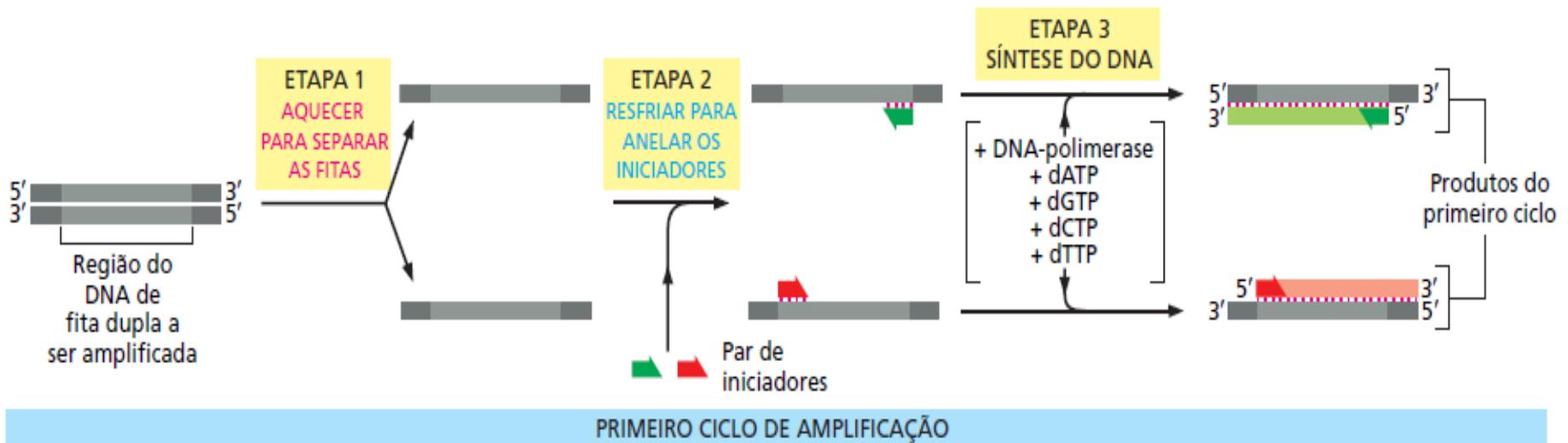
Durante o emparelhamento, os iniciadores (*primers*) ligam-se ao DNA de cadeia simples e a DNA polimerase liga-se aos iniciadores emparelhados.

- **3. Alongamento (polimerização)** (65-80° C, 30-120 segundos).

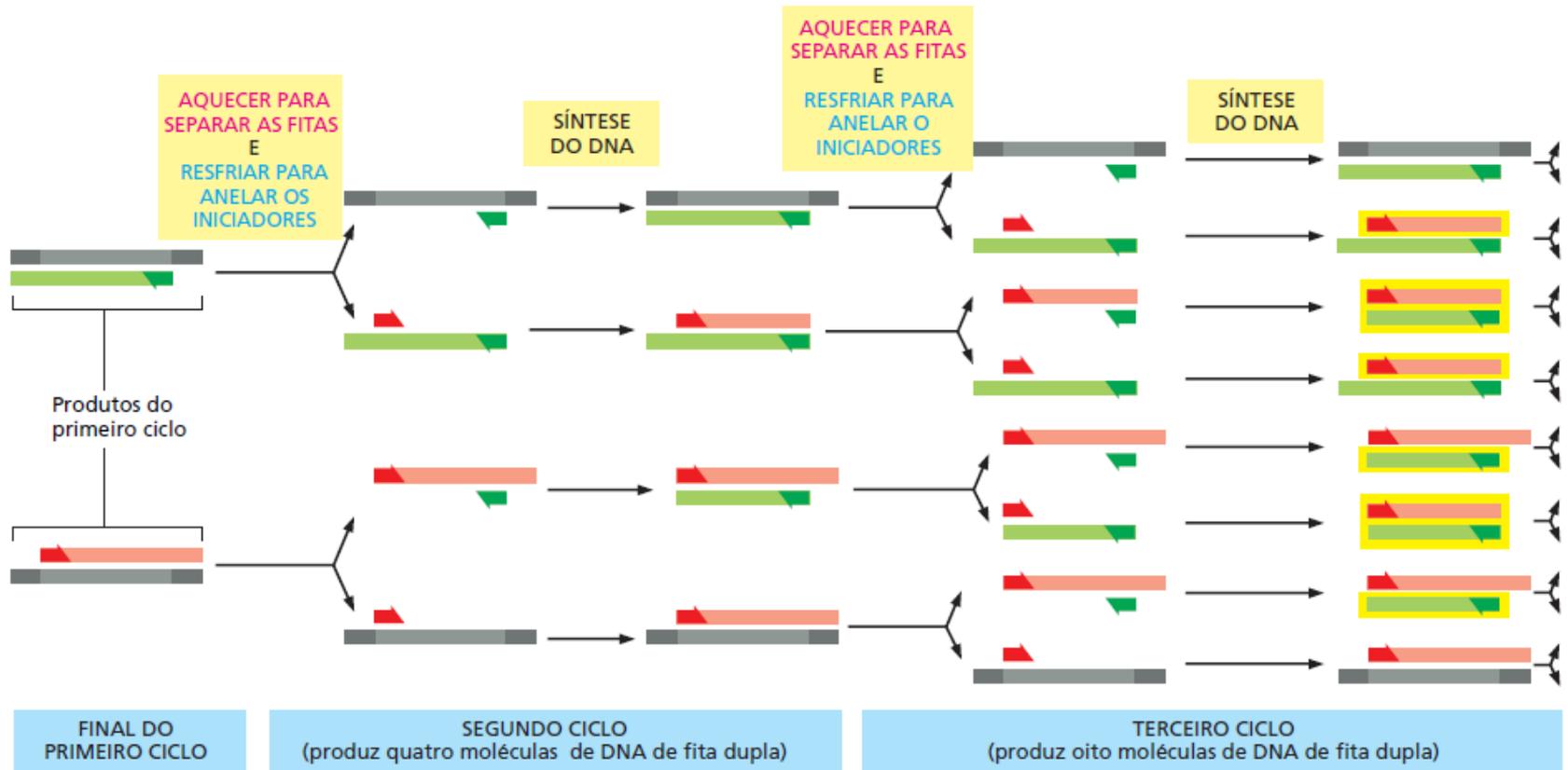
Durante o alongamento, a DNA polimerase cria a cadeia de DNA complementar à medida que percorre o DNA de cadeia simples, incorporando desoxirribonucleótidos presentes na reação.

Após cada ciclo, a quantidade de DNA duplica. Assim, após múltiplos ciclos, o aumento da quantidade de DNA é exponencial de base 2.

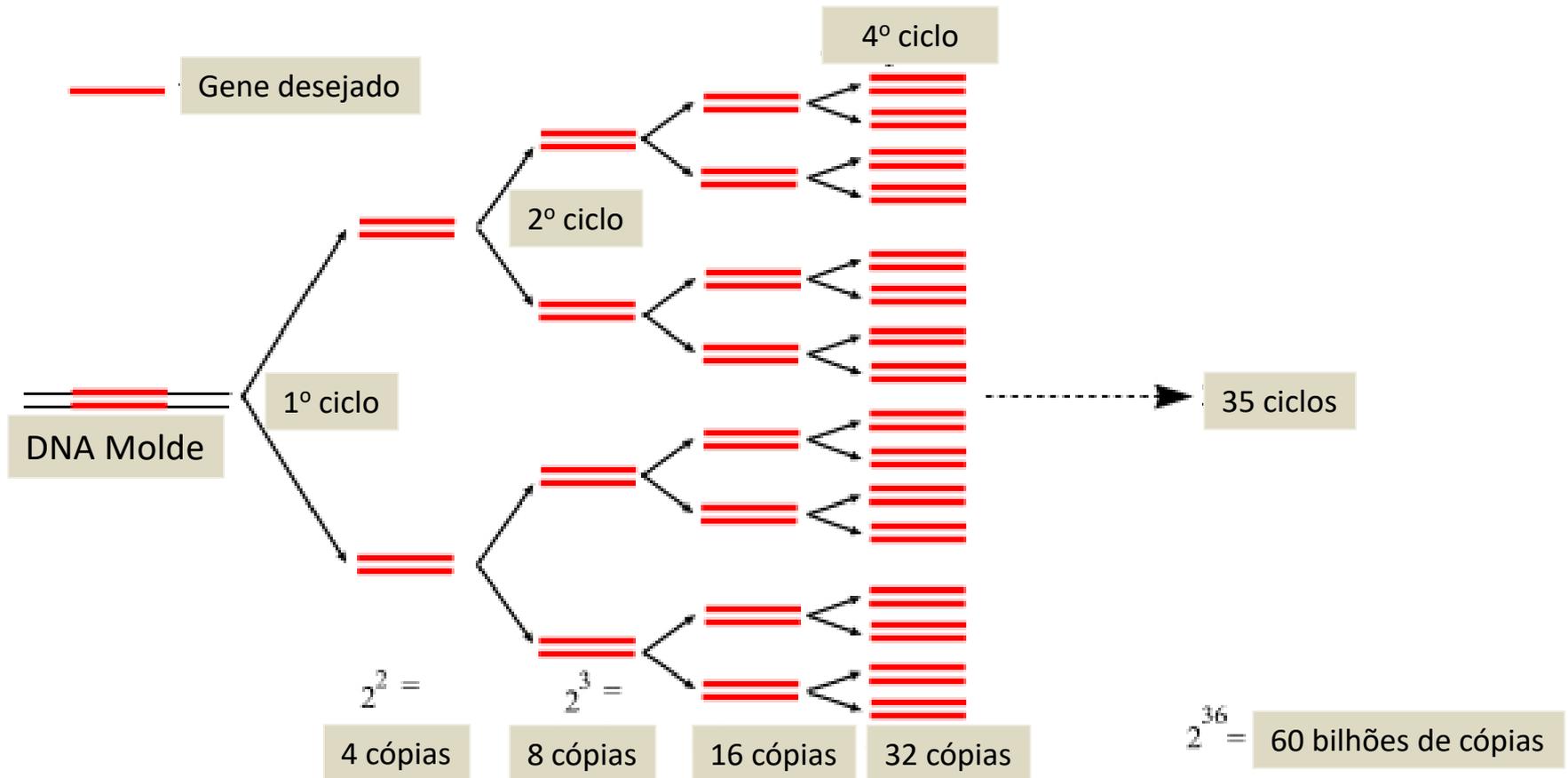
PCR: Polymerase Chain Reaction



PCR: *Polymerase Chain Reaction*



A AMPLIFICAÇÃO É EXPONENCIAL!!



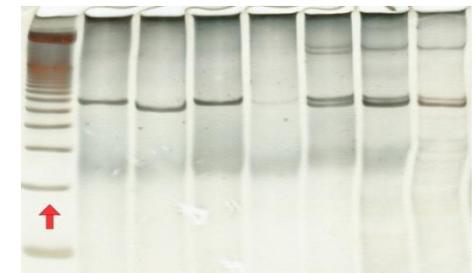
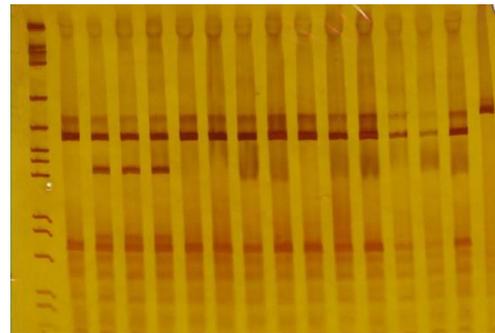
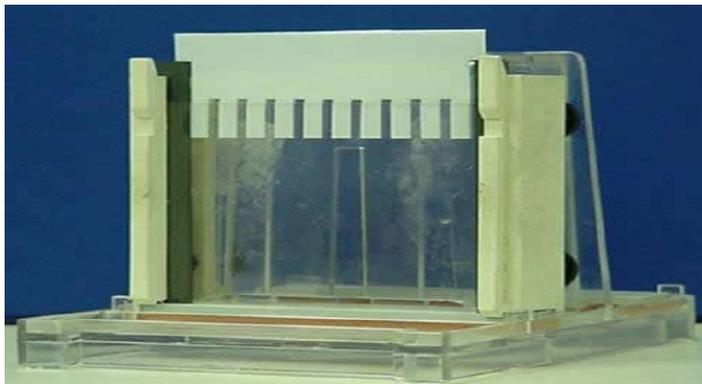
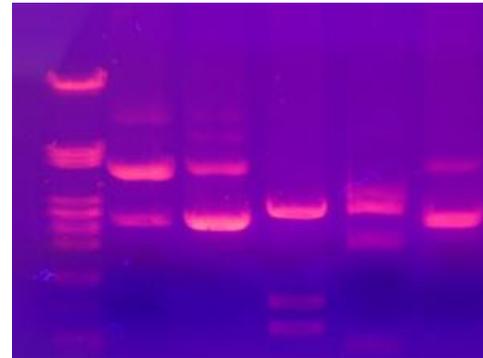
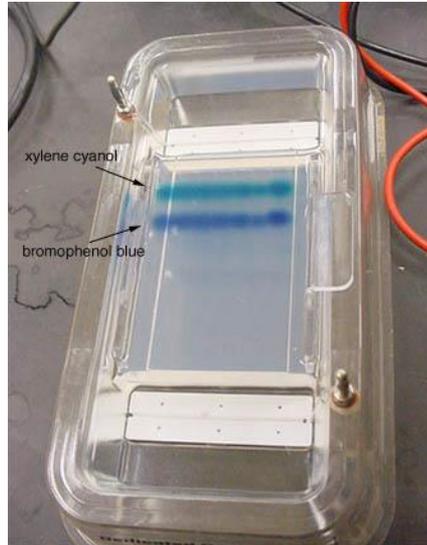
Vídeo: PCR

<https://app.jove.com/embed/player?id=10819&language=Portuguese&t=1&s=1&fpv=1>

TERMOCICLADOR



VISUALIZANDO O MATERIAL AMPLIFICADO





ANIMAÇÕES

<https://www.youtube.com/watch?v=rn40R5w5Fkw>

<https://www.youtube.com/watch?v=iQsu3Kz9NYo>

[https://app.jove.com/embed/player?id=5056&language=Portuguese&t=1
&s=1&fpv=1](https://app.jove.com/embed/player?id=5056&language=Portuguese&t=1&s=1&fpv=1)

SUMÁRIO

- Revisando
- Clonagem
- Princípio da PCR;
- Aplicações da PCR
- Variações muito comuns: RT-PCR e qPCR
- Técnica de sequenciamento de Sanger
- Novas técnicas de sequenciamento
- Estudo dirigido.

EXEMPLOS DE APLICAÇÃO

Evidência molecular da ocorrência de um fitoplasma associado ao lenho mole da macieira

Luiz Fernando Caldeira Ribeiro¹, Ivan Paulo Bedendo¹, Rosa Maria Valdebenito Sanhueza²

¹Setor Fitopatologia / ESALQ-USP, Av. Pádua Dias, 11 CEP 13418-900 Piracicaba /SP, e-mail: ipbedend@esalq.usp.br, ²Embrapa Uva e Vinho, rua Livramento, 515, CEP 95700-000 Bento Gonçalves, RS.

