Title	Plasmid DNA extraction (E. coli)		
Protocol Number	MB21002	Written by	Duha A.
Adapted from	Marczynski Lab	Version	1.0
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Reagents:

- GET
- 1% SDS in 0.2M NaOH solution
- 10M ammonium acetate
- Phenol:Chloroform:Isoamyl (use at room temp, use **bottom**/organic layer for extraction)
- 100% prechilled EtOH
- 70% prechilled EtOH

Cell Lysis:

1. Grow a liquid culture overnight
2. Measure OD (1-2)
3. Palette 5-10mL of cells (5= high copy plasmids, 10= low copy plasmids)
4. Resuspend pallet in 100uL GET (no lysozyme)
5. Add 5uL RNaseA
6. Incubate at 37C for 15-45 mints (vortex every 15 mints)
7. Add 200uL 0.2M NaOH, 1% SDS
8. Add 200uL 10M ammonium acetate
9. Spin for 15 mints
10. Collect the supernatant

Phenol/chloroform extraction:

1. Add 200uL of phenol:chloroform:isoamyl to the supernatant.
2. Vortex for 15 seconds
3. Centrifuge at maximum speed RPM for 15 mints at RT
4. Collect the supernatant (aqueous phase) in a new Eppendorf

Alcohol precipitation:

1. Add 1/10 volume 3M Na acetate
2. Add 2.5 volumes 100% cold ETOH
3. Incubate at -20^{o}C for at least 1 hour
4. Spin at maximum speed at 4^{o}C for 15 minutes
5. Using a pipette (or by decanting), remove the 100% ethanol
6. Add 500µL of cold 75% ethanol to wash the pellet
7. Shake the eppendorfs gently
8. Spin at maximum at 4^{o}C centrifuge for 5 minutes
9. Remove the 75% ethanol by decanting or pipetting
10. Repeat the washing step (total of 2 washes)
11. Allow the pellet to airdry for 10-20 mints (37 ^{o}C heat blocks can speed up the process)
12. Resuspend the pellet in 52µL of low salt TE buffer (2µL to account for nanodrop)

13. Dose RNA using the nanodrop and record RNA quantity, 260/280 and 260/230 ratios
14. Store the RNA at -20°C