Ativadores e Inibidores de PCR

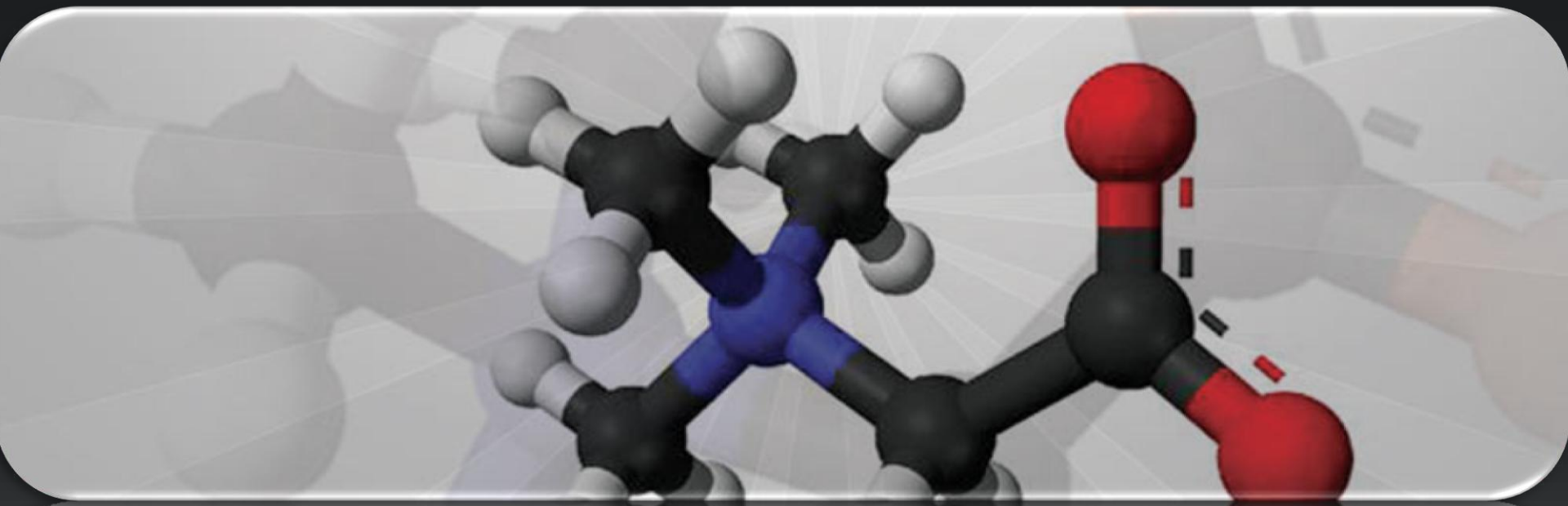
Marina dos Santos
Vitória Gonçalves

Biologia Molecular

Ativadores

- Ativadores, potencializadores ou aditivos, são moléculas que aumentam a eficiência, o rendimento, a especificidade e consistência da reação de PCR.
- Devem ser testados para cada combinação de molde e iniciadores.
- Muitos produtos de potencialização estão disponíveis no mercado.

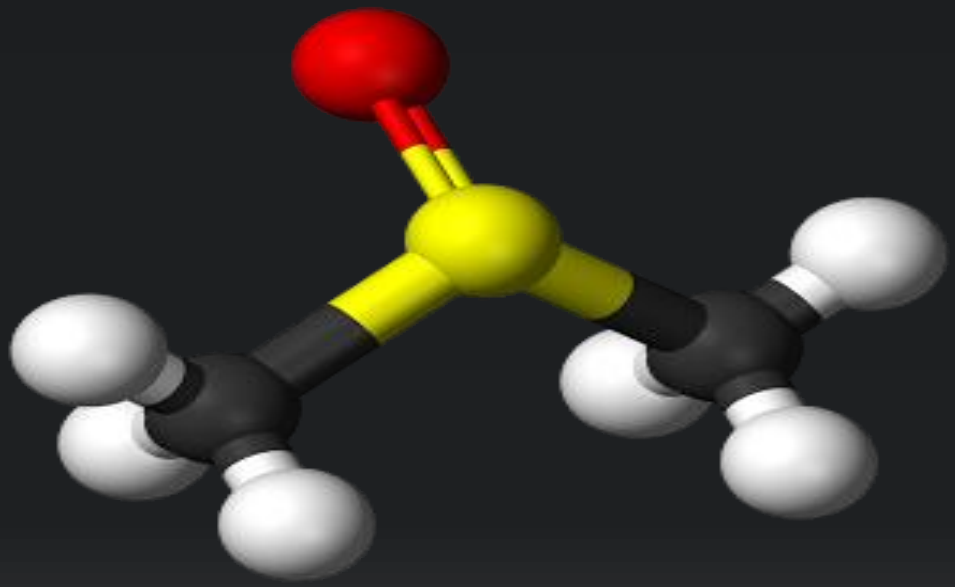
BETAINA



Betaina:

- São aminoácidos;
- Aditivo mais comum usado para aumentar a amplificação de sequências ricas em pareamento GC;
- As vezes é comercializada com outro nome, sem a indicação de presença na composição do produto.

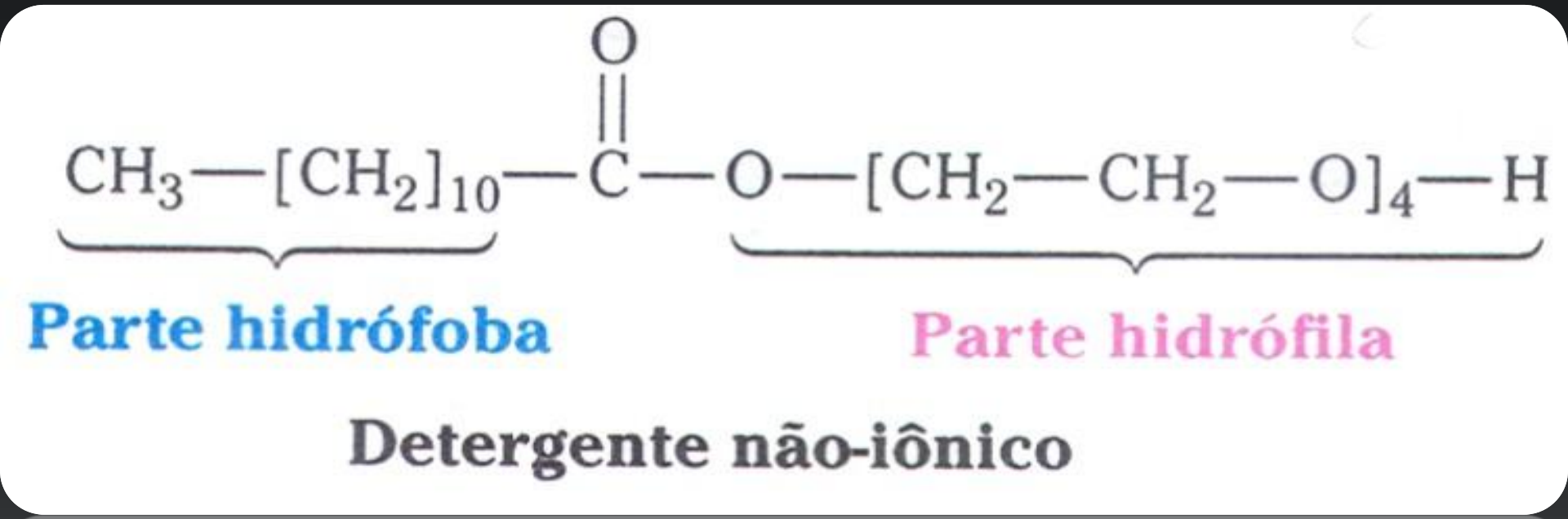
DMSO



DMSO:

- Facilita a separação de reações que são ricas em GC;
- Impede a formação de estruturas secundários no DNA;
- Reduzem a metade a ação da polimerase.

DETERGENTES NÃO IÔNICOS

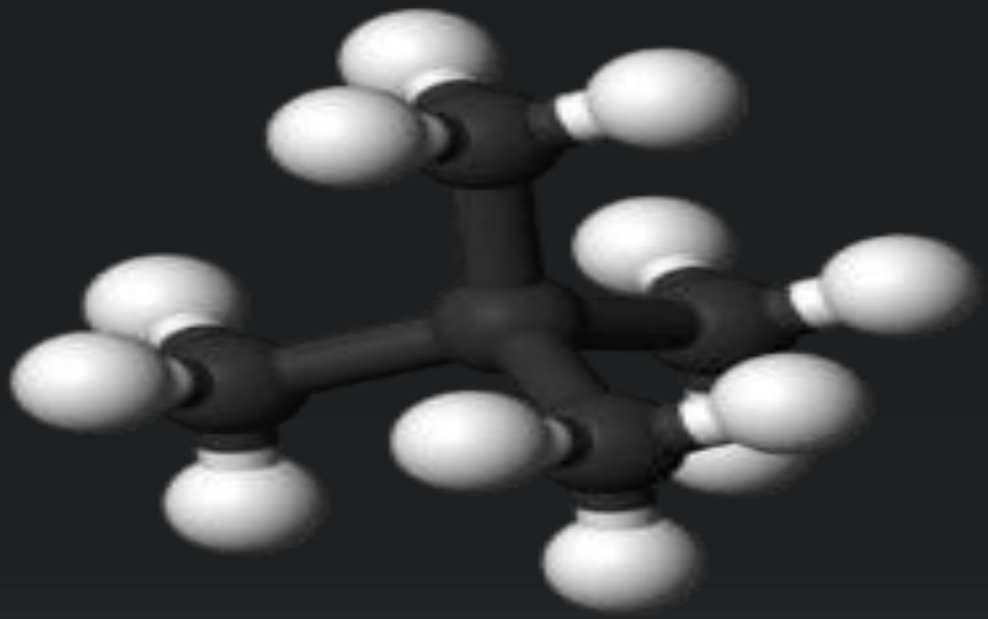


Detergentes não iônicos:

- Estabilizam a taq polimerase;
- Dificultam a formação de estruturas secundárias no DNA.

- Dependendo da escolha do detergente e da concentração, pode inibir a taq e permite a formação de ligações não especificas entre primers e DNA.

CLORETO TETRAMETILAMÔNIO



Cloreto Tetrametilamônio:

- Melhora a união das fitas de DNA;
- Aumenta a compatibilidade de DNA e RNA;
- Diminui as ligaçãoes inadequadas no DNA.

Etilenoglicol e 1,2-propanodio

- São duas substâncias recentemente descobertas, onde estudos apontam que melhora a amplificação de regiões ricas em GC.

Outros ativadores:

- Formamina: Tem efeito benéfica em concentrações entre 1-5% e diminui a inibição em concetrações maiores que 10%;
- Bovine Serum Albumin: facilitam a amplificação de DNA mais antigos, ou que apresentem inibidores;

Artigos de ativadores.

Inibidor-resistente

NOVEL INHIBITION-RESISTANT TAQ MUTANT ENZYMES AND PCR ENHANCERS DESIGNED FOR FORENSICS

Milko B. Kermekchiev[1*], Zhian Zhang[1] and Wayne M. Barnes[1,2]
[1]DNA Polymerase Technology, Inc.
[2]Washington University, Department of Biochemistry and Molecular Biophysics, Saint Louis, Missouri,

Major problem with PCR-based forensic tests of samples containing blood and soil are false negative results and low sensitivity caused by inhibitory substances. The effect of the main PCR inhibitors in such samples, hemoglobin and humic acid, is primarily associated with inactivation of Taq DNA polymerase. Therefore, various protocols and DNA extraction procedures are being used to purify DNA prior to PCR. These extra steps add to cost, are time-consuming, may not completely remove inhibitors, or may lead to losses of target DNA. As a novel alternative these pre-PCR steps we have recently engineered mutants of Taq polymerase (OmniTaq and Omni Klentaq) highly resistant to blood and soil inhibitors (Kermekchiev, M. et al., Nucleic Acids Res., 2009 Apr; 37(5):e40. Epub). We also developed novel PCR enhancer cocktails (PECs) which further improve the performance of the mutant enzymes in crude samples, and increase the specificity and sensitivity of DNA detection. We present data showing that the mutant enzymes, combined with PEC, can amplify human targets, including STR markers from crude samples containing whole blood, soil, or combination of both, where plain Taq and AmpliTaq Gold fail to perform. Identical results are shown with dry blood spots, directly subjected to amplification without DNA extraction. The new enzymes also generate STR profiles from swab specimens without any DNA purification steps prior to amplification.

We also illustrate that OmniTaq and Omni Klentaq in the presence of PEC can outperform AmpliTaq Gold in amplifying nuclease treated partially hydrolyzed DNA. With damaged DNA, extracted from a forty year aged hair shaft, the novel enzymes can produce a STR profile with some spurious alleles, using the PowerPlex 16 kit, while no STR profile is produced with the AmpliTaq Gold enzyme. Development of Taq mutants / PEC combinations optimized for overcoming inhibition in other than blood and soil specimens is underway. The novel enzyme-enhancer systems could eliminate, in many cases, the need to purify DNA prior to PCR and speed-up, lower the cost and increase the efficiency of forensic DNA testing.

The work on this project is founded by an ongoing NIJ Grant Award 2008-DN-BX-K299.

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An efficient and economic enhancer mix for PCR ☆

Markus Ralser [1], Robert Querfurth [1], Hans-Jörg Warnatz, Hans Lehrach, Marie-Laure Yaspo, Sylvia Krobitsch *

Max Planck Institute for Molecular Genetics, Ihnestrasse 73, 14195 Berlin, Germany

Received 19 June 2006
Available online 5 July 2006

Abstract

Polymerase chain reaction (PCR) has become a fundamental technique in molecular biology. Nonetheless, further improvements of the existing protocols are required to broaden the applicability of PCR for routine diagnostic purposes, to enhance the specificity and the yield of PCRs as well as to reduce the costs for high-throughput applications. One known problem typically reported in PCR experiments is the poor amplification of GC-rich DNA sequences. Here we designed and tested a novel effective and low-cost PCR enhancer, a concentration-dependent combination of betaine, dithiothreitol, and dimethyl sulfoxide that broadly enhanced the quantitative and/or qualitative output of PCRs. Additionally, we showed that the performances of this enhancer mix are comparable to those of commercially available PCR additives and highly effective with different DNA polymerases. Thus, we propose the routine application of this PCR enhancer mix for low- and high-throughput experiments.

Keywords: Polymerase chain reaction; GC-rich sequence; Enhancer; Additive; Promoter PCR; Genomic PCR; PCR template; *Taq* DNA polymerase

The polymerase chain reaction (PCR) was developed in the 1980s by Kary Mullis and Fred Faloona [1,2]. Starting with the biotechnological application of thermostable DNA polymerases [3], PCR has become a fundamental technique in molecular biology. There are ever-increasing needs for further improvements of PCR protocols for low-cost and efficient high-throughput approaches in a wide range of applications ranging from quantitative analysis at the genome- or transcriptome levels to routine diagnostic purposes [4,5]. Large-scale PCR experiments require broadly applicable and reliable reaction conditions for establishing cost-effective production pipelines. *Taq* DNA polymerase, originally purified from the thermophilic bacterium *Thermus aquaticus* [6], is widely used, since it can be produced in every standard laboratory at low-cost [7–9]. One major factor limiting the output of PCR routines is that a number of DNA sequences are poorly or not amplifiable under standard reaction conditions, either because of their intrinsic properties to form secondary structures, and/or because of their high GC-content. Improvements of the PCR conditions can be achieved by modifying the classical reaction conditions, for example, by performing "touchdown" PCR, consisting of a stepwise reduction of the annealing temperature for each cycle [10], or by the use of modified DNA polymerases for carrying out "hot-start" reactions [11]. Typically, to overcome amplification problems of GC-rich DNA, the addition of substances that enhance the specificity and/or the yield of the PCR is necessary. The most prominent PCR enhancing additives that are currently used are either betaine [12], small sulfoxides like dimethyl sulfoxide (DMSO, [13]), small amides like formamide [14] or reducing compounds like β-mercaptoethanol or dithiothreitol (DTT, [10]). However, their capacity to significantly improve PCR yields mainly for

☆ *Abbreviations:* BSA, bovine serum albumin; CES, combinatorial PCR enhancer solution; DMSO, dimethyl sulfoxide; DTT, dithiothreitol; PCR, polymerase chain reaction; *Taq*, *Thermus aquaticus*.

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Inibidores

- Compostos que alteram o resultado de uma reação de PCR;
- Podem inibir em diversos níveis, levando a diferentes graus de atenuação, ou até mesmo a inibição completa;
- Propiciam erros na interpretação dos resultados.

Ação

- Podem se ligar diretamente ao DNA;
- Afetar a taq-polimerase;
- Reduzir a disponibilidade de íons Mg++.

Provenientes

- Do próprio material coletado para a amplificação;
- Dos produtos usados da etapa de purificação do DNA.

Componentes do material da amostra:

- Hemoglobina
- Ureia
- Polissacarídeos
- Sais bileares
- Ácidos húmicos
- Cálcio

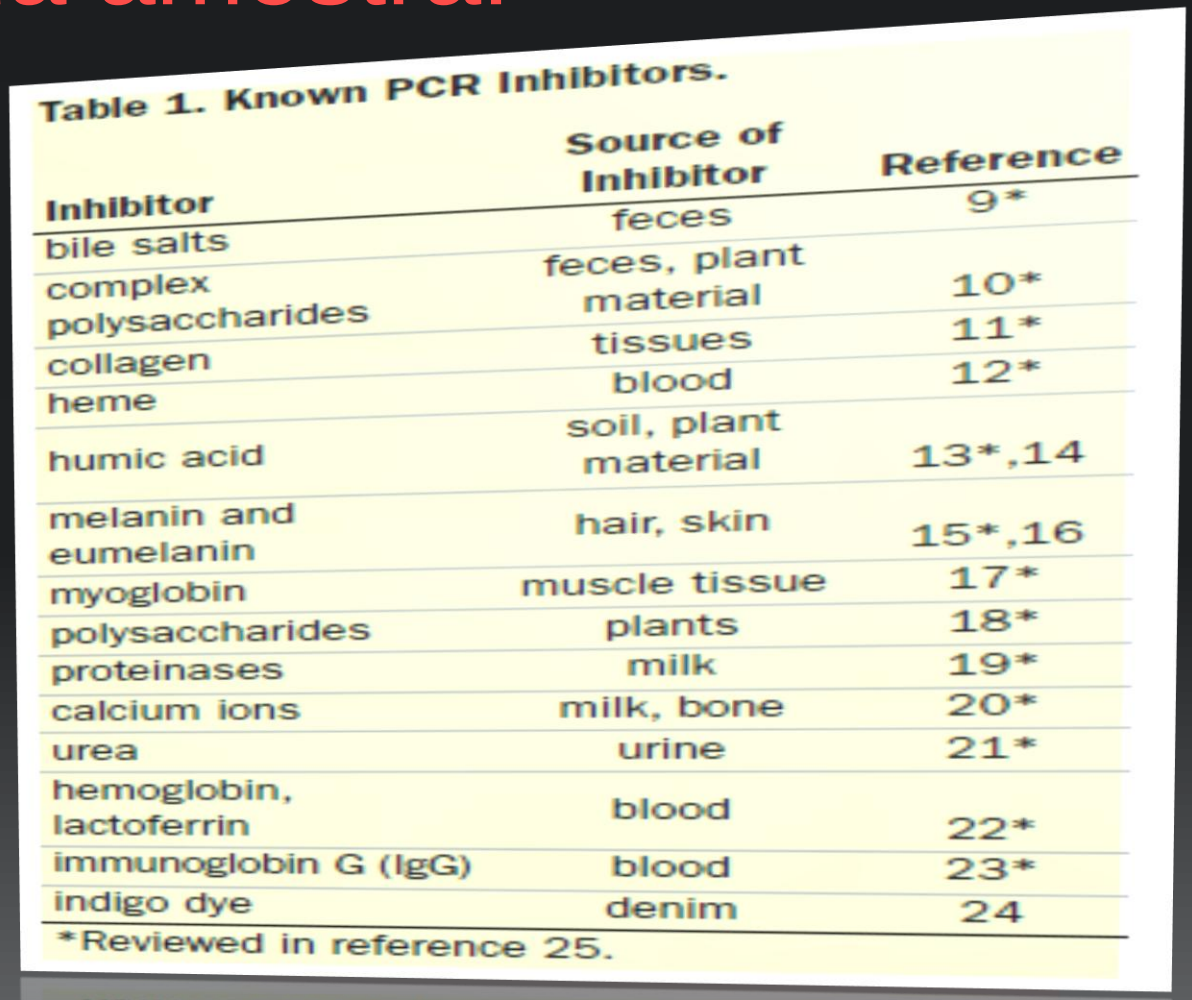


Table 1. Known PCR Inhibitors.

Inhibitor	Source of Inhibitor	Reference
bile salts	feces	9*
complex polysaccharides	feces, plant material	10*
collagen	tissues	11*
heme	blood	12*
humic acid	soil, plant material	13*,14
melanin and eumelanin	hair, skin	15*,16
myoglobin	muscle tissue	17*
polysaccharides	plants	18*
proteinases	milk	19*
calcium ions	milk, bone	20*
urea	urine	21*
hemoglobin, lactoferrin	blood	22*
immunoglobin G (IgG)	blood	23*
indigo dye	denim	24

*Reviewed in reference 25.

Hemoglobina

- Inibidor mais usado;
- O Fe++ age na ligação do Mg++ com a Taq polimerase, inativando a enzima.

Polissacarídeos

- Presentes nas amostras vegetais;
- Interferem na ação da enzima Taq-polimerase.

Cálcio

- Encontrado em ossos e dentes;
- Altera a concentração de Mg++, alterando a atividade da Taq-polimerase.

Produtos ultilizados na purificação do DNA:

- EDTA - Ácido etilenodiamino tetra-acético;
- Heparina;
- SDS;
- Proteinase K;
- Sais em excesso;
- Clorofórmio;
- Fenol;
- Pó das luvas;
- Tubos de PCR.

Detergentes iônicos e alcóois

- SDS, etanol, fenol, isopropanol e outros contribuem com diversos mecanismos na inibição da PCR.

Proteinase

- Degradam a enzima Taq-polimerase.

