Title	Genomic DNA extraction (bacteria)		
Protocol Number	MB21003	Written by	Duha A.
Adapted from	Marczynski Lab	Version	1.0
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Reagents:

- EDTA-Saline (0.01 M EDTA and 0.15 M NaCl; pH = 8.0)
- Lysozyme (10 mg/ml)
- RNase A
- 20% SDS
- 10M ammonium acetate
- 5M Sodium chloride
- 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 OD600 (1-1.5)
3. Palette 5-10 mL of cells (I usually palette 5mL)
4. Resuspend cells in 200uL EDTA saline
5. Add 100uL of lysozyme, vortex
6. Add 7uL of RNAase A, vortex
7. Incubate at 37oC for 1 hour.
8. Add 80uL of 20% SDS, Vortex
9. Incubate at 65oC for 10 mints (vortex once at 5 mints).
10. Spin down for 1 mint at maximum speed
11. Add 200uL of 5M sodium chloride, vortex for 15 seconds (until solution turns white)
12. Spin down for 1 mint at maximum speed

Phenol/chloroform extraction:

1. Add 300uL of phenol:choloform:isoamyl, vortex
2. Shake for 15 mints at 1400 rpm
3. Centrifuge at maximum speed for 15 mints
4. Transfer top layer to a fresh eppendorf.
5. Add 200uL phenol:choloform:isoamyl, vortex
6. Centrifuge at maximum speed for 15 mints
7. Collect supernatant
8. Repeat steps 17-19 once using 100uL P/C/ISO (or repeat until no protein layer is visible)

Alcohol precipitation:

1. Add 1/10 volume 3M Na acetate (for 500uL add 50uL)
2. Add 2.5 volumes 100% cold ETOH (for 500uL sample add 1.5mL)
3. Incubate at -20^{o}C for at least 1 hour (preferably O/N)

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 10 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