**Removal and Washing of proteins from phenol phase**

* 1. Remove phenol phase from extraction step 6 and place in labelled microcentrifuge tube
* 2. Add 1 M sucrose solution (v/v), vortex and centrifuge for 10 min at 16,000 x*g* at RT (=phase separation)
* 3. Extract lower phase (phenol phase) into new microcentrifuge tube and mix with 4x vol of 0.1 M ammonium acetate in MeOH (ice cold) -> incubate this at -20°C for one hour (see step 5)
* 4. Centrifuge samples for 10 min at 16,000 x*g* and 4 °C.
* 5. Repeat step 3 & 4 (can now leave overnight to precipitate if no time for rest of washing)
* 6. Discard supernatant and add 3x vol chilled 80% **acetone** (ie 3 x vol of phenol from step 3)
* 7. Incubate for 15 min at -20 °C
* 8. Centrifuge at 12 000g for 10 min at 4 °C.
* 9. Discard supernatant and again add 3x vol chilled 70 % (v/v) **ethanol**
* 10. Incubate for 15 min at -20 °C
* 11. Centrifuge at 12 000g for 10 min at 4 °C
* 12. Discard supernatant and again add 3x vol chilled 80% **acetone**
* 13. Incubate for 15 min at -20 °C
* 14. Centrifuge at 12 000g for 10 min at 4 °C
* 15. Discard supernatant and again add 3x vol chilled 70 % (v/v) **ethanol**
* 16. Incubate for 15 min at -20 °C
* 17. Centrifuge at 12 000g for 10 min at 4 °C
* 18. Discard supernatant and the samples were incubated at 20 °C under the fume hood until the ethanol was evaporated completely (~ 30mins)
* 19. Resuspend protein pellet in 0.25 - 1 mL urea buffer (7 M urea, 2 M thiourea and 0.01 g/mL dithiothreitol) (resuspending vol depends on how much protein you think you have).