Autor p/ correspondência: Ivan Paulo Bedendo

Data de chegada: 15/08/2005. Aceito para publicação em: 04/05/2006.

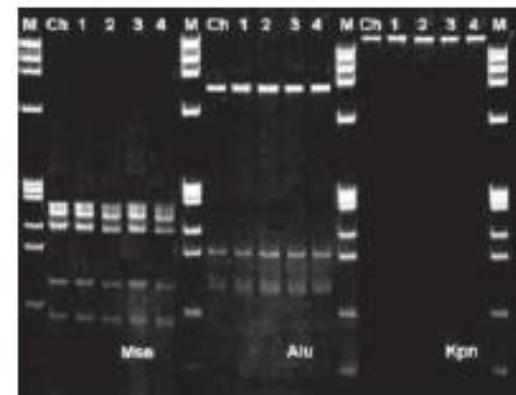
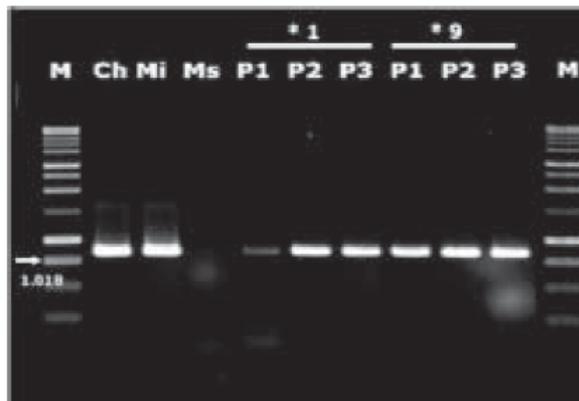
1236

RESUMO

Ribeiro, L.F.C.; Bedendo, I.P.; Sanhueza, R.M.V. Evidência molecular da ocorrência de um fitoplasma associado ao lenho mole da macieira. *Summa Phytopathologica*, v.33, n.1, p.30-33, 2007.

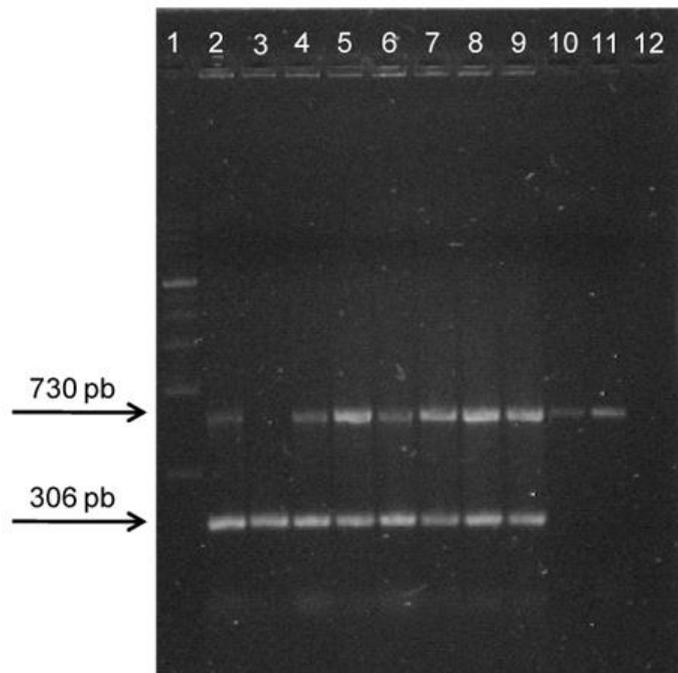
O lenho mole da macieira é uma doença relevante em diversas partes do mundo. Sintomas típicos desta doença têm sido observados em pomares instalados em estados do sul do território brasileiro desde a década de oitenta. Enxertia tem revelado a natureza infecciosa da doença e a observação de corpúsculos filamentosos no floema tem evidenciado possível associação com fitoplasma. No presente trabalho plantas com sintomas de lenho mole foram coletadas em pomar comercial, visando demonstrar a presença de fitoplasma em tecido lenhoso e identificar molecularmente este fitoplasma. Através de PCR com iniciadores universais R16mF2/R1 e R16mR2/R2

foi consistentemente detectado em plantas sintomáticas. A identificação conduzida com duplo PCR usando-se iniciadores específicos R16(III)F2/R demonstrou que o fitoplasma detectado pertencia ao grupo 16SrIII. Análises de RFLP conduzidas com as endonucleases *AluI*, *KpnI*, *HinfI*, *HpaII*, *MseI*, *RsaI* e *SauIII*A confirmaram que o fitoplasma era um representante típico do grupo 16SrIII. A detecção e identificação molecular se constitui numa forte evidência que um fitoplasma está associado ao lenho mole da macieira, complementando os dados já existentes sobre a doença por enxertia e



8 Otimização da técnica de PCR para a detecção de *Xanthomonas axonopodis* pv. *phaseoli* em sementes de feijão

Optimization of PCR technique for detection of *Xanthomonas axonopodis* pv. *phaseoli* in bean seeds



AUTORIA

SCIMAGO INSTITUTIONS RANKINGS

Detecção simultânea de *Xanthomonas axonopodis* pv. *phaseoli* e *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*, em lotes comerciais de sementes

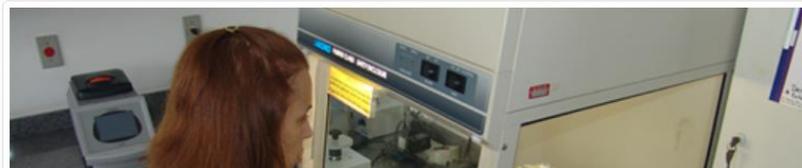
Figura 2 Análise eletroforética em gel de agarose (1%) de 5µL dos produtos amplificados de extratos de sementes comerciais de feijão plaqueados no meio de cultura XCP1 sem antibióticos, utilizando-se os primers específicos para Xap e Cff, em uma reação multiplex. **01** marcador de DNA (Jena Bioscience); **02** Controle (Xap + Cff); **03** Lote-42; **04** Lote-29; **05** Lote-36; **06** Lote-38; **07** Lote-40; **08** Lote-41; **09** Lote-47; **10** Lote-33; **11** Lote-34; **12** Lote-32.

Implementação de diagnóstico por PCR para *Fusarium oxysporum* f. sp. *cabense* (raça 4)

João Nilton Barreto Andrade¹, Shirley Nascimento Costa², Saulo Alves Santos Oliveira³, Edson Perito Amorim³, Fernando Haddad³

LDF realiza análises para diagnosticar a presença de patógenos e tornar mais seguras a produção e comercialização de produtos vegetais

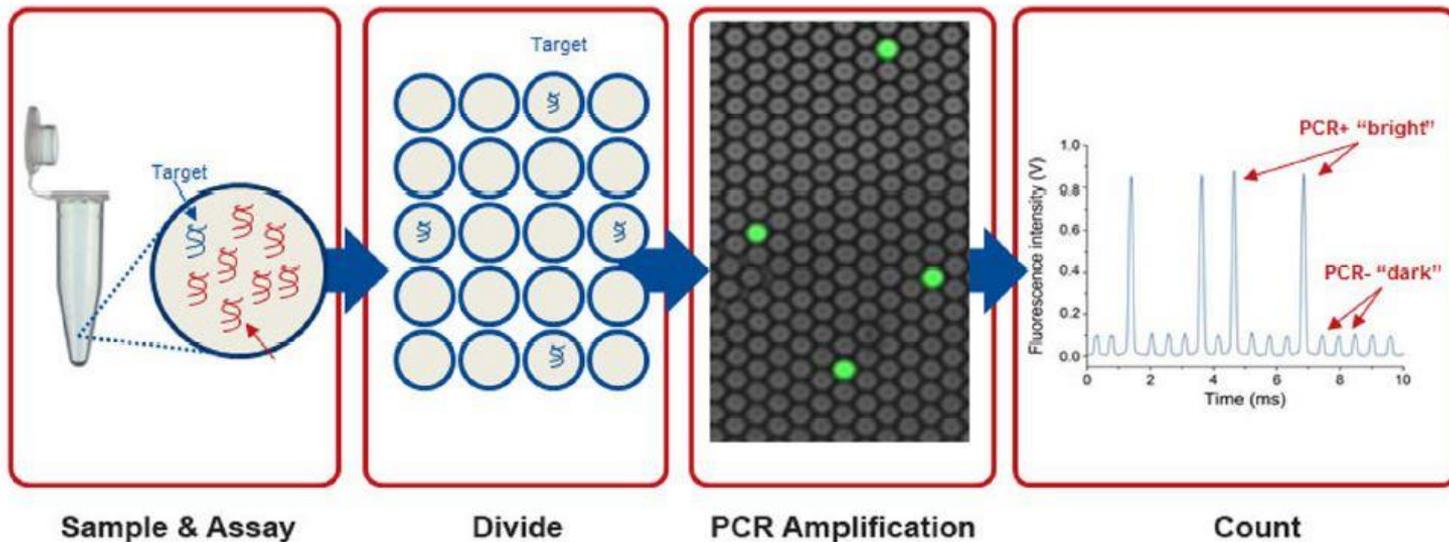
Postado em: 25/10/2022



O Laboratório de Diagnóstico Fitopatológico do Instituto Biológico analisa sementes, bulbos, tubérculos e vários tipos de vegetais, para fins de plantio e comercialização, inclusive em supermercados, multiplicação in vitro e eficácia de fungicidas, em culturas de interesse econômico.

Multiplex Picodroplet Digital PCR to Detect *KRAS* Mutations in Circulating DNA from the Plasma of Colorectal Cancer Patients

Valerie Taly,^{1*} Deniz Pekin,^{1,2†} Leonor Benhaim,¹ Steve K. Kotsopoulos,³ Delphine Le Corre,¹ Xinyu Li,³ Ivan Atochin,³ Darren R. Link,³ Andrew D. Griffiths,^{2,4} Karine Pallier,¹ Hélène Blons,¹ Olivier Bouché,⁵ Bruno Landi,⁶ J. Brian Hutchison,³ and Pierre Laurent-Puig^{1,7}

