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DNA extraction and precipitation

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ABSTRACT

Briefly, the DNA solution is extracted with phenol / chloroform / isoamyl alcohol in a ratio of 25/24/1 to remove protein contaminants and then precipitated with 100% ethanol or isopropanol. It is then washed with 70% ethanol to remove remaining organic molecules and the pellet is resuspended in a volume of interest.

PROTOCOL CITATION

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KEYWORDS

DNA, EXTRACTION, PRECIPITATION

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- 1 Add 1 volume of 25/24/1 phenol / chloroform / isoamyl alcohol.
- 2 Mix by vortexing for  **00:00:10**  **13000 rpm, 00:00:15** or more at  **Room temperature** . 25s
- 3 Take the aqueous phase (the upper one) using a  **200 µl** pipette; transfer to new tube.
- 4 Add 1/10 of 3M sodium acetate,  **pH5.2** . And mix by vortexing or tapping. An alternative way is to extract using chloroform / isoamyl alcohol, this serves to prevent phenol residues, which is not important if you wash well with 70% ethanol.
- 5 Add 2 or 2.5 vol of  **0 °C** 100% Ethanol. Mix well and leave at  **-20 °C** freezer. (minimum  **00:30:00** , one hour 30m
preferable)
- 6  **13000 rpm, 00:05:00** and discard the supernatant. 5m
- 7 Add 70% ethanol (RNase Free)  **1 mL** at  **Room temperature** , invert several times and microcentrifuge as in step 6.
- 8 Discard the supernatant and dry for  **00:05:00** at  **37 °C** (repeat) 5m
- 9 Resuspend the pellet in water.