

Purification of PCR products by means of Microspin columns

1. Subject

After PCR the samples still contain primers, polymerase and dNTP's next to the amplicon. To use the sample for ARDRA or sequencing, only amplicon should be present. With Microspin columns it is possible to get rid of these impurities.

2. Principal

The principal of this purification step is to fix small molecules of primers and dNTPs to the column. After centrifugation the column is user ready. After pipetting the PCR product the column is centrifuged again and the filtrate contains the purified amplicon.

3. Reagents and material

- 3.1 Microspin S-300 HR (Amersham Pharmacia 27-5130-01) of Microspin S-400 HR (Amersham Pharmacia 27-5140-01).
- 3.2 1.5 ml reaction tubes (Sarstedt 72.690)
- 3.3 centrifuge (Eppendorf 5415C of 5417R)
- 3.4 pipets
- 3.5 pipettips

4. Solutions

see 3.1

5. Protocol

- 5.1 Vortex column (3.1) to resuspend resin (6.3).
- 5.2 Loosen cap ¼ turn and snap off the bottom closure.
- 5.3 Place column in a 1.5 ml eppendorfcup [3.2] for support and to collect buffer.
(Cut the cap from a flip-top eppendorfcup or use a screw-cap microcentrifuge tube)
- 5.4 Pre-spin the column for 1 min. at 735xg [6.1, 6.2].
Start the timer at the same time as you start the centrifuge.
- 5.5 Place the column in a new (sterile) 1.5 ml eppendorfcup.
Slowly apply the sample to the top-center of the resin, being careful not to disturb the bed.
- 5.6 Spin the column for 2 min. at 735xg.
The purified sample is collected in the bottom of the support tube.

6. Remarks

- 6.1 The speed of an Eppendorf 5415C with an 18-position fixed-angle rotor is adjusted to 3000 rpm to obtain a 735xg. For the Eppendorf 5417R the rpm adjustment is ≈ 2700 .
- 6.2 For use with other centrifuges the speed can be calculated with the following formula: relative centrifugal force = $1.12 \cdot \text{radius} \cdot (\text{rpm}/1000)^2$. Conclusively, to obtain a 735xg the speed in rpm = $1000 \cdot \sqrt{657/\text{radius}}$. The radius should be given in millimeter.
- 6.3 Depending on the length of the used primers and the amplicon S-300 is used (PCR products ≥ 100 bp, primers ≤ 20 -mers) and a volume of 25-50 μl or S-400 (PCR products ≥ 200 bp, primers ≤ 32 -mers) and a volume of 51-100 μl .

7. Literature

- 7.1 Science Tools from Pharmacia Biotech 1, 3 (1996).

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