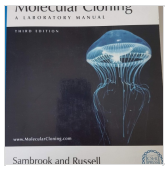

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Ethanol precipitation of nucleic acids (microcentrifuge tubes)

Forked from Ethanol precipitation of nucleic acids (Eppendorf tubes)

Heather Etchevers[1]

[1]Aix-Marseille Université

1 *Works for me* This protocol is published without a DOI.

MMG DevCard - équipe Zaffran

Heather Etchevers
Aix-Marseille Université, INSERM

ABSTRACT

This is a classic technique out of the molecular biologist's playbook of the 1980s, ***Molecular cloning: a laboratory manual***, Volume 1 by Joseph Sambrook, E. F. Fritsch, and Tom Maniatis, Cold Spring Harbor Laboratory Press, 1982, 1989 and beyond. (ISBN 0-87969-136-0).

Nucleic acid precipitation is used to concentrate and/or purify nucleic acids. The below protocol is based on the fact that nucleic acids are less soluble in alcohol than in more polar water. Addition of salt further decreases solubility by competing for water dipoles; as does low temperature. Please see the OpenWetWare website for more details.

Citation: OpenWetWare contributors, 'Ethanol precipitation of nucleic acids', *OpenWetWare, ,* 1 July 2012, 10:48 UTC, <http://openwetware.org/index.php?title=Ethanol_precipitation_of_nucleic_acids&oldid=611420> [accessed 21 January 2015]

EXTERNAL LINK

http://openwetware.org/wiki/Ethanol_precipitation_of_nucleic_acids

PROTOCOL CITATION

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KEYWORDS

ethanol, dna, precipitation

IMAGE ATTRIBUTION

https://openwetware.org/wiki/File:DNA_EtOH_precipitation_small_pellet.jpg by Jakob Suckale

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MATERIALS

NAME	CATALOG #	VENDOR
Ethanol		
Sodium Acetate	AA11554-30	Vwr
Microcentrifuge		
Microcentrifuge tube Safe-Lock write-on 1.5mL Eppendorf Tube	0030 120.086	Eppendorf
Ammonium acetate	7869-A	Euromedex

MATERIALS TEXT

Best done in 1.5 mL microcentrifuge tubes, but will work in 2.0 or 0.5 mL tubes also. The former will tend to have the pellet smear more, vertically, which can present challenges in terms of loss during the rinses. But for large quantities (tens of µg) and volumes, it's also fine.

DISCLAIMER:

Jakob Suckale currently from Universität Tübingen wrote this up for OpenWetWare in the early 2000s from the classic protocol used widely. Kathleen McGinness currently from Unum Diagnostics and I contributed a couple of tweaks, retained here. I'd include these others as authors but truly, this is such a classic that no one is really an author any more. I just put it up as a reminder so it's easy to print for trainees, and hope it is helpful to you in this as well.

Salting-out 1m

1 Add 1/10 volume of 3M sodium acetate, pH 5.2 (or 1/2 volume of 5M ammonium acetate) relative to initial volume A. Mix. 1m

2 Add 2.5 volumes of 100% ethanol relative to the volume in Step 1. (= 2.5 * 1.1 *A) 1m

3 Mix and freeze overnight at -20°C. 10m

(My original comment on OWW: This step some say is unnecessary but others swear by it. If you are in a rush you can also put it in the -80°C for ten minutes to a few hours. Dry ice also works.)

In general, the time you need to incubate in the freezer depends on how much nucleic acid you have, how big it is and the volume it is in. My general protocol is to freeze for 20 min to 1 hr at -80 ˚C. This seems to work well for most things, but you may want to freeze longer if you have only a small concentration of nucleic acid or if it is small in size(<15 nucleotides). (Kathleen)

If you are in a hurry, you can also dip your tube shortly into liquid nitrogen. If you added enough ethanol, the mix won't freeze. Careful with isopropanol - it freezes more quickly. This works well for me and saves me a lengthy incubation in the fridge. (Jasu)

Precipitation and clean-up 48m

4 Spin at full speed in a standard microcentrifuge at 4 degrees for 30 minutes. If isopropanol, favor room temperature to precipitate less salt. 30m

00:30:00

Decant (or carefully pipet off) the supernatant. If your DNA is precious, save this in a labeled, corresponding tube until 1m

5 the pellet has been resuspended and dosed.

6 Rinse with 1-2 volumes A of ice-cold 70% ethanol. Decant once more and add to the reserve tube with the salty ethanol from step 5. 1m

7 Dry the pellet. Laying the tubes on their sides prevents the last ethanol drop from drying on the DNA itself and speeds evaporation. 15m

For this you can air dry (tubes open, ~15 min) or dry in a speedvac. DNA and RNA (if you don't have RNases in your sample) are typically hearty enough for you to air dry at 37 ˚C, if desired.

Overdrying can make DNA hard to re-dissolve. Especially for longer DNA, I avoid vacuum drying and airdry only briefly before re-dissolving. (Jasu)

8 Add your desired quantity of water, or better, TE. Vortex gently and spin down to resuspend. 1m

If resuspending genomic DNA, again, do not dry the pellet entirely, but resuspend to a concentration of greater than 200 ng/µL for quality in terms of length, but keeping viscosity to a minimum for accurate pipetting. Tube inversion at 4° over a weekend or room temperature overnight can help resuspension and homogenization.

Beware of using water unless you are sure of what you are getting in to. The "pH" of water can vary widely (I've seen from pH 5 to pH 8.5), and **depurination of DNA at low pH** or **degradation of RNA at high pH** are possibilities. Water also typically contains trace metals, which can accelerate these reactions.

I typically recommend resuspension in TE (10 mM Tris-HCl, **pH 7.5**, 1 mM EDTA). This makes sure your nucleic acid is at a neutral pH and the EDTA will chelate any trace metals. Since they are in such small amounts, neither the buffer nor the EDTA will affect most downstream reactions. (Kathleen)