

Purifying rRNA or amplicons in solution or agarose with GFX-columns

1. Subject

The disadvantage of a mini-prep extraction is the bad purity of rRNA. The suspension contains RNA, but also other propagules from the cell and cell debris. These substances can inhibit PCR. A possible solution to improve PCR is a purification step. Instead of glassmilk or Microspin, GFX-columns can be used as an alternative.

2. Principal

The principal of this procedure is the ability of the column material to bind template by means of a capture-buffer. After centrifugation the column is washed with buffer containing ethanol. After washing the template is resuspended in sterile water or a Tris c.q. Tris/EDTA-buffer.

3. Reagents and material

- 3.1 pipets
- 3.2 pipettips
- 3.3 eppendorf cups 1.5 ml (Sarstedt 72.690)
- 3.4 microcentrifuge (Eppendorf 5417R)
- 3.5 GFX-columns, RNA and Gelband purification kit (Amersham Pharmacia 27-9602-01)
- 3.6 Eurogentec SmartLadder (Eurogentec MW-1700-02) 200, 400, 600, 800, 1000, 1500, 2000, 2500, 3000, 4000, 5000, 6000, 8000, 10000 bp resp. 20, 40, 60, 80, 100, 15, 20, 25, 30, 40, 50, 60, 80, 100 ng/band
- 3.7 Tris(hydroxymethyl)-aminomethane (Merck 8382)
- 3.8 Na-EDTA (Titriplex III, Bio Rad 161-0729)
- 3.9 1N HCl (Ferak 11448)

4. Solutions

- 4.1 TE-buffer, pH 8:
Add 0.12 g Tris [3.7] and 0.04 g Na-EDTA [3.8] to 80 ml ultrapure water. Adjust pH to 8.0 with 1N HCl [3.9]. Heat the solution if EDTA doesn't dissolve very well. Adjust volume to 100 ml with ultrapure water. Aliquot the buffer in 50 ml tubes. Autoclave the solution during 15 min. at 121°C. Store at roomtemperature.
- 4.2 10 mM Tris-buffer, pH 8:
Add 0.12 g Tris [3.7] to 80 ml ultrapure water. Adjust pH to 8.0 with 1N HCl [3.9]. Adjust volume to 100 ml with ultrapure water. Aliquot the buffer in 50 ml tubes. Autoclave the solution during 15 min. at 121°C. Store at roomtemperature.

5. Protocol

- 5.1 Place a GFX-column [3.5] in a collection tube.
- 5.2 Pipet 500 µl Capture-buffer onto the column and add 50(-100) µl amplicon.
- 5.3 Mix thoroughly by pipetting the sample up and down 4-6 times.
- 5.4 Centrifuge 30 sec. at 11500 rpm [3.4, 6.1].
- 5.5 Discard the flow-through by emptying the collection tube and place the column back onto the collection tube.
- 5.6 Pipet 500 µl wash buffer to the GFX column.
- 5.7 Centrifuge 30 sec. at 11500 rpm [6.1].
- 5.8 Discard the collection tube and transfer the column to a sterile 1.5 ml eppendorf cup [3.3].
- 5.9 Pipet 50 µl TE-buffer [4.1] when eluting rDNA or 50 µl Tris-buffer [4.2] when eluting PCR products for sequencing.

A.H.G. Gerrits van den Ende

- 5.10 Incubate 1 min. at room temperature.
- 5.11 Centrifuge 1 min. at 11500 rpm [6.1] to collect the purified material.
- 5.13 Discard the column.
- 5.14 Store the purified rDNA c.q. PCR product in the refrigerator.
- 5.15 Estimate the concentration of the rDNA/amplicon by gel electrophoresis (protocol 9, p. 33) with the SmartLadder as a reference or by spectrometer measuring OD260 (protocol 20, p. 65).

6. Remarks

- 6.1 The speed of the centrifuge should be adjusted to 10.000-16.000 rcf. With a Eppendorf 5417R 11.500 rpm gives a rcf of 14.000. Start timer when run begins and not when centrifuge has reached the chosen speed.



A.H.G. Gerrits van den Ende