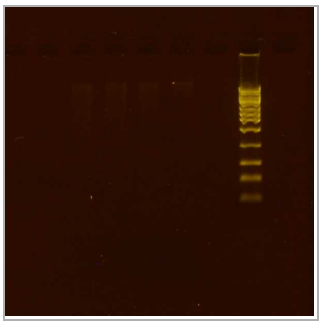

DEC 01, 2022

OTHER

PEG-8000/NaCl Size-Selective DNA Precipitation

COMMENTS 0

This protocol is published without a DOI.

Matt S.A. Penney[1]

[1]Dalhousie University

Matt S.A. Penney: Marine Gene Probe Lab (PI: Dr. Paul Bentzen)

Matt S.A. Penney
Dalhousie University

DISCLAIMER

This is not published work and is basically just me messing with other protocols to see what happens.

ABSTRACT

It is difficult to see in the image (this is heavily diluted gDNA), but this protocol does effectively remove small DNA fragments if you, for example, wanted to remove them prior to preparing Illumina sequencing libraries. I saw this protocol using various NaCl concentrations and different types of PEG:

CITATION

John Tyson. Size Selective Precipitation of DNA using PEG & Salt. protocols.io.
LINK
https://protocols.io/view/size-selective-precipitation-of-dna-using-peg-amp-7erhjd6

My lab (Marine Gene Probe Lab, Dalhousie University) has PEG-8000 and a previous article I read (J.L. Hartley, H. Bowen, PEG precipitation for selective removal of small DNA fragments, have not managed to find this online) tested a 6.7% PEG-8000 solution against MgCl2. Oddly enough, when I tested their protocol I didn't get any DNA back at all but I did when I used NaCl at Tyson's concentrations. Huh. Go figure.

Anyway, I tested four different concentrations of NaCl (450mM, 475mM, 500mM, and 525mM) with 6.7% PEG 8000 in a 25ul reaction. 5ul of this was a ratio of 5M NaCl to 1X TE adjusted to get the aforementioned final concentrations. Another 5ul was 33.5% PEG 8000. Then 15ul of the DNA you want to size select (if you don't have enough, top up with 1X TE). 450mM seems to work the best for precipitating out the HMW band while minimizing the smear, but I have not re-tested this with more concentrated DNA. The 5M NaCl/1X TE ratios (in ul) for the different concentrations are:

Conc: (NaCl/1X TE)
450mM (2.25/2.75)
475mM (2.5/2.5)
500mM (2.75/2.25)
525mM (3/2)

This test was done using gDNA extracted from Three-Spine Stickleback (Gasterosteus aculeatus) which was 1/5 diluted for this test... after being topped up with 10ul 1X TE beforehand. That's why the gel is so faint and why I'm a bit concerned that the 450mM may also be losing HMW DNA. I may re-test this in the future.

PROTOCOL CITATION

Matt S.A. Penney 2022. PEG-8000/NaCl Size-Selective DNA Precipitation. **protocols.io** https://protocols.io/view/peg-8000-nacl-size-selective-dna-precipitation-cjv5un86

KEYWORDS

PEG 8000, NaCl, DNA Precipitation, Size Selection, Quality Control, Salting Out, Organic Solvent, Ethanol

IMAGE ATTRIBUTION

I took that gel image using our super cool EtBr-free camera system.

CREATED

Nov 30, 2022

LAST MODIFIED

Dec 01, 2022

PROTOCOL INTEGER ID

73373

MATERIALS TEXT

5M NaCl
33.5% PEG-8000
1X TE
Microcentrifuge Tubes
1-10ul Pipettor
Pipettor with 25ul in range
Pipette Tips
Somewhere to dump the supernatant

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55m

Kicking Out The Small Fragments

1 5 µL | [M] 5 Molarity (m) (mol/L) | Sodium chloride **P212121**
1X TE buffer (10 mM Tris-HCl pH 8.0 1 mM EDTA) **Contributed by users**
Add the appropriate amounts of 5M NaCl and 1X TE to achieve the desired final concentration of salt (see Description for ratios).

2 5 µL | [M] 33.5 Mass Percent | PEG 8000 **Sigma Aldrich Catalog #81268**

Pipette slowly with this step as the solution is very thick. You can mix by pipetting or by vortexing and centrifuging.

3 15 µL gDNA

If you don't have 15ul of your sample you can top it up with 1X TE to achieve the final volume (25ul). You MUST have a final volume of 25ul to ensure the concentrations of PEG 8000 and NaCl are correct. Mix the sample by vortexing briefly.

20m

4 13000 rpm, Room temperature, 00:20:00

Or whatever the highest setting on your centrifuge is. You can proceed to this step immediately after mixing or you can wait. You will get the same result either way.

NOTE: When you add your tubes into the centrifuge, do so in a way where you can accurately predict where the DNA pellet should form: (https://openwetware.org/wiki/DNA_Precipitation#:~:text=The%20DNA%20pellet%20will%20not,the%20outside%20of%20the%20centrifuge.) You may not be able to see it when the centrifugation is complete, particularly if you are using diluted DNA.

20m

5 25-30 µL | 95% ethanol **Contributed by users** | 13000 rpm, Room temperature, 00:20:00

Decant the pellet carefully, discard the supernatant, and do at least one wash with 95% ethanol (preferably cold ethanol). I recommend re-centrifuging the pellet before decanting the ethanol.

15m

6 Room temperature | 00:15:00

Once you've decided that your pellet is squeaky clean, let it air-dry for at least 15 minutes. Before resuspension, be sure to check the tube for any signs of remaining liquid.

7 15-30 µL | 1X TE buffer (10 mM Tris-HCl pH 8.0 1 mM EDTA) **Contributed by users**

Once the pellet is dry, re-suspend it in 1X TE. 15-30ul of 1X TE will work depending on how dilute you want your final solution to be.

7.1 If you want to assess the efficacy of your precipitation (i.e. if this actually worked), you can run a small volume (1-2ul) of it on a 1% Agarose gel using 0.5X TBE (or TAE) buffer. How you stain your DNA is up to you.