## Overview and Considerations:

This is an old-school method for extracting DNA from E.Coli. The yields tend to be better than the kits.

WEAR GLOVES AND GOGGLES WHEN HANDLING PHENOL/CHLOROFORM/ISOAMYL ALCOHOL AND WORK IN THE FUME HOOD

If you spill phenol on yourself, wash the area with PEG300.

## Reagents:

1. LB
2. Qiagen Buffers: P1 (Fridge), P2 and N3
3. phenol/chloroform/isoamyl (Fridge)

## Protocol:

1. Grow up bacterial overnight culture
   1. 5 mL LB
   2. 10 uL of 50 mg/mL ampicillin or 1.25uL of 120mg/ml Kanamycin
   3. 1 colony from bacteria plate
2. Put 1.4 mL of overnight culture in a 1.5 mL Eppendorf tube
3. Quick spin to pellet bacteria at 14,000 rpm at room temperature
4. Aspirate supernatant
5. Add 200 uL of P1 (Qiagen) to pellet and vortex to resuspend
6. Add 200 uL of P2 (Qiagen), mix by inverting 10 times, incubate 5 minutes max
7. Add 300 uL of N3 (Qiagen), mix by inverting 10 times
8. Spin for 10 minutes at room temperature, 14,000 rpm
   1. While this is spinning, take phenol/chloroform/isoamyl alcohol out of the refrigerator and invert 6 times to mix, let separate into two-phases before use!
9. Save the supernatant
10. Mix the DNA supernatant with Phenol/Chloroform/Isoamyl Alcohol in a 1:1 (v:v) ratio and vortex vigorously
    1. Note: there should be two layers in the phenol/chloroform/isoamyl alcohol bottle. Make sure to **use the lower layer**!
11. Centrifuge for 1 minute at room temperature, 14,000 rpm
12. Allow the phases to separate and carefully remove the upper aqueous layer
    1. Lower organic phase has mostly proteins
    2. Upper aqueous layer contains DNA
    3. Precipitated protein at interface
13. Repeat steps 10-12
14. Add 1/10th volume of 3M sodium acetate (sterile) to the upper aqueous layer
15. Add 2 to 2.5 volumes of 100% ethanol upper aqueous layer and vortex vigorously
16. Place in the -80 degrees C freezer for 1 hour minimum
17. Centrifuge at 20,000 rpm for 30 minutes at 4 degrees C
18. Wash the pellet with 70% ethanol and dry in the hood
19. Resuspend in ~100uL milliQ water