Modified CTAB mini procedure for DNA isolation from (difficult) plant tissues

(1) Disrupt plant tissue to a fine powder/slurry using a suitable method, e.g. one of the following:

(a) Grinding mill: Place ca. 50–75(–100) mg silicagel-dried or herbarium plant tissue in a 2 ml safe-lock microcentrifuge tube, together with 5 glass beads, and grind to a fine powder.

Note: Chilling the tube and tube-holding block of the adaptor set in liquid nitrogen before grinding improves disruption and avoids heating-up of material.

(b) Mortar and pestle, liquid nitrogen: Grind ca. 0.5–1 g fresh or frozen plant tissue under liquid nitrogen to a fine powder and transfer frozen powder into a 2 ml safe-lock microcentrifuge tube. Proceed immediately to step (2) without allowing the sample to thaw.

(c) Mortar and pestle, sorbitol extraction buffer: Grind ca. 0.5–1 g fresh or frozen plant tissue with 1 ml of cold (4°C) sorbitol extraction buffer on ice to a fine suspension and transfer the slurry into a 2 ml safe-lock microcentrifuge tube. Proceed immediately to step (2).

(d) Disperser, sorbitol extraction buffer: Place ca. 0.5–1 g fresh or frozen plant tissue in a 2 ml safe-lock microcentrifuge tube, together with 1 ml of cold (4°C) sorbitol extraction buffer and disperse material on ice to a fine suspension. Proceed immediately to step (2).

Note: Smaller amounts of plant tissue (30–50 mg dried or 0.3–0.5 g fresh or frozen material) are recommended if no sorbitol steps are done and the protocol is continued immediately at step (4).

Note: Alternatively the modified CTAB maxi procedure protocol for greater volumes can be used.

(2) Add cold (4°C) sorbitol extraction buffer up to a maximum of 1,5 ml and mix by brief vortexing and inverting the microcentrifuge tube until the plant material is completely suspended. Incubation for 20 min on ice and centrifuge for 10 min at 10,000 × *g* (rcf) and 4°C. Discard the supernatant carefully without losing material of the loose pellet and keep on ice.

Note: DNA does not dissolve in sorbitol extraction buffer.

(3) Repeat extraction step (2) at least twice. Depending on the amount of compounds within the plant tissue repeat extraction step up to 10 times.

Note: The grinding mill/disperser can be used for some seconds to re-suspend the plant material.

Note: These steps remove most of secondary compounds soluble in sorbitol extraction buffer (e.g., polysaccharides, polyphenols). In cases strongly coloured or mucilaginous supernatant extraction should be repeated until supernatant is only slightly coloured and not viscous anymore.

(4) Add ~~700~~ 800 (500) µl of preheated (70°C) CTAB extraction buffer and 30 µl Sarkosyl, and suspend well by brief vortexing and inverting the microcentrifuge tube. Incubate for 30-60 min at 60–65°C on a thermoblock or in a water bath. Occasionally mix by inverting the microcentrifuge tube.

Note: Do not exceed a total volume of 1.3 (1.5) ml to be able to perform step (5) within the same tube.

Note: Adding 2.5 µl Proteinase K (not affected by Sarkosyl) can improve quality and quantity of DNA.

Note: Adding 5 µl RNase A stock solution 2 (100 mg/µl) and incubating for 30 min at 60°C before (!) adding Proteinase K and Sarkosyl can improve quantity of DNA if a silica membrane based DNA extraction kit is used further after step (6).

(5) Extract with ~~700~~ 600 (500) µl of CIA. Mix well by inverting the microcentrifuge tube and keep at room temperature ~~for 20 min by occasional inverting~~. Centrifuge for 10 min at 10,000 × *g* (rcf) and room temperature and transfer clear, aqueous upper phase (up to 800 µl) to a new 1.5 ml microcentrifuge tube.

Note: Use a 2 ml microcentrifuge tube if a silica membrane based DNA extraction kit is used further after step (6).

Note: This step removes secondary compounds soluble in chloroform and precipitates proteins and cell debris. Do not touch and transfer any chloroform or interlayer material. Alternatively repeat step (5) once again.

Note: Do not exceed a total volume of 800 µl for the aqueous upper phase to be able to perform step (7) within the same tube.

30 µl acetato amônio

(6) Add 1/10 volume (up to 80 µl) of sodium acetate stock solution and vortex briefly.

Note: For very clean extracts proceed e.g. with steps (11) or (13) of the QIAgen DNeasy Plant Mini Kit protocol (or any adequate step of other silica membrane based DNA extraction kits) until end and continue with step (11) of this protocol.

500

(7) Add 2/3 volume (up to 600 µl) of ~~ice-cold (-20°C)~~ isopropanol and vortex briefly. Centrifuge for 20 min at top speed and 4°C and decant the solution.

