RNA isolation

How it works

The aim of this protocol is to isolate RNA from biological samples, for example cultured cells or samples from laboratory animals. For this Thursday we will use mouse brain samples. The general name for this method is “Acid guanidinium thiocyanate-phenol-chloroform extraction”.

The Trizol (or Tri reagent) you will add in the first step contains phenol, water and guanidine isothiocyanate. The last one is a chemical which denatures proteins. This will on the one hand aid in breaking open the cells and separating RNA from bound proteins, and on the other hand inactivate enzymes that would degrade RNA (RNAses). In addition, the Tri reagent contains phenol and water.

Upon adding chloroform in step 5, formation of three phases can be observed: an aqueous upper phase, an interphase and an organic lower phase. In such a system, chemical compounds get separated based on their relative solubility in each phase. Since RNA is more soluble in the aqueous phase and DNA and proteins are more soluble in the interphase and organic phase under these conditions, RNA can be separated by only using the aqueous phase (step 7).

Purification of the RNA is done using filtering columns containing a silica-based matrix. While the sample is passing through the filtering column, RNA will bind to the column. As RNA is not soluble in high concentrations of ethanol, it will stay bound during the washing steps, but will be eluted from the column when adding water during the last step.

Protocol

Work under a fume hood

1. Add 700 µL Trizol to each mouse brain sample. Careful: Trizol is toxic!
2. Dissociate sample by pipetting up and down.
3. Pass the sample 20 times through the 25G needle
4. Incubate 5 min @ room temperature (RT)
5. Add 140 µl chloroform. Vortex 15 sec
6. Incubate 2-3 min @ RT
7. Centrifuge 15 min @ 12000 g, 4°C
8. Transfer upper aqueous phase to new tube
9. Add 1.5 volumes of 100% ethanol. Mix well by pipetting.
10. Pipette 700 µl sample-ethanol mix into a Zymo-Spin IC column (2 ml collection tube)
11. Centrifuge 20 sec @ 10000 g, RT
12. Repeat steps 10+11 for remaining volume
13. Wash the RNA bound to the column by adding 700 µl 70% ethanol
14. Centrifuge for 30 sec @ 10000 g; RT; discard flow through
15. Repeat steps 13 and 14 for two total washes
16. Centrifuge again 30 sec @ 10000g, RT to ensure all ethanol is removed
17. Transfer the column to a fresh RNase-free tube.
18. Elution: Add 25 µl water; Centrifuge 30 sec @ 10000 g, RT
19. Measure RNA concentration at the NanoDrop.