CURSO DE APERFEIÇOAMENTO: TRIAGEM LABORATORIAL E CONTROLE DE QUALIDADE EM SANGUE, TECIDOS, CÉLULAS E ÓRGÃOS

Triagem laboratorial: Biologia Molecular I

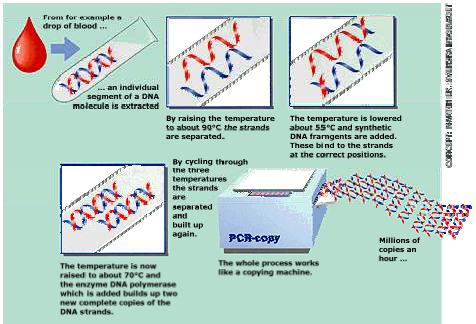
Milena Batista de Oliveira
Farmacêutica Bioquímica

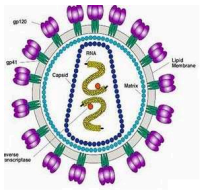
SUS ANVISA Agência Nacional de Vigilância Sanitária Ministério da Saúde Governo Federal

- O que é NAT ou NAAT ?

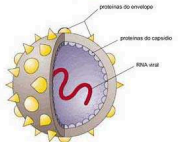
- NAT ou NAAT = *"Nucleic Acid Amplification Testing"*

- Teste de amplificação do Ácido Nucléico





HIV



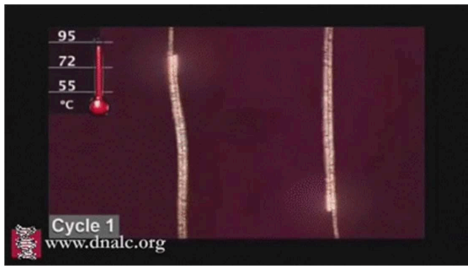
HCV

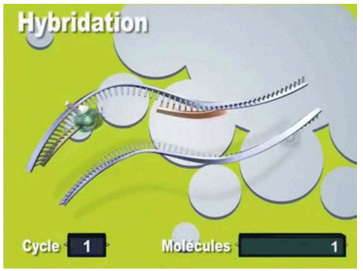
HBV

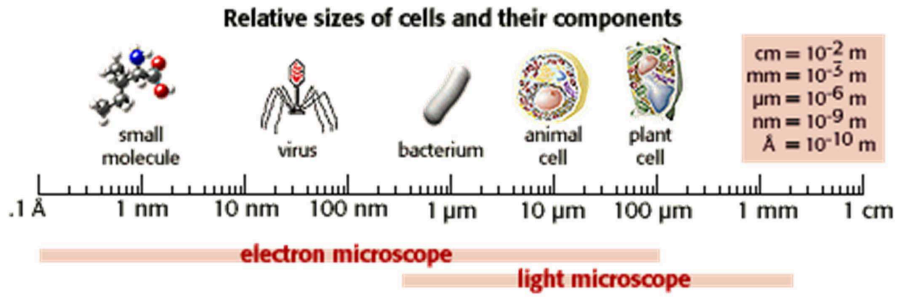
RNA/DNA

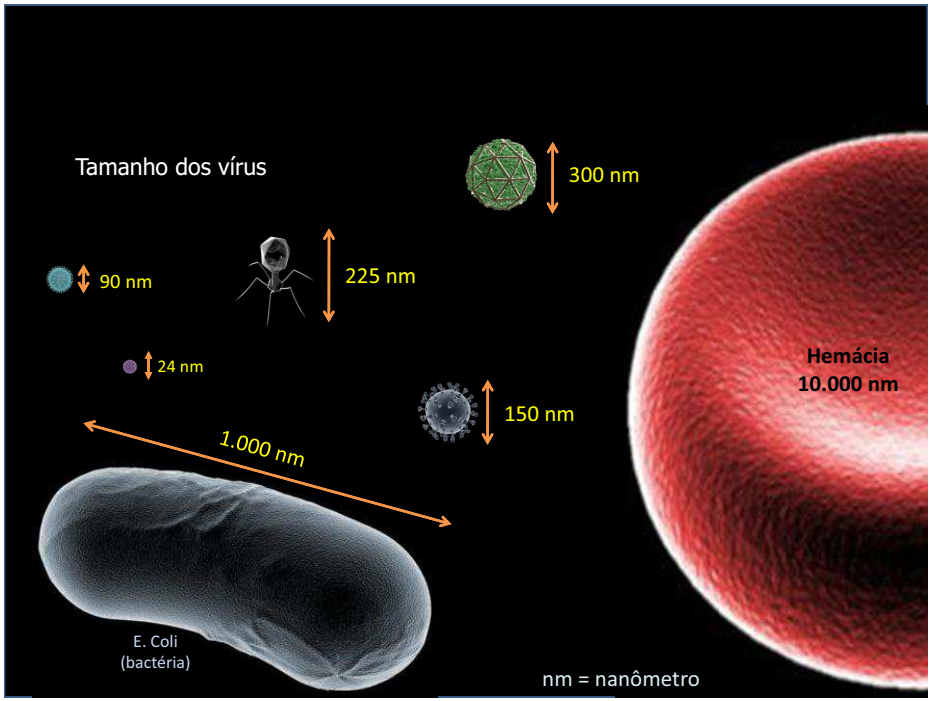
- *PCR – Reação em cadeia da polimerase*

Real Time



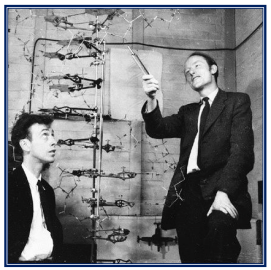


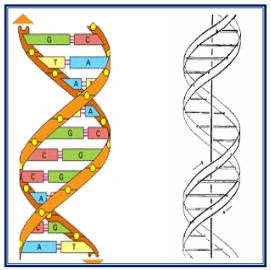




Breve Histórico

- 1952 – James Watson & Francis Crick
- Modelo da molécula de DNA





1950 1960 1970 1980 1990 2000 2010

Breve Histórico

No. 4356 April 25, 1953 NATURE 737

equipment, and to Dr. G. E. R. Deacon and the captain and officers of R.R.S. *Discovery II* for their part in making the observations.

