Boreal Genomics Aurora Nucleic Acid Extraction System
A.K.A: GYPSY DANGER

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DO EVERYTHING IN HOOD, UNLESS OTHERWISE SPECIFIED

<u>0.25x TBE Buffer</u>

- 10x TBE buffer
- Molecular Grade water
- 50 mL Falcon Tubes
- Parafilm

If necessary: heat 10x TBE in microwave until precipitate is dissolved; filter sterilize (0.2 μm). Dispense 1.25 mL 10x TBE into falcon tubes, bring to 50 mL with Molecular grade water (falcon tube 50 mL line is accurate). Do in bulk, with 500 mL or 1000 mL bottles of water. Label and seal with parafilm.

<u>Agarose gel</u>

- 0.2 g Seakem agarose
- 20 mL 0.25x TBE
- Glass bottle (acid washed, sterile)
- Oakridge tube (acid washed, sterile)
- Heat block

Set heat block to 90-95°C. Measure agarose powder with sterile scoop onto sterile foil. Move to hood, dump in glass bottle, then add 20 mL 0.25x TBE. Weigh bottle on scale. Microwave until agarose dissolves (15 seconds at a time; do not allow to boil over). Weigh bottle on scale and replace mass lost with molecular grade water. Move solution to Oakridge tube and place in heat block @ 90-95°C for 1 hour. Reduce temperature to 65-70°C. Allow to equilibrate for ~30 minutes. Makes 20 mL agarose gel. One run uses ~3 mL, and gel should not be allowed to solidify. Use within 3 days.

<u>Setting up Cartridge gel</u>

- Pins
- Well post
- Agarose
- 0.25x TBE
- Molecular grade water
- PCR tape

Place pins according to diagram. Place metal post in extraction well. In space between yellow pins, pipet 1.5 mL agarose, forming concave top. Watch for leaking. If it leaks, pipet agarose back out, remove pins, and remove leftover/solidifying gel with sterile pipet tip. Replace the

pins and try again. Pipet 2 mL agarose into space formed by the leftmost green pin. This will fill the area around the extraction pin. Add more agarose if necessary. Allow to solidify in hood for ~ 20 minutes.

Adding buffer and sample

Remove pins and extraction well post, being careful to not disturb agarose (twist the post carefully to remove). Add 5 mL 0.25x TBE to chambers A, B, C, D, E, and 4 mL to chamber F. Add 60 µL 0.25x TBE to the extraction well. Place a square of sterile PCR tape (cut from 96-well sheet) over the extraction well and press down to secure. Add sample to sample well, bringing to 5 mL with Molecular grade water if necessary. Place lid on cartridge.

Running Sample

Turn on gypsy danger, Thermo cooling block, and computer (Password: research). Ensure that cooling block is set to 20°C and press start ("*" = off, "-" = cooling). Open cartridge drawer, and squirt 1-2 mL on contact surface. Place cartridge on contact surface, with chamber E on the right and F on the left. Close drawer. Select protocol (Soil protocol HMW is commonly used, but other protocols are described in the manual). Save the run to a folder if desired. Hit the start button.

The machine will perform a contact test and injection conductivity test. For failures, see Common Errors section, or RTFM. If tests are passed, the injection charge counter will count down from 5000mC (for soil HMW). When counter reaches 0, the next block begins (~4 hours). Check on the machine occasionally to monitor temperature and operation.

Removing Sample

Open the drawer, remove the cartridge and move to the hood. Use a pipet to remove concentrated sample from the extraction well and place in (preferably) ultra-pure Eppendorf microcentrifuge tube.

Be sure to turn off gypsy and cooler.

Cleaning the cartridge

- 5+ Liters deionized water (sterile)
- Sterile foil
- Cleaning brush (small with rubber tip, in drawer)
- 10% bleach in Tupperware
- Sterile water in Tupperware

Dump contents of cartridge into the sink. Remove agar bits with the cleaning brush. Rinse the cartridge with tap water, removing any discolored liquid/agar debris. Rinse cartridge, pins, and

post with generously sterile water and place in 10% bleach bath for no more than 20 minutes. Remove from bleach, rinse generously with sterile water and place in sterile water bath for 20 minutes. Rinse again with sterile water and place everything on sterile foil. Place under UV in hood for 20 minutes, then flip pins and post, rotate cartridge 180°, and UV for 20 more minutes. Fold foil around everything to set aside for next use.

Common Errors and Issues

Failed contact test: RTE010

Ensure that there is water on contact plate. Reposition cartridge.

Failed conductivity test: RTE020

It is likely that the sample is too conductive (too salty), which reduces the injection efficiency. The sample should be diluted if possible. The ratio of sample conductivity to agar conductivity should be no more than 20%.

Cooling system temperature is out of range: RTE040, RTE041

Make sure you turned the cooling block on (most common). Cooling block may be malfunctioning (repaired in JAN 2018). Tubes going from cooler to gypsy may be kinked or blocked. Also check cooler for "tank level low" error. Add REGULAR TAP WATER to tank if necessary. DO NOT USE ANY FORM OF PURIFIED WATER.