ACGT MICROARRAY FACILITY STANDARD OPERATING PROCEDURE		
TITLE: RNA isolation from woody tissues using CTAB		**PAGE: 1 of 2**
SOP#: MA004	**REVISION LEVEL: .1**	**DATE: 15 November 2005**
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1. PURPOSE

This procedure describes RNA isolation from woody tissues using a CTAB based extraction. This procedure may not be appropriate for all types of tissue and is recommended for samples rich in polyphenols and polysaccharides, such as xylem, phloem and cork scrapings.

2. SCOPE

This procedure may be applied in any laboratory that requires high-quality RNA for preparing microarray probes.

3. MATERIALS

CTAB (Sigma, catalogue number H6269 – 250g)
PVP-40 (Sigma, catalogue number PVP-40-50g)
Spermidine Trihydrochloride (Sigma, catalogue number 50266-1g)
LiCl (Sigma, catalogue number L9650-500g)
DEPC (Sigma, catalogue number D5758)
1M Tris-HCl pH8
0.5M EDTA pH8
NaCl
β-Mercaptoethanol
Isoamylalcohol
Chloroform
50ml sterile plastic screw-cap centrifuge tubes (Falcon, Becton Dickinson, catalogue number 352070)
Liquid Nitrogen
Rnase Away® (Molecular BioProducts, catalogue number #7003)

4. PREPARATION

When working with RNA, ensure all bench surfaces are wiped with RNAse Away and rinsed with distilled water, and gloves are used at all times. RNAses are very stable and active enzymes that generally do not require cofactors to function. Care should be taken not to introduce RNAses into the RNA extraction procedure.

Any plastic containers used in an extraction should be agitated overnight in a 0.01% DEPC solution and then autoclaved and

dried. All glassware should be baked for at least four hours in an oven at 150 °C

Prepare a 0.01% (v/v) solution of DEPC water as follows:
In the fume hood, add 1ml of DEPC to 1L of ddH_2O in a schott bottle.
Allow the mixture to stir overnight.
Autoclave the DEPC water for 20- 40min.

Prepare Chloroform:Isoamylalcohol in a ratio of 24:1
Make up a 10M solution of LiCl and filter sterilise it

Prepare Extraction Buffer and autoclave for 20mins

Volume of EB to be made (ml)	**100**	**200**	**300**	**400**	**500**	**600**	**1000**
CTAB (g)	2	4	6	8	10	12	20
PVP-40 (g)	2	4	6	8	10	12	20
1M Tris pH 8 (ml)	10	20	30	40	50	60	100
0.5M EDTA pH8 (ml)	5	10	15	20	25	30	50
NaCl (g)	11.7	23.4	35.1	46.9	58.5	70.2	117
Spermidine (ul)	54.3	109	163	217	271	326	543

5. PROCEDURE

Day 1

In 50ml centrifuge tubes pre- warm 20ml EB per sample to 65 °C in water bath or oven and add 400ul Mercaptoethanol (work in a fume hood).

Grind 4g of frozen tissue using liquid Nitrogen (For best results grind first in automatic high speed grinder, then to a very fine powder using a pestle and mortar – the finer it is the higher the yields tend to be). If preparing multiple samples store all ground

tissue at –80 °C until use. Any thawing may cause RNA degradation.

Quickly transfer frozen powder to warmed EB and shake vigorously – you want the powdered sample to be completely homogenized with the EB.

Incubate at 65 °C for 10 minutes, taking it out every 2 minutes to shake vigorously.

Add an equal volume CIA and shake vigorously.

Centrifuge at top speed (depending on tubes) for 10 minutes.

Transfer supernatant to a new tube, re-extract with CIA and centrifuge as above.

Collect supernatant & transfer to new tube. Avoid sucking up chloroform or interphase.

Add 0.25 volumes 10M LiCl to the supernatant mix well and incubate at 4°C overnight

Day 2

Recover RNA by centrifuging at top speed for 1 hour.

Discard supernatant, wash pellet twice with 75% ethanol (After pouring off the supernatant I use about 1500ul of 75% ethanol to resuspend the pellet in the 50ml tube, then transfer it into a 1.5ml eppie and spin it down, pour off the ethanol, add another 1500ul ethanol, resuspend gently and spin down before pouring off the ethanol and moving to the next step).

Air-dry for 10 minutes.

Dissolve RNA in 50 – 100ul DEPC-treated water.

Assess concentration and quality by Spectrophotometer and RNA gel analysis