**Modified Protocol for Qiagen’s DNeasy 96 Blood & Tissue Kit**

**for use in McKenzie Lab**

This protocol largely follows the Qiagen DNeasy 96 Blood or Cells Protocol with modifications made for extracting from low biomass swab samples of amphibian skin.

**Prepare stock solutions for a 250 sample kit:**

* Autoclave 50mL of deionized water.
* Make 125mL of 0.2M EDTA by mixing 9.30595g of EDTA into distilled water , adding NaOH pellets until pH=8 (186.119g/L). Filter-sterilize.
* 1M Tris-Cl purchased at pH 8. Already sterile.
* Make 10mL of 10% Triton X-100 by weighing 1.07g (1mL volume) and diluting in 9mL sterile deioinized water. Mix thoroughly.

**Lysozyme solution to lyse gram-positive bacteria:**

* 20mM Tris-Cl, pH 8
* 2 mM sodium EDTA
* 1.2% Triton X-100
* Immediately before use, add lysozyme to 20mg/mL

**Working solution (per reaction; multiply by number of samples + 1 “ghost”):**

* 3.6uL 1M Tris-Cl, pH8 (final concentration: 20mM)
* 1.8uL 0.2M EDTA (final concentration: 2mM sodium EDTA)
* 21.6uL 10% Triton X (final concentration: 1.2% Triton X-100)
* 153uL sterile distilled water (pH 8; NaOH, filter-sterilized)

Right before use, add 0.0036g (3.6mg) of lysozyme and mix thoroughly to get 20mg/mL

Example calculation for 21 rxn of 180uL

* 75.6uL of 1M Tris-Cl, pH8
* 37.8uL of 0.2M sodium EDTA (372.238g/mol)
* 453.6uL of 10% Triton X-100 (1.07 g/cm³ density)
* 3213 of sterile distilled water
* 0.0756g of lysozyme

**Important points and things to do before starting**

* Do NOT add ethanol to Buffer AL
* If necessary, redissolve any precipitates in Buffer AL by warming to 56°C.
* Before using for the first time, add the appropriate amount of ethanol (96-100%) to Buffers AW1 and AW2 to obtain a working solution. Mark bottles as having ethanol added.
* Mix Buffer AW1 before use by inverting several times.
* Preheat bead bath and single heat block to 37°C for use in step 2; then 56°C for step 4.

**Procedure with lysozyme incubation**

1. Resuspend bacteria in 180uL enzymatic lysis buffer (preparation above)
2. Incubate 30min at 37°C (Rebollar et al 2016, ISME); after incubation, change heating block to 56°C for next step.
3. Add 25uL Proteinase K and 200uL buffer AL (without ethanol); mix by vortexing
4. Incubate 56°C for 30 minutes
5. Add 200uL ethanol (96-100%) to sample, and mix thoroughly by vortexing. Ensure that sample is homogenous, and make sure to apply ALL sample (including precipitate) to spin column.
6. Pipet the mixture into a DNeasy mini spin column placed in a 2mL collection tube. Centrifuge at >= 6000 x *g* (8000 rpm) for 1 min. *Keep collection tube and set aside\**.
7. Place the spin column in a new 2mL collection tube. Add 500uL Buffer AW1 (Check: ethanol should be added already). Centrifuge for 1 min at >=6000 x *g*. *Keep collection tube and set aside\*.*
8. Place the spin column in a new 2mL collection tube. Add 500uL Buffer AW2 (Check: ethanol should be added already). Centrifuge for 3 min at 20,000 x *g* (14,000rpm) (or 15 minutes at 6000 x *g*). Discard flow-through, but put spin column back in same 2mL collection tube. (If top speed can’t get that high, do 6000rpm 15 min or 17,000g 5 min)
9. Centrifuge at 20,000 x *g* for 1 minute (17,000 x *g* for 2 minutes), to make sure it is super dry. No ethanol should remain on the membrane. *Keep this centrifuge tube\**
10. Transfer to a new 1.5mL or 2mL microcentrifuge tube (not supplied)—make sure it is clean as this tube will hold DNA elute. Incubate at room temperature for 5 minutes to ensure all ethanol has evaporated.
11. To elute for “E1” (elution 1):
    1. For high-yield samples add 200uL Buffer AE to center of the spin column membrane. Incubate 1 min at room temperature. Centrifuge for 1 min at >=6000 x *g*.
    2. For low-yield amphibian skin samples, add 50uL Buffer AE to centre of spin column and incubate for 1 min at room temperature. Centrifuge for 4 min at 6000rpm.
12. If desired, repeated step 11 in NEW centrifuge tubes. Label this tube “E2 [SampleID]”. Yield will be lower than elution 1. Do not elute more than 200uL into a 1.5mL microcentrifuge tube, otherwise the bottom of the spin tube will touch the DNA elute.
13. Once elutions are done, put spin column back in empty centrifuge tube from step 9.\*
14. Store eluted DNA at -20°C.

\* Keeping these tubes for later in case your extraction failed. It might be possible to “recover” DNA in case you made a mistake somewhere e.g. You forgot to add ethanol to AW1 so the DNA washed out into the collection tube; or the ethanol wasn’t evaporated properly in step 10, so DNA is still in the spin column.