RNA extraction (Kiwi)

(Mortaji et al., 2008)

Reagents for preparation:

- Extraction buffer:

  0,5M Tris-HCl pH=9

  1% (w/v) SDS

- Phenol: chloroform: isoamyl alcohol (25:24:1)
- 3M CH_3COONa pH= 5.2
- Absolute ethanol (100 % (v/v) ethanol)
- 5M LiCl
- 2M LiCl
- 75% (v/v) ethanol
- Sterile H_2O (ddH_2O)

Method:

1) 100 mg of leaf tissue is added in **1ml** of extraction. Vortex sample and add an equal volume of phenol:chlorophorm:isoamyl alcohol (**1ml**) and vortex again for 1 min. Centrifugation at 14000g for 3 min at 4°C.

2) Collect supernatant (600-700µl) and put in fresh chilled tubes on ice. An equal volume (600-700µl) of phenol:chlorophorm:isoamyl alcohol was added and vortex. Centrifugation at 14000g for 7 min at 4°C.

3) The upper phase (500-600µl) is **re-extracted** again with phenol:chlorophorm:isoamyl alcohol in a similar way.

4) After centrifugation at 14000g for 7 min at 4°C, carefully pipette aqueous phase (300-400µl) in to fresh tubes and **do not** collect the white phase at the bottom and centrifuged at 16000 g for 7 min at 4°C (either time g of centrifuge is increase in order to remove whole phenol).

5) Collect supernatant (200-300µl) to fresh chilled tube and add **1/10 volume** 3M CH_3COONa pH= 5.2 and **3 volumes** of absolute ethanol and mix by inversion. Store at -80°C for 20 min.

6) Centrifugation at 16000g for 8 min at 4°C and pellet was dissolved in **100 µl** of ddH_2O, add equal volume of 5M LiCl (**100µl**), invert 4 times to mix. Incubate at -80°C for 2 h (or overnight).

7) Centrifugation at 16000g for 15 min at 4°C and discard supernatant and add **1ml** 2M LiCl. Invert once to mix and centrifuged at 16000g for 2 min at 4°C.

8) RNA is washed with **1ml** 75% (v/v) ethanol (vortex and centrifugation at 16000g for 2 min at 4°C).

9) Discard supernatant and leave until dry (50°C for 2-3 min).

10) Pellet was dissolved in 30µl ddH_2O. Nanodrop and store at -80°C.