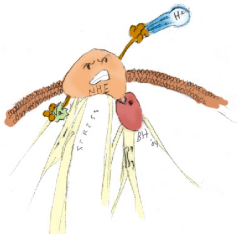
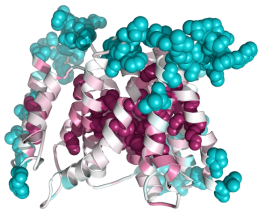


# PROVOST & WALLERT RESEARCH

Investigating the Biochemistry and Cellular Physiology of NHE1

EST. 1998



## Ethanol Precipitation of Plasmid DNA

DNA is polar due to its highly charged phosphate backbone. This polarity, based on the principle of "like dissolves like", makes it soluble in water, which is also highly polar. Ethanol is much less polar than water, its dielectric constant is 24.3 (at 25 °C). This means that adding ethanol to solution disrupts screening of charges by water. If enough ethanol is added electrical attraction between phosphate groups and any positive ions present in solution becomes strong enough to form stable ionic bonds and precipitate DNA. This usually happens when ethanol makes around 64% of the solution. As the mechanism suggests solution has to contain positive ions for precipitation to occur, usually $Na^+$, $NH_4^+$ or $Li^+$ play this role.

Optimal incubation time depends on the length and concentration of DNA. Smaller fragments and lower concentrations will require longer times to achieve the same recovery. For very small lengths and low concentrations over-night incubation is recommended. In such cases use of carriers like tRNA, glycogen or linear polyacrylamide can greatly improve recovery.

During incubation DNA and some salts will precipitate from solution, in the next step this precipitate is collected by centrifugation in a microcentrifuge tube at high speeds (~12,000g). Time and speed of centrifugation has the biggest effect on DNA recovery rates. Smaller fragments and higher dilutions require longer and faster centrifugation. Centrifugation can be done either at room temperature or in 4 °C or 0 °C. During centrifugation precipitated DNA has to move through ethanol solution to the bottom of the tube, lower temperatures increase viscosity of the solution and larger volumes make the distance longer, so both those factors lower efficiency of this process requiring longer centrifugation for the same effect.

After centrifugation the supernatant solution is removed, leaving a *pellet* of crude DNA. Whether the pellet is visible depends on the amount of DNA and on its purity (dirtier pellets are easier to see) or the use of co-precipitants.
70% ethanol is then added to the pellet, and the sample gently mixed to break the pellet loose and wash it. This removes some of the salts present in the leftover supernatant and bound to DNA pellet making the final DNA cleaner. This suspension is centrifuged again to once again pellet DNA and the supernatant solution is removed. This step is repeated once.

Finally, the pellet is air-dried and the DNA is resuspended in water or other desired buffer. It is important not to over-dry the pellet as it may lead to denaturation of DNA and make it harder to resuspend.

Isopropanol can also be used instead of ethanol; the precipitation efficiency of the isopropanol is higher making one volume enough for precipitation. However, isopropanol is less volatile than ethanol and needs more time to air-dry in the final step. The pellet might also adhere less tightly to the tube when using isopropanol.

Taken in part from Wiki and Molecular Cloning: A Laboratory Manual (Third Edition)

**Procedure**:

- Add 1/10 volume of Sodium Acetate (3 M, pH 5.2).
- Add 2.5-3.0 X volume (calculated after addition of sodium acetate) of at least 95% ethanol.
- Incubate on ice for 15 minutes. In case of small DNA fragments or high dilutions overnight incubation gives best results, incubation below 0 °C does not significantly improve efficiency.
- Centrifuge at > 14,000 x g for 30 minutes at room temperature or 4 °C.
- Discard supernatant being careful not to throw out DNA pellet which may or may not be visible.
- Rinse with 70% Ethanol (see note above for details)
- Centrifuge again for 15 minutes.
- Discard supernatant and dissolve pellet in desired buffer. Make sure the buffer comes into contact with the whole surface of the tube since a significant portion of DNA may be deposited on the walls instead of in the pellet.
    - *This last step should be done with sterile TE or water in the hood if you plan to use the plasmid for transfection into animal cells.*

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