[1] Young, F. B., Gerrard, H., and Jevons, W., *Phil. Mag.*, **40**, 149 (1920).
[2] Longuet-Higgins, M. S., *Mon. Not. Roy. Astro. Soc., Geophys. Supp.*, **5**, 285 (1949).
[3] Von Arx, W. S., Woods Hole Papers in Phys. Oceanog. Meteor., **11** (3) (1950).
[4] Ekman, V. W., *Arkiv. Mat. Astron. Fysik.* (Stockholm), **2** (11) (1905).

MOLECULAR STRUCTURE OF NUCLEIC ACIDS

A Structure for Deoxyribose Nucleic Acid

We wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

is a residue on each chain every 3·4 A. in the z-direction. We have assumed an angle of 36° between adjacent residues in the same chain, so that the structure repeats after 10 residues on each chain, that is, after 34 A. The distance of a phosphorus atom from the fibre axis is 10 A. As the phosphates are on the outside, cations have easy access to them.

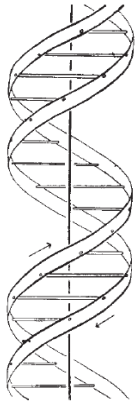
The structure is an open one, and its water content is rather high. At lower water contents we would expect the bases to tilt so that the structure could become more compact.

The novel feature of the structure is the manner in which the two chains are held together by the purine and pyrimidine bases. The planes of the bases are perpendicular to the fibre axis. They are joined together in pairs, a single base from one chain being hydrogen-bonded to a single base from the other chain, so that the two lie side by side with identical z-co-ordinates. One of the pair must be a purine and the other a pyrimidine for bonding to occur. The hydrogen bonds are made as follows: purine position

1950 1960 1970 1980 1990 2000 2010

Breve Histórico

- O DNA é uma hélice dupla composta por duas cadeias complementares que giram para a direita em direções opostas.
- As cadeias estão compostas por milhões de nucleotídeos.

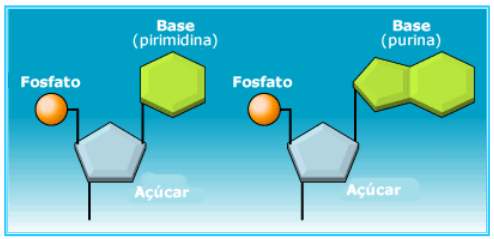


1950 1960 1970 1980 1990 2000 2010

Conceito

– Nucleotídeos

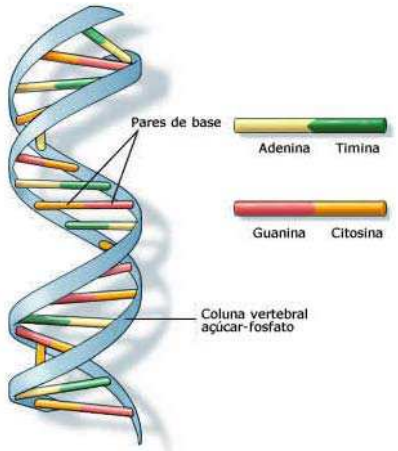
Fosfato + Desoxirribose + Base Nitrogenada



Conceito

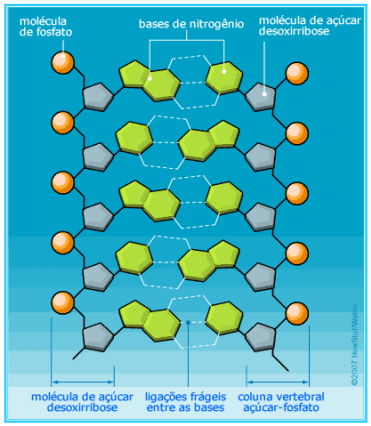
– Bases Púricas: A e G
– Bases Pirimídicas: T e C

Pareamento
das Bases



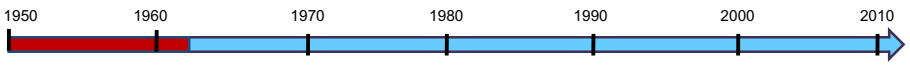
Conceito

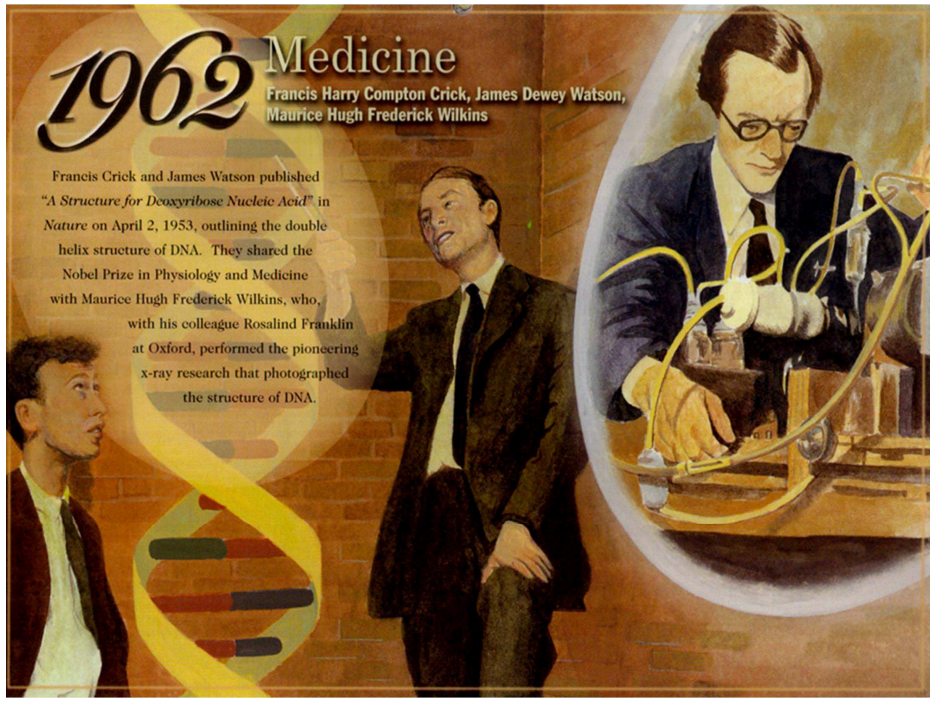
– Ligações de Hidrogênio
  – A = T
  – C ≡ G



