

# Protocol for total RNA extraction from STEM.

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Based on Chang et al. (1993) A Simple and Efficient Method for Isolating RNA from Pine Trees, Plant Mol. Biol. Rep. 11(2) and the Qiagen RNeasy Kit. Modifications by Jarmo Schrader, Umeå Plant Science Centre, 2002.

- Prewarm Extraction buffer in a waterbath to 65°C.
- Grind tissue (0.5 - 1 g) under liquid N.
- Add 2%  $\beta$ -mercaptoethanol (300 $\mu$ l / 15 ml) to the extraction buffer.
- Transfer ground tissue to extraction buffer, mix completely by vortexing briefly. Incubate for 30s (no longer than 5 min).
- Add an equal volume of chloroform:isoamylalcohol and mix by rapidly inverting the tube 5-6 times. When several samples are processed, add Chisam and mix before proceeding to next sample.
- Separate phases at 4500 rpm for 10 min.
- Transfer supernatant to a new tube, be careful not to disturb the interphase, if in doubt, take less supernatant!
- Repeat the Chisam extraction once more.
- Transfer the supernatant to a tube that resists 10.000 rpm. Estimate the remaining volume and add 1/4 vol. 10 M LiCl, mix and precipitate overnight at 4°C (needs at least 6 hours)
- Harvest RNA by centrifuging at 10.000 rpm for 20 min at 4°C.
- Dissolve pellet in 450 $\mu$ l RLT buffer from the Qiagen kit (Incubate for 10 min at RT). Add  $\beta$ -mercaptoethanol to the buffer before using it (10  $\mu$ l/ml).
- Add 225 $\mu$ l (0.5 vol) EtOH, load on pink column.
- Centrifuge 15 s at 10.000 rpm. Discard the flow-through.
- Add 700  $\mu$ l Buffer RW1 to the column. Centrifuge 15 s at 10.000 rpm to wash the column. Discard the flow-through.
- Transfer the column to a new 2 ml collection tube (supplied).
- Add 500  $\mu$ l Buffer RPE. Centrifuge 15 s at 10.000 rpm. Discard the flow-through.
- Add again 500  $\mu$ l RPE. Centrifuge 15 s at 10.000 rpm. Discard the flow-through.
- Centrifuge at max speed during 1 min for removing all RPE.

- ELUTION: transfer the column to a 1,5 ml tube (supplied). Add 30  $\mu$ l of RNase-free water.
- Centrifuge 1 min at 10.000 rpm.
- Add other 30  $\mu$ l of RNase-free water and centrifuge 1 min at 10.000 rpm.

Depending on the tissue, the yield can be  $>200\mu$ g per 0.5g sample. The qiagen columns are specified for up to  $100\mu$ g but they can be overloaded at least two fold if the sample is pretreated as in this protocol. Alternatively one can run two Qiagen columns per extraction, i.e. dissolve the LiCl pellet in  $600\mu$ l RLT.

#### Materials Needed:

- Extraction Buffer

Final concentration	Per 200ml buffer
2% CTAB (hexadecyltrimethylammonium bromide)	4g
2% PVP (polyvinylpyrrolidinone K 30, or similar mol wt)	4g
100mM Tris-Hcl (pH 8.0)	20 ml 1 M
25 mM EDTA	10 ml 0.5 M
2.0 M NaCl	80 ml 5 M
0.5g/L spermidine	0.1g

mix and autoclave, just before use add

- 2%  $\beta$ -mercaptoethanol  $300\mu$ l / 15 ml
- Chloroform:isoamylalcohol (Chisam, 24:1)
- 10M Lithium chloride, RNase free (Treat with DEPC, autoclave)
- Qiagen RNeasy mini kit (the normal, not the plant RNeasy kit)