Heparina e EDTA

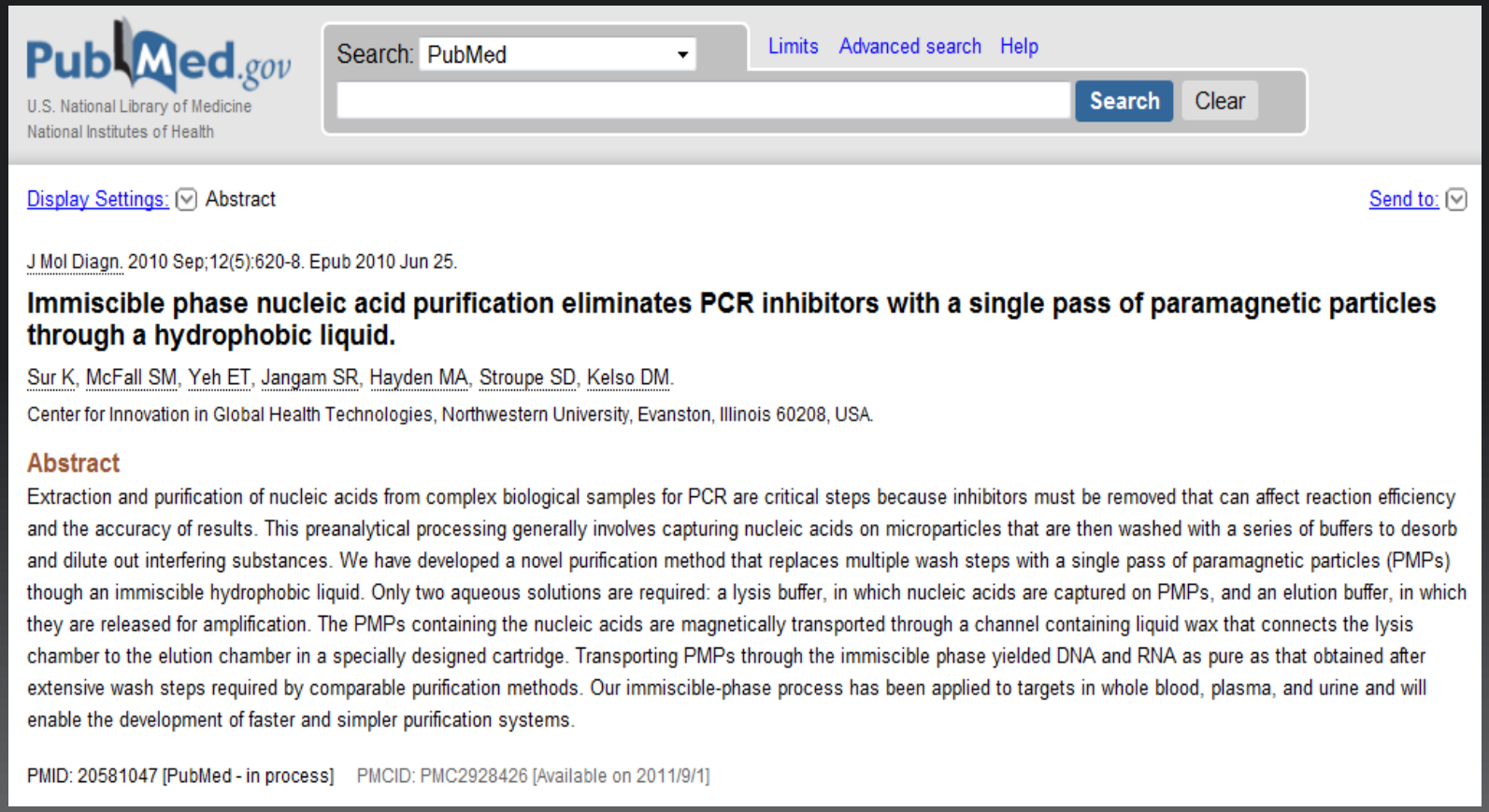
- Anticoagulantes;
- Captam íons metálicos como o Mg e Ca (quelantes).

Tubos de PCR

- Existem relatos de que esses tubos podem conter uma substância inibidora liberada a partir de poliestireno ou polipropileno após exposição a luz ultravioleta.

Artigos de inibidores

Uso de partículas paramagnéticas para retirada de inibidores.



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J Mol Diagn. 2010 Sep;12(5):620-8. Epub 2010 Jun 25.

Immiscible phase nucleic acid purification eliminates PCR inhibitors with a single pass of paramagnetic particles through a hydrophobic liquid.

Sur K, McFall SM, Yeh ET, Jangam SR, Hayden MA, Stroupe SD, Kelso DM.

Center for Innovation in Global Health Technologies, Northwestern University, Evanston, Illinois 60208, USA.

Abstract

Extraction and purification of nucleic acids from complex biological samples for PCR are critical steps because inhibitors must be removed that can affect reaction efficiency and the accuracy of results. This preanalytical processing generally involves capturing nucleic acids on microparticles that are then washed with a series of buffers to desorb and dilute out interfering substances. We have developed a novel purification method that replaces multiple wash steps with a single pass of paramagnetic particles (PMPs) though an immiscible hydrophobic liquid. Only two aqueous solutions are required: a lysis buffer, in which nucleic acids are captured on PMPs, and an elution buffer, in which they are released for amplification. The PMPs containing the nucleic acids are magnetically transported through a channel containing liquid wax that connects the lysis chamber to the elution chamber in a specially designed cartridge. Transporting PMPs through the immiscible phase yielded DNA and RNA as pure as that obtained after extensive wash steps required by comparable purification methods. Our immiscible-phase process has been applied to targets in whole blood, plasma, and urine and will enable the development of faster and simpler purification systems.

PMID: 20581047 [PubMed - in process] PMCID: PMC2928426 [Available on 2011/9/1]

Romoção de inibidores com o uso de DNA em gel agarose.

Nucleic Acids Research, 1998, Vol. 26, No. 13 3309–3310

Efficient removal of PCR inhibitors using agarose-embedded DNA preparations

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Received May 4, 1998; Accepted May 20, 1998

ABSTRACT

The use of agarose blocks containing embedded DNA improves the PCR amplification from templates naturally contaminated with polysaccharides or humic acids, two powerful PCR inhibitors. Presumably, the difference in size between the DNA macromolecules and these contaminants allows their effective removal from the agarose blocks by diffusion during the washing steps, whereas genomic DNA remains trapped within them. In addition, agarose-embedded DNA can be directly used for PCR since low melting

DNA macromolecules (e.g. complete prokaryotic or eukaryotic chromosomes) for pulsed-field gel electrophoresis analysis, has been tested in this work. Basically, whole cells are embedded in low melting point (LMP) agarose blocks and then immersed in a lysis buffer. By this procedure, intact genomic DNA (gDNA) can be obtained avoiding shear damage (4). Several washing steps can be carried out prior to other treatments (such as endonuclease digestion). Taking advantage of the important difference of size between DNA macromolecules and most common soluble PCR inhibitors, I have adapted this method to the preparation of high quality gDNA for PCR amplification. Large DNAs remain trapped within the agarose blocks, whereas cell debris and contaminants are

Empresas relacionadas

- Como PCR é uma técnica que é muito usada na ária de biotecnologia, e é necessário muitos produtos para sua realização, muitas empresas se lançaram no mercado para das assitência a essa área.

Lucigen

- Fundada em 1998;
- Especializada em comérico online;
- Vende produtos ligado a técnicas de biologia molecular;
- Faz pesquisa com microorganismos para descoberta de novas enzimas para comércio.

Zymo Research

- Fundada em 1994;
- Produtos relacionados a DNA, RNA e Epigenéticos;
- Comercializa kits que retiram os inibidores em uma reação de PCR.

Mobio

- Fundada em 1993;
- Oferece produtos para técnicas de biologia molecular além de serviços como remoção de certos tipos de DNA.

Obrigada!

Universidade Federal de Pelotas
Centro de Desenvolvimento Tecnológico
Curso de Graduação em Biotecnologia

Inibidores e aditivos do pcr

Por Amanda Azevedo e Jéssica Stone

Introdução:

•PCR (Polymerase Chain Reaction) é um método rápido e fácil para a geração de inúmeras cópias de qualquer fragmento de DNA.

