Bioplatforms Australia Genomics for Australian Plants Initiative
Activity: Reference genomes
Species: *Acacia pycnantha*
Contact: Gareth Holmes, gareth.holmes@rbg.vic.gov.au

Total RNA isolation protocols for *Acacia pycnantha*

Material from young phyllodes, inflorescences and fruits from the *Acacia pycnantha* reference plant were sampled and placed immediately into chilled RNAlater (Thermo Fisher Scientific, U.S.A.). The samples were then frozen at -20°C until required. For each tissue type, approximately 200 mg of representative material was removed from the RNAlater, blotted to remove excess liquid, and placed in a sterile, pre-chilled mortar. The material was submerged in liquid nitrogen and ground to powder with a pestle. Slightly different RNA isolation protocols were subsequently followed for each of the three tissue types as per below.

Phyllode

Using a sterile, pre-cooled spatula, approx. 80 mg of the still-frozen powdered tissue was placed in a 2 ml Safe Lock tube (Eppendorf, Germany) containing lysis buffer and immediately vortexed. The lysis buffer was based on Ishihara *et al.* (2019) and contained 400 µl of PFL and 20 µl PFR buffers from a NucleoSpin RNA Plant and Fungi Kit (Macherey-Nagel, Germany), 100 µl Fruit-mate for RNA Purification (Takara, Japan) and 5 µl of ß-mercaptoethanol. The standard NucleoSpin RNA Plant and Fungi Kit protocol was then followed with an additional filter column wash with PFW2 buffer, and a final elution using 50 µl RNase-free water. Carryover gDNA was removed from the eluate by adding 1 µl DNase I (NEB), 10 µl DNase buffer, and RNase-free water up to 100 µl total volume. The solution was incubated at 37°C for 10 minutes, 1 µl of 0.5M EDTA pH8 added, then purification undertaken using a Monarch RNA Cleanup Kit (NEB, U.S.A.) with a 30 µl final elution volume. The RNA concentration in the eluate was determined using a Qubit 3 (Thermo Fisher Scientific) with an associated RNA HS kit, and RNA integrity assessed on an agarose electrophoresis gel. The eluates were then sent to the Australian Genome Research Facility (Melbourne) for additional quality checks.

Fruit

Approx. 100 mg of powdered tissue was added to 1.7 ml sorbitol buffer (Table 1) with 1% (v/v) ß-mercaptoethanol in a 2 ml Safe Lock tube. The mix was vortexed well and then centrifuged for 2 mins at 16,000 g. The supernatant was removed, and lysis buffer added to the remaining pellet. The lysis buffer consisted of 400 µl of PFL and 50 µl PFR buffers from a NucleoSpin RNA Plant and Fungi Kit, 100 µl Fruit-mate for RNA Purification and 5 µl of ß-mercaptoethanol. The remaining isolation protocol was as above for phyllode material.

Table 1. Sorbitol buffer components (Inglis *et al.* 2018)

	Final conc. M	**For 40 ml**
D-Sorbitol	0.35 M	2.55 g
PVP-40	1%	0.44 g
EDTA 0.5M, pH8	5 mM	0.4 mL
Tris-HCl (1M)	0.1 M	4 mL
dH_20	-	To volume

Inflorescence

The protocol was as for fruit tissue above except that 50 mg of powdered tissue used and the lysis buffer included 11 µl of ß-mercaptoethanol.

References

Inglis PW, Pappas MdCR, Resende LV, Grattapaglia D (2018) Fast and inexpensive protocols for consistent extraction of high quality DNA and RNA from challenging plant and fungal samples for high-throughput SNP genotyping and sequencing applications. *PLOS ONE* 13(10): e0206085. https://doi.org/10.1371/journal.pone.0206085

Ishihara K, Lee EKW and Borthakur D (2019) An improved method for RNA extraction from woody legume species *Acacia koa* A. Gray and *Leucaena leucocephala* (Lam.) de Wit. *International Journal of Forestry and Wood Science* 3(1): 31–35.