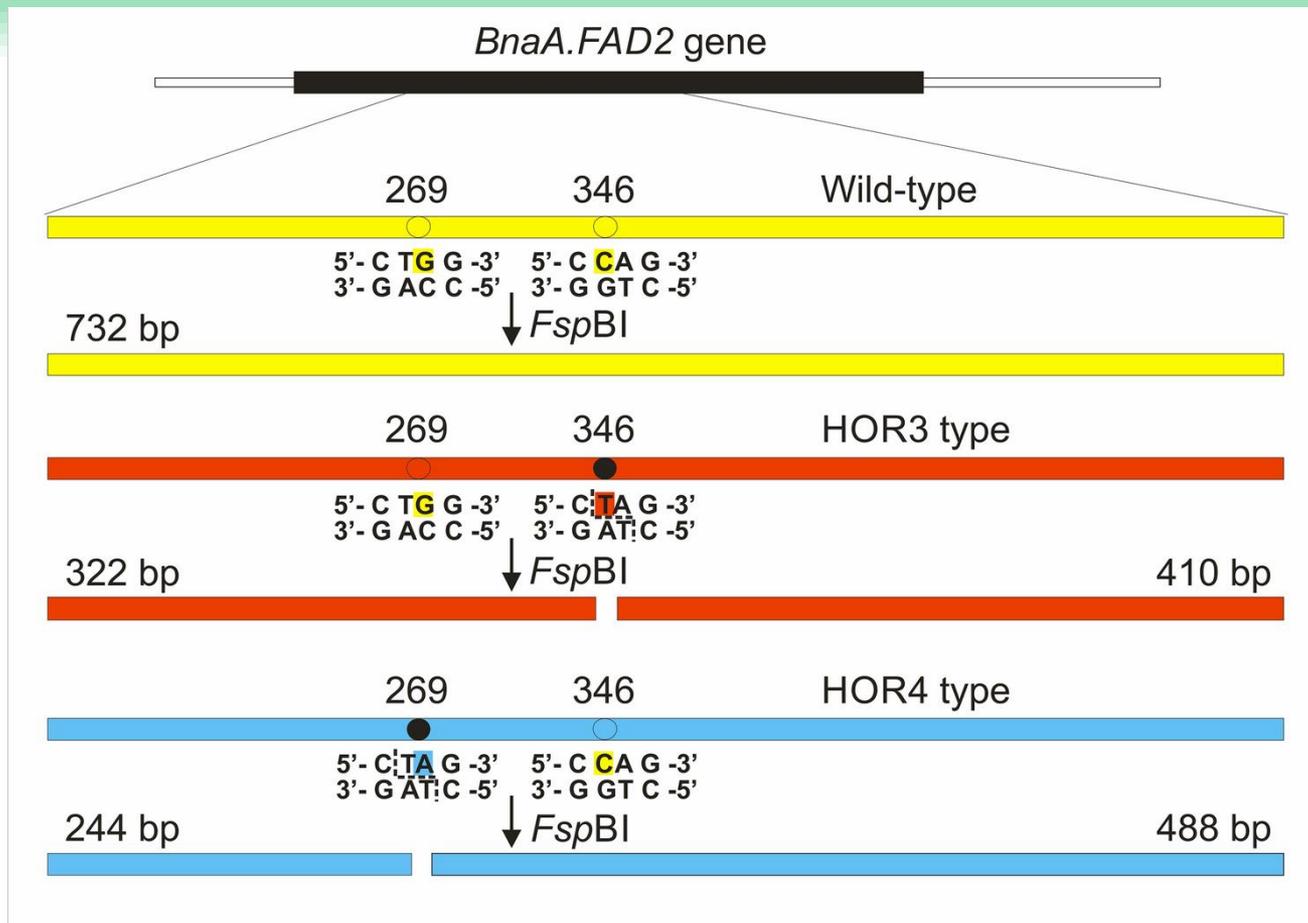


[Home](#) > [Molecular Biology Reports](#) > Article

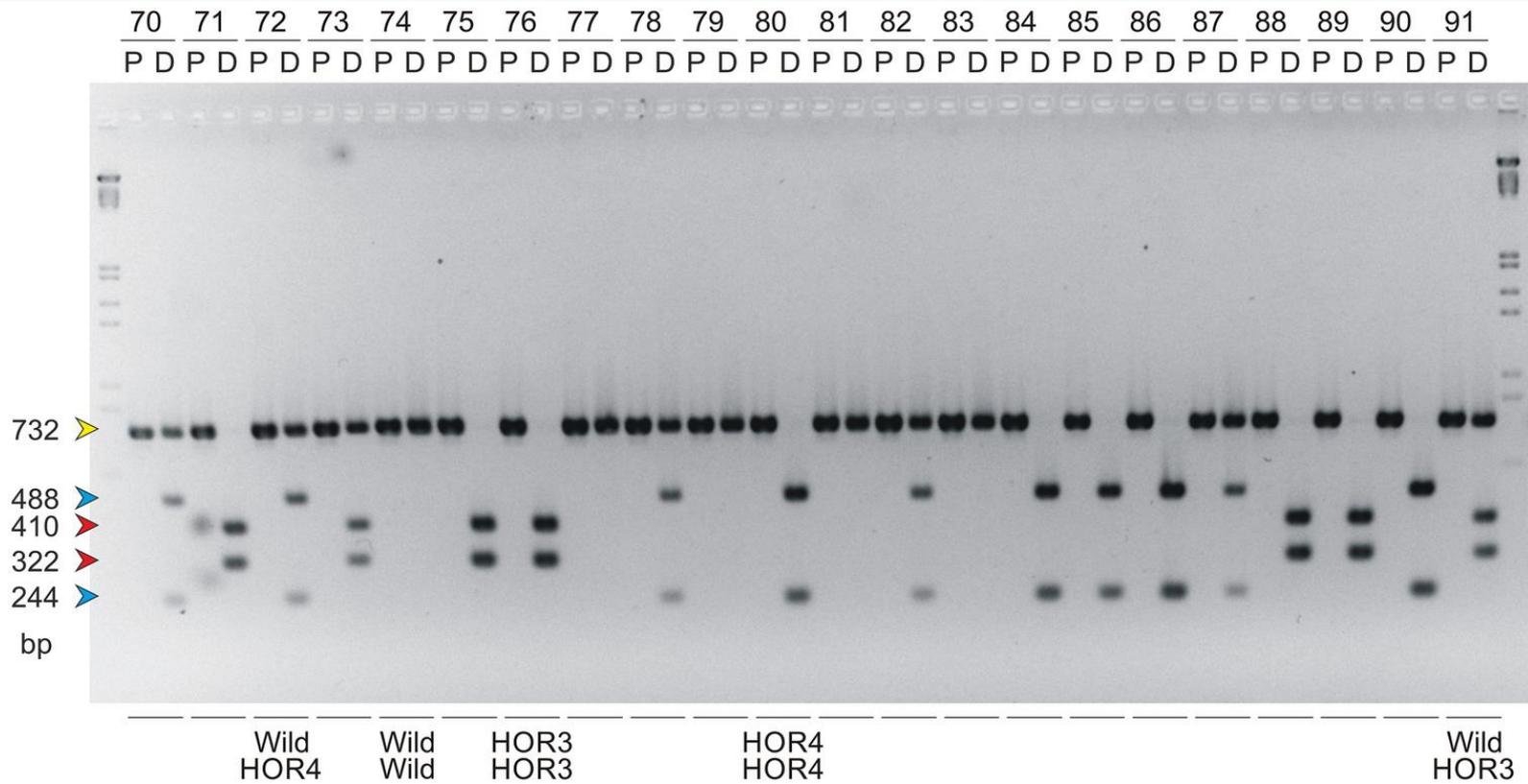
Cleaved amplified polymorphic sequences (CAPS) marker for identification of two mutant alleles of the rapeseed *BnaA.FAD2* gene

Original Article | [Open Access](#) | [Published: 26 September 2020](#) | 47, 7607–7621 (2020)

- Genes FAD: relacionados à qualidade de óleo



HOR3 e HOR4: genótipos criados por mutagênese com alta qualidade de óleo, baixa produtividade

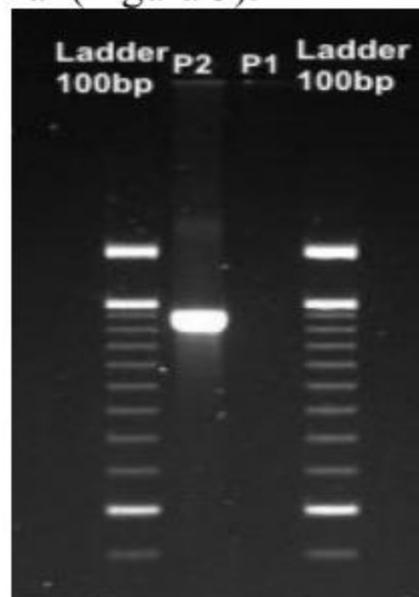


PCR + enzimas de restrição: rastreamento da introgressão de qualidade de óleo

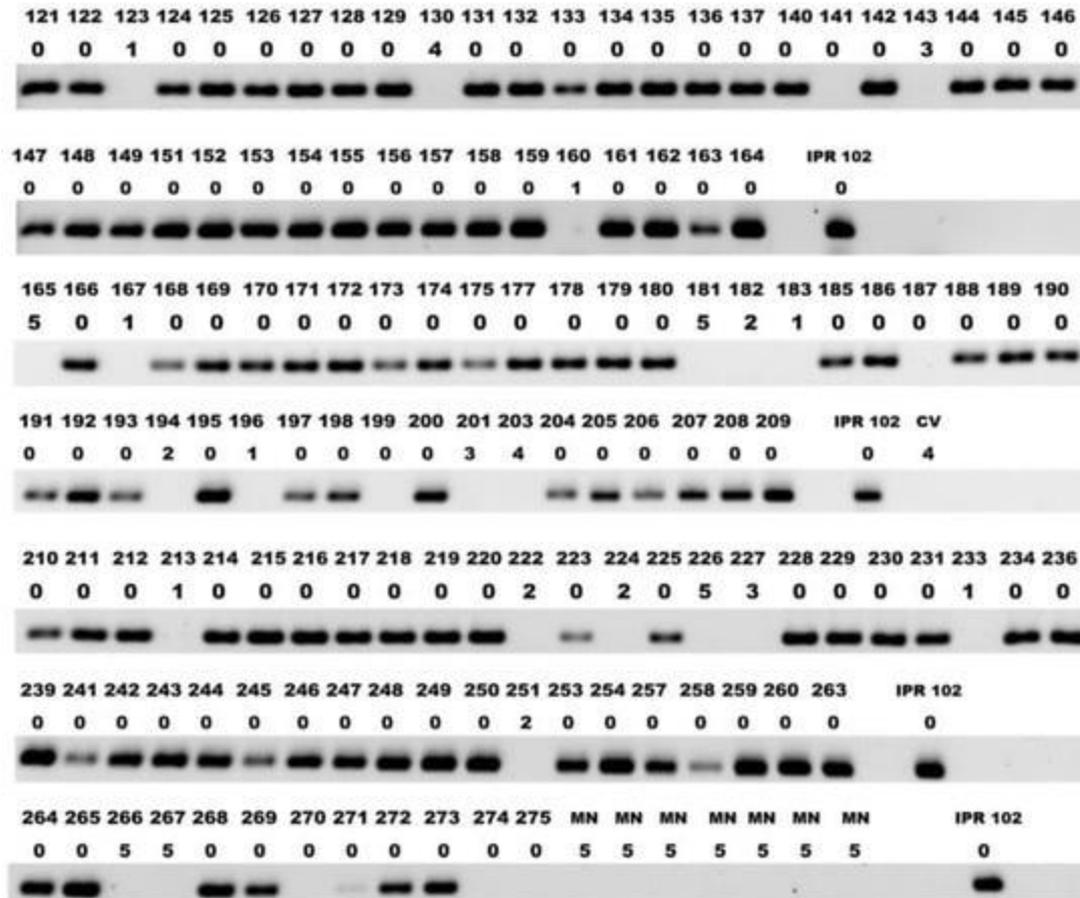
**IDENTIFICAÇÃO DE MARCADOR RAPD E SCAR RELACIONADOS AO CARACTERE
FLORESCIMENTO PRECOCE EM *Eucalyptus grandis*¹**

**IDENTIFICATION OF RAPD AND SCAR MARKER RELATED TO EARLY FLOWERING IN
*Eucalyptus grandis***

Douglas Silva Domingues² Ana Paula Cazerta Farro³ Virgínia Elias Coscrato⁴
Eduardo José de Mello⁵ Shinitiro Oda⁵ Celso Luís Marino⁶



Development and Validation of an Allele-Specific Marker for Resistance to Bacterial Halo Blight in *Coffea arabica*

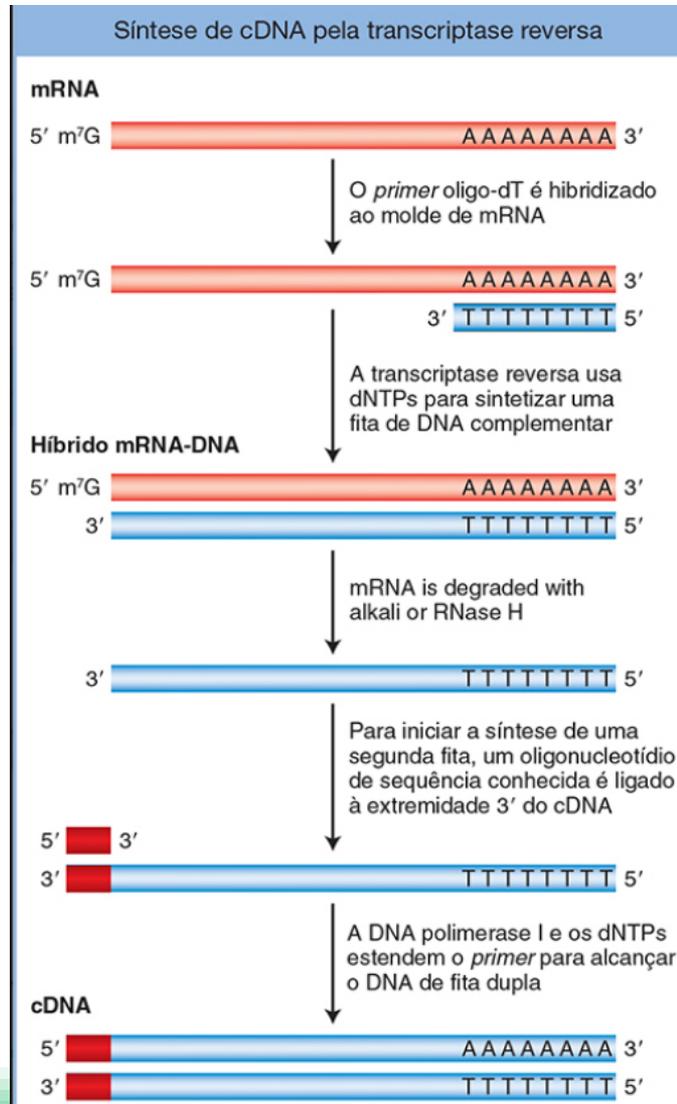


SUMÁRIO

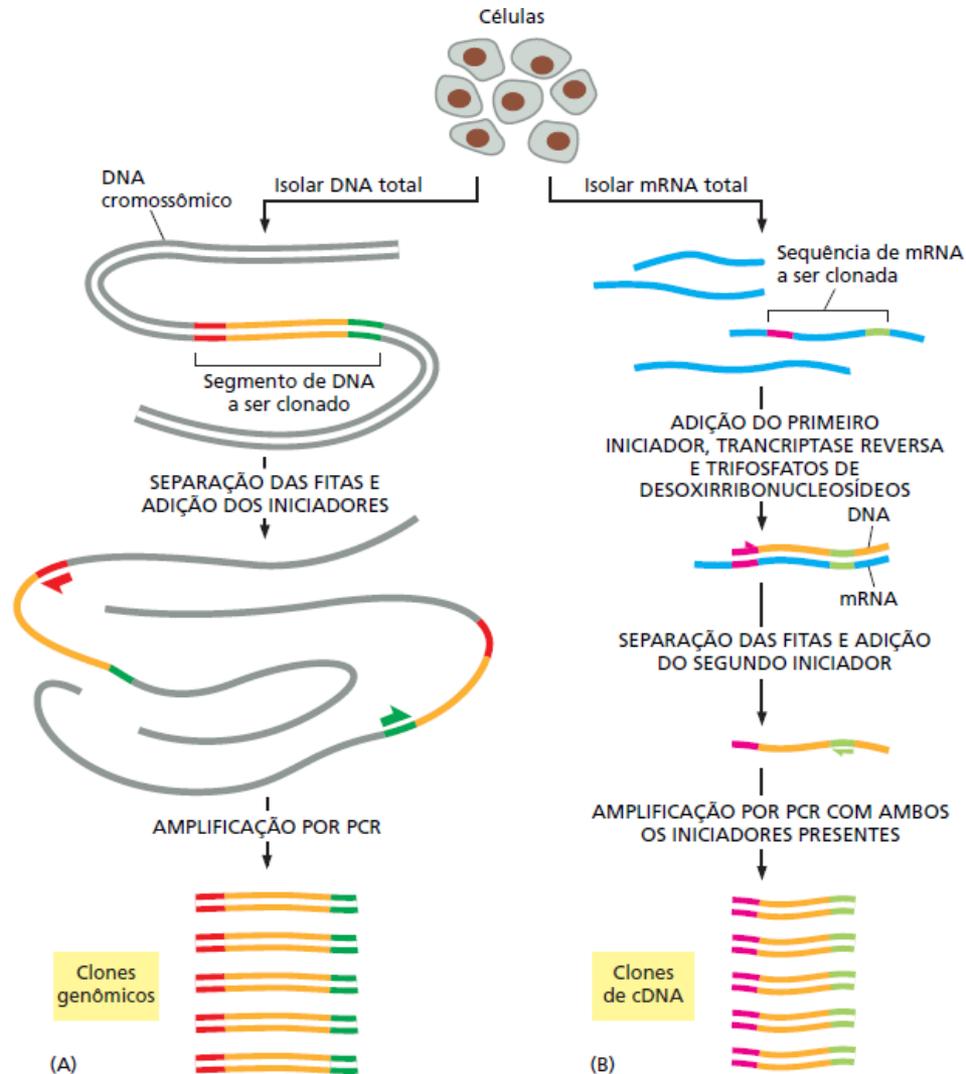
- Revisando
- Clonagem
- Princípio da PCR;
- Aplicações da PCR
- Variações muito comuns: RT-PCR e qPCR
- Técnica de sequenciamento de Sanger
- Novas técnicas de sequenciamento
- Estudo dirigido.

