RNA extraction for plant samples using CTAB-pBIOZOL

Scott Edmunds

Abstract

RNA extraction of plant tissues (in our case Bauhinia leaves) using a pBIOZOL/CTAB lysis buffer

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Materials

- Isopropanol by Contributed by users
- Chloroform by Contributed by users

  Phenol, Saturated, pH 4.3, Liquid BP1751I by Fisher Scientific
- Beta-mercaptoethanol by Contributed by users
- 75% Ethanol by Contributed by users

  CTAB (Hexadecyltrimethylamm onium bromide) CB0108-100g by BBI Biotech

  pBIOZOL Plant Total RNA Extraction Reagent View by Hangzhou bori Technology Co., Ltd. (BIOER))

Protocol

2x CTAB buffer production

Step 1.

To make the 2x CTAB buffer used in the pre-lysis step make up the following b and then autoclave and aliquot.

2% CTAB (w/v)	20g
100mM Tris(PH 8.0 , 1M)	100ml
20mM EDTA(PH 8.0, 0.5 M)	40ml
1.4 M NaCl	81.8g

Then add distilled water to make it up to 1000ml

REAGENTS

CTAB (Hexadecyltrimethylamm onium bromide) CB0108-100g by BBI Biotech

Pre lysis buffer

Step 2.

Add 750µl 2x CTAB buffer to 60µl of beta-mercaptomethanol and 750µl pBIOZOL reagent in 2ml eppendorf tubes. Mix well.

REAGENTS

pBIOZOL Plant Total RNA Extraction Reagent View by Hangzhou bori Technology Co., Ltd. (BIOER))

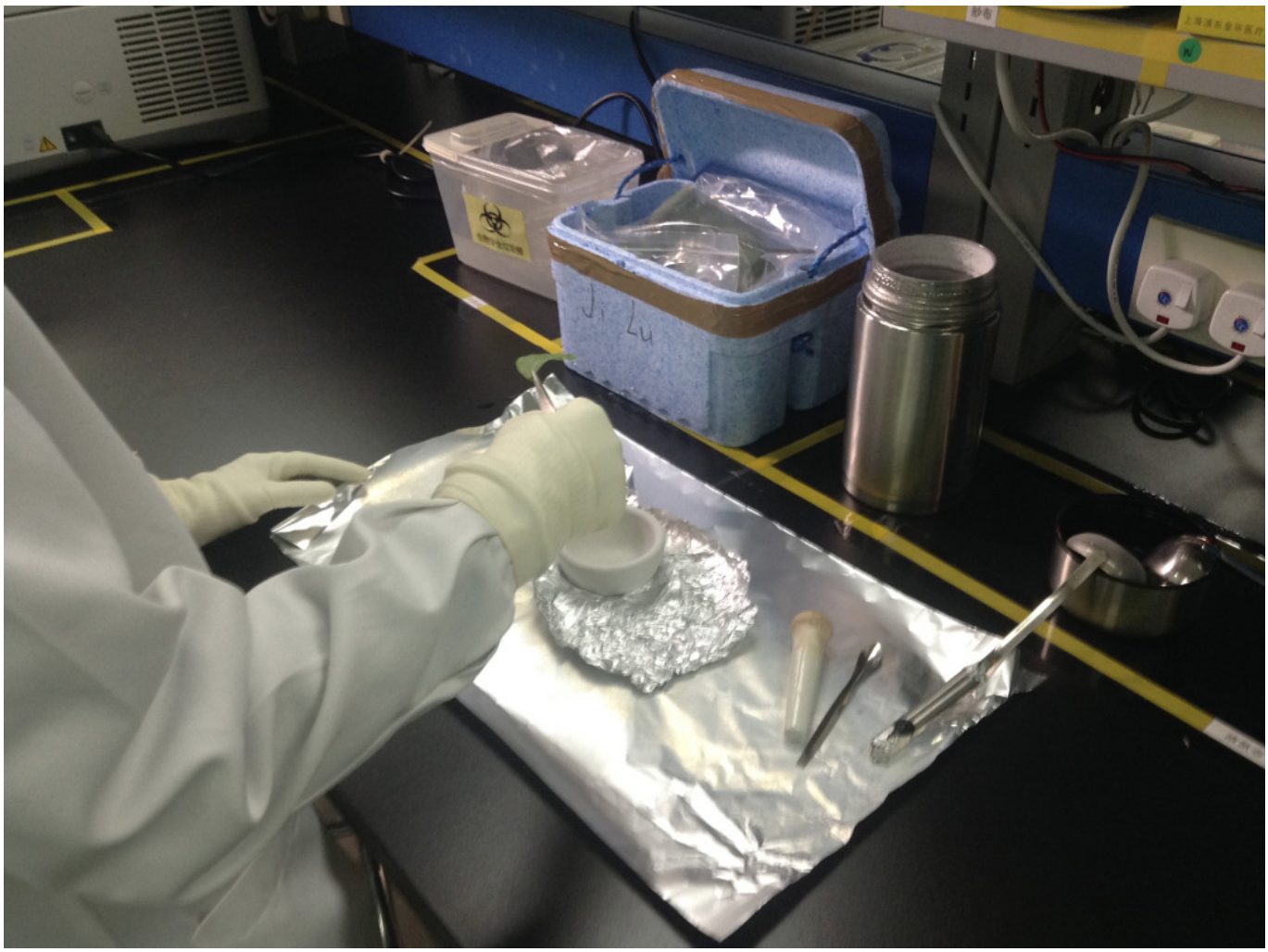
Step 3.