Etapas:

- Relembrando as etapas que compõem um PCR, que são

1. Extração do DNA
2. Desenho dos *primers*
3. Reação da Polimerase
4. Análise dos resultados

Porém...

Durante o processo da técnica de PCR, poderão ser acrescentadas substâncias que promoverão melhor rendimento, especificidade e consistência na amplificação. Em contrapartida, contaminantes podem reduzir drasticamente o sucesso da reação de polimerase, podendo apenas atenuar como também inibir por completo.

Aditivos:

•Moléculas cuja função é a de otimizar a reação de PCR, fazendo-a mais eficiente, específica, consistente e otimizando seu rendimento.

•Podem ser encontrados à venda por empresas especializadas

Porque usar aditivos?

- **Cada aditivo tem a sua particularidade, a sua atuação, durante um PCR. A sua especialidade vai depender da sua fórmula química.**

Betaína:

- Aditivo mais usado atualmente, ajuda na atuação da DNA polimerase em regiões ricas em pares de bases CG.

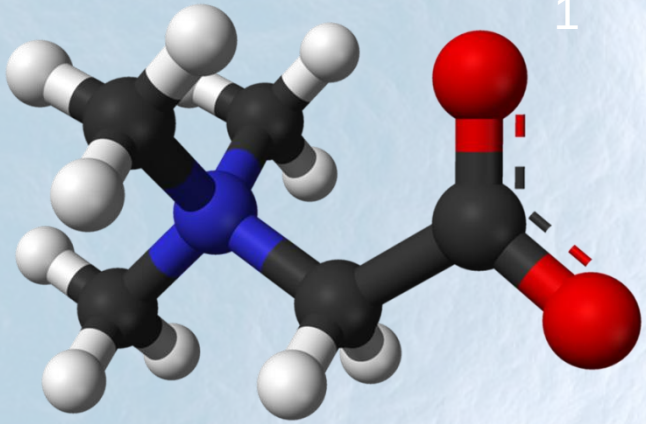


Fig. 1: Fórmula da Betaína.

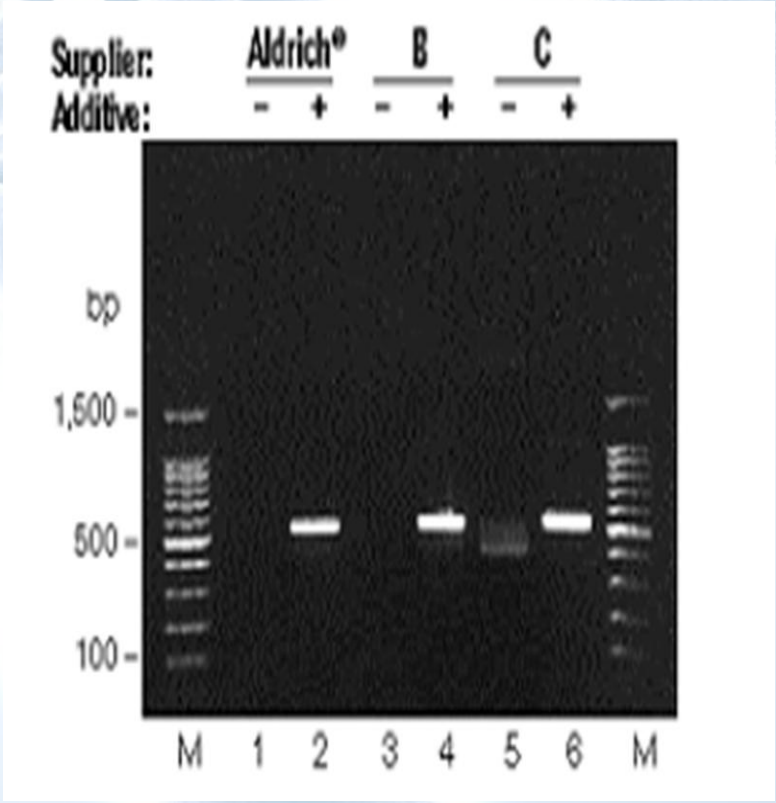
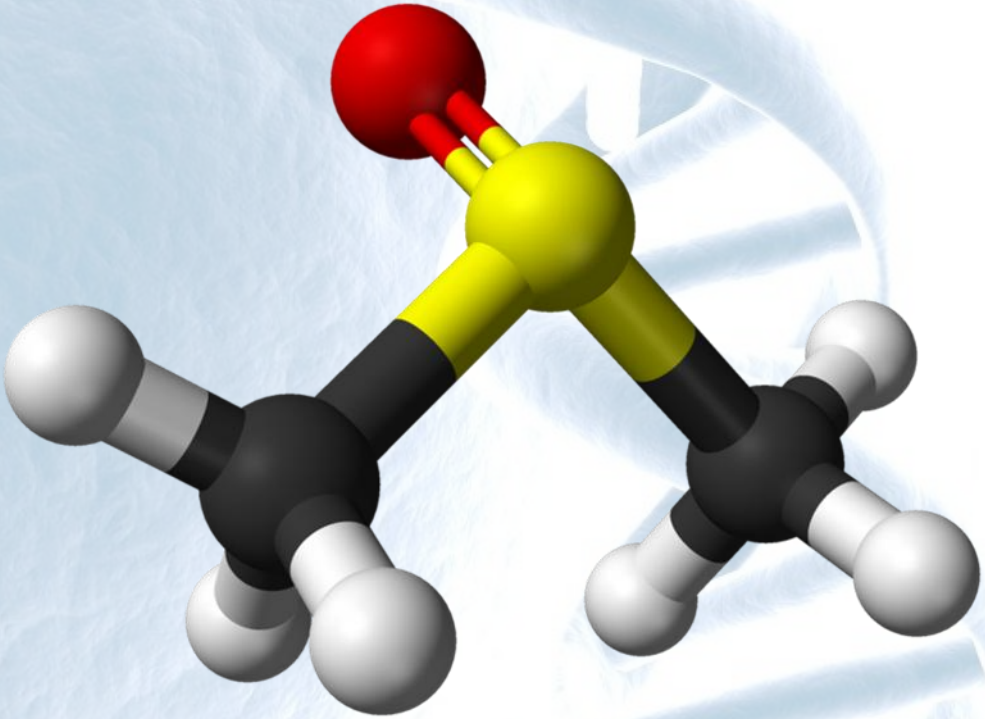


Fig. 2: Amplificação de pa
do gene codificador de u
receptor de fator de
crescimento. É uma
rica em bases C

DMSO (Dimetil sulfóxido):

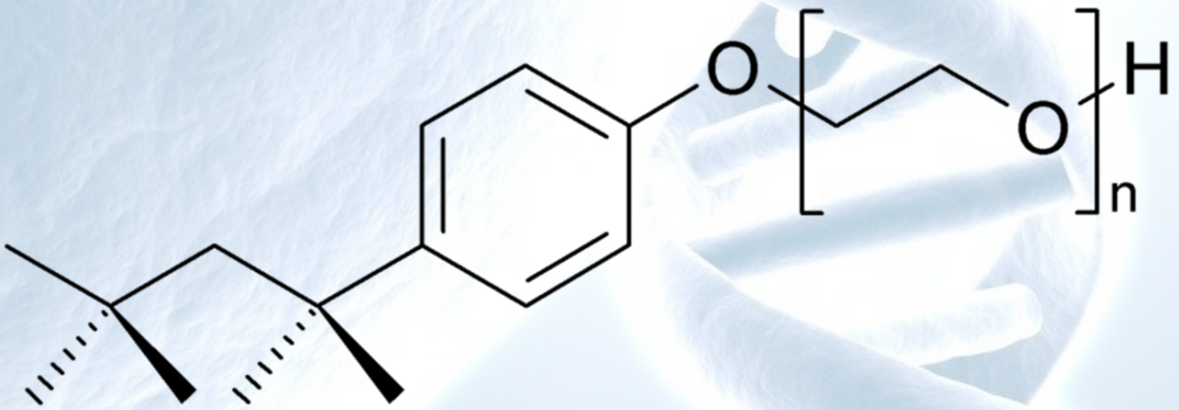
- Quando usado em concentrações entre 2-10% deixam a reação mais eficiente.
- Facilita na hora da desnaturação de regiões com pareamentos CG.
- Quantidades de acima de 10% num PCR dificultam a atividade da Taq Polimerase, ou seja, a atividade da polimerase, fazendo com que caia a 50%.

