## RNA extraction with the Trizol reagent (25/02/2008)

1. organize chloroform, 75% EtOH in DEPC H2O, Trizol
2. switch on centrifuge for cooling down to 4C
3. Weigh eggs – do not use more than 50-100mg for 1 ml Trizol
4. homogenize eggs in 300 µl Trizol reagent in a 1.5 ml RNAase free tube
5. add 700 µl of Trizol and mix by pipetting (do not vortex, might sheer gen. DNA)
6. Spin at 11000g (recommended is below 12000g) for 10 min at 4C
7. Transfer supernatant to new clear 1.5 ml vial
8. Incubate sample for 5 min at RT (15-30C)
9. Add 0.2ml of chloroform to 1ml Trizol
10. Shake tube vigorously be hands for 15 sec (do not vortex)
11. Incubate vial for 3 min at RT
12. Centrifuge vial at 11000g (recommended is below 12000g) for 15 min at 4C
13. Transfer upper phase (RNA) to fresh 1.5 ml vial (aqueous phase should be 60%)
14. Add 0.5 ml isopropyl alcohol
15. Incubate sample for 10 min at RT (put on shaker)
16. Discard tube with lower phase in Phenol waste
17. Centrifuge at 11000g (recommended is below 12000g) for 10 min at 4C
18. Remove supernatant
19. Add 1 ml 75% EtOH in DEPC H2O
20. Tip pellet with the finger that it gets detached from the bottom of the tube, do not vortex the sample!
21. Switch on heat block to 50C and incubate DEPC H2O
22. Centrifuge at **7300g** (recommended is below 7500g) for 5 min at 4C
23. Remove supernatant
24. Air dry pellet for 5 min on heat block at 50C
25. Add *30ul* of DEPC H2O (depending on pellet 10-50ul) and mix by pipetting
26. Incubate at 50C for 5 min to resolve RNA completely
27. Label tube and put on ice
28. Spec 1.5ul of it (if A260/280 ratio < 1.6, the RNA is only partially dissolved)
29. run 300ng in 9 ul DEPC H2O and add 1 ul of Bromphenolblue in 1.2% agarose gel (1% is fine as well, RNase is not a big problem..), use Sybr Safe (Invitrogen)
30. Store at -80C and enter name of RNA sample in digital file

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