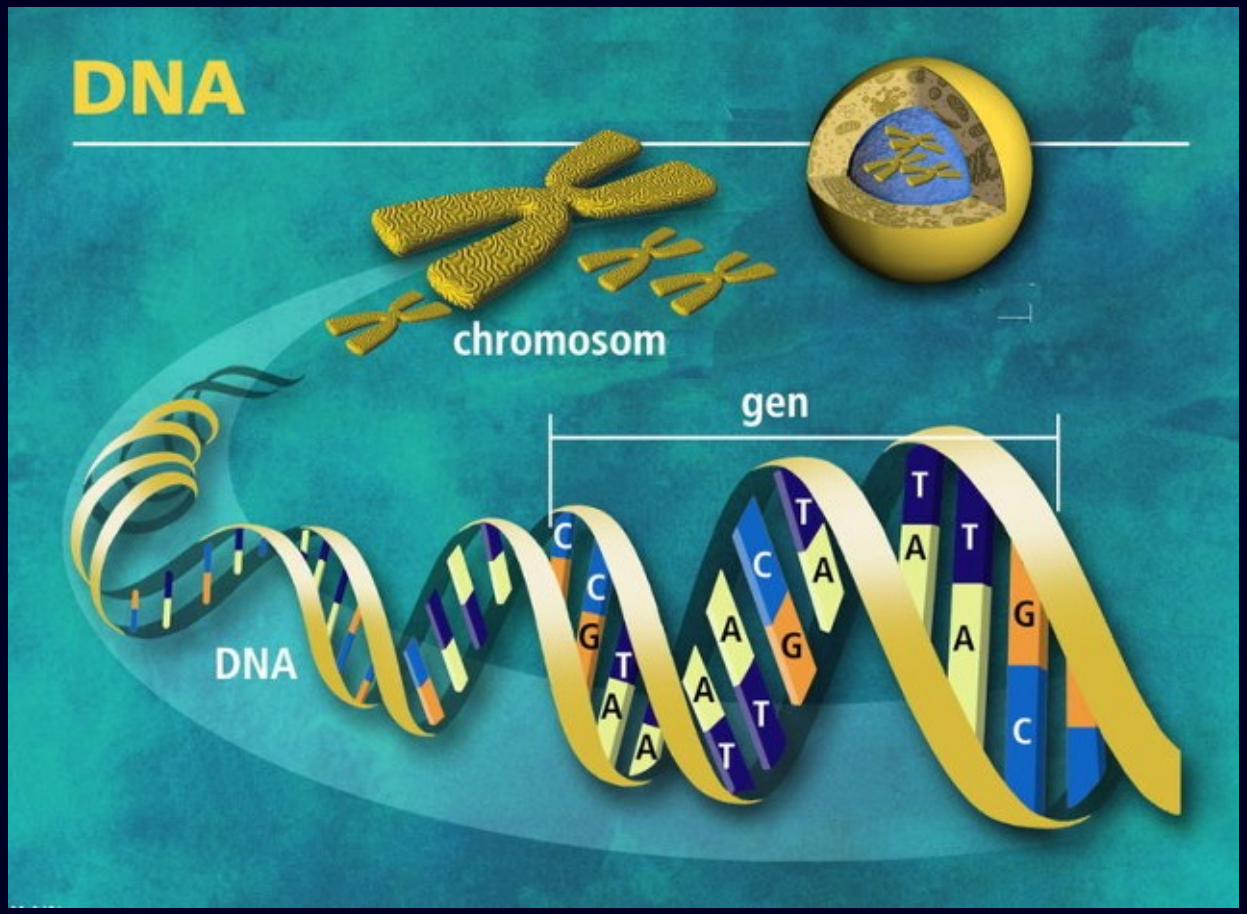
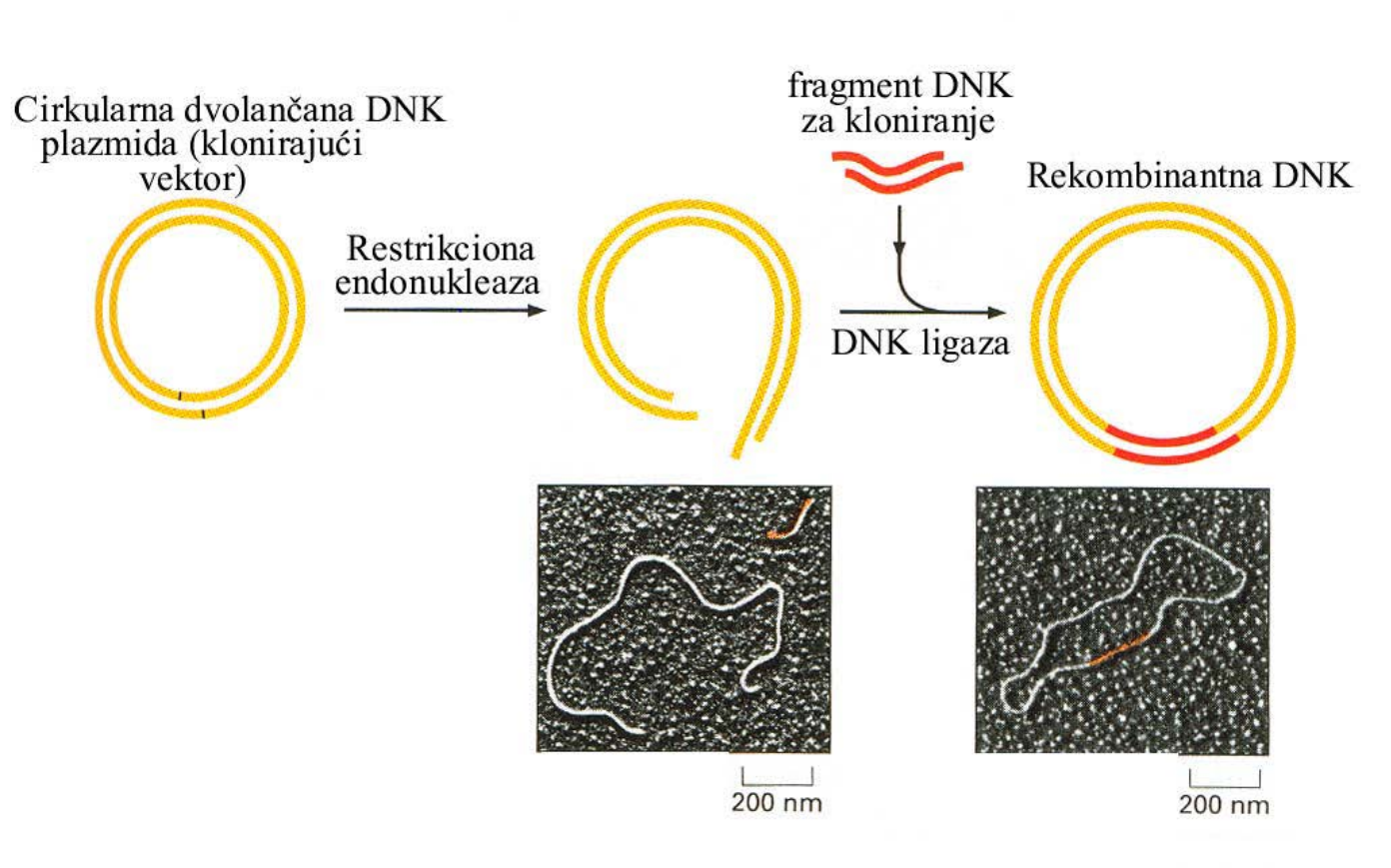
Katedra za biologiju



