RNA extraction (Arabidopsis seed & siliques)
(Luis Oñate-Sánchez and Jesús Vicente-Carbajosa, 2008)

Reagent for preparation:

- Extraction buffer:
  - 0.4 M LiCl
  - 0.2 M Tris pH= 8
  - 25 mM EDTA
  - 1% (w/v) SDS
- Chloroform
- Phenol
- 8 M LiCl
- 3M CH₃COONa pH=5.2
- Absolute ethanol (100% (v/v) ethanol)
- 70% (v/v) ethanol
- Sterile H₂O (ddH₂O)

Method:

1) Transfer 20-50mg ground tissue to a cooled eppendorf and quickly add 550 μl of extraction buffer and 550 μl chloroform. Vortex 10s and keep on ice until all samples are ready. Spin for 3 min (maximum speed) at 4°C.

2) Transfer supernatant to a new eppendorf, add 500 μl of water-saturated acidic phenol, vortex thoroughly, add 200 μl of chloroform and spin 3 min (maximum speed) at 4°C.

3) Transfer to a new eppendorf, add 1/3 volume of 8 M LiCl and mix. Precipitate at -20°C for 1 hour (overnight at -20°C is also possible) and spin for 30 min at 4°C.

4) Dissolve pellet in 26 μl ddH₂O, add 3 μl buffer 10X and 1 μl (10 units) RNase-free DNase I (Roche 10776785001). Incubate 30 min at 37°C.
5) Add 470 μl ddH$_2$O, 7 μl 3 M CH$_3$COONa pH= 5.2 and 250μl absolute ethanol, mix well and spin 10 min at 4°C to precipitate carbohydrates.

6) Transfer supernatant to a new eppendorf, add 43 μl 3M CH$_3$COONa pH=5.2 and 750 μl absolute ethanol, mix well and leave at –20°C for at least 1 hour (or overnight). Spin 20 min at 4°C, wash pellet with 70% (v/v) ethanol.

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

8) Pellet was resuspended in 15 μl ddH$_2$O. Nanodrop and store at -80°C.