Cassava leaf nucleic acid extraction Version 2

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Abstract

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Protocol

Step 1.
Prepare 1.5ml tube by adding a few glass beads.

Step 2.
To each tube add 200mg of leaf material

Step 3.

Freeze in liquid N2 and grind using a homogeniser

Step 4.

Prepare CTAB buffer:

Add 2% beta-mercaptoethanol to the CTAB buffer just before use (200ul/10ml)

CTAB Buffer:

2%	CTAB
2%	PVP-40
100mM	Tris-HCL pH 8.0
25mM	EDTA-Na
2M	NaCl
0.5g/L	spermidine

Make up to required volume with DEPC treated water.

Incubate for 1h at RT and then autoclave

Step 5.

Add 1ml CTAB buffer to the sample, mix, incubate 15min at 50°C

Step 6.
Centrifuge 5min full speed, transfer 900ul supernatant to fresh 2ml tube

Step 7.
Add 900 ul Chloroform:isoamyl alcohol (24:1), mix, centrifuge for 10min full speed

Step 8.

Transfer 800ul supernatant to fresh tube and add equal volume of Chloroform:isoamy alcohol (24:1), mix, centrifuge 10 min full speed

Step 9.

Transfer 650ul supernatant to fresh 1.5 ml tube, add 390 ul (0.6 volume) cold EtOH

Step 10.

Incubate at -80°C for 30 min or at -20C overnight.

Step 11.
Centrifuge 30 min full speed at 4°C

Step 12.
Remove supernatant

Step 13.
Wash the pellet in 1 ml 80% ETOH

Step 14.
Centrifuge 5min and vacuum dry the pellet.

Step 15.
Dissolve the pellet in 100 ul DEPC-treated water. *continue on ice

Step 16.

Measure the DNA concentration using a NanoDrop.

Step 17.

Pipette out as much total NA as required.

Step 18.

Continue with the following steps for RNA precipitation

Step 19.
Add 1/5th volume of 10M LiCl

Step 20.
Incubate at -20 for at least 1 hour

Step 21.
Centrifuge full speed (4C) for 30 min

Step 22.

Wash with 80% EtOH

Step 23.
Centrifuge 5min at 4000 rpm, at 4C, and vacuum dry the pellet.

Step 24.
Resuspend in 30-50ul of H20