Phenol Chloroform DNA Extraction Protocol

Reagents

- Phenol solution, pH 8.0, Equilibrated (CAS 108-95-2)
- Chloroform:Isoamyl Alcohol (24:1, v/v)
- Dow Corning® high-vacuum silicone grease
- Proteinase K 20mg/mL (CAS 39450-01-6)
- Proteinase K Buffer Solution
- 3 M Sodium acetate, pH 5.2 (CAS 127-09-3)
- TLE Buffer Solution

Cost

Approximate per sample cost:

- 2X 1000 &uL; Aerosol Tips: $0.18
- 1.7 mL ETOH: $0.10
- 5 µL Proteinase K: $0.08
- 200 µL Phenol: $0.05
- 600 µL Chloroform:Isoamyl Alcohol: $0.03
- 2X 1.5 mL Microcentrifuge Tubes: $0.04
- 2.0 mL Microcentrifuge Tube: $0.02
- 30 µL 3 M Sodium acetate: $0.02
- 0.25 mL Dow Corning® high-vacuum silicone grease: $0.01

Total: < ~$0.55 per sample

Sources

See post by Alexander Klenov on DIY phase separating tubes

Safety

The following is a cursory set of guidelines to begin familiarizing you with phenol safety but should by no means be considered comprehensive or adequate before beginning work with phenol. Phenol can lethal if simple precautions are not taken and understood so please do not take this warning lightly!

- Use extreme caution when working with phenol which can quickly cause severe burns—possibly without any sensation of pain. It can be lethal due to poisoning at levels of skin exposure or inhalation that could potentially occur when using this protocol.
- Always wear a lab coat, shoes, and safety glasses when working with phenol
- Use ChemTek® 38-214 Viton and Butyl gloves.
- Perform the isolation steps involving phenol and chloroform within a fume hood at least six inches inside of the sash.
- If contact with skin has occurred immediately remove any contaminated clothing and other worn items. Put on goggles if not already on unless this would risk eye exposure. Unless hands have been exposed put on ChemTek® gloves if not already on. Irrigate exposed area with PEG-300 or wipe with gauze soaked in PEG-300. Irrigate with water only after the application of PEG-300 or if no PEG-300 is available. Water will only spread the phenol and potentially expand the area of exposure. Continue application of PEG-300 until there is no detectable odor of phenol.
- If a spill is greater than 50 mL evacuate the laboratory, close the doors, and notify your fire department or environmental health and safety office.
- Visit the following link for a more thorough coverage of phenol safety: https://sp.ehs.cornell.edu/lab-research-safety/documents/phenol_first_aid_and_ppe.pdf

Disposal

All phenol and chloroform contaminated waste must be put into hazardous waste.

Procedure

1) Phase Separating Tube

1. Prepare one phase separating tube per sample by dispensing 0.1-0.2 mL of silicone grease from a 60 mL syringe into a 2 mL microcentrifuge tube.

2) Lysis

1. Prepare lysis master mix containing 5 µL of 20 mg/mL proteinase K and 295 µL proteinase K buffer solution per sample plus a little extra to account for pipetting error.
2. Add 300 µL of lysis master mix to a 1.5 mL microcentrifuge tube containing up to 20 mg of sample tissue (approximately size of a grain of rice)
3. Incubate samples at 55 °C in shaker incubator set to 800 for 4-6 hours

3) Isolation

1. Centrifuge phase separating tubes at 16,100 RCF for 1 minute
2. Add 300 µL of lysis product to phase separating tube

3. Add 200 µL of Phenol to phase separating tube.
4. Add 200 µL of Chloroform:Isoamyl Alcohol to phase separating tube
5. Mix samples by inverting tubes and shaking manually for 1 minute
6. Centrifuge at 16,100 RCF for 5 minutes
7. Add 400 µL of Chloroform:Isoamyl Alcohol to the aqueous layer in the phase separating tube
8. Mix samples by inverting tubes and shaking manually for 1 minute. Do not mix so vigorously as to disrupt the silicone grease layer dividing the aqueous and organic layers
9. Centrifuge at 16,100 RCF for 5 minutes

4) Precipitation

1. Add 30 µL of 3 M sodium acetate to a new 1.5 mL micro centrifuge tube
2. Transfer 300 µL of the top aqueous layer to the tube with sodium acetate. Avoid pipetting any of the silicon grease separating the phases
3. Add 1 mL of 100% ethanol
4. Incubate sample at room temperature for at least 15 minutes
5. Centrifuge at 16,100 RCF for 30 minutes
6. Decant supernatant
7. Add 1 mL of 70% ethanol
8. Centrifuge at 16,100 rcf for 15 minutes
9. Decant supernatant
10. Briefly spin tube to collect residual ethanol
11. Pipette and discard remaining ethanol
12. Dissolve pellet in TLE buffer solution