Phenol-chloroform glass stirring rod genomic DNA extraction protocol (for large amounts of high quality DNA)

Michael Petronio, 2010

1. Grow up isolate culture on plate of choice
2. Heat up 2 water baths, one at 65°C and the other at 50°C
3. Add 100µL lysozyme (100mg/ml) and 5mL molecular grade H_2O to sterile 50mL tube
4. Scrape culture off of plate and add to lysozyme/water mix - vortex well (use about 1 full plate per tube)
5. Incubate in 65°C water bath for 30 minutes
6. Remove from water bath and add 100µL proteinase K (20mg/mL)
7. Vortex tube well then place in 50°C water bath for 30 minutes
8. Remove tube from water bath and vortex until no clumps are visible
9. Add 1mL of 10% SDS (sodium dodecyl sulfate) and mix by inverting tube several times (solution should become more clear and viscous)
10. Incubate in water bath for 30 minutes (by this time no cells should remain in solution)
11. Remove from water bath and tubes to cool to room temperature
12. Create hooked end of a glass stirring rod (or Pasteur pipette) by placing tip in flame and shaping
13. Add 10mL ice cold ethanol to tube while holding at a 45° angle
14. Tilt hooked stirring rod at same angle as tube (45°) and, inserting into solution, twirl rod in a clockwise motion trying not to touch sides of tube. A visible mass of DNA will attach to rod. Carefully remove DNA into sterile 50mL tube.
15. Repeat step 14 until all visible DNA has been collected
16. Add 5mL molecular grade water to tube with DNA and vortex until DNA goes into solution (do not vortex for full-length DNA)
17. Add equal volume of phenol-chloroform to solution and mix tube swirling by hand (until solution becomes cloudy and no longer separated)
18. Centrifuge tubes for 5 minutes at 10,000 RPM at 4°C (if no temperature controlled centrifuge available then get solution as cold as possible before centrifuging)
19. Extract top aqueous layer carefully as to avoid collecting any debris
20. Repeat steps 17-19 (if debris still remains then repeat step once more)
21. Add 1/10 volume sodium acetate (3M) to aqueous layer and mix well
22. Add 2 volumes ice cold ethanol and store overnight at -20°C
23. Centrifuge tubes for 10 minutes at 15,000 RPM at 4°C. DNA will precipitate and form pellet on side of tube
24. Remove excess supernatant and wash pellet 3 times with ice cold 70% ethanol
25. Allow tubes to air dry until no moisture remains (speed vac works best)
26. Once dry add 100-200µL DNA elution buffer to dried DNA pellet and allow to go into solution (may require additional elution buffer, vortexing, heating or all of above to go into solution)
27. Nanodrop
    a. If low 260/280 value (<1.7) or low 260/230 (<2) then RNAse treatment could be needed followed by another phenol chloroform extraction and ethanol precipitation to remove RNAse