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Protocol status: Working
 We use this protocol and it's working

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Purification and quantification from PCR amplification protocol

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GFX PCR DNA and Gel Band
Purification Kit.

GFX™ PCR DNA and Gel Band Purification Kit

kit can be used to purify DNA and concentration of PCR products or DNA fragments ranging in size from 50 bp to 10 kb.

GFX™ columns
collection tubes
Polypropylene tubes of 1,5 mL
Polypropylene tubes of 2,0 mL
Absorbent paper
Tips of 10 µL, 200 µL, 1000 µL
Permanent marker for labeling
Gloves

Reagents

Capture buffer
Washing buffer
Elution buffers (Tris-HCl and sterile water)
SYBER safe at 1:10000
Loading buffer dye (6X)
DNA Ladder 1 Kb (50 ng) 0,5 ng/µL
λ phage (50 ng/µL)
TBE 1X (89mM Tris-borate, 89mM boric acid, 2mM EDTA)

Solutions

DNA PCR amplification (25µL)
Sterile deionized water

Other

Micropipette of 10 µL, 200 µL, 1000 µL
Analytical balance
Freezer
microcentrifugate

Purification steps

- 1 Prepare collection tubes for each sample with their corresponding columns

- 2 Add 500 μ L of capture buffer on each column
- 3 Add 25 μ L of PCR-amplified DNA
- 4 Slowly mix the solution using a micropipette.
- 5 Centrifuge for 1 min at 16000 g
- 6 Keep the DNA in the column and discard the flow through on the tube
- 7 Add 500 μ L of washing buffer on the column
- 8 Centrifuge for 1 min at 16000 g
- 9 Incubate for 1 min at room temperature
- 10 Transfer the column into a new tube

- 11 Add 10 μL of elution buffer
- 12 Incubate for 2 min at room temperature
- 13 Centrifuge for 2 min at 16000 g
- 14 Repeat step 11
- 15 Repeat step 12
- 16 Repeat step 13
- 17 Discard the columns and keep the tubes with purified DNA at 4 °C until visualization

Quantification

- 18 To visualize the amplified products, put 2 μL of the PCR product with 2 μL of loading buffer dye in

agarose gel electrophoresis with 1X TBE stained with SYBER safe at 1:10000.

- 19** To compare, add 1 μL of 1kb DNA ladder (final concentration 0,5 ng/ μL) and observe a band of 1500 pb approximately.