Gram-negative Genomic DNA Extraction (Qiagen):	
Materials	QiaAMP DNA Mini kit: • Buffer ATL • Proteinase K solution • RNase • AL Buffer • AW1 Buffer • AW2 Buffer 10 mM Tris-HCl 100% Ethanol 2 heat blocks
Notes	**Step**
	Place 1.8ml of bacterial suspension into 2 ml tubes
	Find all heat blocks necessary and pre-heat. ____ 56C heat block ____ 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_2O
	____ Pellet bacteria in microcentrifuge tube ____ Centrifuge 13,000 rpm for 7 minutes
	____ Remove supernatant from pellet
	____ Add 180 ul of **Buffer ATL** to the tubes ____ Pipet to homogenize the suspension
	____ Add 20 ul **proteinase K** and mix by pulse-vortexing for 15 s.
	Incubate at 56C for 45-60 minutes (depending on organism); flick tubes or vortex (to mix solution) every 15 minutes of incubation Time in: _____ Time: _____ Flick# _____ (Time: _____ Flick# _____) Time: _____ Flick# _____ Time out: _____ ____ Centrifuge briefly to collect liquid
	____ Add 4 ul **RNase** (100 mg/ml) ____ Gently mix by pulse-vortexing for 15 s ____ Centrifuge for a few seconds to collect liquid Incubate at room temperature for 10 minutes Start incubation: _____ End incubation: _____
	Pre-warm 10 mM Tris-HCL at 56C
	____ Add 200 ul **Buffer AL**, pulse-vortex for 15 s ____ Centrifuge for a few seconds to collect liquid

	Incubate at 70C for 10 minutes Time in heating block: _____ Time out of heating block: _____
	____ Add 200 ul **ethanol (96-100%)** and pulse-vortex for 15 s. ____ Centrifuge for a few seconds to collect drops from inside the lid
	____ Add mixture to spin column in a 2 ml collection tube ____ Centrifuge at full speed (20,000 x g) for 1 minute. When centrifuging each wash, switch the direction of the caps. Tab direction?: _____
	____ Place the mini spin column in a clean 2 ml collection tube Discard the tube containing the filtrate.
	____ Open the spin column. Add 500 ul **Buffer AW1**. Close the cap. ____ Centrifuge at full speed for 1 minute. Tab direction?: _____
	_____ Place the mini spin column in a clean 2 ml collection tube Discard the tube containing the filtrate.
	____ Open the spin column. Add 500 ul **Buffer AW2**. Close the cap. ____ Centrifuge at full speed for 3 minutes. Tab direction?: _____
	____ Place the mini spin column in a clean 2 ml collection tube Discard the tube containing the filtrate.
	____ Open the spin column. Add 500 ul **Buffer AW2**. Close the cap. ____ Centrifuge at full speed for 3 minutes. Tab direction?: _____
	____ Place the mini spin column in a clean 2 ml collection tube Discard the tube containing the filtrate.
	____ Run an empty spin at full speed for 1 minute. ____ Place the mini spin column in a clean 2 ml collection tube Discard the tube containing the filtrate.
	____ 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.
	____ Add 50 ul **10 mM Tris-HCl** directly to the column. ____ Incubate at room temperature for 4 minutes Time start: _____ Time stop: _____
	____ Centrifuge at 8000 rpm for 1 min
	____ Place the mini spin column in a clean 1.5 ml collection tube (labeled elution2)
	____ Add 50 ul **10 mM Tris-HCl** directly to the column. ____ Incubate at room temperature for 4 minutes Time start: _____ Time stop: _____
	____ Centrifuge at 8000 rpm for 1 min
	____ Nanodrop, then store both elutions at -20C