RNA extraction (strawberry)

(Mazzara and James, 2000)

Reagents for preparation:

- Extraction buffer:

50 mM Tris-HCl (pH= 8,9)

150 mM lithium chloride (LiCl)

5 mM EDTA

5 % (w/v) SDS

- Phenol: chloroform: isoamyl alcohol (25:24:1)
- Chloroform
- 8 M lithium chloride (LiCl) (pH= 9,2)
- 3 M sodium acetate (pH= 5,2)
- 70 % (v/v) absolute ethanol in 0,15 M NaCl
- Sterile H_2O (ddH_2O)

Method:

1) The homogenate tissue (100 mg) (~ 2 spatulas) is transferred to a tube and resuspended in 1 ml of extraction buffer. The mixture is vortexed briefly for 2-3 min.
2) An equal volume (1 ml) of phenol: chloroform: isoamyl alcohol **(**25:24:1) is added and the mixture is vortexed for 3 min to mix the phases.
3) Phases are separated by centrifugation at 8,000 g for 15 min at 4 ^{o}C.
4) The upper aqueous phase (~600 µl) is then mixed with an equal volume of chloroform, vortexed for 3 min and centrifuged as above.
5) The upper aqueous phase is transferred to a clean tube and 1/3 of the volume 8M lithium chloride (LiCl) (pH= 9,2) is added to give a final concentration of 2M LiCl.
6) The sample is incubated at -80 ^{o}C for 1 h.
7) Centrifugation at 12,000 g for 30 min at 4 ^{o}C.
8) Pellet is washed **twice** with 0,5 ml 0f 70 % (v/v) ethanol in 0,15 M NaCl.
9) Centrifugation for every washing step at 12,000 g for 10 min at 4 ^{o}C.
10) The pellet is air-dried for 20 min.
11) Pellet is resuspended in 50 µl of freshly sterilized water.

12) After the resuspension, to precipitate contaminants in the aqueous solution we make a spin at low speed (4000-5000 g) and the supernatant containing the total RNA is transferred to a fresh eppendorf tube.
13) Total RNA quantification and purity is calculated using the NanoDrop machine.
14) According with the results obtained, if the product is not the appropriate (i.e. A_{260}/A_{280} and A_{260}/A_{230} are too low) you could optionally either:

- Further clean them with the Nucleospin RNA Clean up protocol
- Or reprecipitate using 0,1 volume of sodium acetate pH= 5,2 and 2,5 volumes of absolute ethanol, placed at -20^{o}C for 1 h and resuspend in freshly prepared water.