Warm up the lysis buffer to 65°C in a heat block

Grind plant tissues

Step 4.

Cut 1-2 cm² sections of plant or leaf tissues and grind up in a pestle and mortar with liquid nitrogen. These roughly 80mg samples are then added into the warmed 1.5ml aliquots of CTAB-pBIOZOL lysis buffer.



Incubate lysis reaction

Step 5.

Incubate the samples in a thermo mixer with gentle mixing (700rpm) for 15 minutes at 65°C to permit the commplete dissociation of nucleoprotein complexes.

DURATION
00:15:00

Step 6.

After incubation centrifuge at 12000xg for 5 minutes at 4°C.

DURATION
00:05:00

Chloroform step

Step 7.

Transfer the supernatant to a new 2.0ml eppendorf tube. Add 400µl chloroform per 1.5ml of CTAB-pBIOZOL reagent. Shake the tubes vigourously for 15s, then centrifuge again at 12000xg for 10 minutes at 4°C.

DURATION
00:10:00

Phenol-Chloroform step

Step 8.

Transfer the supernatant to new tubes. Add 700µl acidic phenol and 200µl chloroform. Shake the tubes vigourously for 15 seconds and centrifuge for 12000xg for 10 minutes at 4°C.

DURATION
00:10:00

Step 9.

Following centrifugation the mixture should seperate into three layers: the phenol chloroform phase, an interphase, and an upper aqueous phase. The RNA remains in the aqueous phase, and if you want to extract DNA later this is in the interphase. Transfer the aqueous phase into a new 2.0ml tube and add an equal amount of chloroform. Shake the tubes vigourously for 15s, then centrifuge again at 12000xg for 10 minutes at 4°C.

DURATION
00:10:00

Isopropyl alcohol Precipitation

Step 10.

Transfer the supernatant to a new 1.5ml tube and add an equal volume of isopropyl alcohol. Mix well and then leave at -20°C for 2 hours to precipitate.

REAGENTS
- Isopropanol by Contributed by users

DURATION
02:00:00

Step 11.

Centrifuge at 13600rpm for 20 minutes at 4°C.

DURATION
00:20:00

Washing

Step 12.

Carefully remove the supernatant and wash the pellet with 1ml of 75% ethanol. Re-suspend the sample and centrifuge at 13600rpm for 3 minutes at 4°C.

REAGENTS
- 75% Ethanol by Contributed by users

DURATION
00:03:00

Step 13.

Repeat the washing step, removing the supernatant, adding 1ml of fresh 75% ethanol, resuspending the pellent, and then centrifuging again at 13600rpm for 3 minutes at 4°C.

REAGENTS
- 75% Ethanol by Contributed by users

DURATION
00:03:00

Resuspension

Step 14.

Completely remove the ethanol without disturbing the pellet, and then air-dry in the biosafety cabinet. Do not overdry the pellet or you won't be able to redissolve it. Once dry you can resuspend in DEPC-treated water to disolve the pellet (note: DEPC inhibits RT reaction). Use 30-50µl of water depending on the concentration needed. To discolve properly you may need to pipet up and down, and heat to 55-60°C for 10 min.

REAGENTS
- DEPC-Treated Water 4387937 by Contributed by users

Warnings

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