DNA Extraction Protocol – Gentra kit

**Cell Lysis**
1. Put 300 µl of Enzymatic Lysis Buffer into 1.5 ml pestle tube.
2. Place insect tissue in tube after dissection.
3. Crush this mixture with pestle.
4. Incubate at 37°C for 30 minutes.
5. Note: get 80°C incubation ready
6. Centrifuge at 14,000 rpm for 5 minutes and remove the supernatant and put in another 1.5mL tube.
7. Add 300µl of Cell Lysis Solution, pipet up and down (lyse cells).
8. Incubate at 80°C for 5 minutes (for complete cell lysis).
9. Add 5 µl of Proteinase K.
10. Vortex and incubate for 30 minutes at 55°C.

**RNase Treatment**
1. Add 1 µl of 100 mg/ml RNase A, mix by inverting 25 times.
2. Incubate at 37°C for 30 minutes.

Note: Make 1.5 ml tube that has 300 µl isopropyl alcohol for each extraction during incubation.

**Protein Precipitation**
1. Cool sample to room temp in ice.
2. Add 100 µl of protein precipitation solution.
3. Vortex 20 seconds, ice 5 minutes, centrifuge at 4°C 5 minutes (14,000 rpm), ice 5 minutes.
   After the centrifuge step handle tubes carefully. You DO NOT want to disturb the pellet.
4. Transfer the supernatant to a new tube with a pipet. (Keep spinning and removing to avoid floating proteins)
5. Spin this again at 14,000 rpm.

**DNA Precipitation and Elution**
1. Pipette the supernatant into the 1.5 ml tube containing 300µl isopropyl.
2. Invert tubes to mix. Place in freezer for at least 15 minutes. Overnight is best.
3. Centrifuge at 4°C, 5 minutes (14,000 rpm).
4. Discard supernatant.
5. Add 400 µl of 70% ethanol. Invert tubes gently to mix.
6. Let sit on ice 5 minutes. Centrifuge 5 minutes (14,000 rpm).
7. Discard supernatant. Tap tube on a clean Kim-wipe to remove remaining liquid.
8. Invert tube on a clean paper towel for one hour to dry. Pellet may not be visible at end of procedure.
9. Add 50 µl of low TE.
10. Flick tube and let sit ~1 hour at room temperature.

**Storage**
Short-term = 4°C                      Long-term = -20°C or -80°C

**Materials required**
- Gentra puregene tissue kit
- Low TE
- Isopropyl alcohol, 100% (isopropynol)
- Ethyl alcohol, 70% (ethanol)
10, 20, 200 and 1000 µL pipette tips
1.5 ml microcentrifuge tubes
1.5 ml pestle tubes
mini pestles

**Equipment required**

10 µL micropipette
20 µL micropipette
200 µL micropipette
benchtop centrifuge capable of 20,000 x g