**eDNA Preservation Experiment II**

**Longmire’s RT – DNeasy Kit**

Filters preserved in Longmire’s Buffer solution are stored at room temperature and were extracted using the DNeasy Kit protocol of Spear et al. 2015 with modifications: Proteinase K was added to sample and incubated overnight on day 1; sample was divided into two Qiashredder spin columns on day 2 moving filter into one spin column;

**Day 1**

1. Turn shaker to 55℃
2. Add 22.5 µL Proteinase K to each sample (final concentration 0.5 mg/mL [Longmire et al. 1997])
3. Incubate overnight at 55℃

**Day 2**

1. Move 225 µL sample into 4 labeled Qiashredder spin columns, moving whole filter into one of them
2. Continue with steps 11-29 in Ethanol-DNeasy extraction protocol
   1. Combine 4 samples during steps 17-19