DNA EXTRACTION FROM WATER SAMPLES

Last week, we collected samples to establish a long-term sampling site for a time-series that subsequent Carleton students will be analyzing. This week, we're going to be extracting DNA to learn about how that process works, and to think about how the steps of DNA extraction might affect your downstream genomics or bioinformatics analyses. You'll be working in the wet lab today in groups of 2.

Each group (pair) should have:

- Pipettes (1ml, 200 µl, and 10 µl)
- Pipette tips (1ml, 200 µl, and 10 µl)
- A microcentrifuge rack
- A tube holder
- Two sharpies (one thick and one thin)
- A Sterivex filter
- A 3 ml syringe
- A 5 ml syringe
- A vortexer
- A timer
- Solutions ST1B, ST2, ST3, ST4, ST5, ST6, ST7
- Inlet and outlet caps
- Bead tubes
- Binding columns
- 5 ml collection tubes
- 2.2 ml collection tubes
- A heating block with a thermometer
- A microcentrifuge

Wear gloves at all times when handling tubes and solutions.

1. Make sure that both ends of the Sterivex are capped with the Inlet and Outlet caps and place the Sterivex vertically on a tube rack.

2. Remove the inlet cap and add **900 µl of Solution STI1B** (stored in the refrigerator) using a pipette tip. Insert pipette completely into the inlet so that the pipette tip is visible inside the unit above the membrane.

3. Re-cap the inlet and secure the Sterivex filter horizontally, with the inlet facing out, to a vortexer.

4. Vortex at **minimum speed** for 5 minutes.

5. While vortexing, put solutions ST2 and ST4 into the tube rack in the 65˚C oven to warm them.

6. While still attached to the vortex adapter, use a Sharpie to make a mark on the underside of the Sterivex. Then rotate the Sterivex 180 degrees so that the mark is facing up.

7. Vortex at **minimum speed** again for 5 minutes.
   (What's happening: solution ST1B releases the cells and helps pull the microbes off of the Sterivex filter and into the solution so we can pop them open and take out their DNA.)

8. Set the Sterivex with the inlet facing up on your tube rack and remove the inlet cap.

9. Add **900 µl of ST2** using a pipette tip. Re-cap the inlet.
   (What's happening: Solution ST2 is a strong lysing agent that includes a detergent that can break cell walls, and it will remove non-DNA organic and inorganic material. NOTE! *It should be warm when you use it.)*

10. Incubate the Sterivex at **90˚C for 5 minutes**. If they don't fit all the way in the bath, flip them over halfway through your 5 minute incubation time.
    (What's happening: Heating the Sterivex with these solutions will help pop open the cells, especially the really stubborn ones. Like archaea or fungi. NOTE! These Sterivexes might warp or leak. Watch them like a hawk! Make sure the bath doesn't get much above 90˚C. If they start to warp too much, take them out of the water bath. The 5 minutes isn't a do-or-die amount of time.)

11. Cool the Sterivex at room temperature for **2 minutes**. After cooling, make sure the caps are on tight.

12. Stick the Sterivex on to the vortexer, with the inlet facing out. Vortex at **maximum speed for 5 minutes**.
    (What's happening: Vortexing helps shake the microbes off the filter and helps lyse the cells.)

13. Set the Sterivex on the tube rack with the inlet facing up, and remove the inlet cap.

14. Pull back the plunger of a 3 ml syringe to fill the barrel with 1 ml of air, and then screw it on to the inlet of the Sterivex. Flip the whole thing upside down so the syringe is on the bottom.

15. Push the air from the syringe into the Sterivex slowly, and then release the plunger. The air will displace the fluid, and the fluid will start to push into your syringe. (Cool, huh?) Gently pull back the plunger to pull as much fluid as possible into the syringe. Detach the syringe from the Sterivex.
    (What's happening: you've shaken and lysed all the cells that were on your filter, and now they're in the fluid. You're removing the fluid from the Sterivex for further processing.)

16. Add the fluid from your syringe into a 5 ml bead tube. Screw the cap on tight.

17. Attach the bead tube with your fluid on to a vortexer.

18. Vortex at **maximum speed for 5 minutes**.
    (What's happening: the bead beating step is really important—it's kind of like a blender for your cells. It homogenizes the mixture and pops the cells open (see a pattern here? These cells can be very stubborn.) The cells have now been lysed using both chemical and mechanical means.)

19. Centrifuge the tube at **4000 x g for 1 minute** at room temperature. (Use the centrifuge on the 3rd floor. Team up with other groups, and make sure the centrifuge is balanced!)

20. Transfer the supernatant to a clean 2.2 ml collection tube.
    (NOTE! Get as much supernatant as possible. It's okay if you get some beads into your pipette tip—it won't affect subsequent steps.)

21. Add **300 µl of solution ST3** and vortex briefly to mix. Incubate at **4˚C for 5 minutes**. (Use the fridge.)

(What's happening: Solution ST3 removes additional non-DNA organic and inorganic material including humic acid, cell debris, and proteins. Some of these compounds can inhibit downstream DNA applications, like PCR.)

22. Centrifuge the tube at **13,000 x g for 1 minute**.
(NOTE! This equates to 12621 RPM on an Eppendorf 5415C microcentrifuge. If we aren't using the same centrifuge, and the x g → RPM conversion isn't on the unit, Google it!)

23. Transfer the supernatant to a clean 5 ml collection tube. Try to avoid the pellet.
(What's happening: The pellet at this point contains additional non-DNA organic and inorganic material. This can mess with downstream applications, so try to avoid it.)