Polymerase Chain Reaction - **PCR**

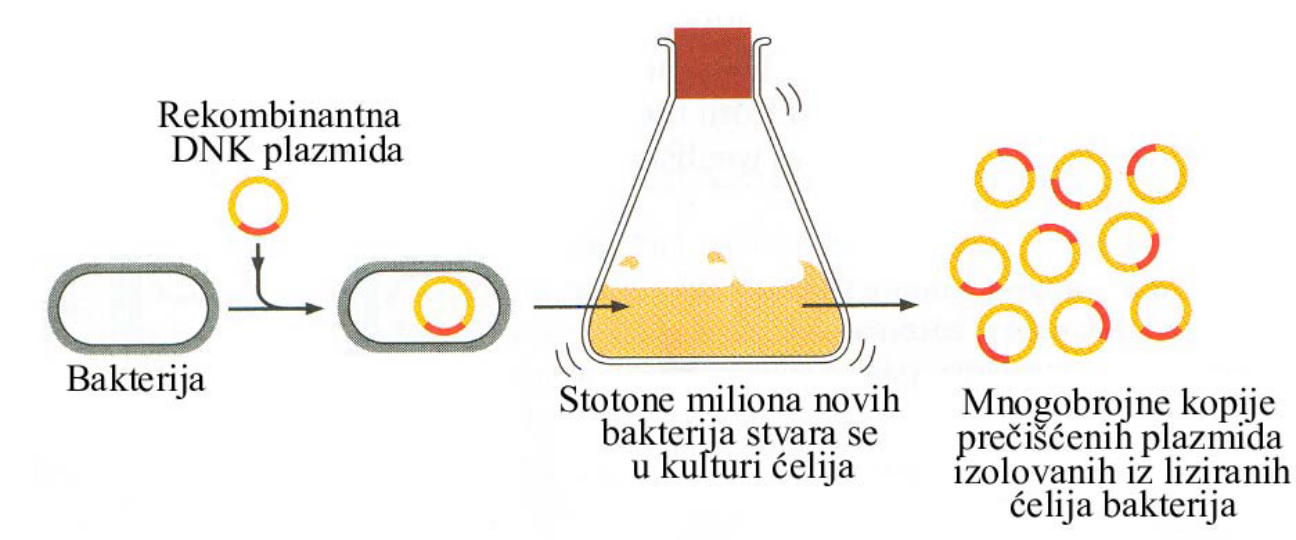
Nakon izolacije DNK (ili RNK) iz ćelije, sledeći korak je **amplifikacija (kloniranje) željenog (ciljanog) gena ili fragmenta DNK**, što se može obaviti ***in vivo*** i ***in vitro***.

