

Phenol-Chloroform Extraction of DNA

1. Add 0.5 ml phenol:chloroform:isoamylalcohol pH 8.0 (25:24:1) to DNA extracts in Phase-lok gel tubes (Eppendorf 2.0 ml Heavy). Shake tubes gently by hand.
2. Tubes are centrifuged at 12000 x g for 5 min.
3. The aqueous layer is removed and combined with an equal volume of chloroform:isoamylalcohol (24:1) in another phase-lok gel tube.
4. Tubes are mixed well and centrifuged at 12000 x g for 5 min at room temperature. The aqueous layer is removed into a sterile 1.5 ml microcentrifuge tube.

You can try this extract in PCR (should be clean enough now) or alternatively put this extract through a spin column clean up like a PCR product clean up kit. You can also follow with an ethanol precipitation to further concentrate your DNA.

Ethanol Precipitation of DNA

1. Add 0.25ul glycogen (20mg/ml – Roche), 1/10 volume 3M sodium acetate (pH 5.2) and mixing gently. 2.5 volumes ice cold ethanol (95%) are then added and tubes well-mixed.
2. Incubate at -20°C for at least 30 minutes to overnight.
3. Centrifuge at 16,000 x g for 15 minutes at 4C. Position the tube so the hinge is facing outward, as the resulting pellet might not be visible.
4. Being careful not to dislodge the pellet, remove the supernatant.
5. Carefully rinse the pellet with 200 ul of ice-cold 70% ethanol while centrifuging at 16,000 x g for 2 minutes at 4C between each wash.
6. Dry the pellet for ~5 minutes in a vacuum desiccator.
7. Resuspend the pellet in 10ul water or 10 mM Tris buffer.

Isopropanol Precipitation of DNA

1. Add 0.25ul glycogen (20mg/ml – Roche), 1/10 volume 3M sodium acetate (pH 5.2) and mixing gently. 1 volume isopropanol is then added and tubes well-mixed.
2. Incubate at -20°C for 30 minutes
3. Centrifuge at 16,000 x g for 15 minutes at 4C.
4. Being careful not to dislodge the pellet, remove the supernatant.
5. Carefully rinse the pellet twice with 200 ul of ice-cold 70% ethanol while centrifuging at 16,000 x g for 2 minutes at 4C between each wash.
6. Dry the pellet for ~5 minutes in a vacuum desiccator.
7. Resuspend the pellet in 10ul water or 10 mM Tris buffer.

Notes

Considerations on using ethanol versus isopropanol, in order of importance:

Volume: Ethanol requires room for 2.5 volumes of alcohol, while isopropanol requires room for only 1 volume

Salts: Many salts are less soluble in isopropanol than in ethanol, so a second 70% alcohol rinse of the pellet is recommended to more efficiently desalt the DNA pellet. NaCl is not as soluble as NH_4OAc , NaOAc or LiCl in ethanol/water or isopropanol/water; it can be replaced by the latter salts when feasible.

Volatile: Isopropanol is less volatile than ethanol, so pellet requires more time to air-dry at the end.

Time: Isopropanol ppt is faster, requiring less time to precipitate the DNA in the 2nd step than ethanol.

Precipitations are commonly performed at -70°C , -20°C , 0°C , or room temp for periods of 5 min to overnight. For low DNA concentrations, higher final concentrations of alcohol, longer precipitations (1 hr to overnight), lower temperatures (-20°C to -70°C) and longer centrifugation times (up to 30 min) may improve recovery.

At the lower temperatures, the viscosity of the alcohol is greatly increased and centrifugation for longer times may be required to effectively pellet the precipitated DNA. The efficiency of precipitation for small concentrations or amounts of DNA may be increased by incubation at -70°C , but these reactions should be brought to 0°C before centrifugation.