Breve Histórico

- 1962 – Watson, Crick e Maurice Wilkins
  Prêmio Nobel de Medicina



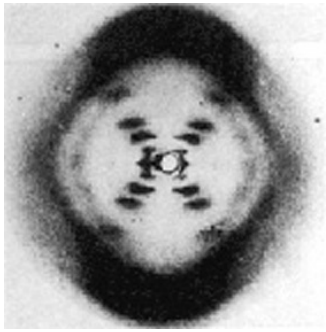


Breve Histórico

Rosalind Franklin

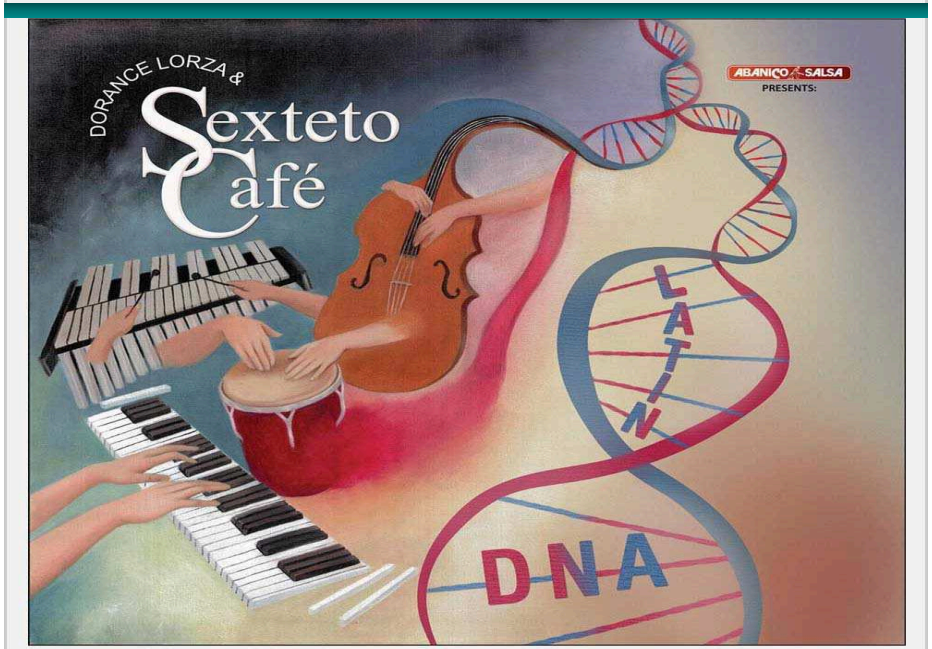
Breve Histórico

- Rosalind Franklin morreu de cancer em 1958 aos 37 anos, possivelmente como resultado da exposição ao Raio-X usado nas suas pesquisas.

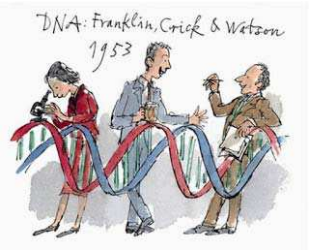


O Poder do DNA

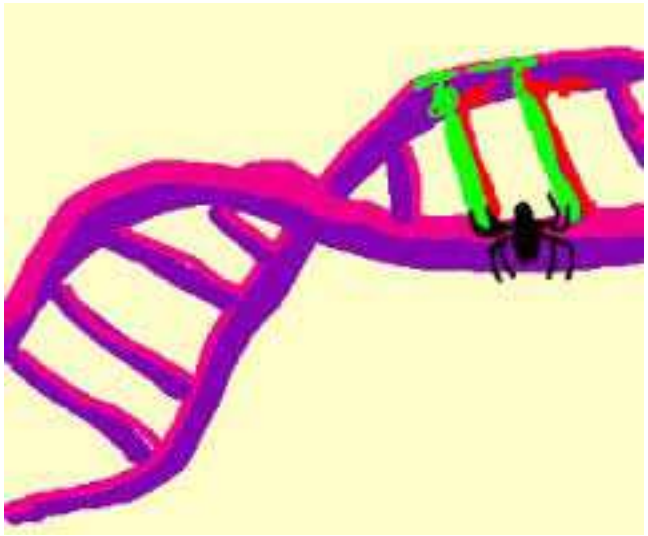
Curso de aperfeiçoamento: triagem laboratorial e controle de qualidade em sangue, tecidos, células e órgãos



Curso de aperfeiçoamento: triagem laboratorial e controle de qualidade em sangue, tecidos, células e órgãos



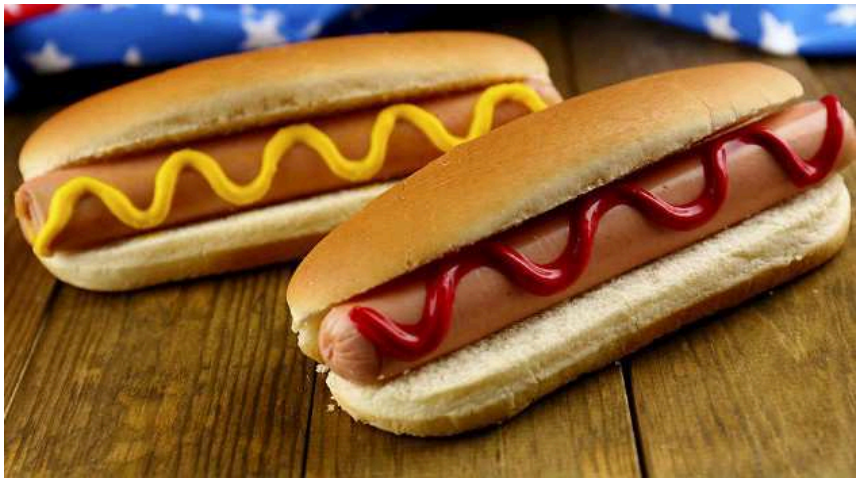
Curso de aperfeiçoamento: triagem laboratorial e controle de qualidade em sangue, tecidos, células e órgãos



