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T-RFLP Sephadex purification and sample preparation

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

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Abstract

Purification of PCR-product after restriction digest. Samples proceeded on the capillary sequencer.

Guidelines

The Sephadex solution is stored in a glass bottle in the fridge. Before you start the purification let the Sephadex solution come to room temperature and shake well before each usage!

Materials

MATERIALS

MultiScreen-HV Filter Plate, 0.45 µm, clear, non-sterile **Merck Millipore Catalog #**MAHVN4550

Sephadex G-50 M DNA Grade, 100 g **Ge Healthcare Catalog #**17004502

Before start

Materials:

- digested PCR-product (100-200ng in 20ul)
- 96 well filter plates
- 96 well collection plates (IBL 96*0.2ml frosted Subkirted thin well)
- Sephadex G50 (GE, 37.5g into 500ml autoclaved MilliQ H_2O)
- Multipette 10ml/1ml
- Multichanel 200ul/10ul
- HiDi Formamide (Prepare a master mix: 10ul HiDi Formamide + 0.3ul Size Standard, GS_1200 LIZ, AB per sample nebo 165ul HiDi Formamide + 5ul Size Standard for one run = 16 samples)

Sephadex purification

1
- Load 200µl Sephadex solution onto the 96 well filter plates (shake the Sephadex solution well before each usage!) (use the EppendorfMultipette with a 10ml tip), the no. of wells you load depends on the no. of samples,
- load filter plate on 96 well collection plate,
- place the lit on top of this 'plate sandwich' and
- place in swing out rotor base.

200 µL Sephadex solution

2 Spin at 230rcf(g) for 1 min 15sec (Eppendorf centrifuge 5810). Don't forget to balance the centrifuge by placing a dummy 'plate sandwich' on the rotor.

Command

420 x g

CENTRIFUGE

00:01:00

3 Discard flow through.

4 Repeat step 2. (Shake Sephadex!)

go to step #2 repeat

5 Place Sephadex G-50 containing 96 well filter plate on the collection plate.

6 Load 20µl sample in the centre of the Sephadex column. Just drop the 20ul on tj top of the gel. **Avoid to destroy the Sd. gel by penetrating it with the pipette tip.**

20 µL sample

7 Spin at 420 rcf (g) for 1 min (Eppendorf centrifuge 5810). Note: There will be some extra buffer that comes through from the Sephadex resulting in a total sample volume of ~ 35-40 µl.

00:01:00

Command
420 x g CENTRIFUGE

8 Transfer the purified samples back to the original caps.

9 Remove the Sephadex from the filter plate and rinse the filter plate and collection plate as well (with MQ). Both plates could be reused several times.

10 Go immediately to sample preparation or store the samples at -20°C.

Sample preparation for capillary sequencing

11 Aliquot 10µl of HiDi Formamide master mix into a **new** 96 well plate.

12 Add the 4µl of your purified sample per well.

4 µL purified sample

13 Denature samples for 3min at 95°C.

00:03:00 denaturation

95 °C

14 Transfer samples immediately on ice and let the chill for 5 min.

0 °C on ice

00:05:00

15 Store samples in the fridge, prepare the sample sheet and run the samples at ABI 3130XL Capillary Sequencer.