ALGUMAS VARIAÇÕES DA PCR: RT-PCR E PCR EM TEMPO REAL

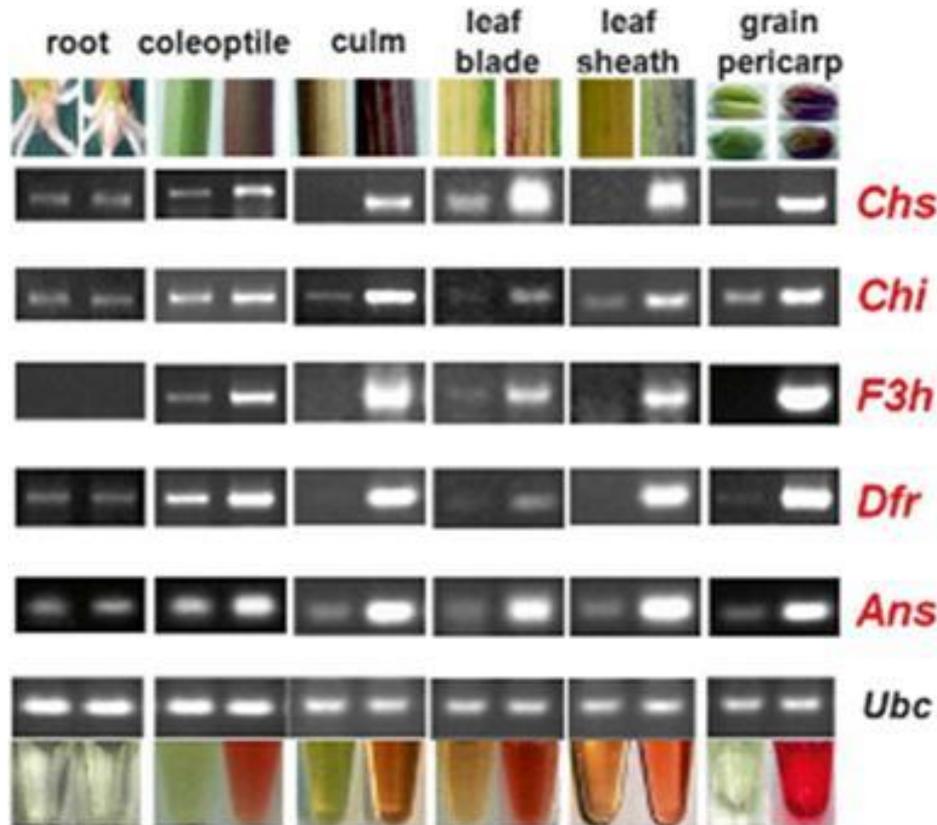
Transcrição reversa do RNA (RT)



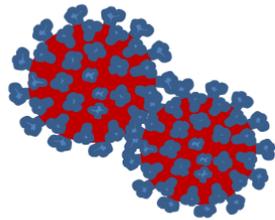
RT-PCR: PCR baseada em produtos da RT (cDNA)



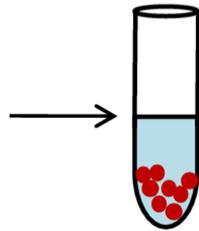
Transcriptional Analysis of Anthocyanin Biosynthesis Structural Genes in Different Wheat Organs



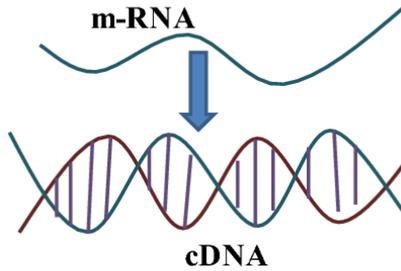
**EXEMPLO DE RT-PCR + PCR EM TEMPO REAL:
COVID**



Corona virus

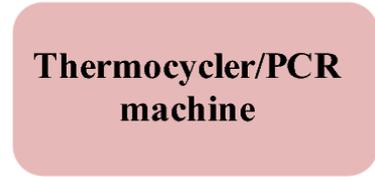


Isolation of the viral RNA

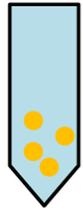


Reverse transcription

Addition of target-specific primers



Amplification



Amplified products

Gene Target	Example of Probe
ORF1ab	FAM-CCGTCTGCGGTATGTATGTGGAAAGTTATGG-BHQ1
N	FAM-TTGCTGCTGCTTGACAGATT-TAMRA
RdRp	FAM-CAGGTGGAACCTCATCAGGAGATGC-BBQ
E	FAM-ACACTAGCCATCCTTACTGCGCTTCG-BBQ

Gene targeted identified using specific probes



**Quantitative/
Qualitative
results**

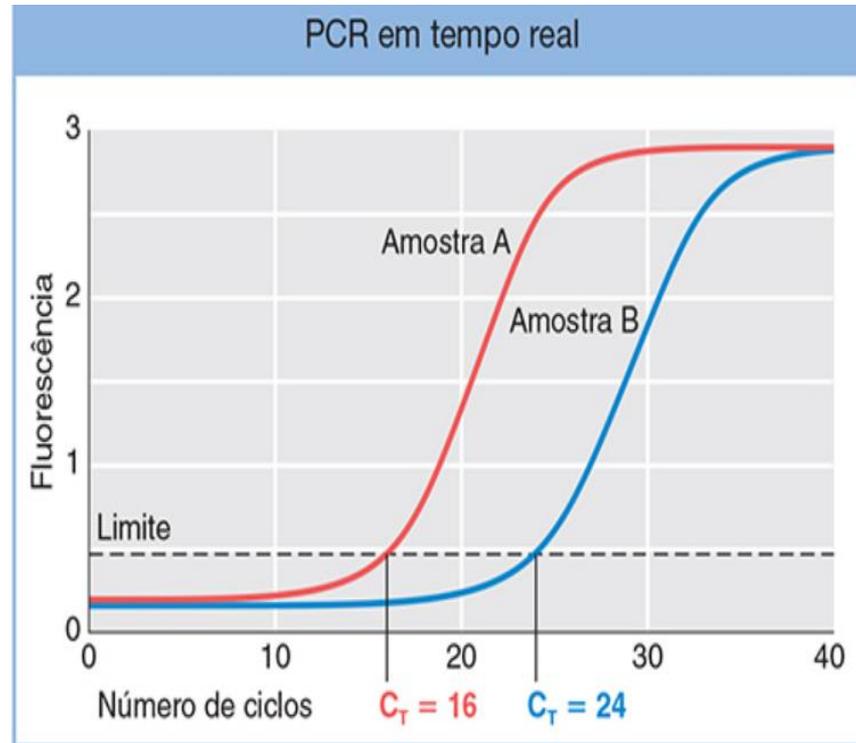


Figura 10.8 A quantidade de uma molécula de DNA específica em uma amostra pode ser quantificada por PCR em tempo real. Uma máquina de PCR em tempo real mede o sinal de fluorescência em cada ciclo de PCR. Neste exemplo, a mesma molécula de DNA foi analisada em duas amostras, Amostra A e Amostra B. O limite de ciclo (C_T) é o ciclo de PCR no qual a fluorescência atingiu um limite. Os valores de C_T para as duas amostras são usados na fórmula $2^{-\Delta C_T}$ para calcular a quantidade relativa da molécula de DNA específica nas amostras.

SUMÁRIO

- Revisando
- Clonagem
- Princípio da PCR;
- Aplicações da PCR
- Variações muito comuns: RT-PCR e qPCR
- Técnica de sequenciamento de Sanger
- Novas técnicas de sequenciamento
- Estudo dirigido.

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)
 - Atualmente, uma variação da PCR



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

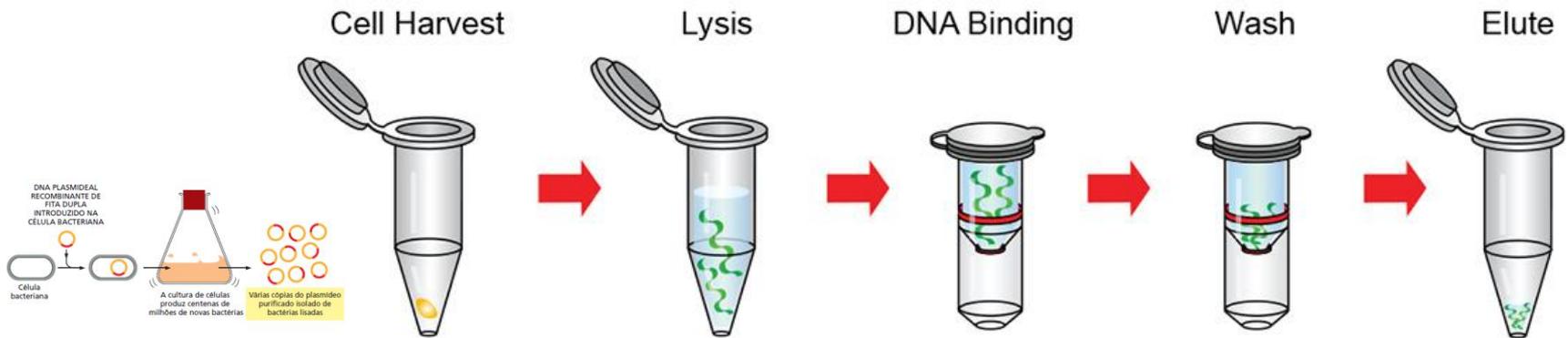
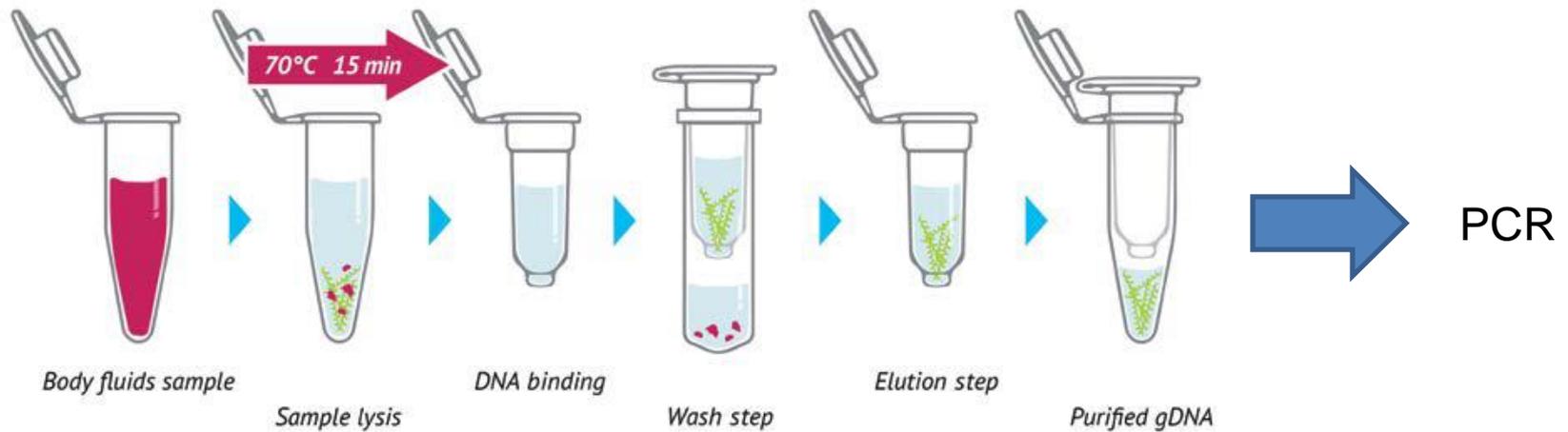


Eletroforese capilar



Análise computacional

PREPARAÇÃO DO DNA



ETAPAS DO SEQUENCIAMENTO DE DNA

Preparação do
DNA



Reação de sequenciamento

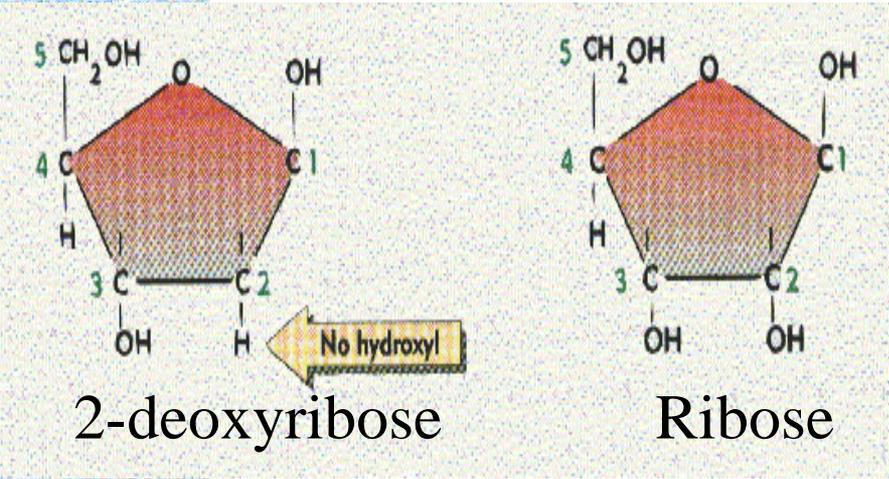
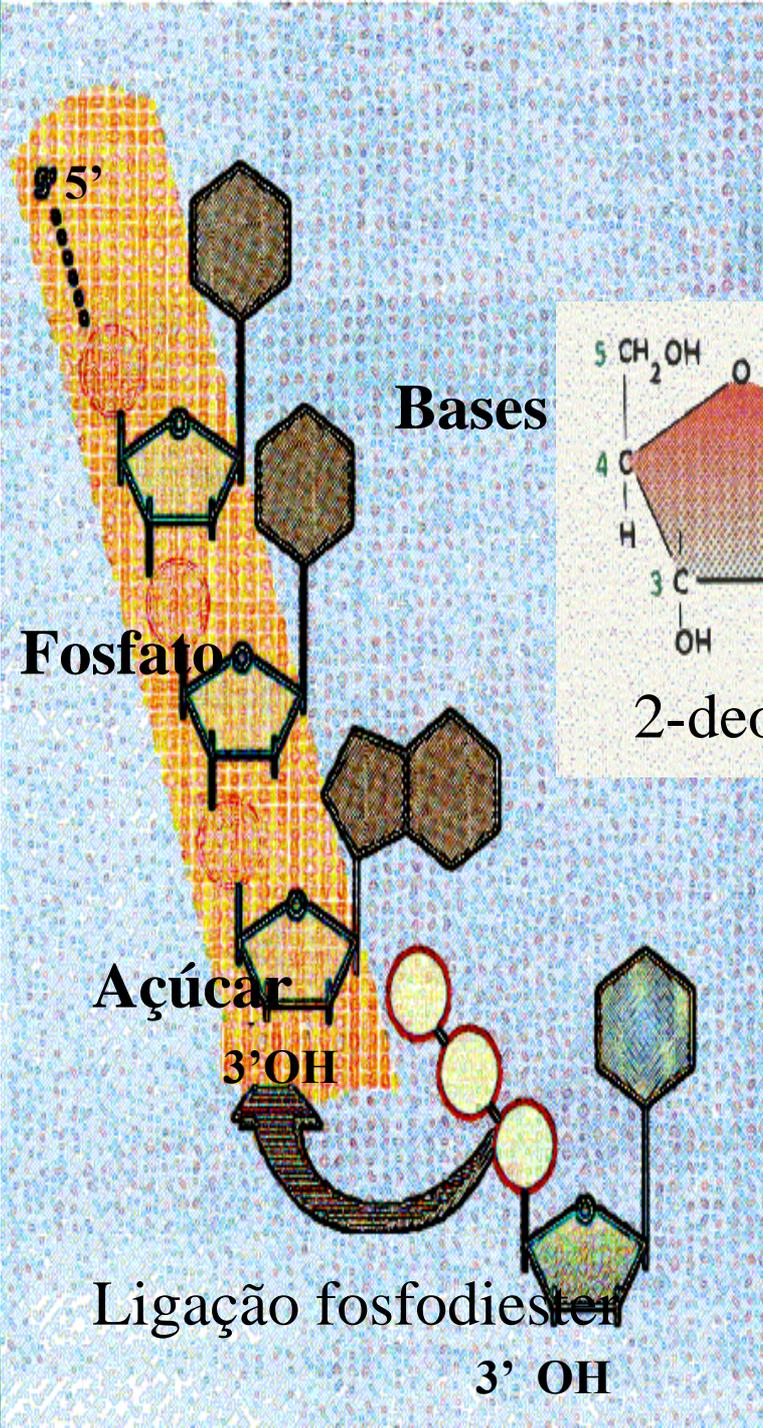


