

Gram + Genomic DNA Extraction (Qiagen):

Materials	<p>QiaAMP DNA Mini kit:</p> <ul style="list-style-type: none"> • Proteinase K solution • RNase • AL Buffer • AW1 Buffer • AW2 Buffer <p>10 mM Tris-HCl 20 mg/ml Lysozyme (can prepare the day before and store at 4C)</p> <ul style="list-style-type: none"> • Lysozyme powder • Tris-HCl • EDTA • Triton <p>100% Ethanol Water bath 2 heat blocks</p>
Notes	Step
	Place 1.8ml of bacterial suspension into 2 ml tubes
	Find all heat blocks/water baths necessary and pre-heat. _____ 37C water bath _____ 56C heat block (which will be bumped up to 57C) _____ 70C heat block
	Prepare 1.5mL of 10 mM Tris-HCl in a 2 ml tube: _____ 15 ul 1 M Tris-HCl _____ 1485 ul dH ₂ O
	Prepare 6mL (for ~30 samples) of 20mg/mL Lysozyme in a 15 ml Falcon tube: _____ 120 mg lysozyme (use lab stock in -20C) _____ 120 ul 1M Tris-HCl _____ 96 ul 125 mM EDTA _____ 72 ul Triton _____ 5,712 ul dH ₂ O
	_____ Pellet bacteria in microcentrifuge tube _____ Centrifuge 13,000 rpm for 7 minutes
	_____ Remove supernatant from pellet
	_____ Add 180 ul of lysozyme solution to the tubes. _____ Pipet to homogenize the suspension.
	Incubate at 37C for 45-80 minutes (depending on your organism); flick tubes (to mix solution) every 15 minutes of incubation Time in: _____ Time: _____ Flick# _____ (Time: _____ Flick# _____) Time: _____ Flick# _____ (Time: _____ Flick# _____) Time out: _____

	Pre-warm 10 mM Tris-HCL at 56C
	<input type="checkbox"/> Add 20 ul proteinase K and mix by pulse-vortexing for 15 s. <input type="checkbox"/> Centrifuge to collect liquid
	Incubate at 56C for 10 minutes Time in heating block: _____ Time out of heating block: _____
	<input type="checkbox"/> Let cool to room temperature. <input type="checkbox"/> Add 4 ul RNase (100 mg/ml). <input type="checkbox"/> Gently mix by pulse-vortexing for 15 s and <input type="checkbox"/> centrifuge for a few seconds to collect liquid. Incubate at room temperature for 10 minutes Start incubation: _____ End incubation: _____
	<input type="checkbox"/> Add 200 ul Buffer AL , pulse-vortex for 15 s. Incubate for 30 min at 57C Time in heating block: _____ Time out of heating block: _____
	Incubate at 70C for 10 minutes Time in heating block: _____ Time out of heating block: _____ <input type="checkbox"/> Cool to room temperature and centrifuge for a few seconds to collect liquid
	<input type="checkbox"/> Add 200 ul ethanol (96-100%) and pulse-vortex for 15 s. <input type="checkbox"/> Briefly centrifuge the microcentrifuge tube to remove drops from inside the lid
	<input type="checkbox"/> Add mixture to spin column in a 2 ml collection tube <input type="checkbox"/> Centrifuge at full speed (20,000 x g) for 1 minute. When centrifuging each wash, switch the direction of the caps. Tab direction?: _____
	<input type="checkbox"/> Place the mini spin column in a clean 2 ml collection tube Discard the tube containing the filtrate.
	<input type="checkbox"/> Open the spin column. Add 500 ul Buffer AW1 . Close the cap. <input type="checkbox"/> Centrifuge at full speed for 1 minute. Tab direction?: _____
	<input type="checkbox"/> Place the mini spin column in a clean 2 ml collection tube Discard the tube containing the filtrate.
	<input type="checkbox"/> Open the spin column. Add 500 ul Buffer AW2 . Close the cap. <input type="checkbox"/> Centrifuge at full speed for 3 minutes. Tab direction?: _____
	<input type="checkbox"/> Place the mini spin column in a clean 2 ml collection tube Discard the tube containing the filtrate.
	<input type="checkbox"/> Open the spin column. Add 500 ul Buffer AW2 . Close the cap. <input type="checkbox"/> Centrifuge at full speed for 3 minutes. Tab direction?: _____
	<input type="checkbox"/> Place the mini spin column in a clean 2 ml collection tube Discard the tube containing the filtrate.
	<input type="checkbox"/> Run an empty spin at full speed for 1 minute.

	<p>___ Place the mini spin column in a clean 2 ml collection tube Discard the tube containing the filtrate.</p>
	<p>___ Run an empty spin at full speed for 2 minutes. ___ Place the mini spin column in a clean 1.5 ml collection tube (labeled elution1) Discard the tube containing the filtrate.</p>
	<p>___ Add 50 ul 10 mM Tris-HCl directly to the column. ___ Incubate at room temperature for 4 minutes Time start: _____ Time stop: _____</p>
	<p>___ Centrifuge at 8000 rpm for 1 min</p>
	<p>___ Place the mini spin column in a clean 1.5 ml collection tube (labeled elution2)</p>
	<p>___ Add 50 ul 10 mM Tris-HCl directly to the column. ___ Incubate at room temperature for 4 minutes Time start: _____ Time stop: _____</p>
	<p>___ Centrifuge at 8000 rpm for 1 min</p>
	<p>___ Nanodrop then store both elutions at -20C for "long term"</p>