6-6-08

*DNA Isolation Protocol

A. Lysis

1. Suspend sample in 150 μl of TE Buffer.
2. Add 1 μl of Ready-Lyse Lysozyme to each sample.
3. Incubate at 37°C overnight.
4. Add 150 μl of 2X T & C Lysis Solution to each sample and pipette up and down when adding.
5. Add 1 μl Proteinase K to each sample.
6. Incubate at 65°C for 30 minutes, vortexing briefly every 5 minutes.
7. Cool the samples to 37°C.
8. Place the samples on ice for 3-5 minutes and then proceed with Part B.

B. DNA Clean-up

1. Add 175 μl of MPC Protein Precipitation Reagent to 300 μl of lysed sample and vortex mix vigorously for 10 seconds.
2. Pellet the debris by centrifugation at 4°C for 10 minutes at ≥10,000 x g in a microcentrifuge. Place on ice immediately after centrifugation.
3. Transfer the supernatant to a clean microcentrifuge tube and discard the pellet.
4. Add 500 μl of isopropanol to the recovered supernatant. Invert the tube 30-40 times.
5. Place on ice for 10 minutes
6. Pellet the DNA by centrifugation at 4°C for 10 minutes at ≥10,000 x g in a microcentrifuge.
7. Pour off isopropanol, being careful not to lose pellet. Use a pipet tip to remove remaining isopropanol without dislodging the DNA pellet.
8. Rinse the pellet with 500 μl 75% ethanol. Centrifuge briefly if the pellet is dislodged. Repeat rinse with additional 500 μl 75% ethanol.
9. Remove residual ethanol.
10. Resuspend the DNA in 25 μl of TE Buffer.

***Protocol amended from Epicentre MasterPure™ DNA Purification Kit and Epicentre MasterPure™ Gram Positive DNA Purification Kit