Curso de aperfeiçoamento: triagem laboratorial e controle de qualidade em sangue, tecidos, células e órgãos

Curso de aperfeiçoamento: triagem laboratorial e controle de qualidade em sangue, tecidos, células e órgãos

Curso de aperfeiçoamento: triagem laboratorial e controle de qualidade em sangue, tecidos, células e órgãos

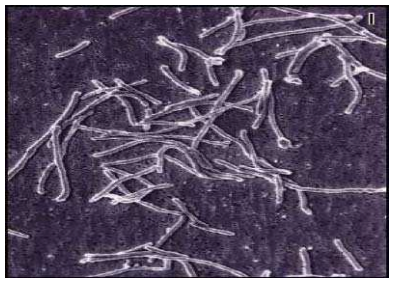


- Vídeo DNA

http://backboneanimation.com/

Breve Histórico

- 1969 – T. Brock e H. Freeze– ***Thermus aquaticus*** **–** sobrevive na água a temperatura média de 75ºC



JOURNAL OF BACTERIOLOGY, Aug. 1969, p. 289-297
Copyright © 1969 American Society for Microbiology
Vol. 98, No. 1
Printed in U.S.A.

Thermus aquaticus gen. n. and sp. n., a Non-sporulating Extreme Thermophile

THOMAS D. BROCK AND HUDSON FREEZE
Department of Microbiology, Indiana University, Bloomington, Indiana 47401

Received for publication 15 January 1969

The isolation of a new thermophilic bacterium, *Thermus aquaticus* gen. n. and sp. n., is described. Successful enrichment requires incubation at 70 to 75 C, and the use of nutrient media relatively dilute with respect to the organic components. Strains of *T. aquaticus* have been isolated from a variety of thermal springs in Yellowstone National Park and from a thermal spring in California. The organism has also been isolated from man-made thermal habitats, such as hot tap water, in geographical locations quite distant from thermal springs. Isolates of *T. aquaticus*

1950 1960 1970 1980 1990 2000 2010

Curso de aperfeiçoamento: triagem laboratorial e controle de qualidade em sangue, tecidos, células e órgãos

Breve Histórico

- 1971- Professor Har Gobind Khorana

Professor Har Gobind Khorana

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Curso de aperfeiçoamento: triagem laboratorial e controle de qualidade em sangue, tecidos, células e órgãos

Breve Histórico

– Princípio: replicação de um fragmento de DNA através do uso de 2 primers

J. Mol. Biol. (1971) 56, 341–361

Studies on Polynucleotides

XCVI.† Repair Replication of Short Synthetic DNA's as catalyzed by DNA Polymerases

K. KLEPPE,‡ E. OHTSUKA,§ R. KLEPPE,‡ I. MOLINEUX‖ AND H. G. KHORANA‖
Institute for Enzyme Research of the University of Wisconsin Madison, Wisc. 53706, U.S.A.

(*Received 20 July 1970*)

Repair replication of short synthetic DNA's corresponding to parts of the gene for the major yeast alanine transfer RNA has been studied. The enzymes used were the *Escherichia coli*, *Micrococcus luteus* and T4 DNA polymerases, similar results being obtained with all the three enzymes. The DNA's used (Fig. 1) were: four double-stranded DNA's with the termini containing the 5'-hydroxyl group protruding by one, three, four or ten nucleotide units and two single-stranded DNA's 22 units long which are capable of folding back on themselves. The aspects investigated were: (1) completion of repair, (2) the minimum size of the polynucleotide chains required as primers and those which can serve as templates and (3) the minimum size of the primers which can abolish hairpin formation so as to give the "normal" repair replication. Repair replication of DNA's (DNA-I to DNA-IV; Fig. 1) was characterized to be essentially complete. The minimum size of the primer for repair replication of an extended single stranded deoxypolynucleotide was ascertained to be about five to seven units long, while the primers required to overcome the hairpin formation in DNA-V and DNA-VI were found to be about twelve

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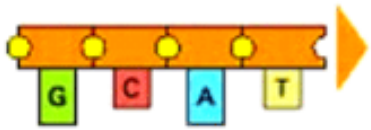
Curso de aperfeiçoamento: triagem laboratorial e controle de qualidade em sangue, tecidos, células e órgãos

Conceito

– Primers, sequência de aproximadamente 15 a 25 nucleotídeos.



Conceito

– Os primers definem o alvo que se deseja amplificar.

DNA

TACGCCCGATCTCGTCCGATCTCGGAAGCTAAGCAGGCTCAGGCGTGGTTAGTACTTGGATGGGAGA
CCGCCTGGGAATACCAGGTGCCGTAGGCTTTTGCTGCTGCTGCTGCTGCTCCTCTGAACAGATGTCCC
CGCAGTAGGAGCTGCTCTCTTCTTTTCAGTCCGCCTCTGACATAGACTCTCGGCACTGCGCTTCTCAC
ATCAGCCCAGAAACGCAAGCACCCTGCTCCTGCTGTCAAGGCACAGGAATACAAGAATGTGCTTTTG
GTGGGCATCCTAGTGCTGCAAGTGCTTCCTCCATACCGCCGCGCTTCGCCACTCGCCTACTGCCGCCGG
ATCTCCCTCAGTCTCAGCCAGGCAGCAAGATGCAAGGGGCTCAAGCGTTCTTGCGCCTCTCTTCTGCG
CCTTGCGCAAACCGTCCATTCTCAGTTGAGGCTTACCTTTGCCTTCTGAGCAGGCATCAGCAAAGGCG
GCAGCTGCTTCCTGCGGCCAGCGGGCTGGCCGAGTAGCCTCTGATGGCTGAGACTGAGCAGCCTGTAT

Conceito

– Os primers definem o alvo que se deseja amplificar.

