Half-Semester

DNA Extraction for high-throughput sequencing

Instructor's Notes

Below are equipment, supplies, and prep instructions for DNA extraction for high throughput sequencing for a lab class of 24 students, assuming 6 lab groups of 4 students. We assume that each group will perform 2 extractions (one for each treatment). In addition, one group from each lab section will complete a negative control DNA extraction (all of the steps of the DNA extraction process, but without a beetle sample.) In total, 13 extractions will be performed per class. The DNA extraction supplies that we send to you will include enough materials for a total of 16 extractions, so there are supplies for 3 extra extractions to accommodate student errors.

Equipment

- Vortex
- 1.5 or 2mL microcentrifuge tubes (nuclease free) (from Emory)
- P100 micropipette and sterile filtered tips (if available, if not, a P200 can be used)
- P200 micropipettes and sterile filtered tips (filtered tips preferred to prevent cross contamination)
- P1000 micropipettes and sterile filtered tips (filtered tips preferred to prevent cross contamination)
- racks for microcentrifuge tubes
- sterile tweezers
- Fine tip sharpies to mark tubes
- Parafilm

Reagents and Supplies

- Qiagen DNeasy Blood and Tissue, ATL Buffer and Proteinase K reagents (from Emory)
- Disposable pestles (from Emory)
- 70% Ethanol (molecular/reagent grade, not denatured)
- 10% Bleach
- Sterile Water

Prepare tips and microcentrifuge tubes

- At least one day prior to lab, autoclave 1.5-2mL microcentrifuge tubes and micropipetter tips on a standard dry cycle to insure that they are not cross-contaminated with bacteria that will be co- extracted, amplified, or sequenced.

Prepare aliquots of all extraction kit reagents

- Because the DNA extraction reagents will be used by several different groups, it is important to prevent accidental cross-contamination. The best way to prevent this

is by creating aliquots of all reagents for each group.

- Reagents should be a room temperature and shake all reagents well prior to aliquoting to individual microcentrifuge tubes
- Aliquot the following reagents into individual 1.5-2mL microcentrifuge tubes. Each group should get 1 tube of each of the following:
    - 400uL of Buffer ATL
    - 50uL Proteinase K

For more safety information on all reagents, check the Qiagen DNeasy Blood and Tissue Handbook available with kit.

Before class

- Prepare a series of tubes containing 10% Bleach, Sterile Water, and 70% Ethanol for students to surface sterilize beetles. The surface sterilization procedure is identical to that used previously before crushing beetles to harvest bacteria for streaks on agar plates (Culturing Microbial Communities protocol).

Important reminders for students

- All microcentrifuge tubes should be labelled so that they can be connected with sample information.
- Aseptic technique is important to avoid cross-contamination with either external bacteria that could obscure sequencing results or nucleases that could degrade DNA and result in low quality sequences.
- For each extraction, students should record the following data in their notes. This information is critical to pair the beetle sequence data with the correct group/treatment. Please collect this information from each group and send as a spreadsheet (hard copy or email) when returning the samples back to Emory.
    - Group name
    - Beetle ID #
    - Beetle life cycle stage (egg, larva, pupa, adult)
    - Beetle sex (if adult)
    - Beetle host bean type
    - Experimental Treatment Group

Remind students to save the plastic microtube pestles, as these can be sterilized and re- used in future labs (specifically the Culturing Microbial Communities lab).

After class

- Ensure that the tubes are sealed with parafilm to avoid the lids from opening during shipment.
- DNA should be stored at -20°C until shipment back to Emory.

Also, place the blue ice packs that came with the insulated box in -20°C so that they are frozen and ready to use for shipping samples back to Emory.

Shipping samples to Emory

- When you are ready to ship your samples, place the microcentrifuge tubes containing frozen DNA in a 50mL screw cap conical tube that is properly labeled with institution name and experimental treatment information).

Then, place the 50mL screw cap conical tubes containing microcentrifuge tubes in the insulated box with frozen blue ice packs. Bubble wrap may be added to the inside of the insulated box to pad the contents during shipping.