Eletroforese capilar



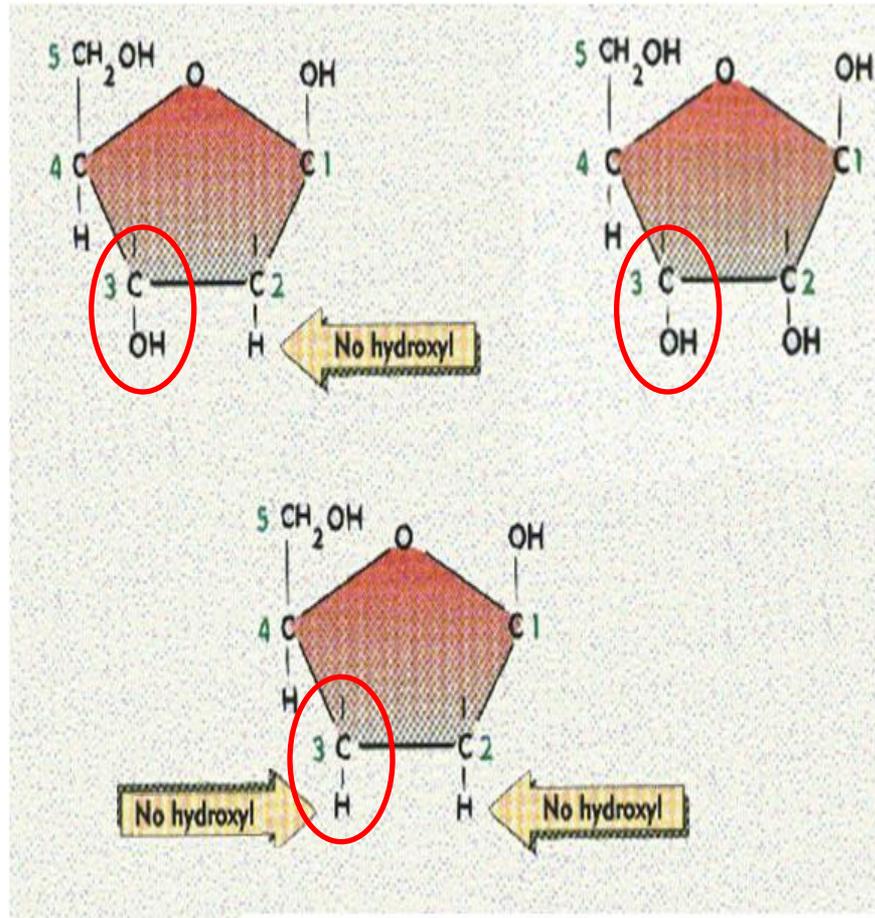
Análise computacional

REAÇÃO DE SEQUENCIAMENTO



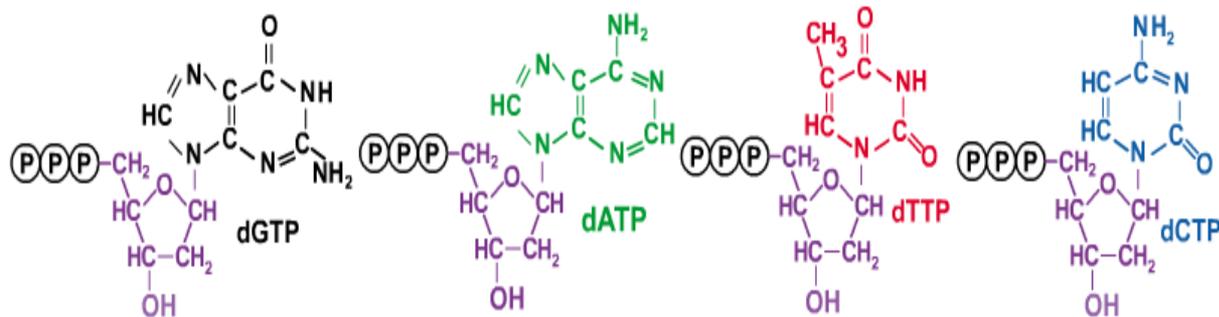
DESOXIRIBOSE

RIBOSE



DIDESOXIRIBOSE

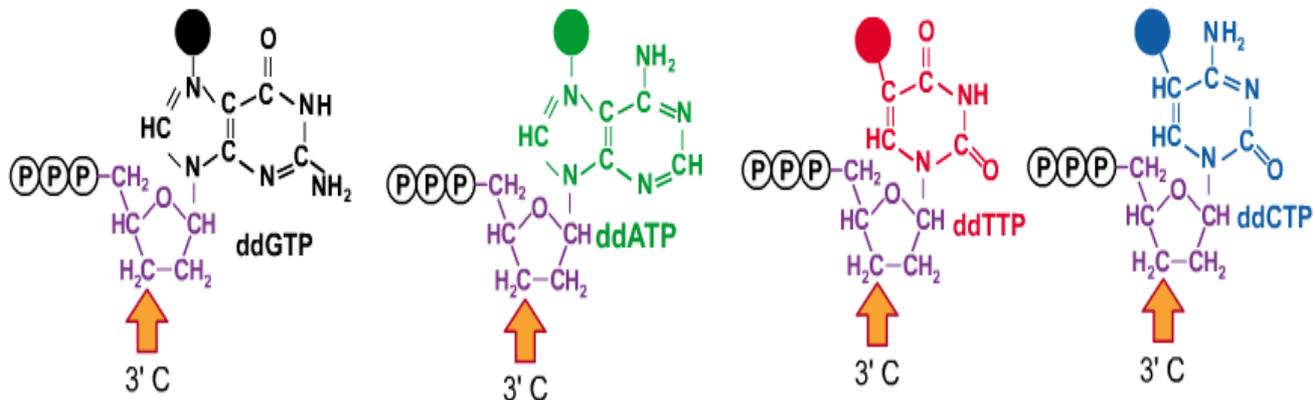
NUCLEOTÍDEOS dNTPs



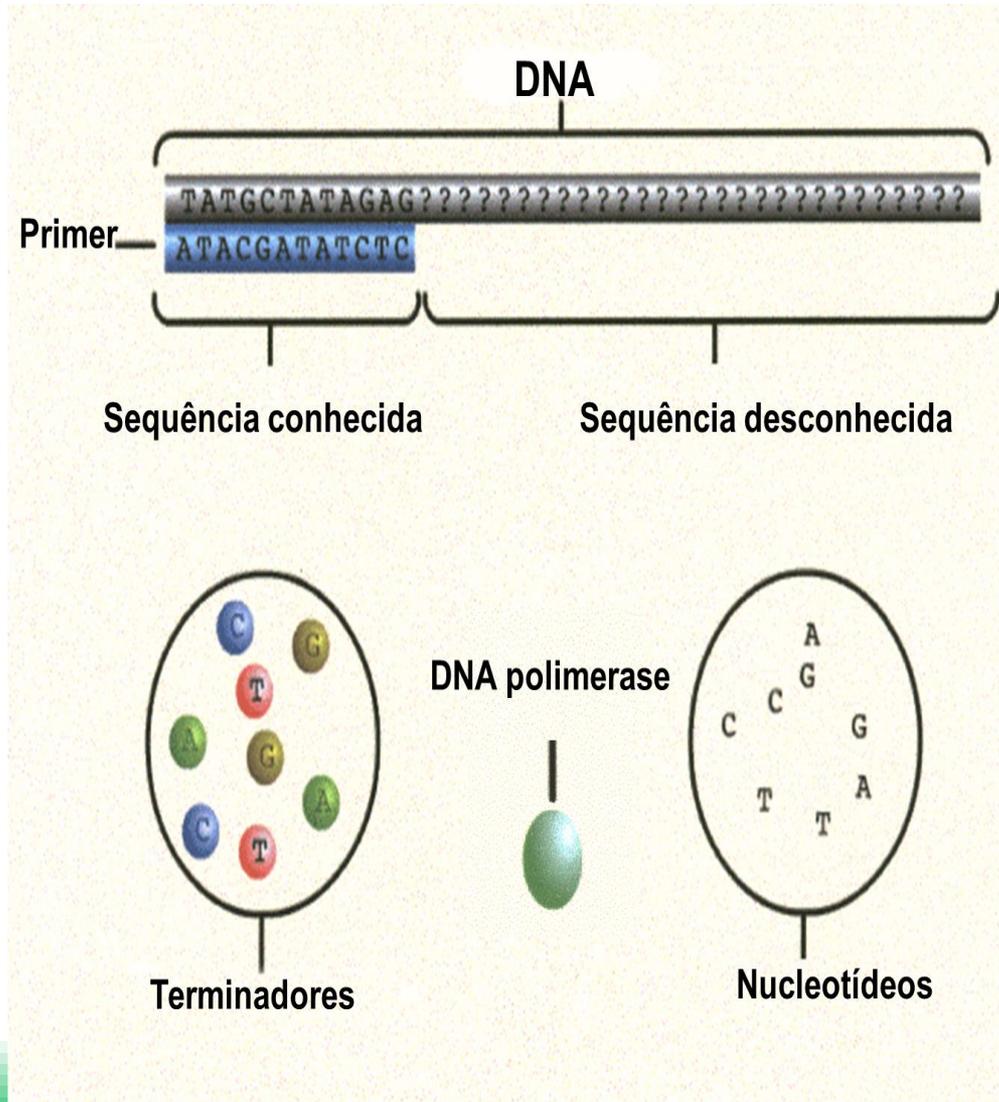
NUCLEOTÍDEOS ddNTPs (TERMINADORES)

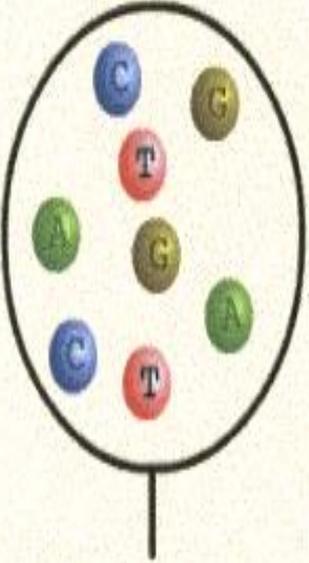
CORANTE FLUORESCENTE

FALTA 3' OH



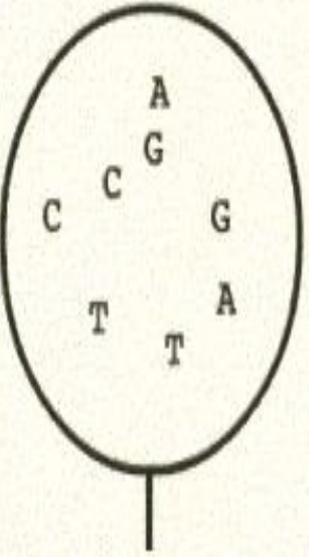
REAÇÃO DE SEQUENCIAMENTO





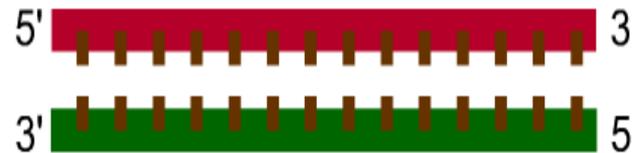
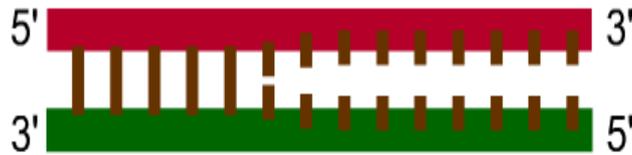
Terminadores

DNA polimerase



Nucleotídeos

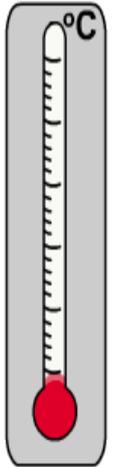
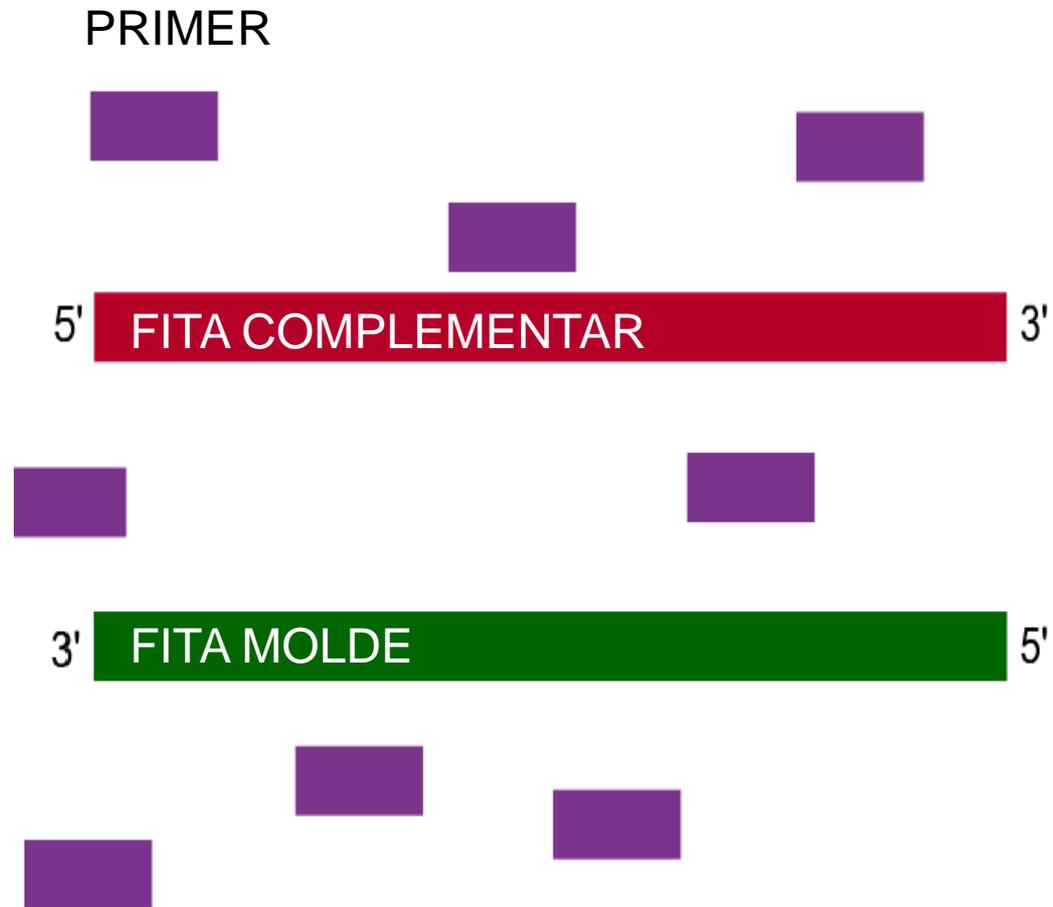
DESNATURAÇÃO DO DNA



