

DNA extraction from plants

Remco Stam

Abstract

Widely used protocol to extract DNA from plant leaves.
Many versions circulate on the web, this is the version as we use it.
It works well on maize, tomato and probably many other plants.

Citation: Remco Stam DNA extraction from plants. **protocols.io**

dx.doi.org/10.17504/protocols.io.eusbewe

Published: 11 Apr 2016

Before start

Prepare all reagents and materials.

TE Buffer, pH 8.0

For 250 ml:

2.5 ml 1 M Tris HCl, (10 mM)

0.5 ml 0.5 M EDTA, (1 mM)

Add H₂O to 250 ml

CTAB Extraction Buffer

For 500 ml:

10 g CTAB (1.5% w/v) (Cetyltrimethylammonium bromide or Certridium Bromide: C₁₉H₄₂BrN)

50 ml 1 M Tris HCl, pH 8.0 (75 mM)

20 ml 0.05 M EDTA, pH 8.0 (100 mM)

140 ml NaCl (5M)

290 ml H₂O

Materials

- ✓ RNase A by Contributed by users
- ✓ Isopropanol by Contributed by users
- ✓ Chloroform by Contributed by users
- ✓ Liquid nitrogen by Contributed by users
- ✓ Ethanol by Contributed by users
- ✓ Isoamylalcohol by Contributed by users
- ✓ Beta-mercaptoethanol by Contributed by users
- ✓ Cetrimonium bromide by Contributed by users

- ✓ Tris by Contributed by users
- ✓ EDTA by Contributed by users

Protocol

Step 1.

Grind frozen plant material (100mg) in liquid Nitrogen

Step 2.

In a 2 ml tube, add 800 uL of 1,5x CTAB and 1 ul of Beta-mercaptoethanol to the ground leaf material

Incubate 1 hour at 60-65 degrees (C)

Step 3.

Cool at Room Temp

Add 1 volume of Chloroform/Isoamylalcohol (24:1) mixture

Mix on overhead shaker for 10 minutes

Centrifuge at 3000 rmp for 25 minutes

Step 4.

Transfer supernatant to a new tube

Use wide pipette tips (cut point of tips if needed)

Step 5.

Add 10 uL RNase (10 mg/ml)

incubate at 37 degrees (C) for 30 minutes

Step 6.

Add one volume 100% Isoopropanol (pre-chilled at -20)

Allow sample to preprecipitate for up to 20 minutes at -20

Step 7.

Centrifuge: full speed, 10 minutes

Remove supernatant

Step 8.

Add 1 ml 70% Ethanol

Centrifuge: full speed, 10 minutes

Remove Supernatant

Repeat once

Step 9.

Dry pellet in air or approx 20 minutes in vacuum centrifuge

Step 10.

Dissolve pallet in 1x TE (10 mM Tris, pH8, 1 mM EDTA)

Warnings

When working with Beta-Mercaptoethanol or Chloroform, always work in a fume hood.