CTAB-DNA POLLEN EXTRACTIONS

Adapted from Lalhmangiahi et. al & Guertler et al. by Benkendorf, Fant, & Noble.

SAMPLE PREPARATION AND GRINDING

a1) Add 380 µl extraction buffer (100 mM Tris-HCl, 50 mM EDTA, 50 mM NaCl, 10% SDS, pH 7.5). This solution will need to be warm enough for the SDS to be in solution, requires heat and stirring.

a2) Vortex samples at speed > 2000, until pellet breaks apart, ca. 20-30 seconds.

a3) Use the Pellet Pestle Motor (Kontes) for ca. 15 seconds to macerate samples.

a4) Add 100 µL extraction buffer to wash the tip of the pestle into the centrifuge tube, and burst bubbles.

a5) Allow to sit at 35°C for 1 hour, use vortex occasionally if sedimentation of pollen occurs.

EXTRACTION AND ISOLATION OF DNA

b1) Warm CTAB buffer to remove any precipitants if present.

b2) Add 480 µL 10% CTAB buffer.

b3) Add 10 µL RNAse (10mg/mL); invert by hand, incubate for 40 minutes at 37°C, increase heat to 60°C wait 20 minutes before continuing to b4.

b4) Add 15 µL proteinase K (20mg/mL) & 12.5 uL DTT (1 molar in H2O); invert by hand, incubate for 1 hr. at 60°C.

b5) Incubate overnight at 40°*C (note: this is a hard stopping point)*

b6) Add 500 µL of Phenol-chloroform-isoamyl alcohol vortex samples, centrifuge at 10,000 rpm (10 min.)

b7) Transfer the uppermost aqueous layer to a new 2 mL centrifuge tube.

DNA PRECIPITATION

c1) Add slightly chilled Isopropyl alcohol & Sodium Acetate 3mM 5:1, equivalent to ca. 2/3 of the removed layer. Store at -20C, 1 hour to allow precipitation.

(Note: potential stopping point for a day or more, samples can stay at -20°C for days)

c2) Centrifuge at 13,000 rpm for 10 minutes.

c3) Pour supernatant into new 2mL centrifuge tube, add 400 uL 70% EtOH. Store at -20°C for 20 minutes.

c4) Spin at 13,000 rpm for 10 minutes, discard supernatant.

for both tubes the following steps apply

c3) Add 400 µL of 75% EtOH, invert tube x3, centrifuge at 13,000 rpm for 4 minutes; discard supernatant

c4) Add 400 µL of 95% EtOH, invert tube x3, centrifuge at 13,000 rpm for 4 minutes, discard supernatant

c5) Dry tubes in vacuum centrifuge for 30 minutes on medium heat at 15 mmHG.

RESUSPENSION OF DNA

d1) Add 40 µL of dna free H_2O to sample.

d2) place on heat block at 37°C until pellet resuspends with occasional use of vortexes.

NOTES: a 10% CTAB preparation will not readily stay in solution, maintain it on heatblock until you are ready to use it. After adding it to extraction tubes move them to heat block immediately (*i.e.* in batches of 5-10).

Solutions

Extraction buffer (100 mM Tris-HCl, 50 mM EDTA, 50 mM NaCl, 10% SDS - pH 7.5, ca. 32 mL H2O)

For 100 samples (50 mL solution)

10 grams SDS (Sodium Dodecyl Suflate , d = 1.01 g/cm3)
146.1 mg Sodium Chloride (NaCl, mw = 58.4 g/mol)
930.6 mg EDTA (EthyleneDiamineTraacetic Acid Disodium Salt dihydrate, mw = 372.24 g/mol)
Add 20 mL deiH2O
5 mL Tris-HCl pH 8.0 (1 molar- kept in fridge)
Fill to 50 mL with deiH2O
Auto clave on 'Liquid' setting for 15 minutes.
Dissolution may require heat and stirring (3 & 4 settings respectively, ca. 15 min.)

10% CTAB solution (20 mM Tris-Cl pH. 8.0, 1.4 M NaCl, 10 mM EDTA pH 7.5, 10% CTAB, 5% PVP, 40 mL DiH2O)

For 100 samples (50 mL solution)

add ~30 mL diH2O,
1 ml Tris-HCl pH 8.0 (1 molar- kept in fridge; 2-Amino-2-(hydroxymethyl)propane-1,3-diol)
4.08 g Sodium Chloride (NaCl, mw = 58.4 g/mol)
4 mL EDTA pH 7.5 (0.125 molar – kept in fridge; 2,2',2",2"'-(Ethane-1,2-diyldinitrilo)tetraacetic acid)
5 g CTAB (hexadecyl(trimethyl)ammonium bromide, mw = 364.45, FYI this is 274 mM)
Auto clave on 'Liquid' setting for 15 minutes.

2.5 g PVP-40 (1-ethenylpyrrolidin-2-one) – add after autoclave
Fill to 50 mL with deiH2O
Dissolution of PVP will require 2-3 hrs, at 65°c with stirring. Before use allow one hour of stirring and heat to resuspend all salts in the solution.

Sodium acetate solution (3mM)

For 100 samples (10 mL solution)

20.4 mg Sodium Acetate trihydrate (mw = 136.08 g/M)
to 50 mL deiH2O
Auto clave on 'Liquid' setting for 15 minutes.

Phenol-chloroform Isoamyl alcohol (25:24:1) Saturated with 10 mM Tris pH 8.0, EDTA

For 100 samples (50 mL solution) (no need to make, is bought)

25 mL Phenol
24 mL Chloroform (Trichloromethane)
1 mL Isoamyl alcohol

Literature cited

Lalhmangaibi, R., Ghatak, S., Laba, R., Gurusubramian, G., Jumar, N.S. *Protocol for Optimal Quality and Quantity Pollen DNA Isolation from Honey Samples.* 2014. Journal of Biomolecular Techniques 25:92-95

Guertler, P., Eicheldinger, A., Muschler, P. Goerlich, O., Bursch, U. *Automated DNA extraction from pollen in honey* 2014. Food Chemistry 149:302-306