DMSO:



Detergentes não-iônicos:

- Estabilizam a Taq-polimerase e podem impedir a formação de estruturas secundárias.
- Embora aumentem o rendimento, podem aumentar a amplificação inespecífica.



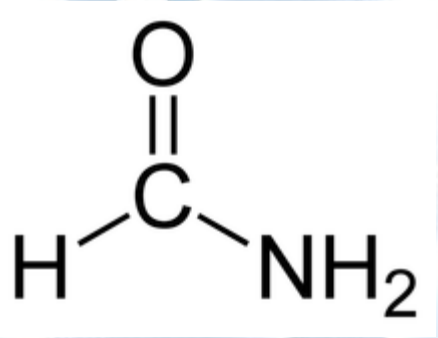
Inconveniências em seu uso:

- De acordo com o detergente e a concentração, a *Taq* pode ser inibida por completo.
- Podem gerar ligações inespecíficas entre o DNA e os *primers*.

Outros aditivos:

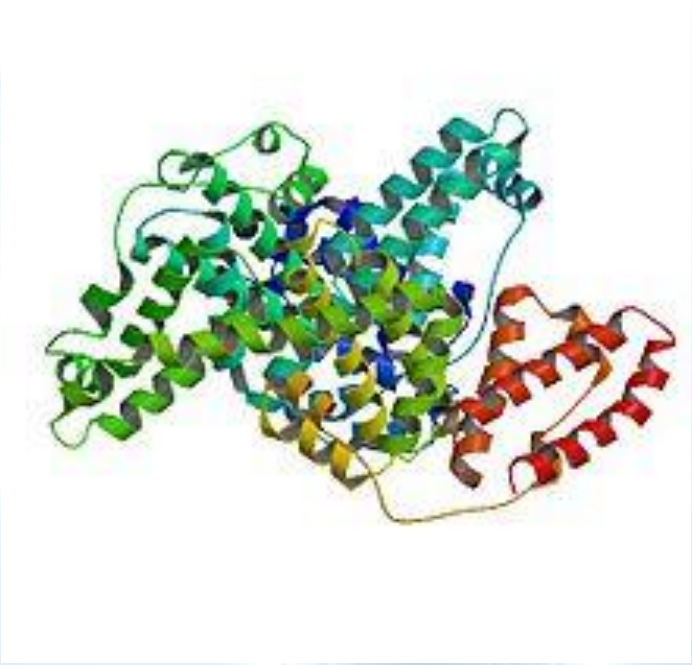
- 7-desaza-2'-desoxiguanosina e análogos amplificam DNAs que possam formar estruturas secundárias.

- Formamida: deve-se ter cuidado no uso dessa molécula, em relação ao seu efeito no PCR. Se utilizada nas porções de 1-5%, tem grande benefício na reação. Mas quando ultrapassa de 10%, não costuma demonstrar amplificação



- **BSA (Bovine serum albumin):**

É eficiente em amplificação de DNAs antigos (*ancient DNA*) ou que apresentam inibidores, como por exemplo a melanina.



Glicerol:

- **Por ser uma substância que não congela, ele ajuda na estabilidade da Taq- Polimerase, e com isso, na sua conservação.**

Artigos relacionados aos ativadores de PCR

Novas enzimas Taq mutantes inibidor[illegible]sistentes e aditivos de PCR feitos para a Foren[illegible]

NOVEL INHIBITION-RESISTANT TAQ MUTANT ENZYMES AND PCR ENHANCERS DESIGNED FOR FORENSICS

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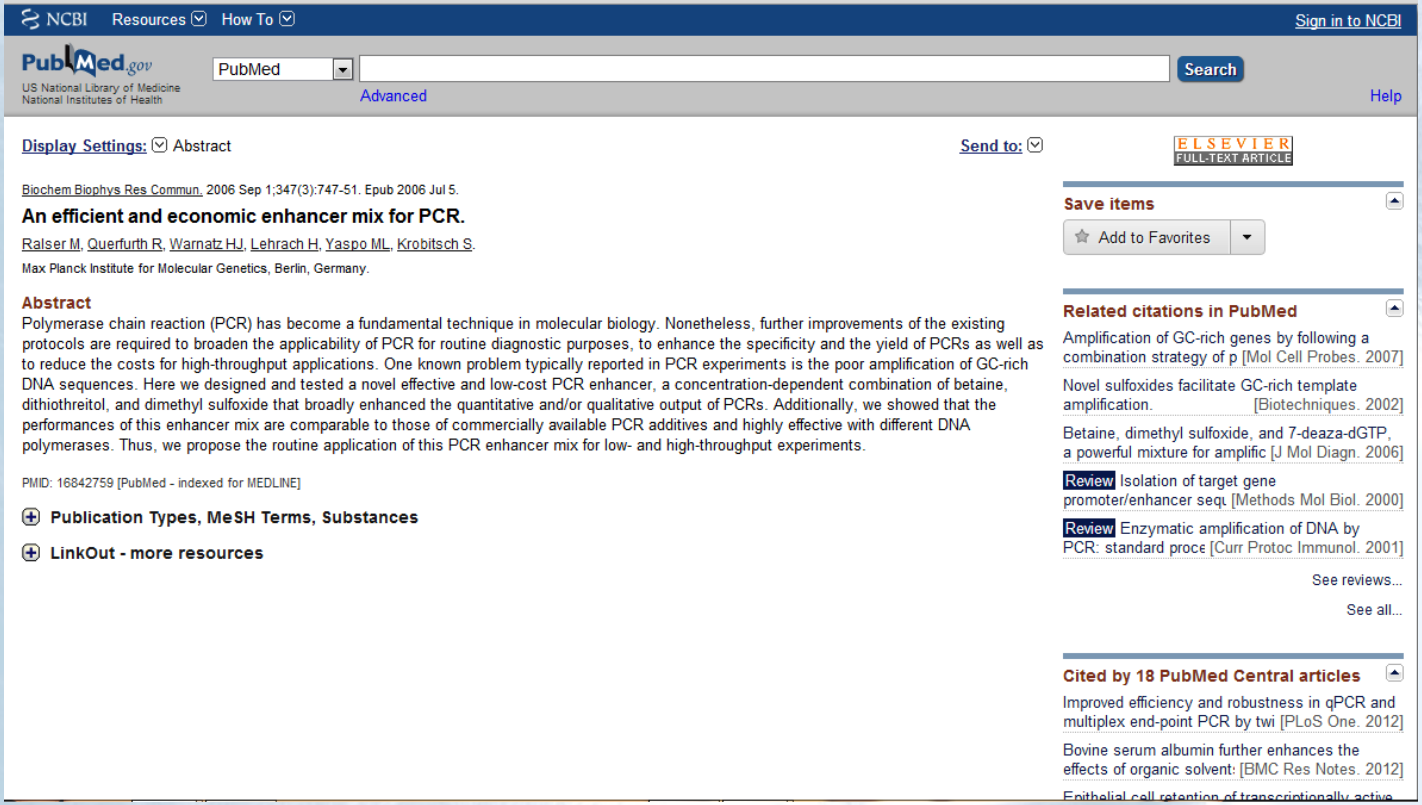
"O novo sistema de [illegible]na-aditivo" pode eliminar, em muitos casos, a necessidade [illegible]car DNA ant[illegible] da PCR e acelerar, diminuir o [illegible]to e aumentar a efic[illegible]ncia dos testes de DNA foren[illegible]se."

