



Sep 16, 2024

T-RFLP Sephadex purification and sample preparation

DOI

<https://dx.doi.org/10.17504/protocols.io.36wgqwyogk57/v1>

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Protocol Citation: Eva Petrova, Roey Angel 2024. T-RFLP Sephadex purification and sample preparation. **protocols.io**

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Protocol status: Working

We use this protocol and it's working

Created: May 24, 2018

Last Modified: September 16, 2024

Protocol Integer ID: 12442

Keywords: sample preparation purification of pcr, rflp sephadex purification, sample preparation purification, purification, product after restriction digest, capillary sequencer, restriction digest, pcr

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 (EMD 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 H₂O)
- 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!)
- 5 Place Sephadex G-50 containing 96 well filter plate on the collection plate.

 go to step #2 repeat

- 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.

