Lab 2: DNA Extraction for Microbiome

Introduction: The microbiome of an environment consists of all microbiological organisms. These relative populations of these organisms can have a significant effect on the environment.

In this experiment, we will study the bacterial content of water from the three rivers of Pittsburgh. The overall protocol is as follows:

1. Collect water samples
   Approximately 1 L of water was collected from each of five locations as well as ultrapure filtered water (for control).
2. Filter organic material from water samples
   Approximately 300 mL of water from each location and control was filtered yielding three saturated filters per location and control. Filter papers were stored at -80 C in preparation for the lab.
3. Extract DNA from organic material
   You will do this step according to instructions below.
4. Amplify 16S gene using PCR
   The 16S gene is highly conserved across multiple species of bacteria which means the gene is present across all of the genomes. However, mutations have caused differences between the 16S genes of different organisms allowing for the identification of the source organism based on the 16S sequence.
5. Prepare Sequencing Library and sequence samples
   This step will be outsourced to ensure quality of sequencing results.
6. Analysis
   We will perform various analyses in the next computational lab (February 15).

Each student will choose one or more samples and execute the following protocol on each sample chosen. Your lab notebook only needs to cover this protocol and the QC following.

Quick-Start Protocol June 2016

DNeasy® PowerWater® Kit

The DNeasy PowerWater Kit can be stored at room temperature (15–25°C) until the expiry date printed on the box label.

Further information

- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Solution PW1 must be warmed at 55°C for 5–10 minutes to dissolve precipitates prior to use. Solution PW1 should be used while still warm.
- If Solution PW3 has precipitated, heat at 55°C for 5–10 minutes to dissolve precipitate.
- Shake to mix Solution PW4 before use.

1. Filter water samples using a filter funnel attached to a vacuum source. The volume of water filtered will depend on the microbial load and turbidity of the water sample.
   Note: Please see Types of Water Samples in the Hints and Troubleshooting Guide.
2. If using a reusable filter funnel, remove the upper portion of the apparatus.
3. Using two sets of sterile forceps, pick up the white filter membrane at opposite edges and roll the filter into a cylinder with the top side facing inward.
   Note: Do not tightly roll or fold the filter membrane. To see a video, please visit the DNeasy® PowerWater® Kit product page at www.mobio.com.
4. Insert the filter into a 5 ml PowerWater DNA Bead Tube.
5. Add 1 ml of Solution PW1 to the PowerWater DNA Bead Tube.
   Note: For samples containing organisms that are difficult to lyse (e.g. fungi, algae) an additional heating step can be included. See Alternate Lysis Method in the Hints and Troubleshooting Guide.

You will start on Step 5 with previously prepared filters.

We will show how to do this in class.

6. Secure the tube horizontally to a vortex adapter (Cat. # 13000–V1–15/13000–V1–5).
7. Vortex at maximum speed for 5 min. Centrifuge the tubes ≤ 4000 x *g* for 1 min at room temperature. (This centrifugation step is optional if a centrifuge with a 15 ml tube rotor is not available, but will result in minor loss of supernatant).
8. Transfer the supernatant to a clean 2 ml collection tube (provided). Draw up the supernatant using a 1 ml pipette tip by placing it down into the beads.
   Note: Placing the pipette tip down into the beads is required. Pipette until you have removed all the supernatant. Expect to recover 600–650 µl of supernatant.
9. Centrifuge at 13,000 x *g* for 1 min at room temperature.
10. Avoiding the pellet, transfer the supernatant to a clean 2 ml collection tube (provided).
11. Add 200 µl of Solution IRS and vortex briefly to mix. Incubate at 2–8°C for 5 min.
12. Centrifuge the tubes at 13,000 x *g* for 1 min.
13. Avoiding the pellet, transfer the supernatant to a clean 2 ml collection tube (provided).
14. Add 650 µl of Solution PW3 and vortex briefly to mix.
15. Load 650 µl of supernatant onto a MB Spin Column. Centrifuge at 13,000 x *g* for 1 min. Discard the flow-through. Repeat until all the supernatant has been processed.
16. Place the MB Spin Column Filter into a clean 2 ml collection tube (provided).
17. Add 650 µl of Solution PW4 (shake before use). Centrifuge at 13,000 x *g* for 1 min.
18. Discard the flow-through and add 650 µl of ethanol (provided) and centrifuge at 13,000 x *g* for 1 min.
19. Discard the flow through and centrifuge again at 13,000 x *g* for 2 min.
20. Place the MB Spin Column into a clean 2 ml collection tube (provided).
21. Add 100 µl of Solution EB to the center of the white filter membrane.
22. Centrifuge at 13,000 x *g* for 1 min.
23. Discard the MB Spin Column. The DNA is now ready for downstream applications.

This approach is different from what is normally done.

Use 40 uL elution buffer to increase the final concentration of DNA.

Quality Control and Extraction Assessment:

After the lab, your instructors will perform various quality control steps to assess your assessments. These results will be deposited on the shared course data spreadsheet (find the link on Canvas).

Quantity – We are looking for as much DNA as possible.

Quality/Purity – The A260/A280 ratio will tell us how pure the resulting samples are. A sample with excellent purity will have a ratio of approximately 1.8.

Figure Assignment:

Please generate a publication grade figure showing the mean and standard deviation of DNA quantity and quality yields for all locations and the negative control. You need a caption describing the figure as well. Refer to Canvas rubrics for requirements.