Acid nucleic extraction from rice dried leaves

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ABSTRACT

Acid nucleic extraction of rice dried leaves by a CTAB method adapted from Li et al 2008.


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MATERIALS

- **TE buffer** Contributed by users
- **Isopropanol** Contributed by users
- **NaCl** Sigma-Aldrich Catalog #S-3014
- **Hexadecyltrimethylammonium bromide (CTAB)** Sigma-Aldrich Catalog #H9151
- **1 M Tris/HCl Stock Solution** (dissolved Tris base adjusted to pH 8.0 with HCl) Contributed by users
- **Chloroform: Isoamyl Alcohol (24:1)** Contributed by users
- **Polyvinylpyrrolidone** Sigma-Aldrich Catalog #PVP40
- **ethanol** Contributed by users
- **sodium bisulfite** Sigma Aldrich
- **Na2EDTA** Contributed by users

Waterbath at 65°C
Qiagen TissueLyser II
Microcentrifuge at 4°C

SAFETY WARNINGS

working with chloroform: isoamyl alcohol under a fume hood

BEFORE STARTING

- Put 20-50 mg of dried rice leaves sample into a safe lock tube 2.0 ml containing two stainless steel beads, 5mm.
- Prepare CTAB Extraction Buffer (warm up the buffer for the CTAB dissolution):

  For 100 ml
  - 2 g CTAB (2% w/v)
  - 2 g PVP-40
  - 10 ml 1M Tris-HCl, pH8.0
  - 8.18 g NaCl
  - 744.48 mg EDTA
  - 0.5 g sodium bisulfite (add just before to use)
  - qs 100 ml H2O

  1 Grind the dried leaves with the Qiagen TissueLyser II until obtaining a fine powder

  2 Add 1 ml CTAB extraction buffer (see ‘before start’ for the buffer content) and homogenize by vortexing.

  3 Incubate at 65°C for 30 min. Periodically, mix gently the tubes during the incubation.  

     65 °C 00:30:00

     #10000 x g, 4°C, 00:10:00

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4  Transfer the supernatant (650µl) to a 1.5 ml microcentrifuge tube.

    Add equal volume of chloroform/isoamyl alcohol (24:1)

5  After shaking, 15000 x g, 4°C, 00:10:00

6  Transfer the supernatant (500µl) in a new 1.5 ml tube containing 350 µl of isopropanol (pre-chilled at -20°C).

    Mix gently

    -20 °C  00:30:00

    15000 x g, 4°C, 00:10:00

7  Discard the supernatant. Wash the pellet with 70% ethanol.

    15000 x g, 00:05:00

8  Remove the ethanol and air-dried the pellet.

9  Dissolve the pellet in 50 µl sterile water or TE buffer and conserve the DNA at -20°C.