DNA

TACGCCCGATCTCGTCCGATCTCGGAAGCTAAGCAGGCTCAGGCGTGGTTAGTACTTGGATGGGAGA
CCGCCTGGGAATACCAGGTGCCGTAGGCTTTTGCTGCTGCTGCTGCTGCTCCTCTGAACAGATGTCCC
CGCAGTAGGAGCTGCTCTCTTCTTTTCAGTCCGCCTCTGACATAGACTCTCGGCACTGCGCTTCTCAC
ATCAGCCCAGAAACGCAAGCACCCTGCTCCTGCTGTCAAGGCACAGGAATACAAGAATGTGCTTTTG
GTGGGCATCCTAGTGCTGCAAGTGCTTCCTCCATACCGCCGCGCTTCGCCACTCGCCTACTGCCGCCGG
ATCTCCCTCAGTCTCAGCCAGGCAGCAAGATGCAAGGGGCTCAAGCGTTCTTGCGCCTCTCTTCTGCG
CCTTGCGCAAACCGTCCATTCTCAGTTGAGGCTTACCTTTGCCTTCTGAGCAGGCATCAGCAAAGGCG
GCAGCTGCTTCCTGCGGCCAGCGGGCTGGCCGAGTAGCCTCTGATGGCTGAGACTGAGCAGCCTGTAT

Conceito

– Os primers definem o alvo que se deseja amplificar.

DNA

ATGCGGGTAGAGCAGGCTA
TACGCCCGATCTCGTCCGATCTCGGAAGCTAAGCAGGCTCAGGCGTGGTTAGTACTTGGATGGGAGA
CCGCCTGGGAATACCAGGTGCCGTAGGCTTTTGCTGCTGCTGCTGCTGCTCCTCTGAACAGATGTCCC
CGCAGTAGGAGCTGCTCTCTTCTTTTCAGTCCGCCTCTGACATAGACTCTCGGCACTGCGCTTCTCAC
ATCAGCCCAGAAACGCAAGCACCCTGCTCCTGCTGTCAAGGCACAGGAATACAAGAATGTGCTTTTG
GTGGGCATCCTAGTGCTGCAAGTGCTTCCTCCATACCGCCGCGCTTCGCCACTCGCCTACTGCCGCCGG
ATCTCCCTCAGTCTCAGCCAGGCAGCAAGATGCAAGGGGCTCAAGCGTTCTTGCGCCTCTCTTCTGCG
CCTTGCGCAAACCGTCCATTCTCAGTTGAGGCTTACCTTTGCCTTCTGAGCAGGCATCAGCAAAGGCG
GCAGCTGCTTCCTGCGGCCAGCGGGCTGGCCGAGTAGCCTCTGATGGCTGAGACTGAGCAGCCTGTAT
ACTCTGACTCGTCGGACATA

Conceito

– Os primers definem o alvo que se deseja amplificar.

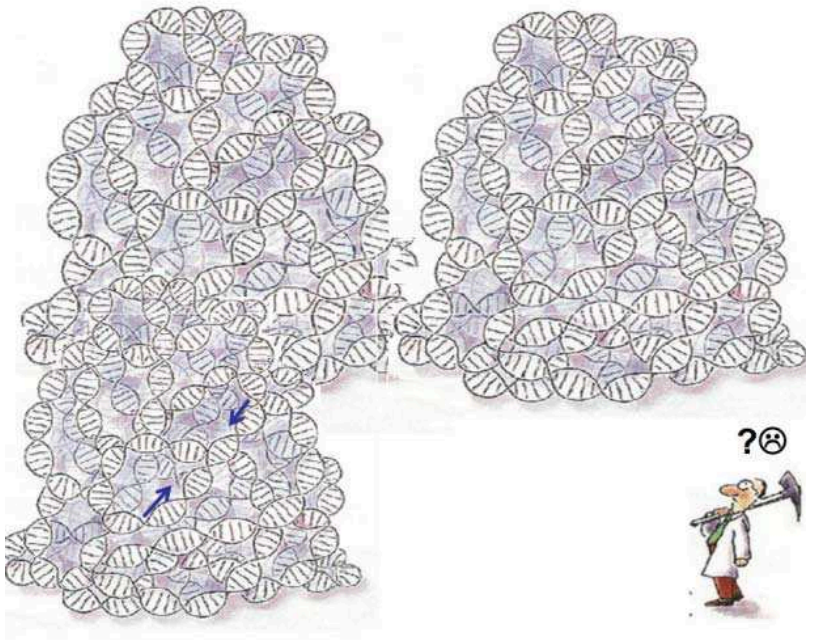
DNA

ATGCGGGTAGAGCAGGCTA
TACGCCCGATCTCGTCCGATCTCGGAAGCTAAGCAGGCTCAGGCGTGGTTAGTACTTGGATGGGAGA
CCGCCTGGGAATACCAGGTGCCGTAGGCTTTTGCTGCTGCTGCTGCTGCTCCTCTGAACAGATGTCCC
CGCAGTAGGAGCTGCTCTCTTCTTTTCAGTCCGCCTCTGACATAGACTCTCGGCACTGCGCTTCTCAC
ATCAGCCCAGAAACGCAAGCACCCTGCTCCTGCTGTCAAGGCACAGGAATACAAGAATGTGCTTTTG
GTGGGCATCCTAGTGCTGCAAGTGCTTCCTCCATACCGCCGCGCTTCGCCACTCGCCTACTGCCGCCGG
ATCTCCCTCAGTCTCAGCCAGGCAGCAAGATGCAAGGGGCTCAAGCGTTCTTGCGCCTCTCTTCTGCG
CCTTGCGCAAACCGTCCATTCTCAGTTGAGGCTTACCTTTGCCTTCTGAGCAGGCATCAGCAAAGGCG
GCAGCTGCTTCCTGCGGCCAGCGGGCTGGCCGAGTAGCCTCTGATGGCTGAGACTGAGCAGCCTGTAT
ACTCTGACTCGTCGGACATA

Curiosidade

- O genoma humano tem cerca de 6 milhões de pares de base de comprimentos . Cada par de base tem 3,4×10^-10 m de comprimento (3,4 Angstrom). Portanto, cada célula terá cerca de 2 m de DNA.
- Multiplicando pelo número de células do corpo humano (1×10^13), obtemos o valor de 2×10^10 km. Dividindo pelo dobro da distância ao Sol (ida e volta), concluímos que temos DNA suficiente para ir e vir ao Sol… 66,8 vezes.

