Full-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

- heat-block, incubator, thermomixer or water-bath that can be set to 56°C
- Vortex
- 1.5 or 2mL microcentrifuge tubes (nuclease free) (from Emory)
- Microcentrifuge
- 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 Kit (from Emory)
- 96-100% Ethanol (molecular/reagent grade, not denatured)
- Disposable pestles (from Emory)
- 70% Ethanol (molecular/reagent grade, not denatured)
- 10% Bleach
- Sterile Water
- Molecular grade water (nuclease free preferred)

Prepare tips and microcentrifuge tubes

- At least one day prior to lab, autoclave 1.5-2mL microcentrifuge tubes and micropipette 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 kit 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.
- Buffer AW1 and AW2 are supplied as concentrates. Before aliquoting into individual tubes, add the appropriate volume of ethanol (96-100%) to the stock (as indicated on the bottle) and shake thoroughly.
- Reagents should be at room temperature and shake all reagents well prior to aliquoting out into individual microcentrifuge tubes.
- Aliquot the following reagents into individual 1.5-2mL microcentrifuge tubes. Each group should get 1 tube of each buffer.
    - 400uL of Buffer ATL
    - 500uL of Buffer AL*
    - 1.1mL of Buffer AW1 (make sure ethanol has been added)*
    - 1.1mL of Buffer AW2 (make sure ethanol has been added)+
    - 50uL Proteinase K
    - 500uL 96-100% ethanol (reagent grade, not denatured)
    - 300uL molecular grade, sterile, nuclease free water

*Contains a harmful irritant and chaotropic salt, guanidine hydrochloride. Not compatible with disinfecting agents containing bleach as it can form a highly reactive compound when combined. If liquid containing this buffer is spilt, clean with suitable laboratory detergent and water.

+Contains sodium azide as a preservative.

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

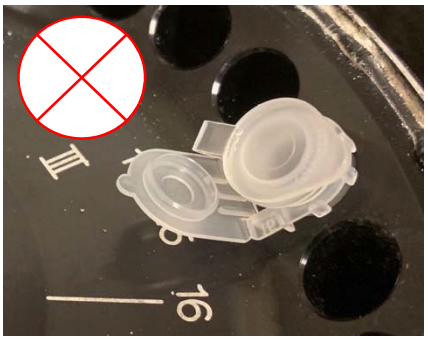
Before class

- Set heat-block, incubator, water bath, or thermomixer to 56°C
- 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).
- Each group should have 2 DNeasy Mini spin columns and 6 collection tubes.
- If nano-drop, qubit, or other DNA quantitation device is available, prepare materials for students to be able to quantify their DNA yields and ensure that extraction was successful. If these materials are not available, DNA can be quantified at Emory. Upon receipt of shipment, DNA will be quantified, and an email will be sent with notifications as to any extractions that did not succeed so students can attempt extraction one more time.

Important reminders for students

- All microcentrifuge and PCR 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).
- The final spin step (elution) is tricky because students will be eluting their DNA into a 2mL tube whose lid cannot fully close due to the spin column placed inside. These open tubes may make it difficult for centrifuges that require lids to properly secure prior to spinning. Students may need help loading the centrifuge to ensure that centrifuge lids are properly secured. If tubes are not loaded correctly, the tube lids may break off during centrifugation. To prevent this, tube lids should be loaded as shown in the photos below, with the outside of the hinge and lid facing up.



- Additionally, tubes should be labeled on both the tops of the lids and on their sides in case the lids do break off during centrifugation.

Troubleshooting

- If DNA extraction is unsuccessful at producing DNA yields, the Qiagen DNeasy Blood and Tissue Kit Handbook, which should be enclosed in your shipment, contains some helpful troubleshooting tips. Refer to the tips if needed.

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.

Note about timing: Undergraduates can successfully perform this DNA extraction in a 2-hour laboratory period. However, if you do not expect students to complete the full extraction, you should stop **after adding proteinase K**. Vortex for 10 seconds and seal each the microfuge tube with parafilm. Refrigerate the samples until ready to complete the extraction. Stopping at this point is appropriate if a sample is to be extracted later by a laboratory assistant, another laboratory group, or in another laboratory meeting.