In vivo amplifikacija



In vivo amplifikacija

Kod *in vivo* kloniranja, najpre se restrikcionim enzimima „iseče” ciljani deo DNK, a zatim taj deo ugradi u DNK vektora (plazmida ili virusa), odnosno stvori se rekombinantna DNK. U cilju umnožavanja rekombinantne DNK, vektor se ubacuje u ćeliju domaćina (najčešće u bakteriju *Escherichia coli*). Pri svakoj replikaciji rekombinantne DNK, umnožava se i ugrađeni (ciljani) deo DNK.



In vitro amplifikacija

In vitro kloniranje željenog (ciljnog) fragmenta DNK obavlja se putem reakcije lančane polimerizacije, odnosno putem **PCR amplifikacije (*Polymerase Chain Reaction* – PCR)** u aparatu koji ima mogućnost brze promene temperaturnih uslova.

Za PCR amplifikaciju potrebne su sledeće komponente:

- **izolovana DNK** čiji određeni fragment želimo da amplifikujemo,
- **prajmeri** (oligonukleotidne sekvence) koji se dizajniraju tako da budu komplementarni sekvencama koji okružuju ciljani region DNK (npr. gen) koji želimo da umnožimo,
- ***Taq* polimeraza**,
- **slobodni nukleotidi**, odnosno gradivne jedinice za sintezu novih lanaca DNK, u vidu mešavine deoksiribonukleozid trifosfata (dNTP): adeninskih (dATP), timinskih (dTTP), guaninskih (dGTP) i citozinskih (dCTP),
- **Magnezijumovi joni** (Mg^{2+}), kofaktori neophodni za aktivnost Taq polimeraze i polimerizaciju (vezuju se za slobodne nukleotide i obezbeđuju njihovo ugrađivanje u rastući lanac DNK).
- **PCR pufer**,
- sterilna dejonizovana **voda**

PCR Setup

A comprehensive guide to PCR

PCR Setup

https://www.thermofisher.com/rs/en/home/life-science/cloning/cloning-learning-center/invitrogen-school-of-molecular-biology/pcr-education/pcr-reagents-enzymes/pcr-component-considerations.html

PCR | PCR Setup—Six Critical Compone

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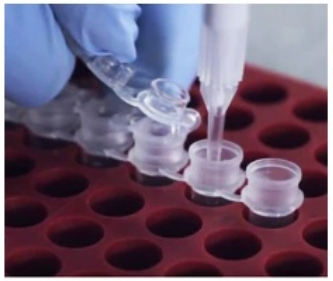
PCR Setup—Six Critical Components to Consider



The success of PCR depends on a number of factors, with its reaction components playing critical roles in amplification. Key considerations in setting up the reactions include the following and are detailed on this page:

- Template DNA
- DNA polymerase
- Primers
- Deoxynucleoside triphosphates (dNTPs)
- Required cofactor: Mg^{2+}
- Buffer

Featured video: Basics of PCR

Give Feedback

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A comprehensive guide to PCR

https://www.qiagen.com/us/knowledge-and-support/knowledge-hub/bench-guide/pcr/

PCR | PCR Setup—Six Critical Compon | PCR

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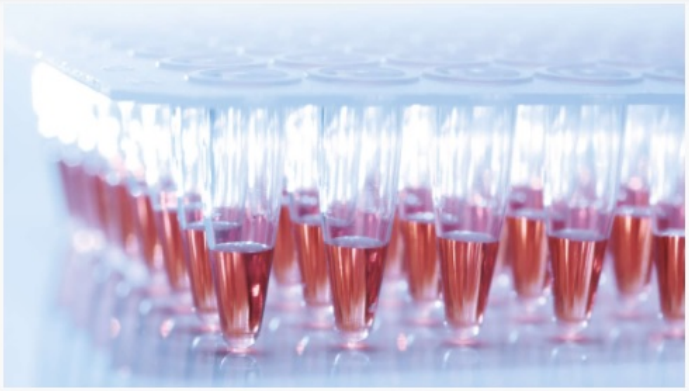
PCR

A comprehensive guide to PCR, including how to maximize your results.

