Phenol/Chloroform extraction/purification of RNA

Use two layers of nitrile gloves, as chloroforms dissolves nitrile fast.
While working with phenol and chloroform, always work under the fume hood. Discard material contaminated with chloroform in an special container.

Aim of the Experiment

This experiment can be used to purify RNA from cell material or an RNA-containing solution in high purity.

Materials

- Lysis buffer:

Table 1: Lysis Buffer

Concentration	Chemicals
1x	100x TE Buffer (Applichem Germany)
0.06%	SDS (Carl Roth)
0.6 mg/ml μM	Reverse primer

- ice-cold 100% Ethanol (Carl Roth, Germany)
- ice-cold 70% Ethanol (Carl Roth, Germany)
- Roti-Aqua-P/C/I (X985, Carl Roth, Germany)
- 3 M sodium acetate (Riedel da Haen, Germany)
- Phase Lock Gel Heavy (733-2478, VWR)
- Lysozyme (NEB, Germany)
- nuclease free H_2O (Carl Roth, Germany)

Procedure

Cell lysis

Skip this step if you are not using cell material as starting sample e.g. you are purifying from an in-vitro TX assay.

1. Centrifuge 1000 μl cells, 4 min, 3000 g.
2. Discard supernatant.
3. Add lysozyme to the lysis buffer to a final concentration of 3 mg/ml.
4. Resuspend pellet it 600 μl lysis buffer with lysozyme.
5. Incubate, 10 min, 90 $°C$.

RNA purification

1. Centrifuge the Phase Lock tubes, 5 min, 16000g, RT.
2. Add 100-300 μl of sample. For in-vitro TX, 100 is usually enough.
3. Add equal volume of Roti-Aqua-P/C/I.
4. Mix by inverting.
5. Centrifuge, 5 min, 16000g, RT.
6. Add equal volume of chloroform.
7. Mix by inverting.
8. Centrifuge, 5 min, 16000g, RT.
9. Transfer supernatant to a fresh 1.5 ml tube. Be careful not to poke the gel.
10. Add 0.3 volumes of 3 M sodium acetate.
11. Add 3 volumes of 100% EtOH.
12. Vortex.
13. Incubate for at least 1 h, -80 $°C$;
14. Centrifuge, 15 min, 16000g, 4 $°C$;

15. Discard supernatant. Be careful not to lose or poke the pellet.
16. Carefully add 1 ml of 70% EtOH.
17. Centrifuge, 5 min, 16000g, 4 $°C$;
18. Discard supernatant. Be careful not to lose or poke the pellet.
19. Dry 5-10 min at RT in a concentrator. Do not overdry.
20. Elute in 50 μl nuclease free H_2O.