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# Ethanol precipitation of small DNA fragments

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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 **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 **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$^{o}$ 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 Molarity (M) sodium acetate

[M] 0.01 Molarity (M) MgCl2

3 Add 1 µl of Pellet Paint NF.

1 µL Pellet Paint NF

Pellet Paint® NF Co-Precipitant **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
```

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 $H_2O$.