Um mix eficiente e econômico [illegible] para PCR



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Biochem Biophys Res Commun. 2006 Sep 1;347(3):747-51. Epub 2006 Jul 5.

An efficient and economic enhancer mix for PCR.

Ralser M, Querfurth R, Warnatz HJ, Lehrach H, Yaspo ML, Krobitsch S.

Max Planck Institute for Molecular Genetics, Berlin, Germany.

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PMID: 16842759 [PubMed - indexed for MEDLINE]

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Review Enzymatic amplification of DNA by PCR: standard proce [Curr Protoc Immunol. 2001]

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Improved efficiency and robustness in qPCR and multiplex end-point PCR by twi [PLoS One. 2012]

Bovine serum albumin further enhances the effects of organic solvent: [BMC Res Notes. 2012]

Epithelial cell retention of transcriptionally active

"Aqui foi desenhado e testado um [illegible] eficaz e de baixo custo para PCR, uma combinação dependente da concentração de betaína, ditiotreitol, e sulfóxido de dimetilo, que largamente melhoram a saída quantitativa e / ou qualitativa dos PCRs."

Amplificação direta de [illegible] de amostras clínicas em bruto, utilizando um cocktail de [illegible] de PCR e novos mutantes de Taq

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J Mol Diagn. 2010 Mar;12(2):152-61. Epub 2010 Jan 14.

Direct DNA amplification from crude clinical samples using a PCR enhancer cocktail and novel mutants of Taq.

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Abstract

PCR-based clinical and forensic tests often have low sensitivity or even false-negative results caused by potent PCR inhibitors found in blood and soil. It is widely accepted that purification of target DNA before PCR is necessary for successful amplification. In an attempt to overcome PCR inhibition, enhance PCR amplification, and simplify the PCR protocol, we demonstrate improved PCR-enhancing cocktails containing nonionic detergent, l-carnitine, d-(+)-trehalose, and heparin. These cocktails, in combination with two inhibitor-resistant Taq mutants, OmniTaq and Omni Klentaq, enabled efficient amplification of exogenous, endogenous, and high-GC content DNA targets directly from crude samples containing human plasma, serum, and whole blood without DNA purification. In the presence of these enhancer cocktails, the mutant enzymes were able to tolerate at least 25% plasma, serum, or whole blood and as high as 80% GC content templates in PCR reactions. These enhancer cocktails also improved the performance of the novel Taq mutants in real-time PCR amplification using crude samples, both in SYBR Green fluorescence detection and TaqMan assays. The novel enhancer mixes also facilitated DNA amplification from crude samples with various commercial Taq DNA polymerases.

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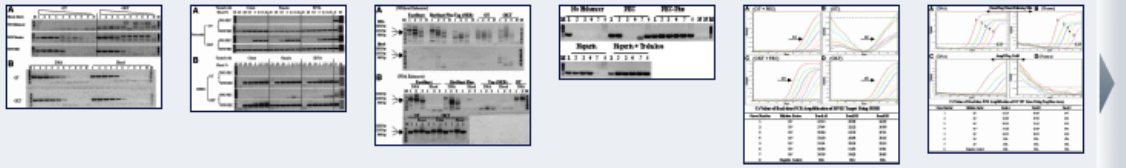

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“Estes cocktails, em combinação com dois mutantes resistentes ao inibidor de Taq, OmniTaq e Omni Klentaq, permitiu a amplificação eficiente exógena, endógena, e o alto teor de GC do ADN tem como alvo diretamente a partir de amostras em bruto, contendo o plasma humano, o soro, o sangue total e sem purificação de ADN. Na presença desses cocktails de aditivos, as enzimas mutantes foram capazes de tolerar pelo menos 25% de plasma, soro ou sangue total e tão elevada como 80% de conteúdo de GC em modelos de reações de PCR. Estes cocktails potenciadores também melhoraram o desempenho dos novos mutantes de Taq em tempo real a amplificação por PCR utilizando-se amostras em bruto, quer na SYBR Green fluorescência de detecção e ensaios de TaqMan.”

Inibidores:

- Compostos orgânicos ou inorgânicos que quando presentes num PCR podem atenuar ou inibir por completo a reação da polimerase, interferindo na amplificação do DNA desejado.
- São comuns em amostras de DNA e podem ter das mais variadas origens.
- Causam a leitura errônea dos resultados.

Ação dos inibidores:

- Ligam-se diretamente ao DNA;
- Afetam a Taq-polimerase;
- Reduzem a disponibilidade de íons Mg^{2+}

Existem componentes naturais numa amostra ou até mesmo que estejam presentes numa das etapas de purificação que podem funcionar como inibidores do processo de PCR:

- Hemoglobina, ureia, ácidos húmicos, polissacarídeos, sais bileares e cálcio (naturais)

- EDTA (ácido etileno diamino tetra-acético), heparina, SDS (detergente iônico), proteinase K, sais em excesso, clorofórmio, fenol, pó de luvas, tubos de PCR (para a purificação do DNA)
- Álcoois como o etanol, fenol, isopropanol também contribuem para a inibição do PCR.

Hemoglobina:

- É o principal inibidor em amostras para PCR.
- O íon Fe^{2+} atrapalha na ligação do íon Mg^{2+} à Taq-Polimerase, inativando a enzima.

Cálcio:

- Encontrado em abundância nos dentes e ossos, ele interfere na concentração de Mg^{2+}.

- Afeta diretamente a ação da Taq polimerase.

Polissacarídeos:

- Contaminação por polissacarídeos são mais comuns em amostras vegetais

- Age diretamente na Taq polimerase

Proteinases:

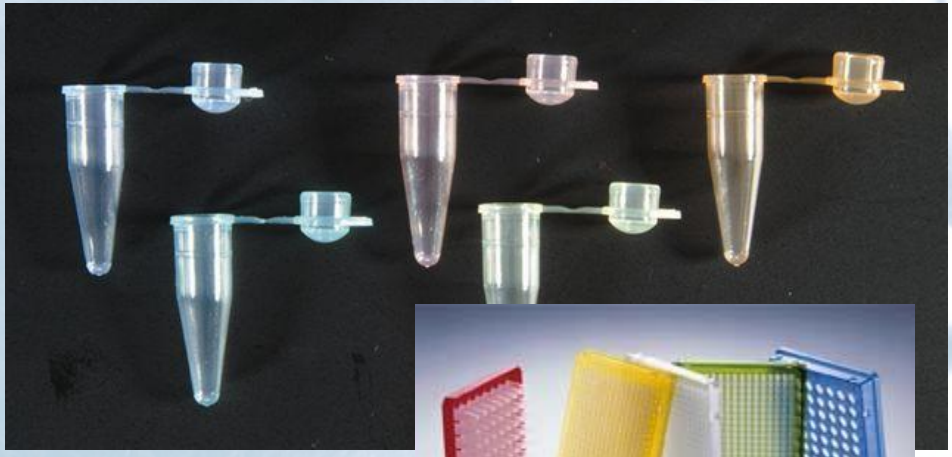
- **Fazem a degradação da Taq-polimerase.**

Heparina e EDTA:

- **Anticoagulantes com comportamento [illegible] pois captam [illegible] como o Ca^{2+} e o [illegible]**

Tubos de PCR:

- Há quem diga que estes tubos possam conter uma substância inibidora que seria liberada pelo poliestireno ou polipropileno quando estes materiais se expõem à luz UV.