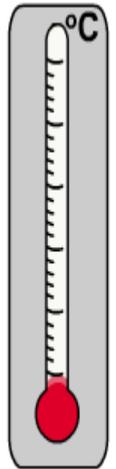
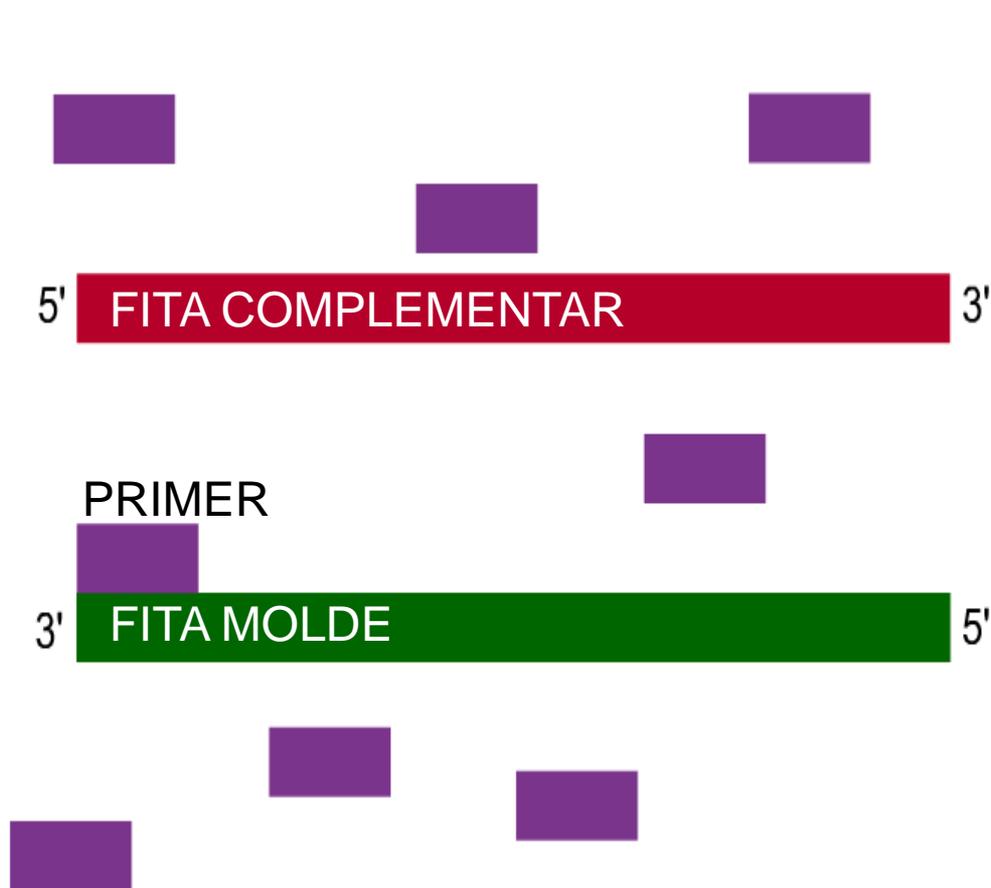
5' FITA COMPLEMENTAR 3'

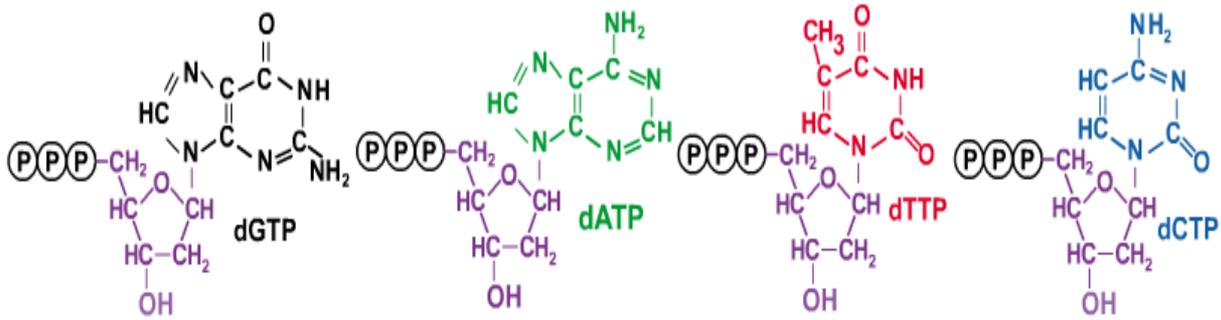
3' FITA MOLDE 5'

ANELAMENTO DOS PRIMERS

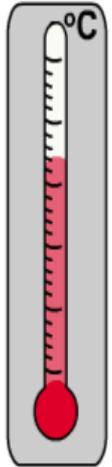
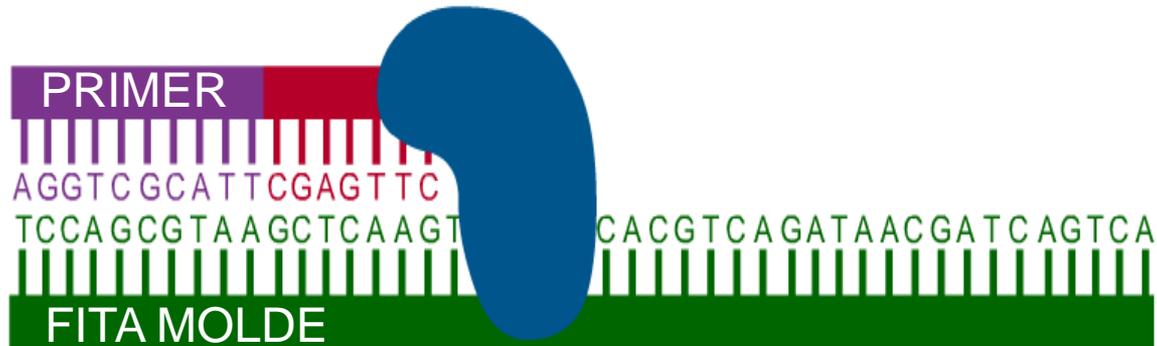


ANELAMENTO DOS PRIMERS





DNA POLIMERASE

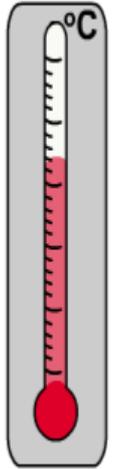


POLIMERIZAÇÃO

PRIMER
AGGTCGCATT

TCCAGCGTAAAGCTCAAGTACTCGCACGTCAGATAACGATCAGTCA

FITA MOLDE



DNA POLIMERASE

PRIMER
AGGTCGCATT

TCCAGCGTAAAGCTCAAGTACTCGCACGTCAGATAACGATCAGTCA

FITA MOLDE



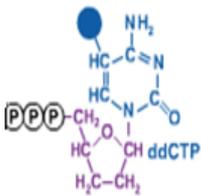
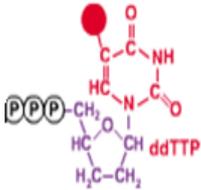
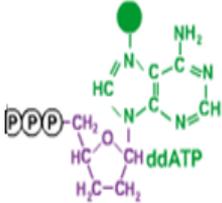
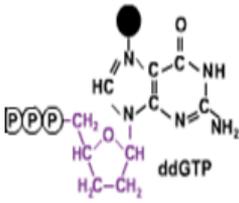
DNA POLIMERASE

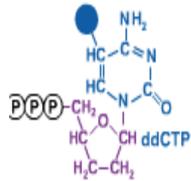
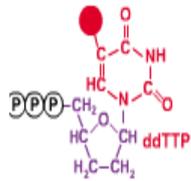
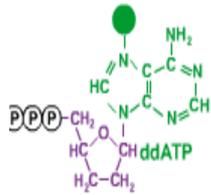
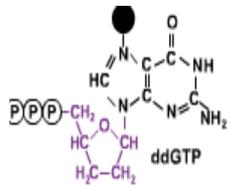
PRIMER
AGGTCGCATT CGAG

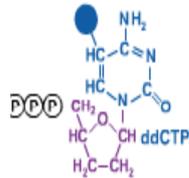
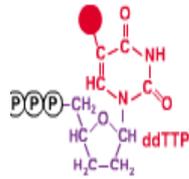
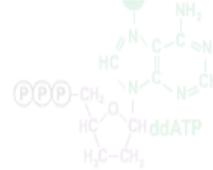
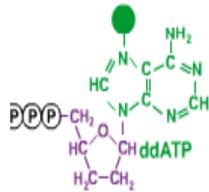
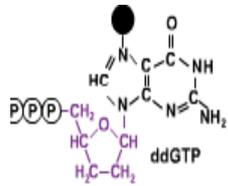
TCCAGCGTAAAGCTCAAGTACTCGCACGTCAGATAACGATCAGTCA