Curso de aperfeiçoamento: triagem laboratorial e controle de qualidade em sangue, tecidos, células e órgãos



Curso de aperfeiçoamento: triagem laboratorial e controle de qualidade em sangue, tecidos, células e órgãos

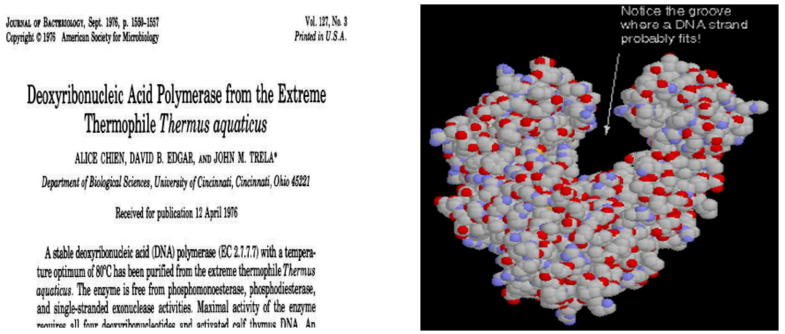
- **Alvos genômicos no HIV, HCV e HBV**
- **HIV Integrase – HCV 5'UTR – Proteína S**

- Regiões genômicas mais conservadas com cerca de 100pb;
- - Menor risco de perda de sensibilidade devido a variantes virais (genótipos e subtipos);

Curso de aperfeiçoamento: triagem laboratorial e controle de qualidade em sangue, tecidos, células e órgãos

Breve Histórico

- 1976- Taq DNA polimerase termoestável é isoldada da bactéria *Thermus aquaticus*.



Yellowstone National Park

Breve Histórico

- 1985 - Kary B. Mullis – Sugeriu a utilização da Taq DNA polimerase termoestável para PCR.

1950 1960 1970 1980 1990 2000 2010

Kary Mullis

Breve Histórico

Science AAAS

RESEARCH ARTICLE

1985; 230 (4732): 1350-4

Enzymatic Amplification of β-Globin Genomic Sequences and Restriction Site Analysis for Diagnosis of Sickle Cell Anemia

Randall K. Saiki, Stephen Scharf, Fred Faloona, Kary B. Mullis
Glenn T. Horn, Henry A. Erlich, Norman Arnheim

Recent advances in recombinant DNA technology have made possible the molecular analysis and prenatal diagnosis of several human genetic diseases. Fetal DNA obtained by aminocentesis or chorionic villus sampling can be analyzed by restriction enzyme digestion, with subsequent electrophoresis, Southern transfer, and specific hybridization to cloned gene or oligonucleotide probes. With

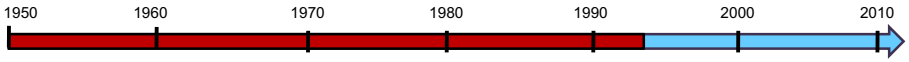
This disease results from homozygosity of the sickle-cell allele (β^S) at the β-globin gene locus. The S allele differs from the wild-type allele (β^A) by substitution of an A in the wild-type to a T at the second position of the sixth codon of the β chain gene, resulting in the replacement of a glutamic acid by a valine in the expressed protein. For the prenatal diagnosis of sickle cell anemia, DNA ob-

Abstract. *Two new methods were used to establish a rapid and highly sensitive prenatal diagnostic test for sickle cell anemia. The first involves the primer-mediated enzymatic amplification of specific β-globin target sequences in genomic DNA, resulting in the exponential increase (220,000 times) of target DNA copies. In the second technique, the presence of the β^A and β^S alleles is determined by restriction endonuclease digestion of an end-labeled oligonucleotide probe hybridized in solution to the amplified β-globin sequences. The β-globin genotype can be determined in less than 1 day on samples containing significantly less than 1 microgram of genomic DNA.*

1950 1960 1970 1980 1990 2000 2010

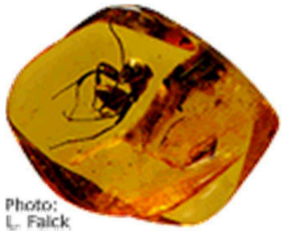
Breve Histórico

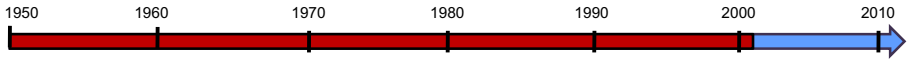
- 1993 – Prêmio Nobel em Química



Curiosidade

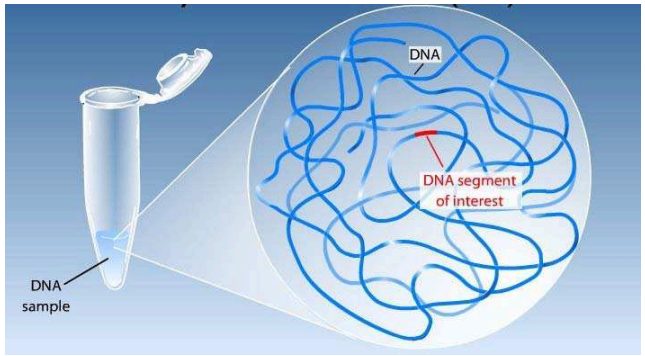
- 1993- Michael Crichton – Jurassic Park



