DNA Extraction for plant samples by CTAB

Ting Yang,Chenyu Wu

Abstract

DNA extraction of −80◦C stored leaves by CTAB.

Citation: Ting Yang,Chenyu Wu DNA Extraction for plant samples by CTAB. **protocols.io** dx.doi.org/10.17504/protocols.io.pzqdp5w
Published: 17 May 2018

Materials

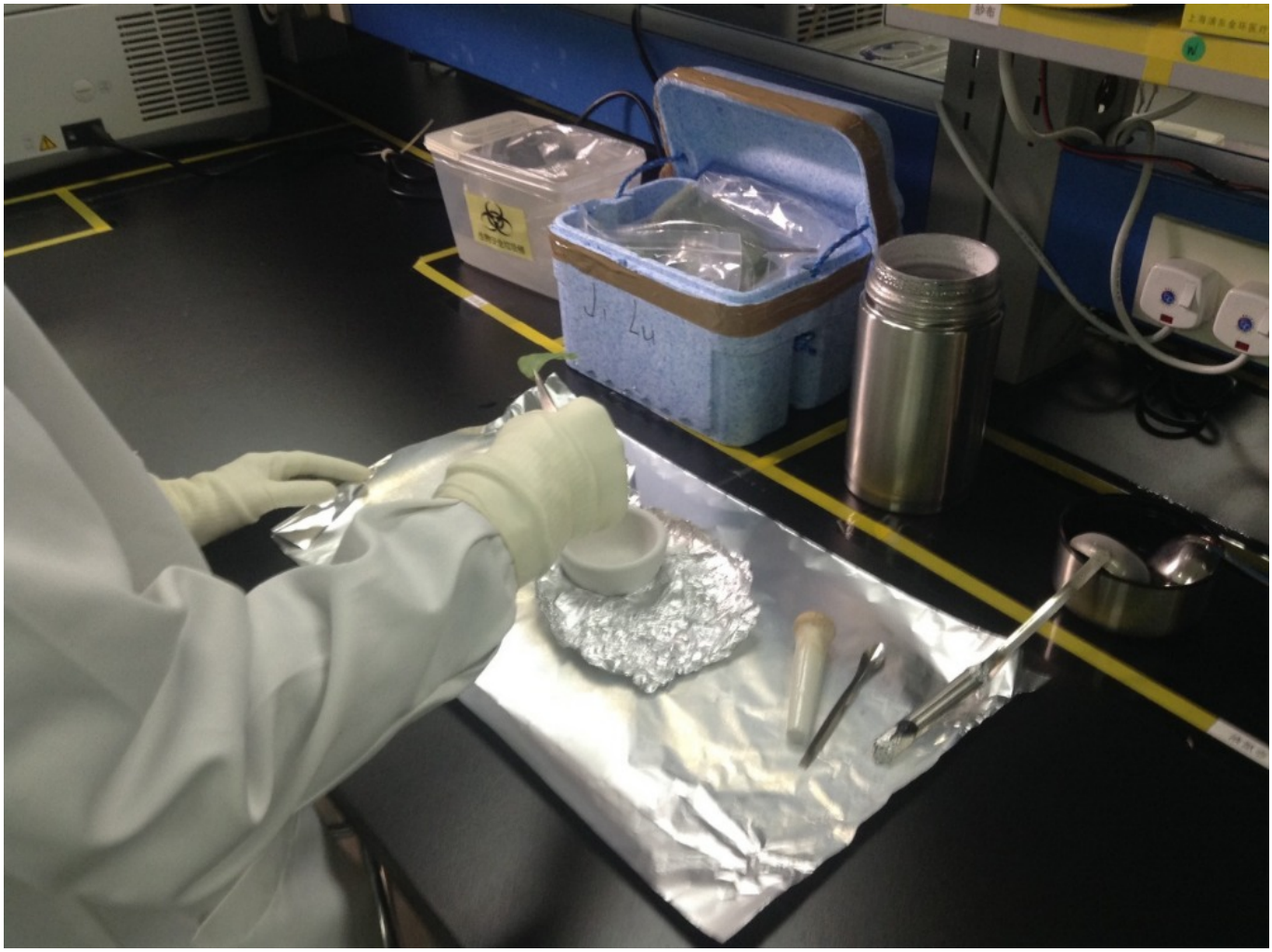
PVP by Sigma
20 mM EDTA by Sigma
100 mM Tris-HCl by Sigma
1.5 M NaCl by Sigma
2% CTAB by Sigma
1% β-mercaptoethanol by BBI Biotech
Chloroform: Isoamyl alcohol by Sangon Biotech
isopropanol by Sangon Biotech
sodium acetate by Qiagen
ethanol by BBI Biotech
TE buffer by Thermo Fisher Scientific

Protocol

Step 1.

Grind −80◦C stored leaves (100-200 mg) to fine powder using liquid nitrogen in the presence of 50 mg PVP (PolyVinyl Pyrrolidone, Mr 10,000). Note: It' s very important that the tissue doesn't thaw.

This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited



REAGENTS

PVP by Sigma

Step 2.

Add 1.5 mL of freshly prepared 2x CTAB buffer and incubate at 60◦C for 45 min with intermittent shaking every 10 minutes.

20 mM EDTA (PH 8.0, 0.5M)	40 ml
100 mM Tris-HCl (PH 8.0, 1M)	100 ml
1.5 M NaCl	87.6 g
2% CTAB (w/v)	20 g
1% β-mercaptoethanol	10 ml (add before use)

Then add distilled water to make it up to 1000 ml.

This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

REAGENTS

20 mM EDTA by Sigma

100 mM Tris-HCl by Sigma

1.5 M NaCl by Sigma

2% CTAB by Sigma

1% β-mercaptoethanol by BBI Biotech

Step 3.
Centrifuge at 12,000 rpm for 15 min at room temperature.

Step 4.

Carefully transfer the aqueous phase into a new tube. Note: Use wide-bore tips for transferring the aqueous phase to avoid mechanical damage to DNA.

Step 5.

Add double volume of Chloroform: Isoamyl alcohol (24 : 1) under fume hood, and invert gently 15 to 20 times and centrifuge at 12,500 rpm for 15 min. Note: If the aqueous layer appears translucent, repeat the stepuntil the solution is transparent. Be careful to avoid transferring any chloroform.

REAGENTS

Chloroform: Isoamyl alcohol by Sangon Biotech

Step 6.

Add double volume of chilled isopropanol followed by 1/3rd volume of 3 M sodium acetate (pH 5.2) and keep at -20◦C for one hour to precipitate the DNA. Note: The longer the chilled incubation, the more the precipitation.

REAGENTS

isopropanol by Sangon Biotech

sodium acetate by Qiagen

Step 7.
Centrifuge at 12,000 rpm for 15 min and discard the supernatant.

Step 8.

To the pellet, add 70% chilled ethanol and spool out the pellet carefully and centrifuge again at 12,000 rpm for 15 min, and repeat the step again.

REAGENTS

ethanol by BBI Biotech

Step 9.

Discard the supernatant and vacuum dry or air dry the pellet at room temperature. Note: Make sure that there is no residual ethanol, this is very critical especially if the DNA is to be used directly for PCR. Overdrying should also be avoided as it makes the pellet difficult to resuspend.

This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Step 10.
Add 40-50 µL TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8), and quantify the DNA using Qubit flurometer/Nanodrop, and run an 0.8% Agarose gel electrophoresis.

REAGENTS
TE buffer by Thermo Fisher Scientific

Step 11.
Store at -20◦C/-40◦C till further use.

This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited