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Chloroform-free DNA Extraction - Ammonium Acetate Precipitation Method V.1

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Protocol status: Working
We have been regularly using this method for decades.

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Abstract

A chloroform-free, cost-effective DNA extraction method for a variety of sample types.

Materials

Digsol: To make 500ml

- 20ml 0.5M EDTA (pH 8.0)
- 3.425g NaCl
- 25ml 1M Tris-HCl (pH 8.0)
- 430ml ddH2O

Autoclave then add 25ml of 20% SDS.

Low TE ($Tris_{10mM}$,$EDTA_{0.1mM}$): To make 500ml

- 5ml 1M Tris-HCl (pH 8.0)
- 100µl 0.5M EDTA (pH8.0)
- 495ml ddH2O

Autoclave.

Protocol materials

Proteinase K, 2mL **Qiagen Catalog #**19131

1M DTT **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**43816

1M DTT **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**43816

Ammonium Acetate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**A1542-500G

Troubleshooting

Safety warnings

Use GLP and wear protective equipment. Avoid contact with skin.
All chemicals can be disposed of down the sink with copious amounts of water to dilute the ethanol down to >20% of the waste.

If inhaled: If unconscious, place in recovery position and seek medical advice. Keep the respiratory tract clear. If symptoms persist, call a physician.
In case of skin contact: Wash off immediately with soap and plenty of water while removing all contaminated clothes and shoes. If symptoms persist, call a physician.
In case of eye contact: Remove contact lenses. Protect unharmed eye. Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.
If swallowed: If accidentally swallowed, obtain immediate medical attention. Rinse mouth with water. Never give anything by mouth to an unconscious person.

1 Add 250 µL Digsol buffer (see Materials for recipe) and 10 µL Proteinase K, 2mL **Qiagen Catalog #**19131 to a lablelled 1.5ml tube. 1m

2 **Tissue:** Cut into small pieces (<1cm^2) with a sterile razor blade on a sterile glass plate before adding to the 1.5mL tube. 5m
Blood: Centrifuge blood sample at 13,000rpm for about 1 min (to pellet sample). Remove sample from ethanol with toothpick and blot onto tissue. When almost dry, transfer the toothpick into the 1.5mL tube and jiggle to dislodge the blood. Remove toothpick and place in disinfectant.
Swab: Dry of ethanol and place into the 1.5mL tube. Snap off the end of the swap so the lid may close.
Feather: Cut the calamus of 1-3 feathers into small pieces with a sterile razor blade on a sterile glass plate before adding to the tube. If feathers are very small and blood spots are present on the tips, feathers can be added whole.
Hair: Place hair(s) into the 1.5mL tube preferably with the largest root and add 5 µL 1M DTT **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**43816
Insect: Degut and add to 1.5mL tube. If the insect is very small, starve for 48hrs before freezing. Add 5 µL 1M DTT **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**43816. Crush with a pestle or add a metal lysing bead and place on TissueLyser (Qiagen) for 2min, 30/s.

3 Vortex and place samples in a rotating oven at 55 °C for 03:00:00 or Overnight for maximum digestion. 3h

4 Add 300 µL 4M Ammonium Acetate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**A1542-500G . 1m

5 Vortex several times over a period of at least 00:15:00 at Room temperature. 15m

6 13000 rpm, 00:10:00 10m

7 Aspirate the supernatant (clear liquid containing the DNA) into a new labelled 1.5ml tube. Discard tube containing the pelleted protein debris. 1m

8 Add 1 mL 100% ethanol and invert the tube gently several times to precipitate DNA. 1m

9 13000 rpm, 00:10:00 10m

10 Pour off ethanol in a smooth motion, taking care not to lose the DNA pellet. 1m

11 Add 500 µL 70% ethanol and invert gently several times to clean the pellet. 1m

12 15000 rpm, 00:05:00 5m

13 Pour off ethanol in a smooth movement or using a pipette gently draw off the supernatant if fear of losing the pellet. Stand tubes upside-down on clean tissue until dry (approx. **30-60 minutes**). This can be sped up by using the heat of a lamp from above. 30m

14 Once fully dry add approx. 100 µL Low TE (see Materials for recipe). Add less if a very tiny pellet or no pellet is observed. Flick sample to dislodge pellet. 1m

15 Place tubes in a thermomixer or shaking oven at 50 °C for 00:30:00 to dissolve the pellet. If the pellet has not completely dissolved add more Low TE. 30m

16 Store at -20 °C (long term) or 4 °C (short term).

Protocol references

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