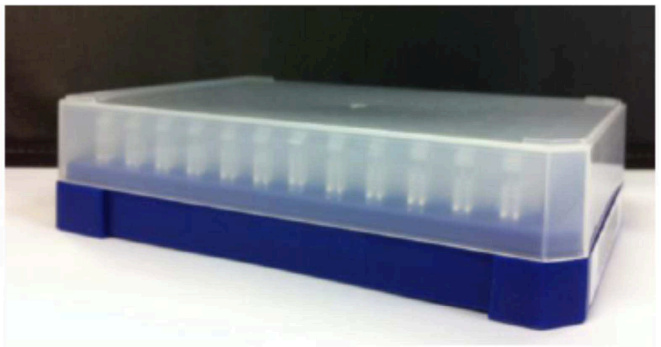


Componentes da PCR

- A PCR requer 6 Componentes essenciais:
  1) DNA molde (Amostra/Sample/Template)



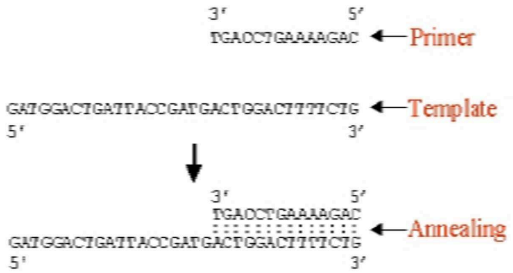
Componentes da PCR



Extração conjunta de DNA (HBV) e RNA (HIV/HCV)

Componentes da PCR

- A PCR requer 6 Componentes essenciais:
  2) Iniciadores/Primers



Componentes da PCR

1 par para HIV
1 par para HCV

1 par para HBV
1 par para PC

Componentes da PCR

- Primer
  - Comprimento de 15 – 25 nucleótidos;
  - Os dois primers devem ter temperatura de fusão(Tm) próxima

$$Tm = 2(A+T) + 4(C+G)$$

  - A temperatura de anelamento é aproximadamenteTm-5ºC

Componentes da PCR

- Exemplo

  CTA GCT ACA TGG ACT CCC

  A + T = 08 X 2 = 16

  C + G = 10 X 4 = 40

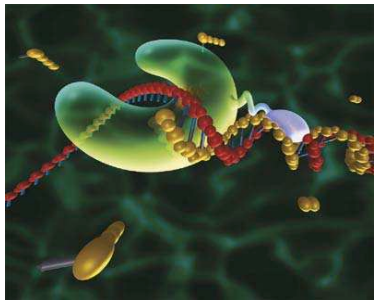
  Tm= 56º

  Tanelamento = 51º

Componentes da PCR

- A PCR requer 6 Componentes essenciais:

3) DNA Polimerase termoestável (Taq)



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Componentes da PCR

- A PCR requer 6 Componentes essenciais:

4) dNTP (Desoxinucleotídeos Trifosfatos)

ATP, TTP, CTP e GTP

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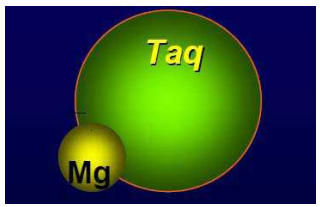
Componentes da PCR

- A PCR requer 6 Componentes essenciais:

5) $MgCl_2$

Fornece íons Mg^{2+} que são cofatores indispensáveis para o funcionamento da Taq.

Aumenta a interação do DNA *template* com o *primer*



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Componentes da PCR

- A PCR requer 6 Componentes essenciais:

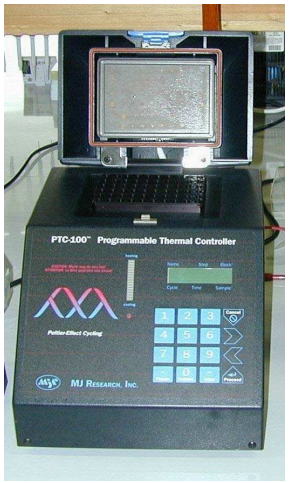
6) Tampão

Mantém o pH ótimo para a atividade enzimática.

Componentes da PCR

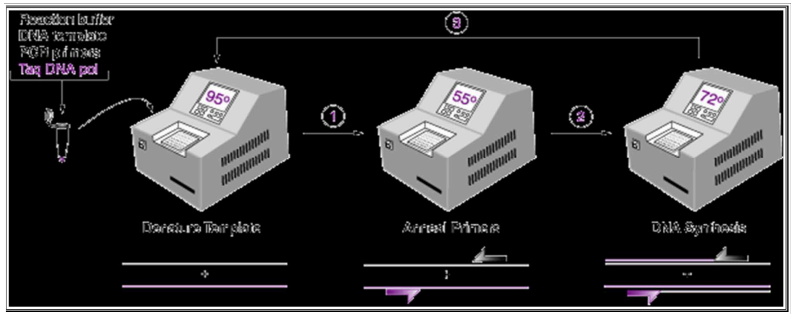
- Taq Dna polimerase
- DNTP
- $MgCl_2$
- Tampão

Termocicladores



Etapas da Reação de PCR

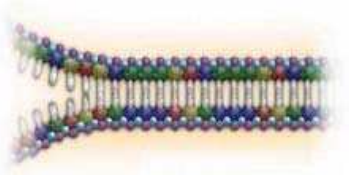
1) Desnaturação
2) Anelamento
3) Extensão



Etapas da Reação de PCR

1) Desnaturação

- Separação das duas cadeias de DNA por ação do calor



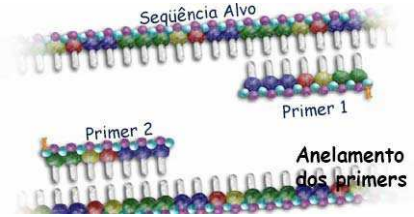
92-94ºC

- As ligações de hidrogênio entre nucleotídeos de cada cadeia são quebradas
- Originam-se duas cadeias simples de DNA

