

RNA extraction (small samples)

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First day

Sample preparation

- Material: 0,2 - 0,4 g. Grind sample in a small mortar. Volume of extraction buffer: 1,75 ml. Use 15 ml Falcon tubes for the incubations. You should increase the extraction buffer if the sample is not well dissolved (add sets of 250 μ l of extraction buffer)
- Material: one leaf (0,01 g). Grind it in 1,5 ml epp tubes with the blue pestle. Volume of extraction buffer: 200 μ l.

Extraction

- Remember to warm up the extraction buffer to 65°C and add the B-mercaptoethanol just before use it (2,67% 47 μ l/1.75 ml extraction buffer).
- Add the extraction buffer and dissolve well the sample.
- Add the same volume of CHISAM.
- Mix (you can use the vortex but not very energetic).
- Centrifuge at RT max speed 15 min. For Falcon tubes you use the swing-out rotor.
- Remove the upper phase to a new clean tube.
- Make this step two times.

Precipitation

- Add $\frac{1}{4}$ of volume of 10M LiCl.
- Mix and put in ice in the cold room overnight.

Second day

- Switch on the bath in the morning (60°C).
- Centrifuge 10000-14000 rpm, 20 min at 4°C.

- Add 300 μl of SSTE at 60°C for dissolving the pellet and put it on 2ml-ependorf. Rinse the big tube with 300 μl of the same buffer and put in the same eppendorf.
- Hold the eppendorf tube in the bath for a few seconds until the liquid is clear (SSTE buffer precipitate at RT). THIS STEP IS IMPORTANT.
- In these steps, dont use cold temperature because RNA would precipitate.
- Add the same volume of CHISAM (600 μl) (TUBE A). Mix and centrifuge 10000 rpm, 10 min at RT. Remove the upper phase and put it in a new eppendorf (TUBE B).
- In this second tube (tube B) add 600 μl of CHISAM and repeat the last step. Remove the upper phase and put it in a new tube (TUBE 1).
- Now you have 3 tubes: TUBE A and TUBE B with CHISAM. Add 300 μl of SSTE at each tube and repeat the extraction. If you remember put the tubes for a few seconds in the bath at 60°C.
- Mix the two upper phases in one tube (TUBE 2).
- Now you have 2 tubes with RNA (TUBE 1 and TUBE 2), one for using and other for storage. If the volume is small enough you can combine both in the same tube.
- Precipitation: Add 2 volumes of chilling 99,5% ethanol. Leave at least 30 min (first time 2 h at -70°C) or overnight at -20°C.

Third day

- Centrifuge 20 min, maximum speed at 4°C in a microfuge.
- During this time cast a 1% agarose/TAE/EtBr
- Remove the supernatant and add 70% ethanol (not forget to use DEPC-treated water). Mix gently and centrifuge again. You can use a Falcon tube for preparing the ethanol. This step is for washing the salt.
- Air-dried the pellet until the ethanol disappears. Dissolve the dried pellet with 50 μl of DEPC-treated water. RNA is hard to dissolve then leave the tubes in the fridge (4°C) for a while. It makes easier to dissolve the RNA.
- Run the gel for checking preparation. Less than 1 μg RNA per well is enough. Measure the concentration using the NANO DROP.
- The gel has to be fresh (same day or day before). 1% agarose/TAE/EtBr (2 μl of the stock for 100 ml of gel). 1 μg RNA.
- Trick: Pre-run the gel during 5-10 min before loading it for removing the RNase from the wells. You usually dont need to do the trick.