Method: 20mL Emulsion Breaking Protocol (Part 2 of 3)

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Principle:

There are 3 main steps to breaking emulsions after PCR cycle is complete:

1. Remove oil phase using a combination of diethyl-ether and ethyl acetate washes, marking aqueous PCR phase with blue loading dye

2. Use phenol-chlorophorm to extract residual proteins, such as Polymerase, from PCR reaction to prevent degradation of short oligos later on

3. Precipitate short oligos to a smaller, more concentrated volume using ethanol, glycogen, and NH_4OAc

Time Required:

Depending on the number of 20mL emulsion bags setup, the protocol can either be done in 1 full day (8 hours) or broken up over 2 days.

Solutions / Reagents:

- 6X Loading Dye (Thermo-Fischer)
- Diethyl-ether (Sigma #296082-1L)
- Ethyl Acetate (Sigma #494518-1L)
- Phenol-chloroform
- 0.4M NH_4OAc
- 100% EtOH
- Glycogen, Molecular Biology Grade (Fermentas #R0561)
- Oak Ridge Tube (Thermo-Fischer #3119-0030 PPCO)
- Sealing Cap (Thermo-Fischer #DS3132-0020)

Procedure:

1. Transfer contents of each emulsion bag to a clean 30mL oak ridge tube with sealing cap

2. Add 30μL of 6X loading dye and 3mL mineral oil to each oak ridge tube, vortex for 30 seconds

3. Spin for 10 min at max speed (15,300 rpm)

4. Remove and discard upper oil phase

5. Add 20mL water-saturated diethyl ether using dispenser in fume hood
6. Vortex for 2 min on at 3,000rpm
7. Spin for 2 min at max speed (15,400rpm)
8. Remove the top ether phase, and discard ether waste container
9. Add 20mL water-saturated ethyl acetate using dispenser in fume hood
10. Vortex for 2 min at 3,000rpm
11. Spin for 2 min at max speed
12. Remove top acetate phase, and discard in acetate waste container

[Repeat Steps 5-8]

13. Add 20mL water-saturated diethyl ether using dispenser in fume hood
14. Vortext for 2 min on red vortexer at 3,000rpm
15. Spin for 2 min at max speed
16. Remove the top ether phase, and discard ether waste in bottle
17. Pellet will be loose – try to remove last of ether phase using Pasteur pipette or pipetman
18. Evaporate residual ether at 37/42/ or 55ºC on heat block in hood
    (*This may take minutes to hours depending on step 17, you may also leave O/N at RT to completely evaporate residual ether*)
19. Add 2.8mL of acid phenol-chloroform to aqueous phase in oak ridge tube (1:1 volume of aqueous phase:phenol-chlorophorm)
20. Invert conical 6 times to extract aqueous phase; afterwards spin in centrifuge at 2,000 rpm for 2 minutes
21. Transfer top aqueous phase to a 50mL conical; ***at this step you should pool aqueous phase from 2 oak ridge tubes together per 50mL conical***
22. Add 1.4mL dH2O to phenol to re-extract residual PCR product in oak ridge tube
23. Repeat steps 21-22; 50mL conical should now have total volume of 8.4mL

24. Now add 800μL of 4M NH_4OAc to each 50mL conical

25. Incubate for 30 min at 37^0C

26. Vortex for 3 min at 3000rpm

27. Spin for 1 min at max speed

28. Each 50mL conical should now contain 9.5mL aqueous mix; **First**, add 230μL of Glycogen to each conical, then add 25mL of 100% ice cold EtOH, for a total volume of ~35mL (*Be careful not to add EtOH before glycogen, as you will precipitate glycogen out of solution before it can act as a carrier!*)

29. Vortex mix very well with at least 6 short pulses per conical and 6 inversions

30. After mixing, use automatic pipetman to transfer solution to 2mL eppendorf tubes, 2mL each (Should have ~20/22 tubes per conical)

31. Invert 2mL eppendorfs in rack, and place in freezer O/N to precipitate

Notes: