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Sephadex/sephacryl purification of AFLP products

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1 Works for me dx.doi.org/10.17504/protocols.io.rq8d5zw

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GUIDELINES

It is the best to gather two full 96x plates (or at least one 96x plate).
All the volumes are given for two plates.
Never leave dry wells in a purification plate (membrane deterioration).

MATERIALS

NAME	CATALOG #	VENDOR
EDTA		
NaCl	53014	Sigma Aldrich
MultiScreen HTS DV 0.65µm Clear Non-sterile plates	MSDVN6550	Millipore
Sephadex G-50 Superfine	17 0041 01	Ge Healthcare
Sephacryl S-200	17 0584 01]	Ge Healthcare
Twin.Tec PCR Plate 96 semi-skirted, colourless wells, 25 pcs	0030128575	Eppendorf
Tris		

BEFORE STARTING

Solutions to prepare:

NaCl 0.5 M:
5.84 g NaCl in 200 ml ddH2O

EDTA-Na2 100 mM pH 8.0:
Dissolve3.72 g EDTA-Na2 in 90 ml ddH2O
Adjust pH to 8.0
Fill up with ddH2O to 100 ml

TRIS-HCl 1 M pH 7.0:
Dissolve 12.114 g Tris in 80 ml dH2O.
Adjust pH to 7.0 with the appropriate volume of concentrated HCl.
Bring final volume to 100 ml with deionized water.
Stock at 4°C

5% Sephadex G50:
2 ml NaCl 0.5 M
2 ml EDTA-Na2 100 mM pH 8.0
2 ml Tris-HCl 1 M pH 7.0
Add ddH2O up to 200 ml
Add 10 g Sephadex G 50 – mix [attention: it will never become clear]
Add 100 µl Triton X-100 (final conc. 0.05%; attention: viscous solution – cut tip end and pipet slowly!)
Stock at 4°C!
This amount serves ca. 5 purification cycles of two 96x plates each.

Preparation of 96x plates

1 Put the Sephacryl bottle on the shaker for 5-10 minutes, adjust speed to 4-5 to make it liquid. For the last minute add also the Sephadex mixture bottle and decrease the speed to 3-4.

2 Prepare a 1:1 (v/v) solution of pre-prepared 5% Sephadex G50 and Sephacryl S200 (35 ml of each) and mix well.

3 Pipet 300 µl of the mixture to each well of the Millipore purification plate.NOTE: Use the Matrix electronic pipet >> Fill at 1250 µl, Disp at 300 µl).

4 Place the Millipore plates on trash plates.

5 Centrifugate 1 min at 2800 rpm.Purification of AFLP products

Purification of AFLP products

6 Match and place the prepared purification plates (Millipore plates with fresh Sephadex/Sephacryl layer prepared as described above) on new Eppendorf plates.
- describe the contents of the Eppendorf plate beforehand.
- double-check carefully the orientation of the two plates!
- fix the two plates additionally with small pieces of tape on both sides.

7 Pipet the whole volume of the selective PCR product onto the centre of Sephadex/Sephacryl membranes without touching the membranes (using multichannel pipette).

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8 Centrifugate 2 min at 2800 rpm.

9 Remove the Millipore plate and use the filtrated PCR product in the Eppendorf plate for subsequent dilution of the selective PCR product.

10 Prepare the mix of 10 µl of Hi-Di formamide and 0.1 µl of Gene Scan-500 ROX size standard per sample and pipet 1-1.5 µl of the purified and diluted PCR product.

Millipore plates can be reused up to three times. Leave the Sephadex resin to dry in ambient temperature – it will contract and become easy to remove. Then, plates can be loaded with a new Sephadex/Sephacryl mixture.

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