DNA Extraction for high-throughput sequencing
Student Handout for Half Semester

DNA extractions will be performed using the Qiagen DNeasy Blood and Tissue DNA Extraction kit. The protocol has been slightly modified from the kit's standard extraction protocol to optimize results for insect microbiomes. Reagents for this protocol will have been aliquoted by your instructor for each group (4 students) and contains enough material for 2 DNA extractions. Each group of 4 students will process 1 beetle from each treatment (assuming you have 2 treatments), for a total of 2 beetles per group, one beetle per each pair of students. In addition, one group from each lab section also will be asked by your instructor to complete a negative control DNA extraction (consisting of all the steps in the DNA extraction process, but without a beetle sample.) The procedure below is for the DNA extraction of a single beetle.

Procedure

1. With a permanent marker, carefully label a sterile 1.5mL microcentrifuge tube with the following information:
    a. Your group name/number
    b. Beetle ID #
    c. Experimental Treatment Group
2. Select a beetle for DNA extraction and place it in the labelled microcentrifuge tube. Use one beetle per tube.
3. For the beetle selected, make sure to record the following information in your notebook.
    a. Group members names
    b. Beetle ID #
    c. Beetle life cycle stage (egg, larva, pupa, adult)
    d. Beetle sex (if adult)
    e. Beetle host bean type
    f. Experimental Treatment Group
4. Freeze the beetle at -20°C or -80°C for 5 minutes. Then take the tube back to your lab bench.

Surface Sterilization

1. Holding a beetle with forceps, surface sterilize the beetle by dipping beetle in
    a. 10% Bleach (3 seconds)
    b. Sterile Water (10 seconds)
    c. 70% Ethanol (5 seconds)
    d. Sterile Water (10 seconds)

2. Place the surface sterilized beetle in a new appropriate labelled sterile 1.5mL microcentrifuge tube.

Partial DNA Extraction

1. Add 180uL of buffer ATL and crush the beetle using a sterile microtube pestle. When the beetle is fully crushed, the mixture will be cloudy with small fragments of the beetle.

   The microtube pestle should be saved for cleaning, autoclaving and re-use.

2. Put on gloves and safety glasses. Add 20uL proteinase K. Vortex for 10 seconds and seal your tube with parafilm.

3. When finished, check your labels one last time to make sure that they are clearly written in a way that someone else can read them. At a minimum, your tube must be labelled with your group name, experimental treatment and "DNA". Give your tube to your instructor.