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Ethanol precipitation of small DNA fragments

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Eva Petrova¹, Roey Angel¹

¹Soil and Water Research Infrastructure

SoWa RI Anaerobic and Molecular Microbiology (public)

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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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 <u>here</u> for a discussion of theory behind ethanol precipitation of DNA fragments.

Guidelines

3M Sodium Acetate, 0.11M MgCl₂ pH 5.5

24.6 g sodium acetate 0.952g MgCl₂ 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 Sigma Aldrich Catalog #70748-3

Protocol materials

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

Materials, Step 3

Before start

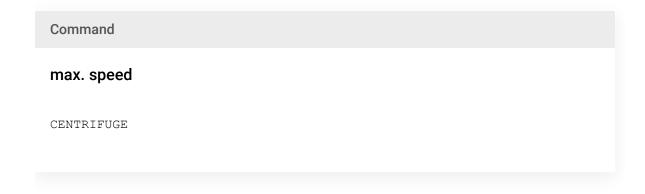
- Absolute Ethanol (100% = 200 proof) at -80° C
- 70% ethanol at -80° C (don't cool ethanol for too long or water will freeze)
- Tabletop centrifuge
- -80°C freezer



- 1 Transfer the sample to 1.5 mL eppendorf tube.
- Adjust the salt conc. of the sample to 0.3M sodium acetate and 0.01M MgCl₂.

[M] 0.3 Molarity (M) sodium acetate
[M] 0.01 Molarity (M) MgCl2

- 3 Add 1 µl of Pellet Paint NF.
 - Δ 1 μL Pellet Paint NF
 - Pellet Paint® NF Co-Precipitant **Sigma Aldrich Catalog #**70748-3
- 4 Add 1 μ I of non labeled genomic DNA e.g. from E.coli (this acts as a carrier and increases precipitation yield).
 - Δ 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).



- **₽** 0 °C
- **(5)** 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.

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





11 Repeat washing procedure and centrifuge again.

≘5 go to step #9

- 12 Let air dry on benchtop.
- 13 Resuspend in an appropriate volume of H_2O .