24. Add **3 ml of solution ST4** to the 5ml collection tube containing your supernatant, and vortex to mix. Put it onto the tube rack.
(What's happening: Solution ST4 is a high concentration salt solution. Once we add the salt solution, it allows the DNA to bind to the silica membrane you're about to push it through, while all the non-DNA organic and inorganic material will flow right through. NOTE! Solution ST4 should be warm when you use it.)

25. Remove the plunger from a 20 ml syringe and place the syringe firmly into the binding column. Put that onto the tube rack.

26. Pour the contents of your 5 ml collection tube in to the syringe/binding column unit.

27. Stick the plunger of your syringe back into the top of the syringe/binding column unit. Hold the syringe/binding column unit above the 5 ml collection tube that you just emptied. Slowly depress the syringe so that the fluid flows through the binding column and into the 5 ml collection tube.

    (What's happening: the DNA is getting bound to the filter membrane of the binding column, while the contaminants pass through the silica filter membrane and into your 5 ml collection tube.)

28. Remove the syringe from your binding column. Place the binding column into a clean 2.2 ml microcentrifuge tube.

29. Add **700 µl of solution ST5** to the binding column. Close the cap on to the top of the binding column.
(What's happening: Solution ST5 is a prewash to help remove residual contaminants, which will result in higher DNA purity and yield.)

30. Centrifuge for **1 minute at 13,000 x g**. Remove the binding column from the tube, discard the fluid that flows through into the 2.2 ml microcentrifuge tube, and put the binding column back in the emptied microcentrifuge tube.
(Remember! Right now your DNA is attached to the silica membrane in the binding column, it's not in the fluids.)

31. Shake to mix solution ST6. Open the cap and add **700 µl of solution ST6** to the binding column. Close the cap on to the top of the binding column.

32. Centrifuge for **1 minute at 13,000 x g**. Remove the binding column from the tube, discard the fluid that flows through into the 2.2 ml microcentrifuge tube, and put the binding column back in the emptied microcentrifuge tube.

33. Open the cap and add **700 µl of solution ST5** (yes, again) to the binding column. Close the cap on to the top of the binding column.

34. Centrifuge for **1 minute at 13,000 x g**. Remove the binding column from the tube, discard the fluid that flows through into the 2.2 ml microcentrifuge tube, and put the binding column back in the emptied microcentrifuge tube.

35. Centrifuge the tube again at **13,000 x g for 2 minutes** to completely dry the membrane. *(What's happening: the second spin helps to remove residual solution from the membrane. It's important to remove all of the solution because there is ethanol in solution ST5, and that can interfere with downstream DNA applications like PCR, restriction digests, and gel electrophoresis.)*

36. Transfer the binding column to a new 2.2 ml collection tube and add **100 µl of solution ST7** to the center of the while filter membrane. Close the cap on to the top of the binding column.
(What's happening: Solution ST7 does not have any salt, and it will release the DNA from the silica filter membrane. It will elute from the membrane into the tube below during the next centrifugation step.)

37. Centrifuge at room temperature for **1 minute at 13,000 x g**.

38. The DNA in your tube is now ready for downstream application!

39. Use the Nanodrop on the 3rd floor (same room as the big centrifuge) to quantify your DNA. Bring a 10µl pipettor and clean tips with you, and follow the instructions on the screen. Pipette 1 µl of a blank or of your sample on to the stage of the Nanodrop. Record your final DNA concentrations.

40. Label your sample with your name, date, and sample number. Store your extracted DNA in a -80˚C freezer in a microcentrifuge tube box.

Lab Questions

Submit a Word document with your answers on the Moodle by lab time next week:

1. Describe your sample site and your sample procedure as if you were writing a paragraph for a Methods section for a lab report. Be sure to include all important and relevant details, but don't include a blow-by-blow analysis. (For the DNA extraction, you can simply write: "We extracted DNA from the Sterivex using the MoBio PowerWater Sterivex Extraction kit according to the manufacturer's directions." No further explanation is necessary. This is typical for most scientific publications.)
2. Briefly describe the metadata you took along with your sample as if you were writing a paragraph for a Results section for your lab report. In addition to this, please include the concentration of your final DNA extract.
3. Some of the steps in our DNA extraction procedure, including the chemical and mechanical disruption of the cells, could result in physical shearing of the genome into smaller pieces. Would this have an important impact on downstream analyses if you were conducting a bioinformatics project focusing on genome structure? How about if you were interested in microbial diversity? Explain your reasoning.
4. This sample was collected for the purposes of creating a metagenome. You are interested in comparing and contrasting community diversity and metabolic differences between the samples. Describe what sequencing platform you would select for this project and provide justification for your choice.
5. An important decision that often has to be made for most 'omics projects is the DNA extraction method, because this may have an impact on downstream analyses. Conduct a literature search for papers that specifically tested how DNA extraction procedures affect bioinformatics analyses. Select two papers and describe the following things for each of them:
    a. Which paper did you examine? (Provide a citation.)
    b. What DNA extraction methods did they test?
    c. What kind of sample were they extracting DNA from?
    d. What metrics did they use to assess the performance or effect of the DNA extraction method on downstream analyses?
    e. Which DNA extraction procedures performed the best, according to these metrics?
    f. Describe whether you think these results would be broadly applicable across many sample types (i.e. water, soil, plants, human saliva, feces, tissue, etc.) and explain your reasoning.