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Artigos relacionados aos Inibidores de PCR

Extração de DNA de materiais de arquivos e fontes escassas para utilização em reação de polimerização em cadeia (PCR)

*Rev. bras. hematol. hemoter. 2004;**26**(4):274-281* *Barea JA et al*

Artigo / Article

Extração de DNA de materiais de arquivo e fontes escassas para utilização em reação de polimerização em cadeia (PCR)

Methods of DNA extraction from archived materials and rare sources for utilization in polymer chain reaction

Jaqueline A. Barea[1]
Maria Inês M. C. Pardini[2]
Tsieko Gushiken[3]

*Este trabalho visou a comparação de cinco métodos diferentes de extração de DNA de materiais de arquivo (tecidos incluídos em parafina, esfregaços de sangue periférico – corados e não corados com Leishman, lâminas com mielogramas, gotas de sangue em Guthrie Card) e de fontes escassas (células bucais, um e três bulbos capilares e 2 mL de urina), para que fossem avaliadas a facilidade de aplicação e a facilidade de amplificação deste DNA pela técnica da reação de polimerização em cadeia (PCR). Os métodos incluíram digestão por proteinase K, seguida ou não por purificação com fenol/clorofórmio; Chelex 100® (BioRad); Insta Gene® (BioRad) e fervura em água estéril. O DNA obtido foi testado para amplificação de três fragmentos gênicos: Brain-derived neutrophic factor (764 pb), Factor V Leiden (220 pb) e Abelson (106 pb). De acordo com o comprimento do fragmento gênico estudado, da fonte potencial de DNA e do método de extração utilizado, os resultados caracterizaram o melhor caminho para padronização de procedimentos técnicos a serem incluídos no manual de Procedimentos Operacionais Padrão do Laboratório de Biologia Molecular do Hemocentro - HC - Unesp - Botucatu. Rev. bras. hematol. hemoter. 2004;**26**(4):274-281.*

Palavras-chave: *Materiais de arquivo; fontes escassas; extração de DNA; PCR.*

“Utilização de DNA arquivado em guthrie card, tecidos fixados e incluídos em parafina, bulbos capilares e células bucais e DNA extraído de fios de cabelo, resquícios salivares e outras fontes escassas”

Remoção eficiente de Inibidores utilizando DNA em gel de agarose

Nucleic Acids Research, 1998, Vol. 26, No. 13 3309–3310

Efficient removal of PCR inhibitors using agarose-embedded DNA preparations

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Received May 4, 1998; Accepted May 20, 1998

ABSTRACT

The use of agarose blocks containing embedded DNA improves the PCR amplification from templates naturally contaminated with polysaccharides or humic acids, two powerful PCR inhibitors. Presumably, the difference in size between the DNA macromolecules and these contaminants allows their effective removal from the agarose blocks by diffusion during the washing steps, whereas genomic DNA remains trapped within them. In addition, agarose-embedded DNA can be directly used for PCR since low melting point agarose does not interfere with the reaction. This simple and inexpensive method is also convenient for genomic DNAs extracted by other procedures, and it is potentially useful for samples containing other kinds of soluble inhibitors, overcoming this important problem of current amplification techniques.

Organic and inorganic compounds that inhibit the amplification of nucleic acids by PCR are common contaminants in DNA samples from various origins. They can interfere with the reaction at several levels, leading to different degrees of attenuation and even to complete inhibition. This constitutes an important problem for general research and especially for clinical forensic and

DNA macromolecules (e.g. complete prokaryotic or eukaryotic chromosomes) for pulsed-field gel electrophoresis analysis, has been tested in this work. Basically, whole cells are embedded in low melting point (LMP) agarose blocks and then immersed in a lysis buffer. By this procedure, intact genomic DNA (gDNA) can be obtained avoiding shear damage (4). Several washing steps can be carried out prior to other treatments (such as endonuclease digestion). Taking advantage of the important difference of size between DNA macromolecules and most common soluble PCR inhibitors, I have adapted this method to the preparation of high quality gDNA for PCR amplification. Large DNAs remain trapped within the agarose blocks, whereas cell debris and contaminants are free to diffuse during lysis and washing steps. In this way, the resulting gDNA is highly purified and free of contaminants. In addition, agarose-embedded DNA is useful for PCR, since reactions are not affected by the presence of high quality LMP agarose concentrations even as high as 0.3% in the PCR mixture (data not shown).

This method was tested using gDNA naturally contaminated with polysaccharides and humic acids, which are especially fastidious contaminants and difficult to remove using conventional protocols. gDNA was prepared from the bacterium *Enterobacter aerogenes*, protoplasts of the brown alga *Pylaiella littoralis* (kindly provided by Dr G.Ducreux) and unidentified bacteria naturally

“DNA em gel de agarose pode ser usado diretamente para PCR desde ponto de fusão baixo da agarose não interfira a reação. Este método simples e barato também é conveniente para DNAs genômicos extraídos por outros procedimentos, e é potencialmente útil para amostras contendo outros tipos de inibidores solúveis, para superar este importante problema de técnicas de amplificação de corrente.”

Purificação de um PCR com partículas paramagnéticas utilizando um líquido hidrofóbico

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J Mol Diagn. 2010 Sep;12(5):620-8. doi: 10.2353/jmoldx.2010.090190. Epub 2010 Jun 25.

Immiscible phase nucleic acid purification eliminates PCR inhibitors with a single pass of paramagnetic particles through a hydrophobic liquid.

Sur K, McFall SM, Yeh ET, Jangam SR, Hayden MA, Stroupe SD, Kelso DM

Center for Innovation in Global Health Technologies, Northwestern University, Evanston, Illinois 60208, USA.

Abstract

Extraction and purification of nucleic acids from complex biological samples for PCR are critical steps because inhibitors must be removed that can affect reaction efficiency and the accuracy of results. This preanalytical processing generally involves capturing nucleic acids on microparticles that are then washed with a series of buffers to desorb and dilute out interfering substances. We have developed a novel purification method that replaces multiple wash steps with a single pass of paramagnetic particles (PMPs) though an immiscible hydrophobic liquid. Only two aqueous solutions are required: a lysis buffer, in which nucleic acids are captured on PMPs, and an elution buffer, in which they are released for amplification. The PMPs containing the nucleic acids are magnetically transported through a channel containing liquid wax that connects the lysis chamber to the elution chamber in a specially designed cartridge. Transporting PMPs through the immiscible phase yielded DNA and RNA as pure as that obtained after extensive wash steps required by comparable purification methods. Our immiscible-phase process has been applied to targets in whole blood, plasma, and urine and will enable the development of faster and simpler purification systems.

PMID: 20581047 [PubMed - indexed for MEDLINE] PMCID: PMC2928426 Free PMC Article

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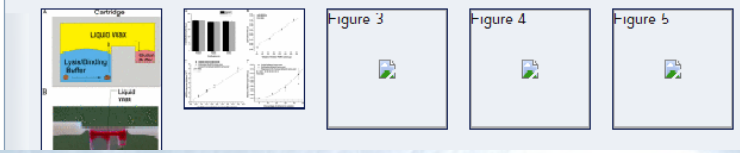

"Os PMPs contendo os ácidos nucleicos são magneticamente transportados através de um canal que contém cera líquida, que liga a câmara de lise para a câmara de eluição em um cartucho especialmente concebido. Transportar PMPs através da fase imiscível produziu DNA e RNA pura como a obtida após os passos de lavagem extensiva necessários por métodos de purificação comparáveis. O nosso processo de fase imiscível tem sido aplicada a alvos em sangue total, plasma e urina, e irá permitir o desenvolvimento de sistemas de purificação mais rápida e mais simples."

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