

FIELD SAMPLING

There are tons of DNA databases containing billions of sequences that we can mine to our hearts' content. But those sequences didn't appear out of thin air. Every sequence was sampled from a human, plant, animal, fungus, microbe or virus, and then the DNA was extracted, prepared for sequencing, and then sequenced on a sequencer.

There are many steps that must be considered to get the best sequences for your project—how much sample do I need? What other data do I need to collect along with that sample? What's the best way to collect the sample? How do I extract the DNA? How do I clean it? What do I need to do to prepare the sample for sequencing? Much of this, of course, depends on the nature of your sample, what sequencing method you're using, and what kinds of questions you're asking.

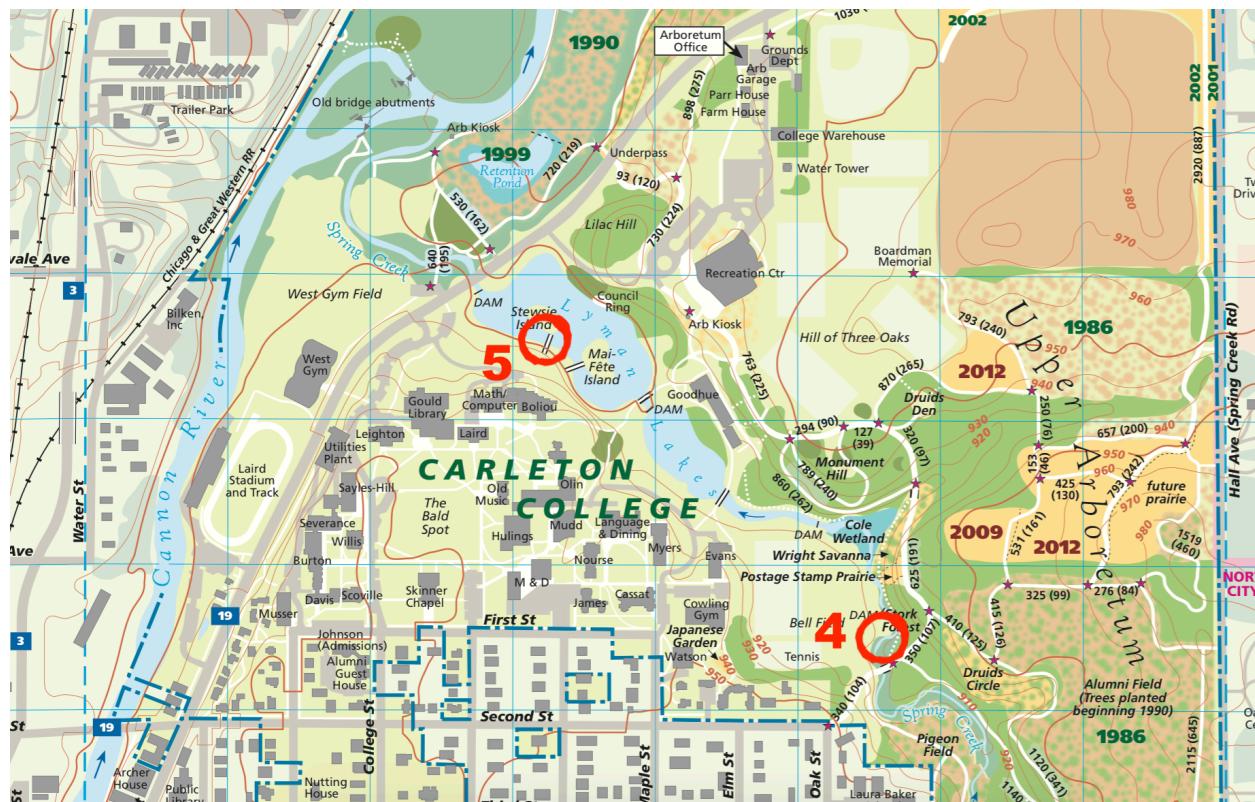
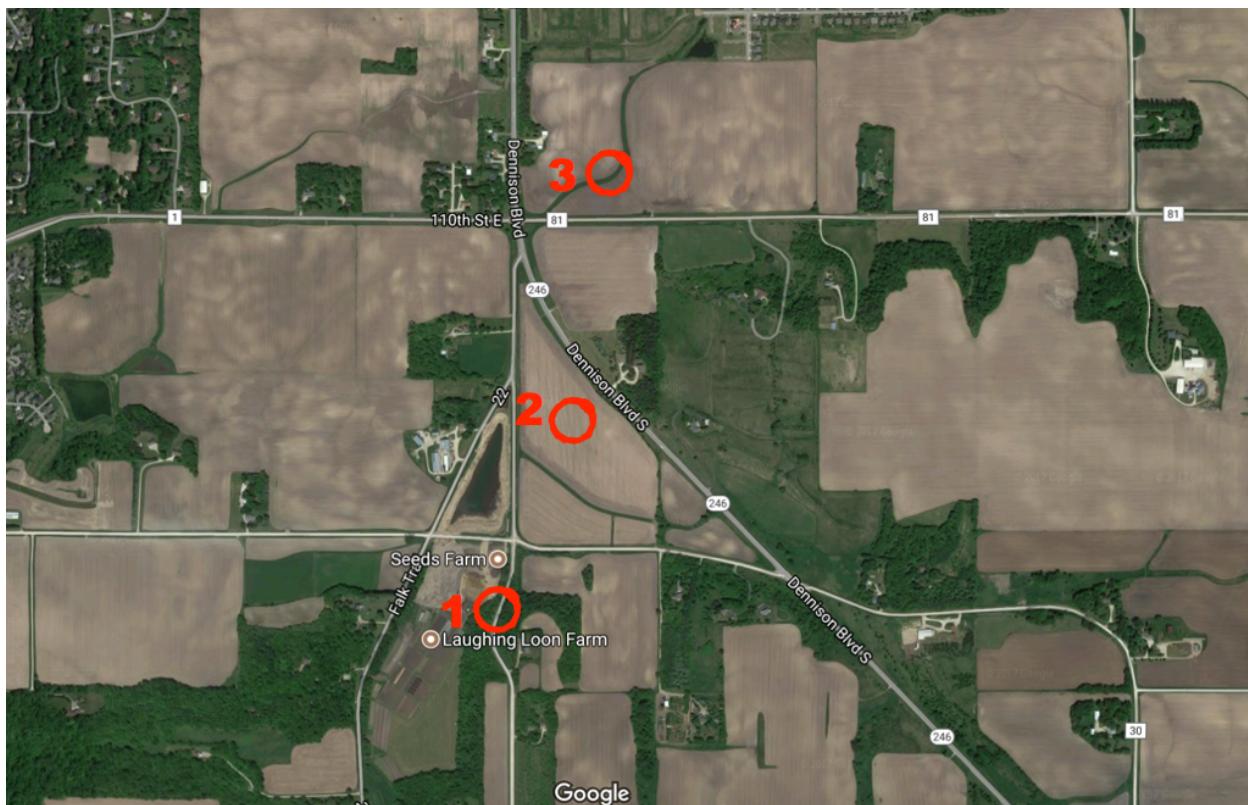
This week and next, we're going to walk through the first few steps in that process—taking a sample, recording metadata, extracting DNA from the sample, and quantifying the DNA. If you were to do the whole process, you'd also prepare a library for sequencing on an Illumina, PacBio, or other platform. We don't have the equipment needed for library prep here, so we will extract the DNA, quantify it, and then send it to a sequencing center. Our aim is to start a long-term sampling site that students in subsequent years will use for their own projects for this course.

