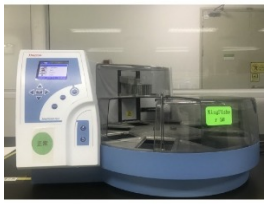
May 16, 2018

# DNA Extraction for plant samples by CTAB



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**Protocol status:** Working
**We use this protocol and it's working**

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**Keywords:** dna extraction for plant sample, ctab dna extraction, dna extraction, leaves by ctab, plant sample, stored leaf, extraction, leaf, ctab, dna

## Abstract

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

## Materials

MATERIALS

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

STEP MATERIALS

- PVP **Merck MilliporeSigma (Sigma-Aldrich)**
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Chloroform: Isoamyl alcohol **Sangon Biotech**

isopropanol **Sangon Biotech**

sodium acetate **Qiagen**

ethanol **BBI Biotech**

TE buffer **Thermo Fisher Scientific**

## Protocol materials

100 mM Tris-HCl **Merck MilliporeSigma (Sigma-Aldrich)**

1% β-mercaptoethanol **BBI Biotech**

isopropanol **Sangon Biotech**

ethanol **BBI Biotech**

2% CTAB **Merck MilliporeSigma (Sigma-Aldrich)**

20 mM EDTA **Merck MilliporeSigma (Sigma-Aldrich)**

TE buffer **Thermo Fisher Scientific**

PVP **Merck MilliporeSigma (Sigma-Aldrich)**

1.5 M NaCl **Merck MilliporeSigma (Sigma-Aldrich)**

sodium acetate **Qiagen**

ethanol **BBI Biotech**

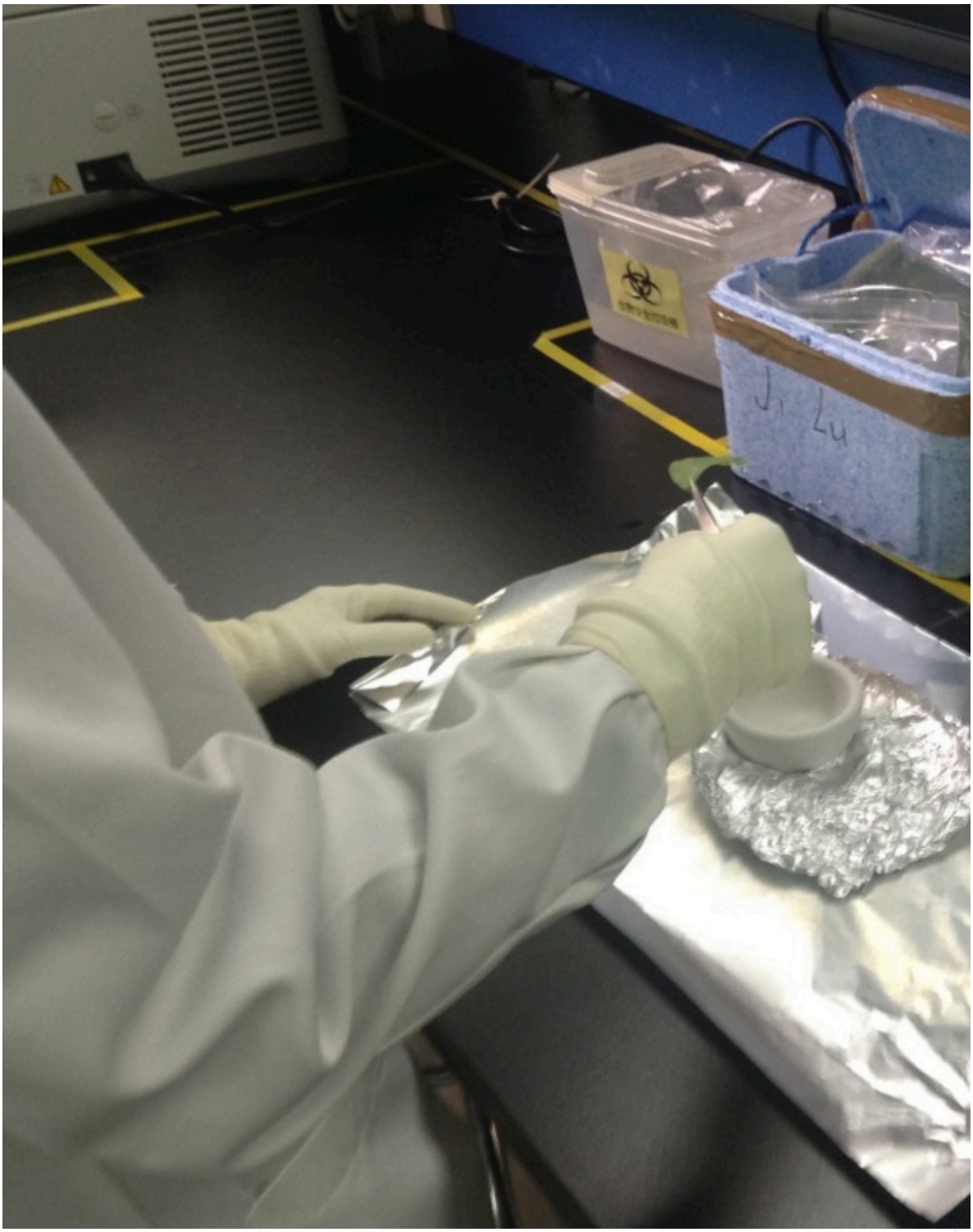
TE buffer **Thermo Fisher Scientific**

isopropanol **Sangon Biotech**

Chloroform: Isoamyl alcohol **Sangon Biotech**

Chloroform: Isoamyl alcohol **Sangon Biotech**

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.



PVP **Merck MilliporeSigma (Sigma-Aldrich)**

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% $\beta$-mercaptoethanol | 10 ml (add before use) |

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

00:45:00

20 mM EDTA **Merck MilliporeSigma (Sigma-Aldrich)**

100 mM Tris-HCl **Merck MilliporeSigma (Sigma-Aldrich)**

1.5 M NaCl **Merck MilliporeSigma (Sigma-Aldrich)**

2% CTAB **Merck MilliporeSigma (Sigma-Aldrich)**

1% β-mercaptoethanol **BBI Biotech**

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

00:15:00

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.

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.

00:15:00

Chloroform: Isoamyl alcohol **Sangon Biotech**

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.

01:00:00

isopropanol **Sangon Biotech**

sodium acetate **Qiagen**

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

00:15:00

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.

00:15:00

ethanol **BBI Biotech**

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.

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.

TE buffer **Thermo Fisher Scientific**

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