Note: Incubation on ice (4°C) or at -20°C for 30 min up to several hours (overnight) before centrifugation can increase DNA yield of lower-quality material. incubar overnight -20°C

centrifugar 15000 rpm - 30 min. -4°C

(8) Wash the pellet twice with 0.5 ml 75% ethanol by brief vortexing, centrifuging for 10 min at top speed and room temperature and removing the ethanol carefully without losing the pellet. 15000 rpm

Note: Step (8) can be repeated several times.

(9) Wash the pellet once with 0.5 ml 96% ethanol by brief vortexing and centrifuging for 10 min at top speed and room temperature. Decant the ethanol carefully and dry the pellet.

Note: This step helps to quickly dry the pellet. Drying can be done at room temperature, on a thermoblock, or in a vacuum centrifuge at a suitable temperature for sufficient time. Do not over-dry the pellet.

15000 rpm

(10) Dissolve the pellet in 50 µl of TE buffer.

Note: Make sure that the pellet is completely dissolved. If not, incubate at 37°C for 30 min and/or incubate at 70°C for 10 min on a thermoblock or in a water bath. Let the tube cool down to 37°C before continuing with step (11).

(11) Add 5 µl of RNase A stock solution 1 (10 mg/µl) and incubate at 37°C for 30-60 min.

(12) Check 5 µl DNA extract mixed with 5 µl loading dye for quality and quantity on 1% agarose gels and store samples at -20°C (short-term) or -80°C (long-term).

Materials and equipment needed

- Grinding mill (for dry material) [e.g. Retsch MM-200, MM-301; QIAgen TisssueLyser]
- Mortar and pestle (for fresh or frozen material)
- Disperser [e.g., IKA ULTRA-TURRAX] (for fresh or frozen material)
- Vortexer
- 2 ml safe-lock microcentrifuge tubes [e.g., from Sarstett]
- 1.5 ml microcentrifuge tubes
- Glass beads (Ø 3mm)
- Cooling microcentrifuge for 1.5 ml / 2 ml microcentifuge tubes, top speed 20,000 × *g*
- Ice bath
- Water bath or thermoblock
- Equipment and materials for agarose gel electrophoresis and detection
- Pipettes and filter tips (P20, P100, P1000)
- [QIAgen DNeasy Plant Mini Kit]

Reagents and stock solutions

- Sorbitol buffer (pH 8): (Autoclaved and stored at 4°C)
  - 100 mM Tris-HCl (pH 8) (12.114 g/L)
  - 5 mM EDTA (1.461 g/L)
  - 0.35 M sorbitol (63.77 g/L)
- High-salt 3× CTAB buffer (pH 8): (Autoclaved and stored at room temperature)
  - 100 mM Tris-HCl (pH 8) (12.114 g/L)
  - 20 mM EDTA (5.844 g/L)
  - 3 M NaCl (175.32 g/L)
  - 3% CTAB (30 g/L)

Note: Dissolve CTAB by heating to 70°C. At room temperature High-salt 3x CTAB is viscous. Before use heating to 70°C is very helpful.

- TE buffer 1x (pH 8):
  - 10 mM Tris-HCl (1.2115g/L)
  - 1 mM EDTA (0.2922g/L).
- 2-mercaptoethanol
- PVP-10 (PVP-40, PVP-30)
- Sarkosyl (30% aqueous solution)
- CIA, chloroform : isoamyl alcohol (24:1 v/v)
- Sodium acetate stock solution (3M, adjusted to pH 5.2)
- Isopropanol (100%)
- Ethanol 75%
- Ethanol 96%
- Agarose, gel stain [e.g., ethidium bromide, GelRed, GelGreen]
- Loading dye (e.g. Orange-G)
- Ribonuclease A stock solution 1 (RNase A, 10 mg/ml)
- [Ribonuclease A stock solution 2 (RNase A, 100 mg/ml)]
- [Proteinase K stock solution (20 mg/ml)]
- [Liquid nitrogen]

Buffers prepared just before use [or kept for short time]

- Sorbitol extraction buffer (can be stored at 4°C for short time):
  Sorbitol buffer **plus 1% 2-mercaptoethanol and 1% PVP added just before use.**
- CTAB extraction buffer (can be stored at room temperature for short time):
  High-salt 3× CTAB buffer **plus 0.2% 2-mercaptoethanol and 1% PVP added just before use.**

Note: Dissolve PVP by heating the buffer to 70°C.

Reference

EL-ZUR, N., S. ABBO, D. MYSLABODSKI, AND Y. MIZRAHI. 1999. Modified CTAB procedure for DNA isolation from epiphytic cacti of genera *Hylocereus* and *Selenicereus* (Cactaceae). *Plant Molecular Biology Reporter* 17: 249-254.