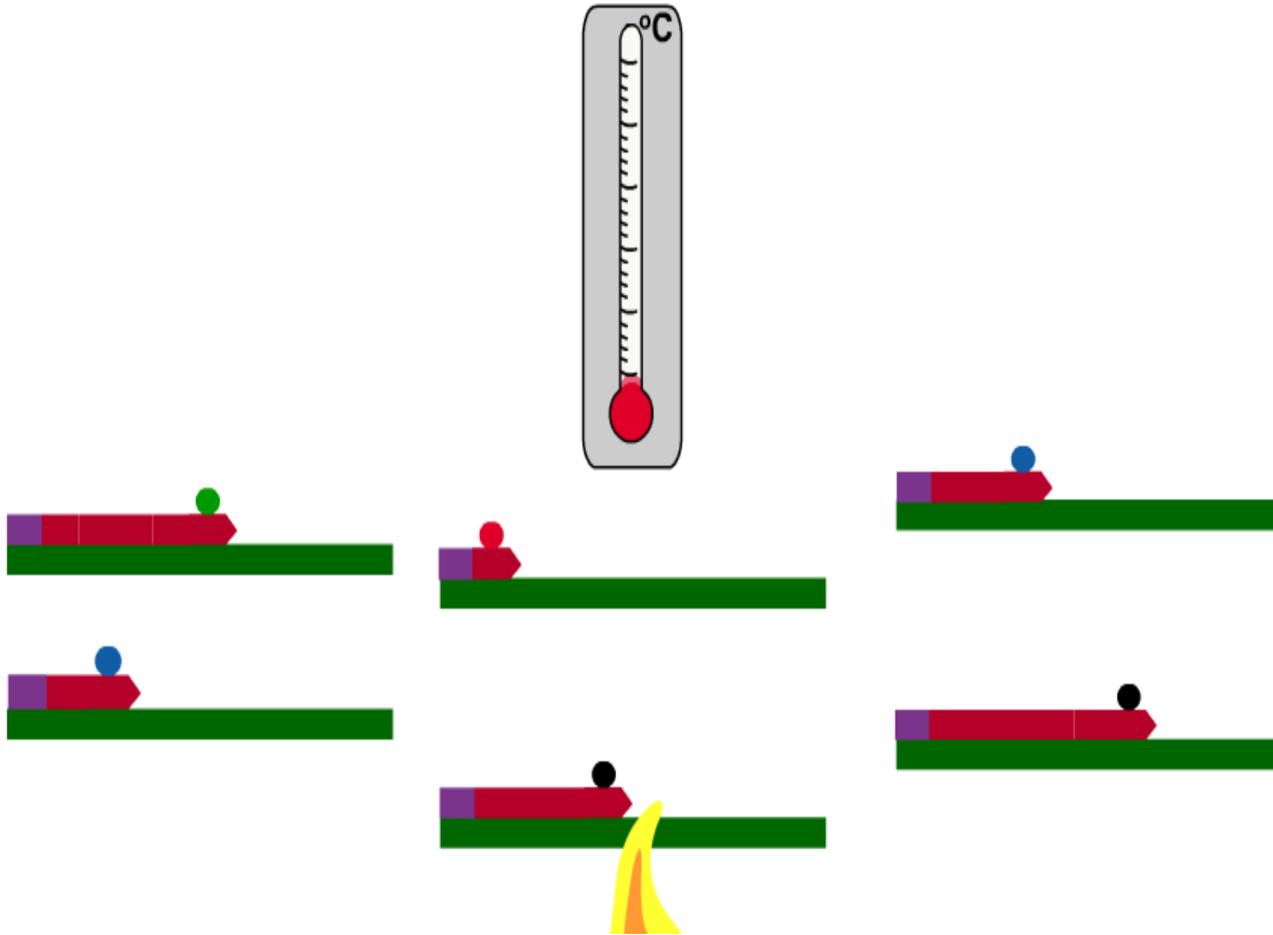
FITA MOLDE

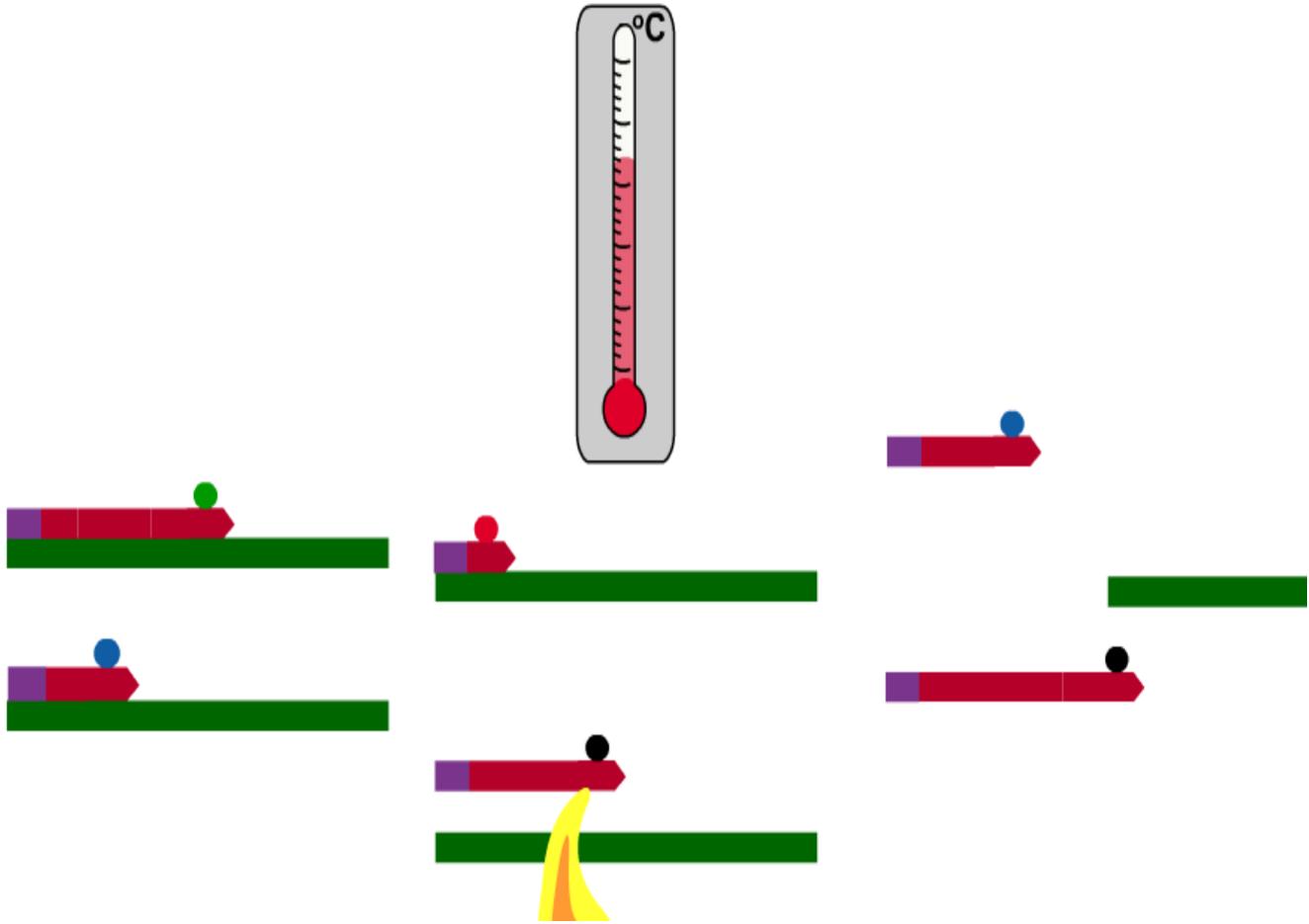


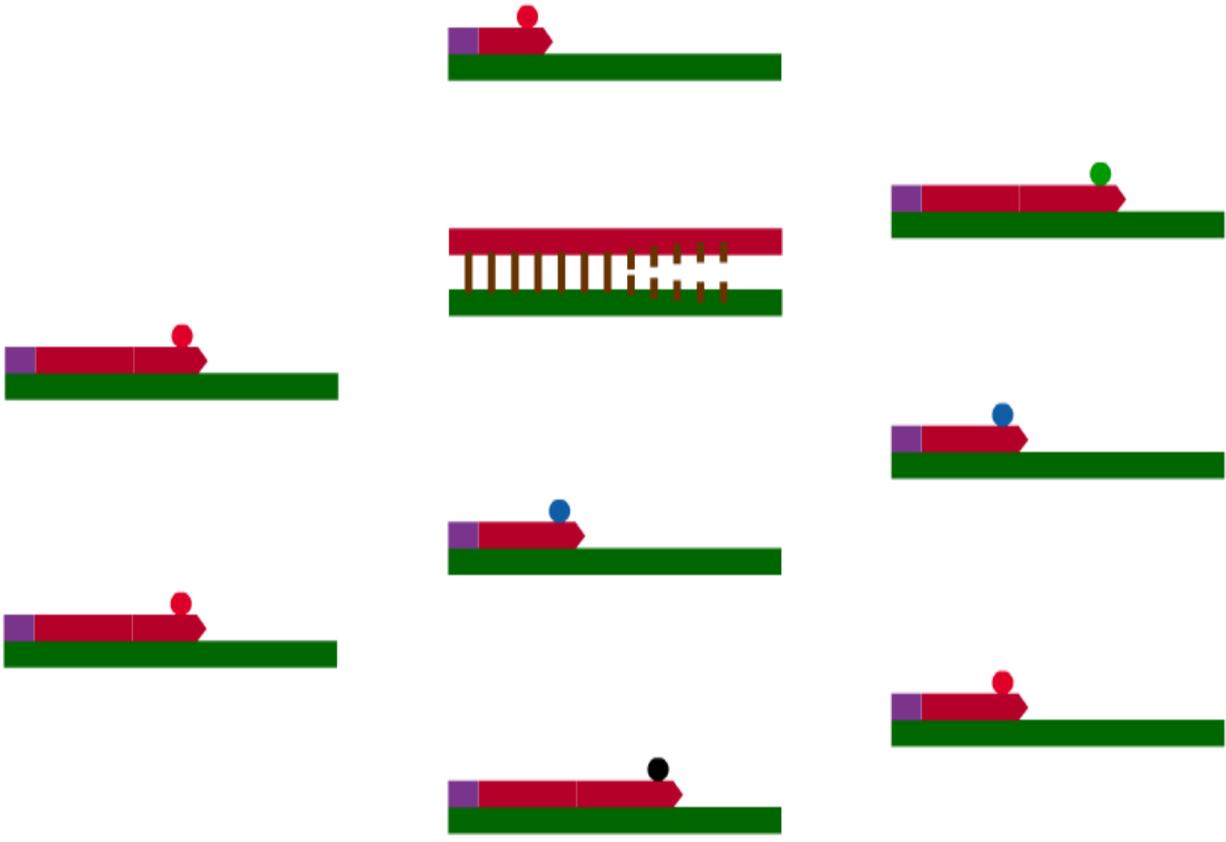




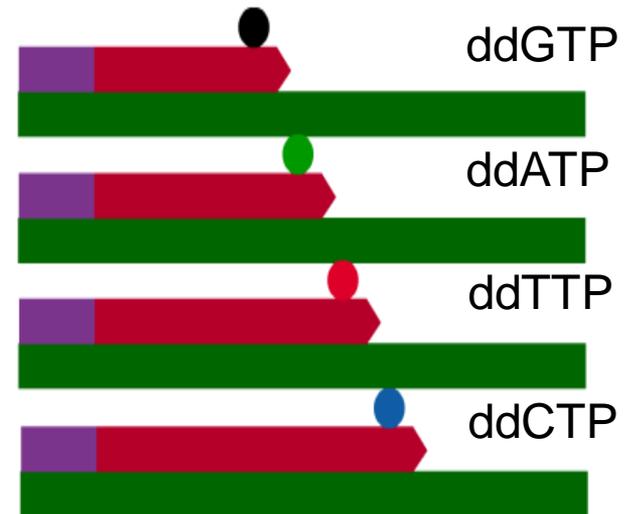








TERMINAÇÃO



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

ATACGATATCTCGACTCTCGAGCTAGAA T

ATACGATATCTCGACTCTCGAGCTAGAATCTTTA A

ATACGATATCTCGACTCTCGAGCT A

ATACGATATCTCGACTCTCG A

ATACGATATCTCGACTCTCGAGCTAGAATCTTT T

ATACGATATCTCGACTCTCGAGCTAGAATCT T

ATACGATATCTCGACTCTCGAGCTAGAATCTTTAAGGCA T

ATACGATATCTCGACTCTCGAGCTAGAATCTTT A

ATACGATATCTCGACTCTCGAGCTAG A

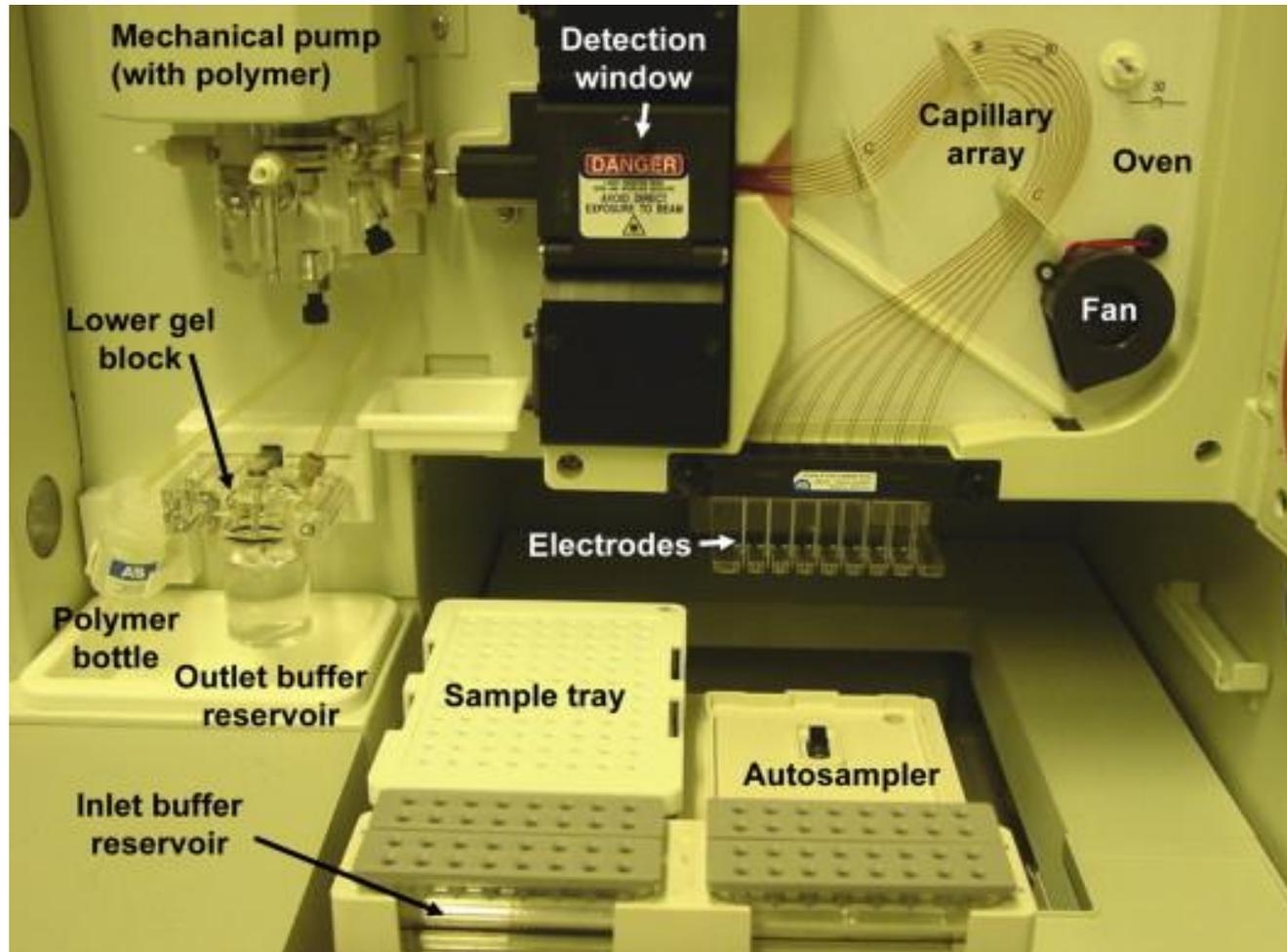
ATACGATATCTCGACTCTCGAGCTAGAATCTTT T

ATACGATATCTCGACTCTCGA A

ATACGATATCTCGACTCT T

ATACGATATCTCGACTCTCGAGCTAGAATCTTTAAGC C

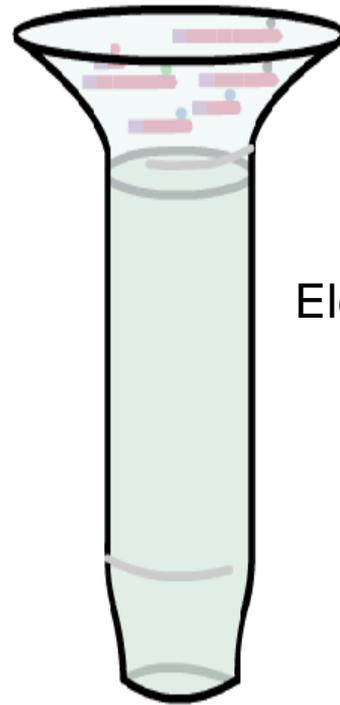
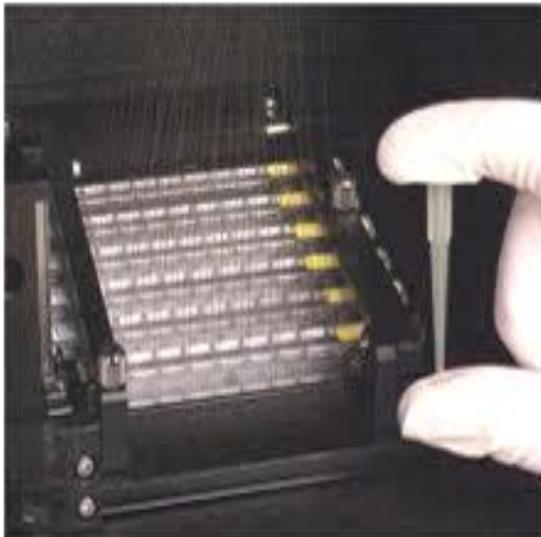
ELETROFORESE CAPILAR



ABI 3700 Applied Biosystems

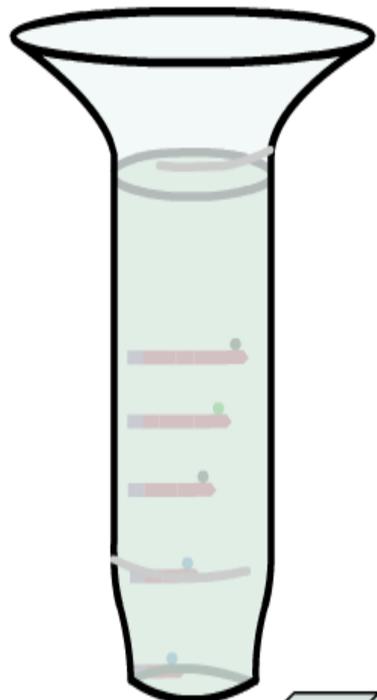
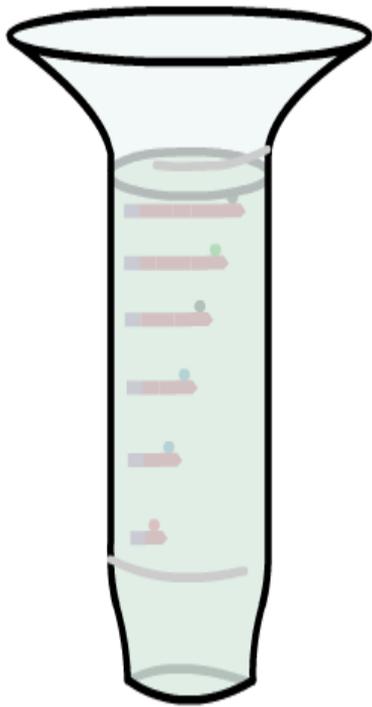


