Solutions:

- 1,5% CTAB: Tris (75 mM), Cetyltrimethylammonium bromide (15 g/l) , EDTA (15 mM, pH8.0), NaCl (1.05 M), pH 8.0

- Chloroform:isoamylalcohol (24:1)

- **10xTE**
  100 mM Tris-Cl (desired pH)
  10 mM EDTA (pH 8.0)
  Sterilize solutions by autoclaving for 20 minutes at 15 psi
  (1.05 kg/cm 2) on liquid cycle. Store the buffer at room temperature.

  - **Tris-Cl**
    Dissolve 121.1 g of Tris base in 800 ml of H_2O. Adjust the pH to the desired value by adding concentrated HCl.
    pH HCl
    7.4 70 ml
    7.6 60 ml
    8.0 42 ml
    (1 M) Allow the solution to cool to room temperature before making final adjustments to the pH. Adjust the volume of the solution to 1 liter with H_2O. Dispense into aliquots and sterilize by autoclaving.
    If the 1 M solution has a yellow color, discard it and obtain Tris of better quality. The pH of Tris solutions is temperature-dependent and decreases approx. 0.03 pH units for each 1°C increase in temperature. For example, a 0.05 M solution has pH values of 9.5, 8.9, and 8.6 at 5°C, 25°C, and 37°C, respectively.

  - **EDTA** (ethylenediamenetetraacetic acid)
    To prepare EDTA at 0.5 M (pH 8.0): Add 186.1 g of disodium EDTA•$2H_2O$ to 800 mL of H_2O. Stir vigorously on a magnetic stirrer. Adjust the pH to 8.0 with NaOH (~20 g of NaOH pellets). Dispense into aliquots and sterilize by autoclaving. The disodium salt of EDTA will not go into solution until the pH of the solution is adjusted to ~8.0 by the addition of NaOH.

Procedure:

1. Cut part of a leaf (~2 cm) into small pieces, freeze in liquid nitrogen and grind leaf tissue to powder in a 2 ml safe-lock reaction tube containing a metal bead with a TissueLyser (Qiagen, Hilden, Germany)
2. Add 900 µl boiled 1.5% CTAB buffer and incubate for 1h at 65°C.
3. Add 630 µl of Chloroform:isoamylalcohol solution (24:1), and shake horizontally for 15 min at 75 rpm at room temperature.
4. Centrifuge at 15,000 rpm in a table centrifuge for 10 minutes.
5. Transfer the supernatant containing the nucleic acids into a new 2 ml reaction tube and precipitate DNA with 2/3 v/v ice-cold isopropanol (e.g. supernatant 700 µl, isopropanol 450 µl).
6. Shake gently and centrifuge at 15,000 for additional 10 min. Remove supernatant (be careful to keep the pellet).
7. Wash the precipitated nucleic acids with 1 ml 70% ethanol, centrifuge and remove supernatant (be careful to keep the pellet).
8. Dry in a vacuum centrifuge for 15 min.
9. Dissolve DNA in 50 µl 1/10 TE buffer containing 5 µg RNase A for RNA digestion. Keep the sample 2 hours at room temperature before freezing at -20°C.