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Ethanol precipitation of small DNA fragments

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Eva Petrova[1], Roey Angel[1]

[1]Soil and Water Research Infrastructure

Anaerobic and Molecular Microbiology Lab, Biology Centre CAS
Tech. support email: **eva.petrova@bc.cas.cz**

Eva Petrova
Soil and Water Research Infrastructure

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

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Protocol Integer ID: 12439

Keywords: ethanol precipitation of dna fragment, ethanol precipitation of small dna fragment, dna fragment, simple ethanol precipitation of small fragment, small dna fragment, ethanol precipitation, simple ethanol precipitation, small amounts of dna, dna, enzymes for ligation, ligation, protocol, digest, small fragment, pellet, enzyme

Abstract

This protocol is for a simple ethanol precipitation of small fragments and/or small amounts of DNA. Note that this protocol simply concentrates your sample and removes enough salts/enzymes for ligation to be successful. All DNA fragments from your digest will still be present in your pellet.
See **here** for a discussion of theory behind ethanol precipitation of DNA fragments.

Guidelines

3M Sodium Acetate, 0.11M $MgCl_2$ pH 5.5

24.6 g sodium acetate
0.952g $MgCl_2$ anhydrous
Dissolve in about 60 ml DDW Adjust pH with glacial acetic acid (>8 ml), Dilute to 100 ml with DDW and filter sterilize.

Materials

MATERIALS

Pellet Paint® NF Co-Precipitant **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**70748-3

Protocol materials

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Before start

- Absolute Ethanol (100% = 200 proof) at -80^{o} C
- 70% ethanol at -80^{o} C (don't cool ethanol for too long or water will freeze)
- Tabletop centrifuge
- -80°C freezer

1 Transfer the sample to 1.5 mL eppendorf tube.

2 Adjust the salt conc. of the sample to 0.3M sodium acetate and 0.01M $MgCl_2$.

[M] 0.3 Mass Percent sodium acetate

[M] 0.01 Mass Percent MgCl2

3 Add 1 µl of Pellet Paint NF.

1 µL Pellet Paint NF

Pellet Paint® NF Co-Precipitant **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**70748-3

4 Add 1 µl of non labeled genomic DNA e.g. from E.coli (this acts as a carrier and increases precipitation yield).

1 µL genomic DNA (E.coli)

5 Add 2 volumes cold absolute ethanol to sample.

6 Incubate 2-3 hr at -80°C. The long incubation time is critical for small fragments.

-80 °C

02:00:00

7 Centrifuge for 30 minutes at 0°C at maximum speed (generally >10000 g at least).

Command

max. speed

```
CENTRIFUGE
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0 °C

00:30:00

8 Remove supernatant.

9 Wash with 750-1000 µL cold 70% ethanol. Another critical step for small fragments under 200 base pairs. Washing involves adding the ethanol and inverting several times.

750 µL cold 70% ethanol

10 Centrifuge for 30 minutes at 0°C at maximum speed (generally >10000 g at least).

Command

max. speed

CENTRIFUGE

0 °C

00:30:00

11 Repeat washing procedure and centrifuge again.

go to step #9

12 Let air dry on benchtop.

13 Resuspend in an appropriate volume of H_2O.