DNA EXTRACTIONS- Arthropod Preparation

Preparation:

1. Lightly bleach work space and put on latex gloves.
2. Get specimens.
3. Get 1.5ml centrifuge tubes (autoclaved), label for each individual specimen. Label should include specimen ID.
4. Get pestles (bleached, rinsed, and autoclaved) if needed.
5. Check heat block is at lysis temperature (56^{o}C).
6. If you are using a new Qiagen kit, read notes and add ethanol to Buffers AW1 and AW2.
7. Write in lab book date/time and what specimens you are working on.

* Optional* Prepare vial with o-ring, label with specimen ID on top and side for archival storage of specimen

Grinding tissue:

1. Pull tissue off a specimen and place in 1.5ml centrifuge tube. Use legs for most insects. If specimen is very soft-bodied or small, use whole body for extraction. *Place rest of specimen in labeled storage vial with o-ring.
2. Use sterile pestle for each individual. Grind against tube wall until legs are thoroughly broken. Alternatively, use scissors or razor blade to dice up tissue, but flame sterilize the instrument for each specimen.
3. Clean instruments. (Soak pestle in 10% bleach solution, rinse with water multiple times, and autoclave)

Cell Lysis:

Qiagen Animal Tissue Protocol:

1. Lysis temperature should be 56^{o}Celsius.
2. Add 180ul Buffer ATL and 20ul of Proteinase-K. Cap tube securely.
3. Vortex sample thoroughly (5 seconds). Spin down briefly (5 seconds) in centrifuge.
4. Incubate 1 hour to overnight, occasionally vortexing sample.

DNA extraction:

Qiagen Animal Tissue Protocol:

1. Remove samples from 56^{o}Celsius and vortex thoroughly.
2. Optional RNAse A treatment (4ul of 100mg/ml solution, or 25ul of 4mg/ml solution): add RNAse A solution to room temp samples, leave at room temp for 2 min.
3. Add 200ul Buffer AL, vortex thoroughly.
4. Add 200ul of 100% ethanol and vortex solution.
5. Pipette this mixture into a DNeasy spin column attached to a collection tube. Centrifuge for 1 minute at 6000 x *g*. Carefully remove collection tub and flow through, and discard in a plastic beaker. This wash will go into the sink.

6. Add 500ul Buffer AW1. Centrifuge for 1 minute at 6000 x *g*. Carefully remove collection tub and flow through, and discard in a plastic beaker. This wash will go into the sink.
7. Add 500ul of Buffer AW2. Centrifuge for 3 minute at 20000 x *g* (full speed). Carefully remove collection tub and flow through, and discard in a plastic beaker. This wash will go into the sink.
8. Place mini-column in a 1.5ml tube.
9. Pipet **50-200ul** of Buffer AE or ddH2O directly onto mini-column membrane. Let sit 1 minute. Centrifuge for 1 minute at 6000 x *g*.
10. Repeat step 9 for large tissue samples.
11. Eluted sample should be stored frozen at -20 Celsius.

Clean-up:

1. Rinse bleached pestles several times in water, and twice in ddH20.
2. Place cleaned pestles in "To autoclave" container, cover with aluminum and autoclave tape.
3. Return pipettor, etc.
4. Pipette tips should be kept separate from other trash (they are "sharps").
5. Columns and centrifuge tubes can go in the trash.
6. Wipe down work area with 95% ethanol.

Additional References:

I suggest the following for more particular methods of extraction, including ancient DNA methods.

Nishiguchi, M.K. P. Doukakis, M. Egan, D. Kizirian, A. Phillips, L. Prendini, H.C. Rosenbaum, E. Torres, Y. Wyner, R. DeSalle, and G. Giribet (2002). DNA isolation Protocols. In: DeSalle, R., W. Wheeler and G. Giribet (eds.), Techniques in Molecular Systematics and Evolution, Birkhäuser, Basel, Germany, pp 243-81.

It is available online at: http://biology-web.nmsu.edu/nish/docs.htm