DNA Protocol
Gram (-): Negative

1.) **Harvest Cells** (max 2 x 10^9 cells) and **centrifuge** for **10 minutes** at 5000 x g (or 7500 rpm) **remove supernatant**.

2.) Added **180 µl** of enzymatic **ATL buffer.**

3.) Add **20 µl** of **proteinase K** and **incubate** for **55°C** until cells completely lysis (**30 minutes**) (**Vortex** lightly between incubation to make sure cells are dispersed.)

4.) After incubation **vortex** for **15 seconds** and add **200 µl** of **AL buffer** to the sample, mix thoroughly by **vortex** again and incubate for **70°C** for **10 minutes**

5.) Add **200 µl** of **Ethanol** (96-100%) to the sample, and **(Vortex)**

6.) Pipet mixture for step 6 into a DNeasy spin column placed in **a 2ml collection tube**. **Centrifuge** at >6,000 x g (or 8000 rpm) for **1 minute**. *Discard flow-through and collection tube.*

7.) Place DNeasy spin column into a **new 2ml collection tube**. Add **500 µl** of **Buffer AW1** and **centrifuge** for **1 minute** at > 6000 x g (or 8000 rpm) *Discard flow-through and collection tube.*

8.) Place DNeasy spin column into a new **2ml collection tube**, add **500 µl** of **Buffer AW2**, and **centrifuge** for **3 minutes** at 20,000 x g (or 14,000 rpm) to dry the DNeasy membrane. *Discard flow-through and collection tube.*

A.) Place DNeasy spin column into a **new 1.5ml microcentrifuge tube**. Pipet **200 µl** of **buffer AE** directly onto the DNeasy membrane. **Incubate** at **room temperature** for **1 minute** and then **centrifuge** for **1 minute** at >6000 x g (or 8000 rpm) to elute. Save **microcentrifuge tube 1**

B.) Using a **new microcentrifuge** tube Repeat step 9A. Save **microcentrifuge tube 2**