RNA extraction from frogs

Preparation the day before the extraction

Materials needed

- Beads
- 2 ml tubes for tissue
- 100% & 70% ethanol
- (2) β-mercaptoethanol[1] and fume hood access
- Qiagen RNA mini kit
- Filter tips (1000 µl)[2]
- Glass beaker for β-mercaptoethanol waste (labelled)
- Glass beaker for other waste (labelled)

Preparation

Once per kit: Add 44 µl ethanol to RPE buffer, and tick the box on the lid.

Collect tissue in RNAlater.

Extraction day

Setup[3]

1. Clean the bench, trays, pipetes, centrifuge and gloves with 70% ethanol.
2. Prepare 2 ml tubes with sample names.
3. Prepare 1 long tray with pink RNEasy mini spin columns, label with numbers.
4. Prepare tubes with 600 µl 70% **ethanol**.
5. Prepare RLT buffer in 2 ml tubes: per 1 sample 600 µl **RLT** + 6 µl **β-mercaptoethanol**. Work in fume hood.
6. Prepare glass beaker in fume hood (RLT buffer + β-mercaptoethanol waste) and glass beacker for other waste[4].

[1] In tissue culture room 4°C.

[2] In qPCR room, stock in end of same corridor.

[3] Since RNA is sensitive, the more time is spent preparing the lab before the RNA is in a sensitive stage, the better the extraction quality.

[4] Could wash down sink but they react badly with bleach, so better to evaporate in fume hood.

Extraction

Work in fume hood until step 10.

1. Place tissue in labelled 2 ml tubes. 30 mg max, about 3 mm^3 cube. Add 600 µl prepared **RLT** and 1-2 glass **beads**. Use a light-coloured tray.
2. Take photo of ordered tubes in tray. Write the names in order in the lab book.
3. Disrupt tissue in beadbeater, 2 min, 25 Hz.
4. Centrifuge for 3 min at full speed.
5. Use filter tips. Remove supernatant to tubes with 70% **ethanol** and mix immediately by pipetting. Transfer 700 µl to pink RNEasy mini spin columns.
6. Centrifuge at 8000 RCF for 15 s.
7. Discard flow through in fume hood beaker for β-mercaptoethanol waste[5]. Transfer remaining supernant to same spin columns.
8. Centrifuge at 8,000 RCF for 15 s.
9. Discard flow through in fume hood beaker for β-mercaptoethanol waste.
10. Add 700 µl **RW**1 solution[6].
11. Spin for 15 sec at 8,000 RFC.
12. Discard flow-through in other waste glass beaker. Add 500 µl **RPE**.
13. Spin for 15 sec at 8,000 RFC.
14. Discard flow-through in other waste glass beaker. Add 500 µl **RPE**.
15. Spin for 2 min at 8,000 RFC.
16. Prepare 1 tray with RNase free kit tubes for RNA elution. Label with names from photo you took.
17. Discard flow-through in other waste glass beaker. Transfer column to RNase-free tubes from kit. Add 50 µl **RNase-free H_20** from kit, directly on the membrane.

[5] Leave beaker to evaporate in fume hood. Wash salt remains.

[6] For all these washes, close the lid and roll and invert the tubes to achieve a thorough wash. DNase treatment done here if needed.

18. Clean centrifuge lid and tube-holding area with 70% ethanol.

19. Spin for 1 min at 8,000 RCF[7].

20. (Optional) Pipette 3 µl to additional tube "Q" to be used for nanodrop, qubit and tape-station. Keep on ice. Order in a box and transfer samples to -80ºC until use.

21. If not already done so, update lab book with extraction names.

22. Store samples at -80ºC.

[7] Single elution yields 200 - 800 ng/µl in 50 µl from g28 embryos.