Etapas da Reação de PCR

2) Anelamento

- Os primers ligam-se especificamente ao DNA



T = Tm–5º

- No local de hibridização forma-se um pequeno fragmento de cadeia dupla
- A partir destes a polimerase começa a síntese das duas novas cadeias

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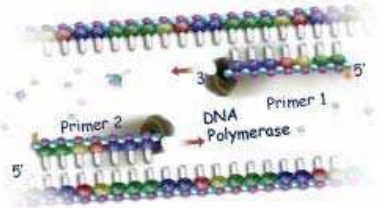
Etapas da Reação de PCR

3) Extensão

- Síntese de uma nova cadeia



T = Tótima Taq
T = 72º

A polimerase procede à elongação da nova cadeia por adição dos nucleotídeos disponíveis no mix

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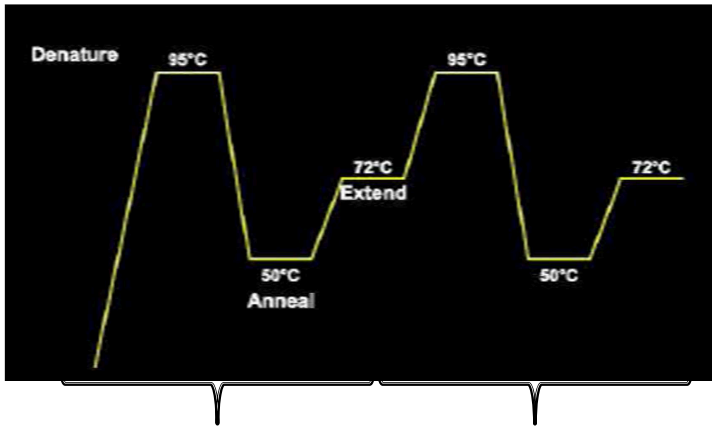
Etapas da Reação de PCR



Ciclo 01 Ciclo 02...

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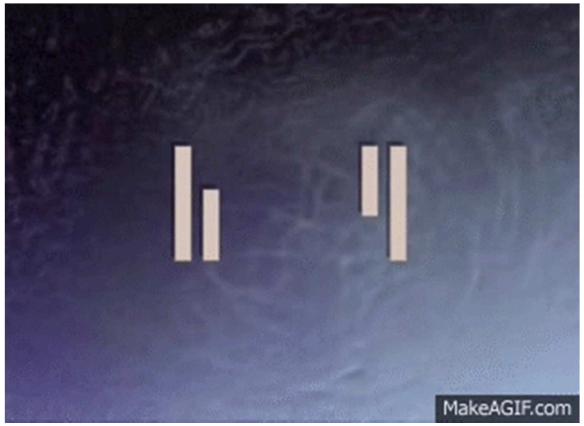
Etapas da Reação de PCR



Etapas da Reação de PCR

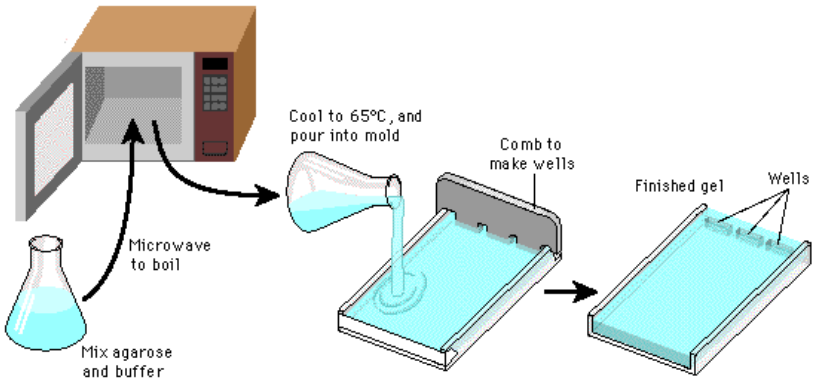
Etapas da Reação de PCR

- Vídeo PCR

http://backboneanimation.com/

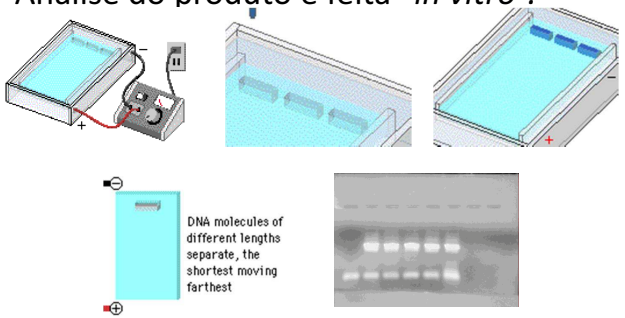
Análise do Produto de PCR

- Gel de Agarose



Análise do Produto de PCR

- Vídeo PCR
- PCR convencional
  - Eletroforese em gel de Agarose ou Acrilamida
  - Análise do produto é feita *"in vitro"*.



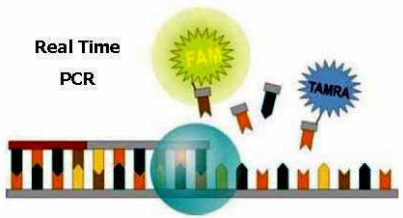
Análise do Produto de PCR

- Vídeo Eletroforese

http://backboneanimation.com/

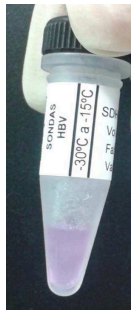
Análise do Produto de PCR

Uso de sondas marcadas que enviam sinal fluorescente diretamente para um software onde a análise é realizada em tempo real.



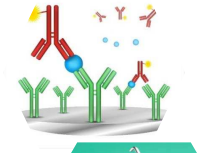
Análise do Produto de PCR

HIV
HCV
PC



HBV
PC

CURSO DE APERFEIÇOAMENTO: TRIAGEM LABORATORIAL E CONTROLE DE QUALIDADE EM SANGUE, TECIDOS, CÉLULAS E ÓRGÃOS



– Contatos

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