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Ethanol precipitation protocol

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DNA precipitation by ethanol can be used to concentrate highly diluted DNA samples and to remove certain impurities that can influence MLPA and digitalMLPA results.

Method

1. Add 1/10 volume of sodium acetate (NaOAc; 3 M, pH 5.2).
2. Add 2.5 volumes (calculated after addition of sodium acetate) of at least 95% ethanol.
3. Incubate at room temperature or on ice for at least 15 minutes. In case of small DNA fragments or high dilutions, overnight incubation gives better results.
4. Centrifuge at > 12,000 × g for 30 minutes at a temperature between 4°C and room temperature.
5. Discard the supernatant carefully, making sure the DNA pellet (which may not be visible) remains in the tube.
6. Rinse with 70% ethanol and centrifuge again (> 12,000 × g) for 15 minutes.
7. Centrifuge the tube to remove all the remaining ethanol.
8. The pellet may be air-dried (leave the tubes open for ~10 min until the pellet borders lose their milky-white colour). It is important not to over-dry the pellet as this may make resuspension harder. SpeedVac should not be used.
9. Dissolve the pellet in $TE_{0.1}$ (10 mM Tris-HCl pH 8.0 + 0.1 mM EDTA). Make sure that the buffer comes into contact with the whole surface of the tube as a significant portion of DNA may be deposited on the walls instead of in the pellet.

Background

DNA precipitation by ethanol requires the correct concentration of positive ions. The presence of too many positive ions results in a lot of salt co-precipitating with DNA, while the presence of too few positive ions results in incomplete DNA recovery. The most effective DNA precipitation may be achieved at room temperature; the optimal incubation time depends on the length and concentration of the DNA. Smaller DNA fragments and lower concentrations require longer incubation times (up to overnight incubation) to achieve a similar recovery. The addition of a carrier (e.g. 10 µg glycogen; Roche; 901393) to the DNA sample can greatly improve recovery without affecting the MLPA or digitalMLPA reaction.

During incubation in ethanol, DNA and some salts precipitate from the solution. This precipitate is collected by centrifugation at high speed (> 12,000 × g) in a microcentrifuge tube. The duration and speed of centrifugation has the biggest impact on DNA recovery rates. Smaller fragments and lower concentrations require longer centrifugation at higher speed. Centrifugation can be done between 4°C and room temperature. During centrifugation, precipitated DNA moves through the ethanol to the bottom of the tube. Lower temperatures increase the viscosity of the solution, and larger volumes increase the distance. In both cases, longer centrifugation will be required to achieve the same effect.

References

- Sambrook J & Russell D. Molecular Cloning: A Laboratory Manual (Third Edition).
- Zeugin JA, Hartley JL (1985). Ethanol Precipitation of DNA. Focus 7 (4): 1–2.
- Crouse J, Amorese D (1987). Ethanol Precipitation: Ammonium Acetate as an Alternative to Sodium Acetate. Focus 9 (2): 3–5.

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