3500 Genetic Analyzer Instrument

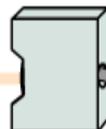


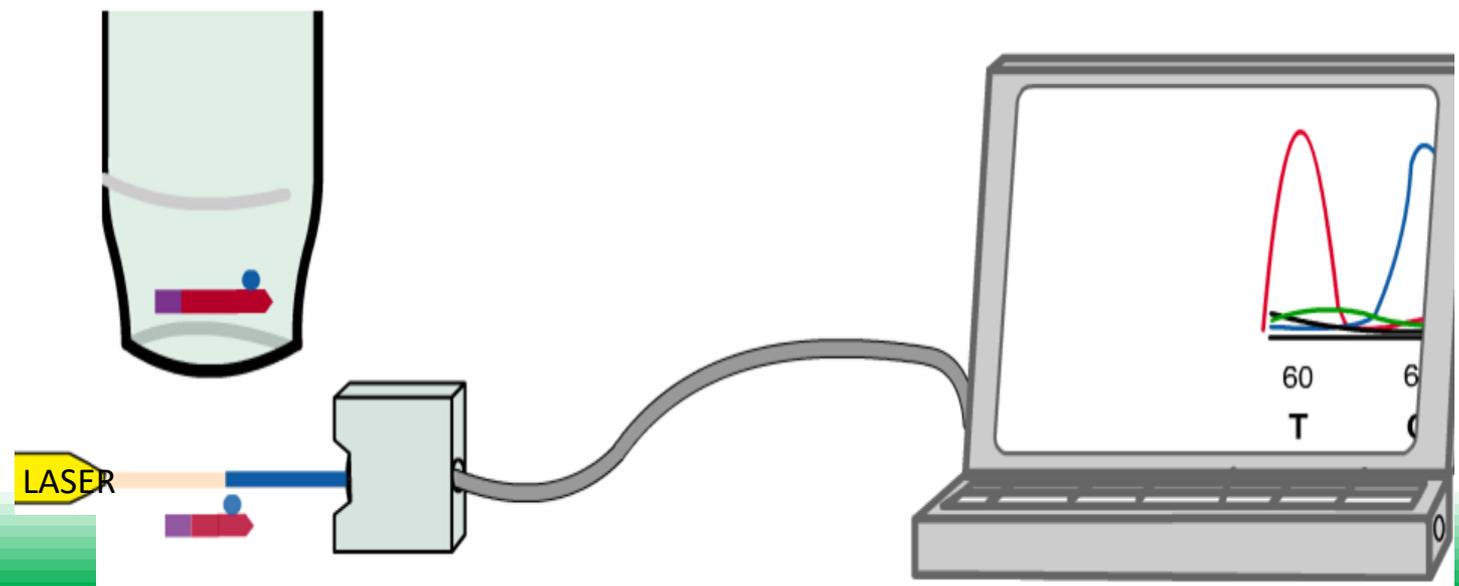
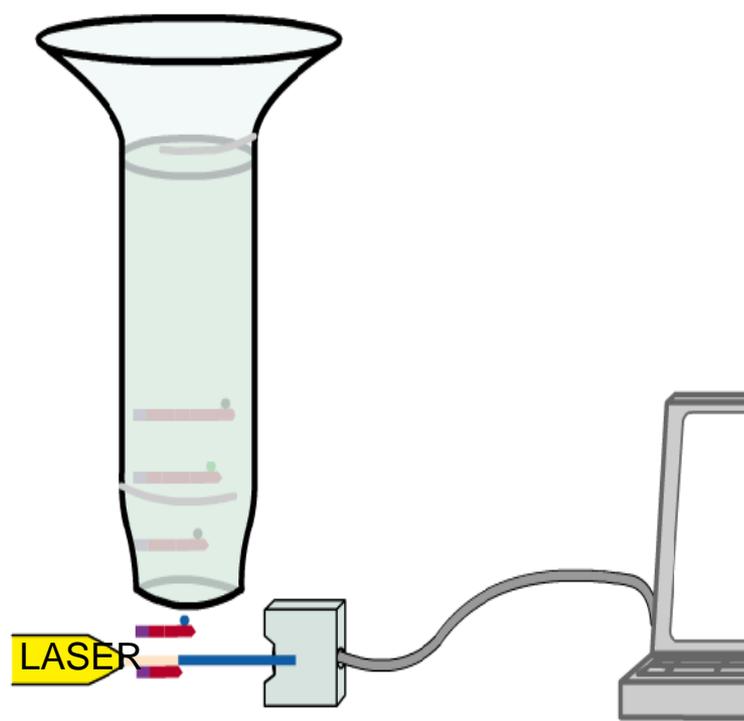
Eletroforese capilar





LASER







**Detecção
a laser**



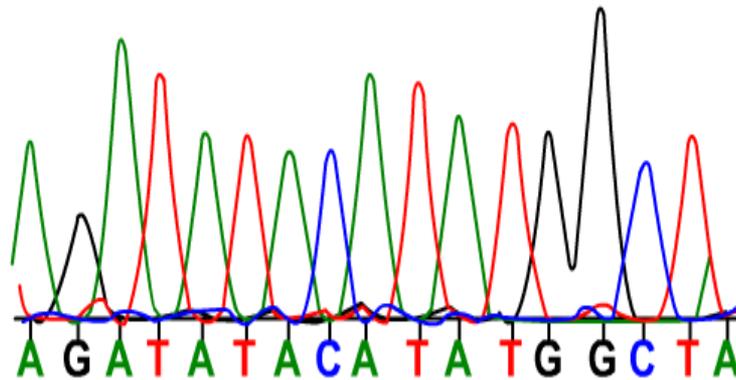


4) Current Task Type (url & prof) - Query Tool

Vídeo: secuenciamento Sanger

- <https://app.jove.com/embed/player?id=12020&t=1&fpv=1>

ANÁLISE COMPUTACIONAL



ACTTGACGTAGCTAC

AGCTACGTTACCTATAGGTACGTTAC

TACGTTACGGAGGCTATCGCGAT

TCGCGATGAGATCAAA

FRAGMENTOS DE DNA SECUENCIADOS

ACTTGACGTAGCTAC

AGCTACGTTACCTATAGGTACGTTAC

TACGTTACGGAGGCTATCGCGAT

TCGCGATGAGATCAAA

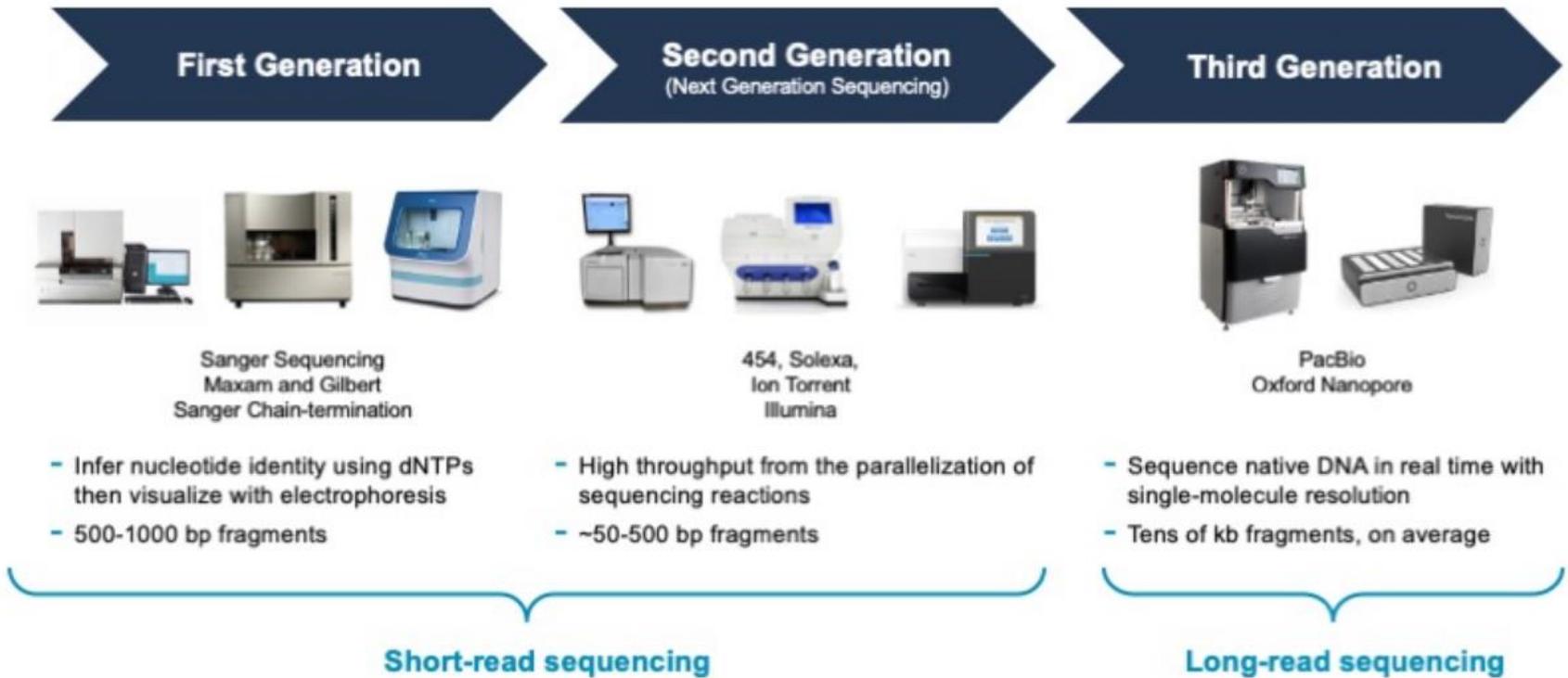
ACTTGACGTAGCTACGTTACCTATAGGTACGTTACGGAGGCTATCGCGATGAGATCAAA

ACTTGACGTAGCTACGTTACCTATAGGTACGTTACGGAGGCTATCGCGATGAGATCAAA

FRAGMENTOS COMPLETOS

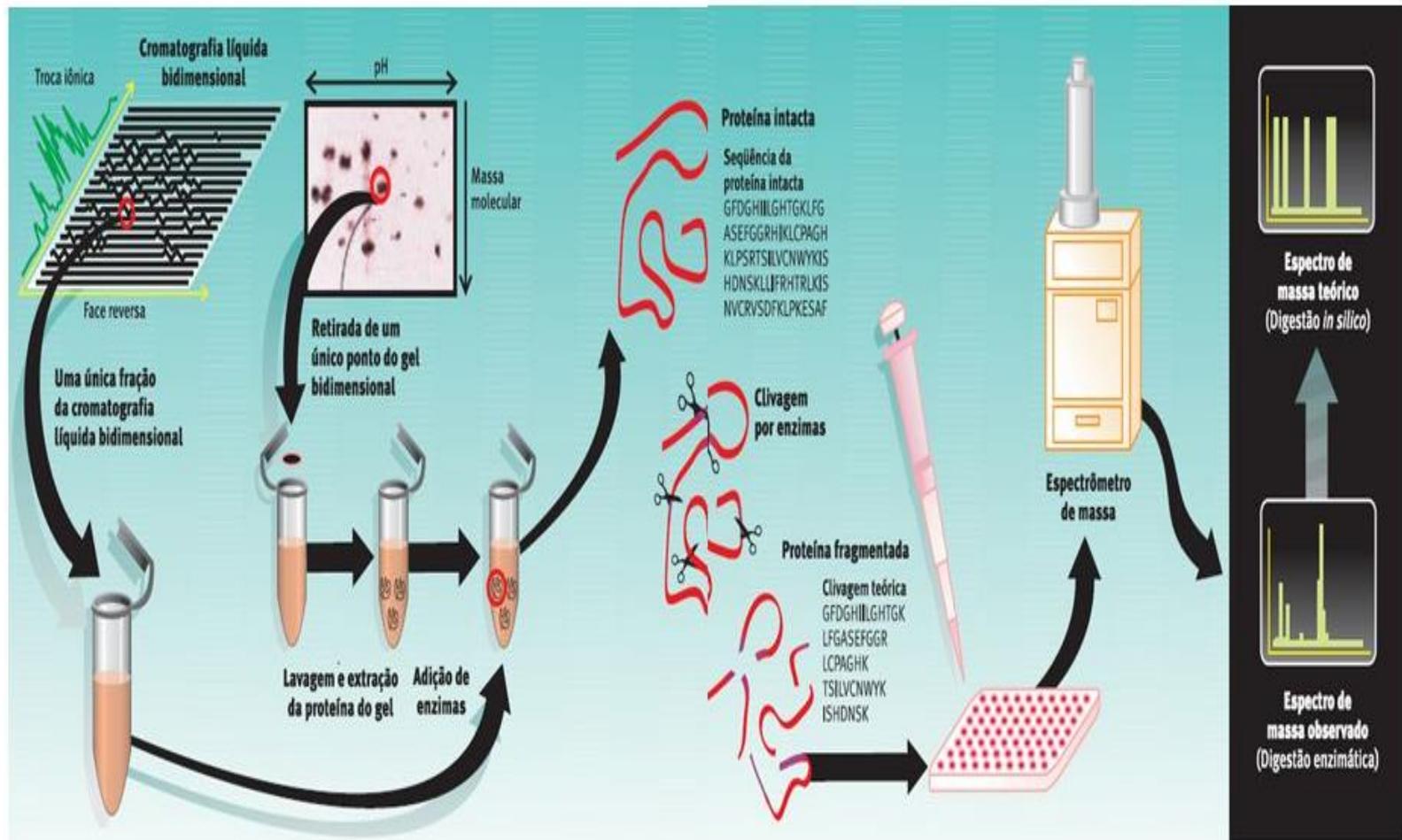
**HOJE EXISTEM OUTRAS TECNOLOGIAS DE
SEQUENCIAMENTO (NGS OU HTS)**

Tecnologias de segunda geração



Tecnologia de primeira geração

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=3l9wzvj0b_A

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

<https://www.youtube.com/watch?v=GUb1TZvMWsw>



SUMÁRIO

- Revisando
- Clonagem
- Princípio da PCR;
- Aplicações da PCR
- Variações muito comuns: RT-PCR e qPCR
- Técnica de sequenciamento de Sanger
- Novas técnicas de sequenciamento
- Estudo dirigido.

ESTUDO DIRIGIDO

1. PCR;
2. Aplicação da técnica de PCR;
3. Sequenciamento por Sanger;
4. Novas técnicas de sequenciamento.

Leitura

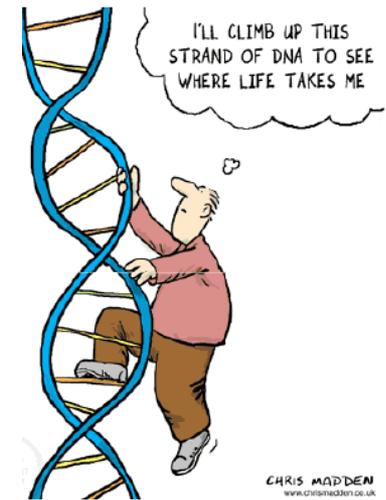
Capítulo 2 – Princípios da PCR convencional e

Capítulo 3 - Aplicações gerais da PCR convencional (páginas 31 a 53).

Pereira, T.C. Introdução às técnicas de PCR convencional, em tempo real e digital. Sociedade Brasileira de Genética, 2018.

Capítulo 10 – Fundamentos de Biologia Celular

LER o texto “Sequenciamento de nova geração”



The image features a central graphic consisting of several concentric circles. The innermost circle is a dark blue color. Surrounding it are several rings of varying shades of red, from a deep, dark red to a lighter, more vibrant red. The outermost ring is a solid dark red. Overlaid on this circular pattern is the text "That's all Folks!" written in a white, elegant cursive font. The text is positioned diagonally across the center of the circles. The entire graphic is set against a solid dark red background. The image is framed by a thin green border at the top and bottom edges.

That's all Folks!