

Mini Hot Phenol Arabidopsis RNA extraction procedure (ver. 3)

- ◆ Thaw phenol and set water bath to 60°C in fume cupboard
- ◆ Prepare hot phenol tubes by adding:
 - 250 µL phenol
 - 500 µL homogenisation buffer (recipe below)
 - 5 µL β-mercaptoethanol
 to a 1.5 mL tube and heat in 60°C water bath
- ◆ Add a pinch of glass beads (Sigma G4649) or sand and grind approximately 0.4 ml frozen tissue to a fine powder (if you use 0.4 ml of leaf tissue, don't pack it into the bottom of the tube - rather, put it on the side with a space so you can get the pestle in and grind the tissue more easily; also once a sample is ground, you can store the tubes in a container of liquid nitrogen until all the sample are ground. If you have pre-ground powder already simply add ~0.4 mL tissue powder to a tube and keep it frozen)
- ◆ Let liquid N₂ completely evaporate and the tube to warm a little (but don't let tissue thaw) then add hot phenol mixture
- ◆ Shake tube well for 15 minutes (beware of leaking if tubes are horizontal, use good quality eppies, best is putting them vertically in a multi-tube mixer and leaving them)
- ◆ Add 250 µL chloroform and shake for a further 15 minutes
- ◆ Spin top speed, room temperature, 10 minutes
- ◆ Transfer 550 µL aqueous layer to a new tube and add 550 µL phenol:chloroform, shake 10 minutes and spin 10 minutes.

From this step onwards you need to be particularly careful to avoid RNase contamination

- ◆ Transfer 500 µL aqueous layer to fresh tubes
- ◆ Add 50 µL 3M sodium acetate and 400 µL isopropanol
- ◆ Incubate at -80°C for 15 minutes (may freeze solid, also at this stage reduce temperature of centrifuge)
- ◆ Spin top speed, 4°C, 30 min
- ◆ Remove all supernatant (can zip spin, a remove with fine tip), invert tube on a tissue, dry ~10 min
- ◆ Resuspend in 500 µL autoclaved water (avoid vortexing if DNA is to be kept)
- ◆ Add 500 µL 4 M LiCl (final concentration = 2M)
- ◆ Incubate at 4°C overnight
- ◆ Spin top speed, 4°C, 30 minutes (the supernatant contains genomic DNA and this can be kept if needed, the RNA pellet may be invisible if very clean but is usually white)
- ◆ Wash pellet in 1 mL 80% ethanol (if it was invisible the pellet should appear with addition of ethanol and be white in colour), spin 5 minutes and remove wash carefully as pellet is very delicate (after initial removal of the wash, give the tubes a quick second spin and remove all wash with a fine tip)
- ◆ Dry in vacuum desiccator
- ◆ Resuspend in 60 µL autoclaved water (although not usually present when this protocol is followed - if insolubles are present, spin 5 minutes at RT and transfer to new tube)
- ◆ Quantify RNA in spectrometer

Homogenisation buffer (For the Hot Phenol Method)

	100 mL	400 mL	
100 mM Tris pH between 8-9	10 mL	40 mL	of 1 M
5 mM EDTA pH 8	1 mL	4 mL	of 0.5 M
100 mM NaCl	2 mL	8 mL	of 5 M
0.5 % SDS	5 mL	20 mL	of 10%

Method Historical (updated 22th February 2012)

- ◆ Jimmy Botella obtained the protocol from Chris Somerville's lab back in the 80's
- ◆ A longer method has been published in Botella *et al.*¹ and later in Etheridge *et al.*²
- ◆ In the Dean lab at the John Innes Centre, Josh Mylne cut the protocol back to a 1.5 mL version, called it "mini hot phenol" and since then it hasn't changed. It's a simple, reliable protocol that works with a wide range of species and tissues.
- ◆ The mini hot phenol method has been mentioned within Mylne *et al.*³ and fully described in Mylne *et al.*⁴ and Mylne *et al.*⁵. In Box *et al.*⁶ mini hot phenol was compared to Trizol.
- ◆ A even shorter hot phenol method that is better suited to 96-well RNA extraction is described in Box *et al.*⁶ The major difference is that the LiCl precipitation was dropped from the protocol.
- ◆ Although Box *et al.*⁶ recommends acidic phenol, Mathew Box found (unpublished observations) that acidic or neutral pH phenol did not make that much of a difference (DNA is said to dissolve in low pH phenol).
- ◆ Importantly the protocol is an excellent alternative to Trizol, which for reasons still unknown cannot isolate mRNA from young *Arabidopsis* tissue. For proof see "Figure 1. TRIZOL is not suitable for extracting RNA from very young tissues" in Box *et al.*⁶ (<http://www.plantmethods.com/content/7/1/7>).
- ◆ In the published papers the mini hot phenol was used for a range of tissues and species including challenging tissues like dry *Arabidopsis*⁵ or sunflower⁵ seeds.
- ◆ A final note: before you bake your mortar and DEPC treating everything ... for your own sanity bear in mind, plant tissue is full of RNAses. The buffer prevents them from working and phenol-chloroform extractions deactivate and precipitate them. Ultrapure water contains no RNAses and most plasticware comes with a guarantee of RNase-free so baking, autoclaving and DEPC treatments really can be just a waste of time. The homogenisation buffer lasts for years. Enjoy!

Bibliography

- 1 Botella, Arteca, Schlagnhauer, Arteca & Phillips. (1992) Identification and characterization of a full-length cDNA encoding for an auxin-induced 1-aminocyclopropane-1-carboxylate synthase from etiolated mung bean hypocotyl segments and expression of its mRNA in response to indole-3-acetic acid. *Plant Mol. Biol.* **20**: 425-436.
- 2 Etheridge, Trusov, Verbelen & Botella. (1999) Characterization of ATDRG1, a member of a new class of GTP-binding proteins in plants. *Plant Mol. Biol.* **39**: 1113-1126.
- 3 Mylne, Barrett, Tessadori, Mesnage, Jacobsen, Johnson, Bernatavichute, Franz & Dean. (2006) *LHP1*, the *Arabidopsis* homologue of *HETEROCHROMATIN PROTEIN1* is required for epigenetic silencing of *FLC*. *Proc. Natl. Acad. Sci. U.S.A.* **103**: 5012-5017.
- 4 Mylne, Wang, van der Weerden & Craik. (2010) Cyclotides are a component of the innate defense of *Oldenlandia affinis*. *Biopolymers (Pept. Sci.)* **94**: 635-646.
- 5 Mylne, Colgrave, Daly, Chanson, Elliott, McCallum, Jones & Craik. (2011) Albumins and their processing machinery are hijacked for cyclic peptides in sunflower. *Nat. Chem. Biol.* **7**: 257-259.
- 6 Box, Coustham, Dean & Mylne. (2011) Protocol: A simple phenol-based method for 96-well extraction of high quality RNA from *Arabidopsis*. *Plant Methods* **7**: 7.