ACGT MICROARRAY FACILITY STANDARD OPERATING PROCEDURE		
TITLE: RNA isolation using Qiazol		**PAGE: 1 of 2**
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1. PURPOSE

This procedure describes RNA isolation using Qiazol. This procedure may not be appropriate for all types of tissue and is recommended for soft plant tissue such as Pearl Millet and *Arabidopsis thaliana*.

2. SCOPE

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

3. MATERIALS

Qiazol (Qiagen Scientific, catalogue number 79306)
DEPC (Sigma, catalogue number D5758)
0.8M sodium citrate/ 1.2M NaCl
Isopropanol
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 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.

Prepare a 0.1% (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 20min.

Prepare 75% EtOH in DEPC water.

Prepare 0.8M sodium citrate/ 1.2M NaCl by dissolving
Xg of sodium citrate and yg of NaCl in 500ml of DEPC-treated water and autoclave.

5. PROCEDURE

Grind tissue in liquid nitrogen using homogeniser.

Transfer 1g of ground tissue to 50ml tube containing 15ml Qiazol

Incubate samples at 60°C for 5min.

Shake tubes intermittently

Centrifuge samples at 12,000xg at 4°C for 10min.

Transfer supernatant to a new tube and discard pellet

Repeat last two steps if debris remains.

Add 3ml chloroform to each tube in fume hood.

Shake tubes vigorously with vortex for 15sec.

Let tubes sit at room temperature for 2-3min.

Centrifuge tubes at 10,000xg at 4°C for 15min.

Carefully pipette aqueous phase into clean 50ml tube and discard interphase and lower phase.

Precipitate the RNA by adding isopropanol and 0.8M sodium citrate/1.2M NaCl, half the volume of the aqueous phase each.

Cover tube and mix by gentle inversion.

Allow the tube to sit at room temperature for 10min.

Centrifuge samples at 10,000xg at 4°C for 10min.

Discard supernatant take care not to lose the pellet.

Add 10ml of 75% EtOH (ice cold) and vortex gently for 15sec.

Centrifuge samples at 10,000xg at 4°C for 10min.

Discard supernatant take care not to lose the pellet

Centrifuge samples at 10,000xg at 4°C for 2min.

Remove all the remaining liquid with a pipette.

Dry the samples at 37°C for 5-10min – not longer.

Add 200ul RNase- free water and resuspend the pellet by scraping the pellet with the pipette tip.

Pipette up and down

Transfer the RNA to a 1.5ml tube.

Place the sample at 55^{o}C for 5min to allow the RNA to dissolve.

Assess concentration and quality by Spectrophotometer and RNA gel analysis