**E.Z.N.A.® TISSUE DNA KIT**

**PROCEDURE**

**Benjamin Gallo & Brian Leydet**

[**bdgallo@syr.edu**](mailto:bdgallo@syr.edu)[**bfleydet@esf.edu**](mailto:bfleydet@esf.edu)

***This method is suitable for DNA extraction from tissue up to 30 mg. Yield vary depending on source. The protocol can be scaled up to accommodate larger samples*.**

1. Mince 20 mg tissue (+/-1 mg) and transfer to 1.5 mL microcentrifuge tube
2. Add 200 µL TL buffer
3. Add 25 µL OB Protease Solution. Vortex to mix thoroughly
4. Incubate at 55˚C overnight (Average 17 hours +/- 3 hours)
5. Centrifuge at maximum speed (≥10,000 x g) for 5 minutes
6. Transfer the supernatant to a sterile 1.5 mL microcentrifuge tube
7. Add 220 µL BL Buffer & Vortex (A wispy precipitate may form)
8. Incubate at 70˚C for 10 minutes
9. Add 220 µL 100% EtOH & Vortex
10. Transfer the ENTIRE sample to HiBind® DNA minicolumn in 2 mL collection tube. Centrifuge at max speed for 1 minute. Discard the filtrate and reuse the collection tube
11. Add 500 µL HBC Buffer (w/ proper dilution). Centrifuge at max speed for 30 seconds. Discard filtrate + collection tube.
12. Add 700 µL DNA Wash buffer (w/ proper dilution) into minicolumn inserted into new collection tube. Centrifuge at max speed for 20 seconds. Discard filtrate + reuse tube.
13. Repeat step 12 for a 2nd DNA wash buffer step.
14. Centrifuge the empty minicolumn at max speed for 2 minutes (removes trace ethanol)
15. Transfer minicolumn into nuclease-free 1.5 ml microcentrifuge tube. Add **200 µL Elution Buffer heat to 70˚C.** Let sit at room temperature for 2 minutes before centrifuging samples at max speed for 1 minute (Optional: repeat step).
16. Discard minicolumn. Store eluted DNA at -20˚C.
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**TAKE NOTE OF FOLLOWING MODIFICATIONS FOR SAMPLES:**

Final elution adjusted based on starting tissue mass where samples:

* <25 mg were eluted in 50µL and samples
* >25mg in 100 µL of EB buffer.
* >30mg and < 40 mg = 25% increase in the following reagents: TL Buffer (250 µL); BL Buffer (275 µL); 100% ethanol (275 µL).
* > 40mg processed according to the supplementary information provided by the manufacturer: 400 µL for TL Buffer, 420 µL each for BL Buffer and 100% ethanol respectively.

**16S rRNA PCR PROTOCOL**

**V6 – V8 PRIMERS**

**F: 5’-ACGCGHNRAACCTTACC-3’ (B969F)**

**R: 5’-ACGGGCRGTGWGTRCAA-3’ (BA1406R)**

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**Primers derived from Comeau et al. 2011. PCR methods closely follow procedures outlined in Comeau et al. (2017) and Microbiome Helper.**

**Link to Microbiome Helper:** [**https://github.com/LangilleLab/microbiome\_helper/wiki/Microbiome-Amplicon-Sequencing-Workflow**](https://github.com/LangilleLab/microbiome_helper/wiki/Microbiome-Amplicon-Sequencing-Workflow)

**A. PREPARING MASTER MIX:**

1. Order and prepare the PCR primers according to Comeau et al. (2017)
2. Prepare Master Mix as follows:

**Reaction Volume: 25 µL  
Sample added: 2.5 µL**

**Q5® Taq polymerase: 0.25 µL/sample (Taq = 2 U/µL)**

**Forward Primer (Ultramer): 5 µL / sample (primer = 1µM)**

**Reverse Primer (Ultramer): 5µL / sample (primer = 1µM)**

**PureStrand dNTPs: 0.5 µL/sample (dNTPs = 40 mM)**

**Q5® Taq polymerase buffer: 5µL/sample (buffer = 5X)**

**Nuclease free water: 6.75 µL/sample)**

* **Note: Addition of Forward/Reverse primer should be made PRIOR to adding in the additional reagents. Unlike routine PCR, primers are unique for each sample (given the barcode sequence for later NGS). It is advised to mix the non-primer reagents first, and then add 12.5 µL of this mix to the 10 µL of forward/reverse primer. Then simply add 2.5 µL of the sample to achieve the final reaction volume.**

**B. PCR Cycling Protocol;**

1. Initial Denaturation (95˚C) – 30 seconds
2. **35X** Denaturation (95˚C) – 30 seconds
3. **35X** Annealing (55˚C) – 30 seconds
4. **35X** Elongation (72˚C) – 30 seconds
5. Final extension (72˚C) – 300 seconds
6. Hold (4˚C)

**C. GEL ELECTROPHORESIS PROTOCOL**

1. Mix 75 mL 1X TAE buffer with 1.5 g of LE Quick Dissolve Agarose in a 150 ml Erlenmeyer flask. This will form a 2% agarose gel.
2. Cover the mouth of the flask with Chemwipes and microwave at intervals of 30 seconds. It usually takes approximate 75-80 seconds to liquefy the agar.
3. Carefully remove the molten agar solution from the microwave and allow it to cool on the lab bench for 1-2 minutes.
4. Pipette 7.5 µL of SYBR-SAFE solution into the agar solution. The solution may turn a light orange color. Mix the flask thoroughly.
5. Pour the agarose into the loading tray of the gel electrophoresis chamber. Make sure the tray is put sideways so that the gel does not run off the edges. Insert the gel comb into the appropriate slot when the agarose is still molten.
6. Allow the gel to solidify for 15 – 20 minutes. Carefully remove the comb and re-orient the tray so that the exposed well are closest to the negative anode of the chamber.
7. Add adequate 1x TAE buffer to the chamber so that it just covers the openings of the wells set by the gel comb.
8. Prep for loading the samples by taking a short piece of clean wax paper and pipette 1 µL dots of 9x loading dye for each one of your samples ( + the DNA hyperladder).
9. Begin loading the gel with the 100 bp DNA hyperladder. Load 1 µL of the hyperladder and mix it by pipetting up/down on the wax paper with the loading dye. Pipette this mixture into the first well of the gel.
10. Pipette 5 µL of your PCR sample and similarly mix with the 1 µL of the loading dye. Once thoroughly mixed, load onto the next well of the gel. Continue for the rest of your samples.
11. Place the lid on the gel electrophoresis chamber and run the gel for 40 minutes at 95 V (note the gel runs from - 🡪 +).
12. After completion, view the gel under a UV box to track the visible DNA bands.

**References:**

Comeau, A. M., Li, W. K. W., Tremblay, J-É., Carmack, E. C., Lovejoy, C. 2011. Artic Ocean Microbial Community Structure before and after the 2007 Record Sea Ice Minimum. *PLoS ONE* **6**(11): e27492. doi: 10.1371/journal.pone.0027492.

Comeau, A. M., Douglas, G. M., & Langille, M. G. I. 2017. Microbiome Helper: a Custom and Streamlined Workflow for Microbiome Research. *mSystems* **2**(1), e00127-16. doi:10.1128/mSystems.00127-16.