Center for Anchored Phylogenomics, Lemmon Lab
Florida State University
September 2015

Initial Sample Preparation and Shipment:

DNA Extraction:

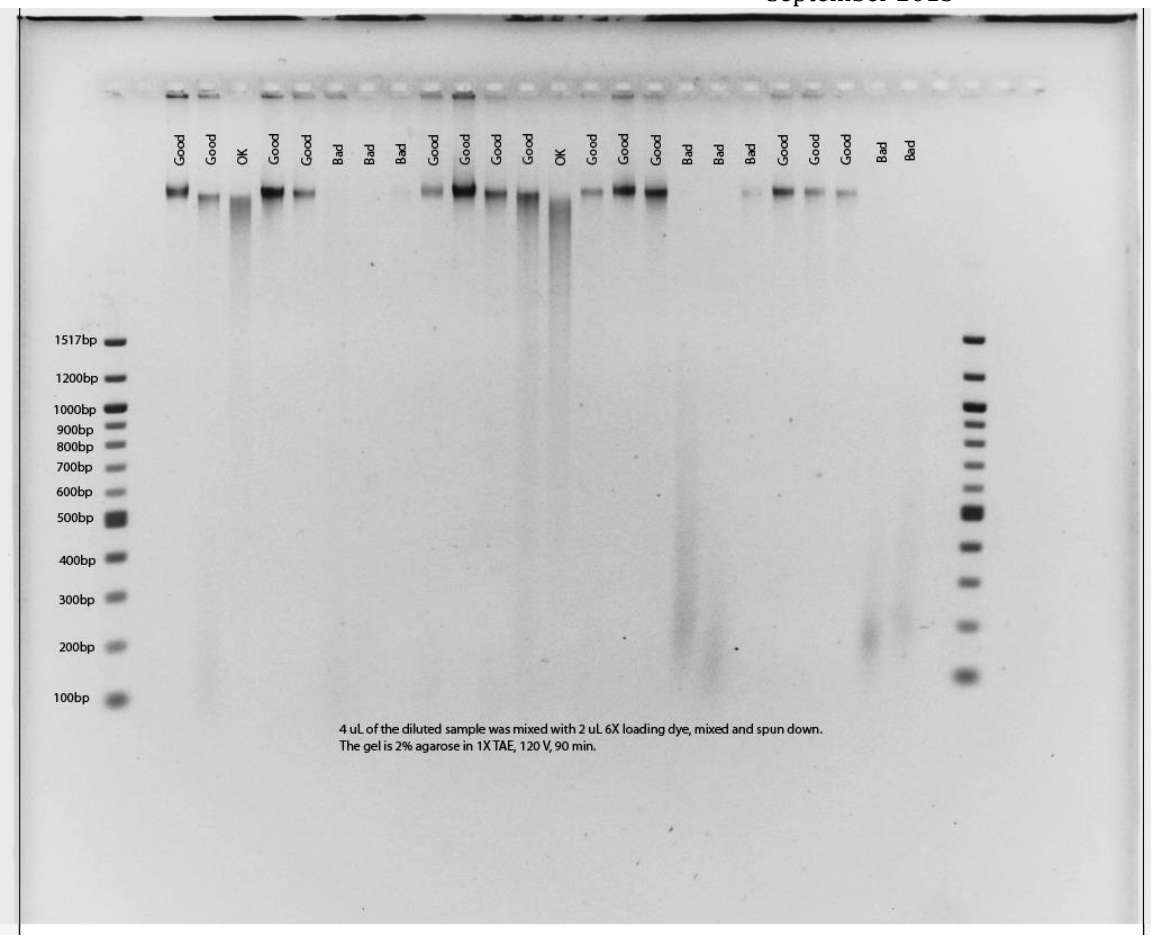
- Samples should be extracted with Qiagen DNeasy kit (or equivalent)
- Samples should be eluted into Tris EDTA, Tris, Qiagen EB, or equivalent (pH 8-9)
- **RNase should be used during extraction!**

Quantification:

- All samples should be quantified with a Nanodrop Spectrophotometer
- Preferred sample conditions: 2.3 micrograms in 130 microliters of buffer
- 260/280 and 260/230 ratios should both be above 1.8
- If you can not obtain this much DNA for a critical sample, contact the project manager to see if samples can still be processed
- For insects and other samples with low DNA quantities, we prefer to receive at least 200 nanograms, but contact us if you have lower quantities to discuss options

Running a gel:

- BEFORE shipping, a gel must be run and approved
- For final approval, send a picture of the gel to mk09g@my.fsu.edu and chorusfrog@bio.fsu.edu
- The gel should be a 2% agarose gel in 1x TAE (or equivalent)
- Samples should be prepared with 4 uL of sample and 2 uL of loading dye
- The gel electrophoresis should run for 60-90 min at 110 V.
- A ladder should be run for each row of samples (please send ladder description in gel approval email)
- An example of gel suitability is shown below: ("bad" quality samples will not be suitable for library preparation, and will need to be replaced)



- If the gel contains bright, low molecular weight smears, the sample might still contain RNA. Make sure that RNase was added earlier

Shipping:

- Samples should be shipped overnight in individually labeled tubes
- The "tissue template" spreadsheet should be filled out completely and both emailed, and sent in the sample box
- Please email the template to the following addresses: alemmon@fsu.edu , chorusfrog@bio.fsu.edu , and mk09g@my.fsu.edu
- Sample tubes should be placed in a box, then in a Ziploc bag, on dry ice
- Ship samples so they arrive on or before Friday to avoid weekend delivery **Do not ship on Thursday from West Coast**
- Ship samples to :

Emily Lemmon
89 Chieftan Way, Bio Unit 1
Florida State University
Tallahassee, FL 32306

Additional tips for preparing good quality DNA extracts:

- Muscle tissue usually works best
- Liver has lots of RNA and the DNA is often degraded, in addition, Liver tissue is regenerative so genomes can copy in unusual ways. It can be used if other tissue is unavailable.
- Overnight digestion in a rotary incubator is preferred, best done in 2 mL tube which has a flatter bottom and allows for more mixing
- Heat elution buffer or H_2O to 55 °C before elution step, incubate columns 2 min at room temperature before elution
- Perform two separate elutions with 30-50 uL each time
- To allow samples to be speedvac'd for removing residual EtOH, one can either use H2O to elute the DNA off of columns, or dilute the elution buffer to half strength with H2O. This avoids concentrating any salts in the extracted solution
- Qubit quantitation is preferable to Nanodrop if possible (Nanodrop does not distinguish between RNA and DNA. Anything that has a similar absorbance to DNA will be included in the nanodrop concentration estimate. Qubit targets and quantifies ONLY double stranded DNA)
- Gels should show a bright band close to the top of the lane
- A lane that has a band with some smearing is ok
- A lane that has a large smear without a band should work, but can be re-extracted if tissue is available
- A lane with a smear and no visible high molecular weight DNA probable contains RNA and should be treated with RNAse