

DNA Extraction

DNA extraction solutions:

- 2% CTAB
- Chloroform
- Isopropanol (-20 C)
- 70% cold ethanol
- TE-buffer

Recipe for CTAB - for 40 samples

2% CTAB: Lysis Buffer

	25ml
CTAB	0.5 g
sterile nanopure water	14.45 ml
1M TRIS - HCl	2.5 ml
5M NaCl	7 ml
0.5M EDTA	1 ml
b-mercaptoethanol	50 ul (add just before using CTAB, under hood)

CTAB:

Hexadecyltrimethylammonium bromide.
CTAB, NaCl, EDTA found in glass cabinet
HCl, water in refrigerator
b-mercaptoethanol under hood in cabinet

1. Add CTAB to water.
2. Dissolve CTAB by swirling under warm water.
3. Add Tris-HCl, NaCl, and EDTA.
4. Swirl to mix well.
5. Add b-mercaptoethanol just before ready to add CTAB to samples.

Methods

**Turn on heating block.

1. Before mixing CTAB take 40 samples from the -80oC freezer. Put in refrigerator in lab until ready to use.

2. Add 50 ul CTAB to each sample.
3. Grind with micropestle. Leave pestle in tube after grinding.
4. Add remaining CTAB (550 ul) while rinsing off micropestle.
5. Put eppendorf onto heating block at 65oC for 40min to 1 hr.

**Number new tubes 1-40 for step #7,

***Number tubes with fine sharpy on tube top and pencil on tube side with sample numbers for step #10 transfer.

6. Centrifuge tubes at max speed (13000 rpm) for 7 min at room temp.
7. Transfer upper phase into new sterile 1.5ml tube. Be sure NOT to pipette up any of the pellet or floating debris.
8. Add 1 volume chloroform (600 ul for 600ul CTAB) to each tube. After finished invert entire tray to mix thoroughly. Solution will turn cloudy.
9. Centrifuge at 13000 rpm for 15 min.
10. Transfer upper phase into a new sterile fully labeled 1.5ml eppendorf tube. Be careful not to pipette up any chloroform.
11. Precipitate DNA with 750 ul isopropanol (found in freezer). Mix well by inverting rack. Put in freezer for 30 min (can leave samples in freezer overnight)
12. Centrifuge samples at 13000 rpm for 30 min (room temp).
13. Discard upper phase (by pouring off into a beaker).
14. Add 200 ul of 70% ice-cold ethanol (found in freezer) to wash DNA.
15. Centrifuge the samples at 7000 rpm for 5 min (room temp).
16. Discard (pour off into waste beaker) upper phase into beaker and let the DNA-pellet dry (inverted on Kimwipe under hood).
17. Resuspend the pellet by adding 50 ul TE-buffer (found in refrigerator).

-If doing PCR immediately after leave in hood at room temp for an hour for DNA to dissolve into TE-buffer.

-If not place in refrigerator overnight.

18. Mark the tray of tubes with a sticker indicating DNA extracts, sample run, and date.
Place samples in refrigerator.