This section provides a comprehensive guide to PCR. It also includes guidelines and suggestions for maximizing results from your PCR.



Introduction

Guidelines for PCR

Types of PCR

- Multiplex PCR
- Long-range PCR
- Single-cell PCR
- Fast-cycling PCR

PCR primer design

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Proces **PCR amplifikacije** obuhvata sledeće korake:

1. Inicijalna denaturacija DNK u trajanju od dva do četiri minuta (zavisno od udela GC parova).

2. Denaturacija DNK – rasplitanje i razdvajanje lanaca DNK. Obavlja se na 94-96°C u trajanju od 30 sec do nekoliko minuta (zavisno od udela GC parova),

3. Hibridizacija para prajmera sa komplementarnim sekvencama koji okružuju ciljani region DNK. Obavlja se na temperaturi od 45-65°C u trajanju od 30 sekundi do nekoliko minuta,

4. Elongacija prajmera (ekstenzija), odnosno **sinteza novih DNK lanaca** počev od prajmera, tako što se *Taq* polimeraza vezuje za mesta hibridizacije prajmera i katalizuje ugrađivanje novih nukleotida komplementarih inicijalnim sekvencama. Ovaj proces se obavlja na 72°C i traje od 45 sec do 1 minuta.

Koraci od 2. do 4. ponavljaju se tokom 25 do 40 ciklusa, kako bi se obezbedilo umnožavanje dovoljnog broja kopija ciljnog fragmenta DNK. **Umnoženi ciljni fragmenti DNK nazivaju se amplikoni ili PCR produkti**. U svakom ciklusu količina amplikona se duplicira (u prvom ciklusu nastaju 2 aplikona, u drugom 4, u trećem 8, u četvrtom 16, u petom 32, u šestom 64 ...) tako da na kraju PCR procesa nastaje od milion do bilion amplikona.

5. Elongacija preostalih produkata (na 72 °C, u trajanju od dva do četiri minuta).

http://learn.genetics.utah.edu/content/labs/pcr/

PCR

learn.genetics.utah.edu/content/labs/pcr/

What are these things doing in my PCR reaction?

Primers are short pieces of DNA that are made in a laboratory. Since they're custom built, primers can have any sequence of nucleotides you'd like.

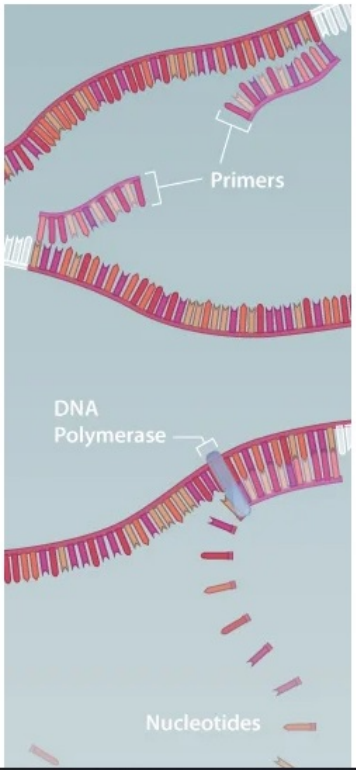
In a PCR experiment, two primers are designed to match to the segment of DNA you want to copy. Through complementary base pairing, one primer attaches to the top strand at one end of your segment of interest, and the other primer attaches to the bottom strand at the other end. In most cases, 2 primers that are 20 or so nucleotides long will target just one place in the entire genome.

Primers are also necessary because DNA polymerase can't attach at just any old place and start copying away. It can only add onto an existing piece of DNA.

DNA Polymerase is a naturally occurring complex of proteins whose function is to copy a cell's DNA before it divides in two. When a DNA polymerase molecule bumps into a primer that's base-paired with a longer piece of DNA, it attaches itself near the end of the primer and starts adding nucleotides. (In nature, these primers are made by an enzyme called primase).

The DNA polymerase in our bodies breaks down at temperatures well below 95 °C (203 °F), the temperature necessary to separate two complementary strands of DNA in a test tube. The DNA polymerase that's most often used in PCR comes from a strain of bacteria called *Thermus aquaticus* that live in the hot springs of Yellowstone National Park. It can survive near boiling temperatures and works quite well at 72 °C (162 °F).

Nucleotides are the building blocks that DNA molecules are made of. You add a mixture of four types of nucleotides to your PCR reaction A's, C's, G's and T's. DNA polymerase grabs nucleotides that are floating in the liquid around it and attaches them to the end of a primer.



Feedback

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Animacije PCR

https://youtu.be/DkT6XHWne6E

https://youtu.be/iQsu3Kz9NYo