Sample sites:

We're going to be collecting samples from ponds, lakes, rivers, and streams near the Northfield area. Our goal will be to establish a time series for this course that will monitor microbial ecology and evolution over time and space. We'll be able to learn about how microbial metabolisms and diversity change from one water body to the next over space and time, how genomes might change and evolve in response to selection pressure, and even how microbial diversity, genome structure, or metabolism might respond to changes in flooding events or climate change.

Here are the samples we'll collect:

1. Brand new flood retaining pond at the outskirts of Northfield: this was built as a way to ease the pressure on streams and lakes during flooding events. We'll track the microbes in this pond as the pond gets established. We also know that flooding events are becoming more common as climate changes, which may affect the way microbes respond and evolve.
2. Upstream of the flood retaining pond
3. Downstream of the flood retaining pond
4. Lyman Lakes: we will monitor the lakes over time to see if the microbial community changes in response to changes in campus architecture, flooding events, and climate change.
5. Upstream of Lyman Lakes
6. Cannon River: we will monitor the microbial ecology and evolution in the Cannon River to see if the response is the same or different from the other water bodies we are studying.
7. Downstream of the sewage treatment plant, Cannon River: we will contrast the microbial community downstream of the outflow of the sewage treatment plant with the microbial communities in the water bodies upstream of the plant, and track those communities over time.





Sample method:

1. If sampling from a deep lake with a boat or a bridge you can stand on, use a Niskin bottle. If sampling from a shallow stream or pond, use boots or waders.
2. Use a sterile, autoclaved plastic bottle to collect a water sample from the surface of the water. Rinse the bottle three times first with the water, then fill it to the top from the surface of the water. Screw the top back on.
3. Move back to dry land. Use a 60ml syringe to filter your water sample through the Sterivex filter. Fill the syringe with water from your sample bottle. Screw the end of the syringe on to the inlet (see image) and push your sample through. Filter as much through as you can. If you can filter 1L that would be ideal; if your sample is turbid and clogs the filter, try to sample at least 500 ml. Discard the water that filters through; keep the filter. Keep track of how much water you have filtered through the Sterivex.
4. Cap both ends of the Sterivex with the provided caps. Put the Sterivex into a Falcon tube, label the Falcon tube with information about your group and your sample site and number, and then place the Falcon tube into the cooler with dry ice.
5. When back in lab, store Sterivexes at -80°C until you are ready for DNA extraction.

INLET



OUTLET

Metadata:

It is crucial to collect metadata (a set of data that gives information about other data) along with any samples you take so that you can incorporate that metadata into your downstream analyses. We will be using YSI meters to record metadata like water temperature, pH, oxygen, and salinity. Since each pair of students will probably sample only one or two locations, use this table to record the following metadata in the field. When you return to lab, enter it into the class spreadsheet on Google Drive (see the Moodle).

Metadata	Sample site	Sample site
Date and time		
Location (latitude/longitude)		
Oxygen		
Temperature		
pH		
Salinity		
Amount of water filtered through Sterivex		
Depth of the water at sample site		
Other description of sample site		
Recent weather events		