LAB MANUAL

Urban #LandBack: Indigenous Peoples, Soil Science, and Disruptive Sequencing Technologies.

SING Canada
Summer internship for INdigenous peoples in Genomics

In partnership with

LifeLabs®

GenomeCanada

Oxford NANOPORE Technologies

Digital Research Alliance of Canada | Alliance de recherche numérique du Canada

TABLE OF CONTENTS

ABOUT SING CANADA 2023

In 2023, a multi-disciplinary team of faculty members from Canada and the US will partner with participants in an unprecedented comparative soil microbiome analysis with the goal of integrating Oxford Nanopore MinION sequencing technologies. Given the growing need to conduct research and training remotely, a focus on portable sequencing technologies is an opportunity to train advanced students in ways that are also well-suited to community-based Indigenous research.

The goals of the workshop will be to introduce participants to mobile sequencing technologies and their role in metagenomic soil science. Participants will learn the techniques of identifying soil microorganisms from their DNA, assessing their relative abundance, and comparing metagenomic composition across different collection sites. This year we are partnering with the Indigenous Knowledge and Wisdom Centre (IKWC) a collective of First Nations peoples which governs kihcihkaw askî (Plains Cree meaning “Sacred Land”), the first urban Indigenous ceremonial ground in Canada. Our metagenomic work will support IKWC by characterizing metagenomic composition across the conservation area.

You will be trained in these approaches through the prism of an Indigenous relational research methodology. We will consider soil composition in a framework that understands differing land-based relations as impactful to humans and more than human beings. Participants will also consider the ways that mobile technologies might expand Indigenous governance in their territories through the use of genomic technosciences. They will assess their potential to unsettle and thus productively transform the conventional genome “lab” by shifting the spatial and disciplinary contours of scientific knowledge production and enabling another pathway for Indigenous-centered genomics. That is, in this workshop you will transform not only where we do science but how.

Eve Tuck and K. Wayne Yang tell us that decolonization is not a metaphor, but rather it is about the return of Indigenous life and land. We acknowledge that there is no outside of colonial power in the genome lab or with genome technologies as we now know them. By evaluating the multiple meanings of #LandBack, we seek to intervene in the ways that the genome lab has been central to colonial processes of Indigenous racializations and ongoing dispossessions, and is therefore a site in need of reinvention. Decolonizing genomics therefore requires us to 1) operate beyond the physical, disciplinary, and methodological bounds of the lab; and 2) orient transformative pathways among STEM fields in ways distinct from increasing calls for equity, diversity, and inclusion (EDI) to, instead, hold Indigenous land, life, and relations (in a word, governance) centrally.

COVID AND SAFETY PROCEDURES

While COVID-19 restrictions and safety measures have been lifted across Alberta over the past year, the safety of our participants and the SING Team remains a priority. If you become sick during the course of the workshop, we ask that you stay home. We will have many COVID antigen tests on hand for those who may need them. The lectures and discussions will also be available via zoom for those who may require the option of joining sessions remotely.

ART, SCIENCE, & LIVING NOTEBOOKS

With Daphne Boyer

Please read the following section. As the workshop commences, begin to think about words, photos, sketches or imaginings that resonate with this gathering and experience.

My name is Daphne Boyer (IG @daphnebboyer). I am a plant scientist and visual artist of Métis descent. Recently I invented three photo-based, digital techniques that mirror and celebrate the spectacular art forms of my Indigenous ancestors - beading, quillwork and moose tufting.

This year, as part of SING 2023, we're going to make some art together. Each of you will receive your own art-making kit that features my beading and quillwork designs. You will be able to use my materials to create a collage that reflects your internship experience. All materials are supplied.

Before the programme begins, please create a safe place where you can record words, photos, sketches or imaginings - digitally or physically - of those things that attract your attention during the week. Perhaps they shimmer, speak to you or make you laugh. Stay open, tune in, and make friends with them when this happens. Capture at least part of them in whatever way you please - consider these as creative 'notes to self'.

You will use these 'notes' as inspiration to create your collage. On Friday, all collages will be assembled in a grid for a group photoshoot in celebration of SING 2023.

This is not a competition, there is no right or wrong. Leave your inner critic at the door...it's play time.

“Mycelium and VOCs” by Daphne Boyer, digital beadwork printed on peel-and-stick vinyl. This is but one example of the type of art we will create together. Bring your own ideas, feelings, and individual styles to this exploratory session.

Art helps to process the world around us, giving body to our thoughts

---

Respectful Research and Sampling

Place-based research involves respecting the beliefs, traditions, values, and protocols of local Indigenous peoples as well as of yourselves. Through engagement with our partners at the Indigenous Knowledge and Wisdom Centre (IKWC; the stewards of kihcihkaw askî), and our invited Elder Betty Letendre, we will be providing a tobacco protocol and engaging in an honouring ceremony to ensure the work and learning we are doing is conducted in good spirit and good relations. We ask that you consider the following during your visits to kihcihkaw askî and when handling the soil samples, DNA extractions, and data from the work you do:

- Treat the land and your greater-than-human relatives with kindness and respect.
- Minimize your impact in the area and try not to disturb the vegetation.
- Be respectful of the sweat lodge circles.If you are uncertain about respectful behavior in this context, please feel welcome to ask Jess, Warren, Kim, or Laurie.
- Soil sampling is a temporary exchange. Tobacco protocol will be provided to allow us to work with the soils so we may learn from them. Upon completion, the soils will be returned to the land. Spilled soils (e.g., in the lab) will be collected and returned rather than put into a waste bin.
- The data produced will be for reciprocal purposes, and will primarily benefit our collaborative partners at IKWC to aid their governance of kihcihkaw askî.
- Data governance agreements are ongoing consensual discussions that prioritize IKWC's interests and stakes in our work, writing, and presentation of this study.

FIELD SITE AND Urban #LANDBACK

This year, SING Canada will be producing an initial baseline survey of the microbial composition of the soils at kihcihkaw askî.

Local Cree, Nakoda, and Métis peoples, and many other travelers and visitors, have been holding relationships with the lands, air, water, and more-than-human kin in Amiskwaciwâskahikan/Edmonton (ᐊᒥᖏᑲ·ᑎᐊ̇·ᖏᑲᐟᐃᑲᐢ), leaving an irrefutable imprint on the local macro- and micro-biodiversity, despite colonial dispossession.

kihcihkaw askî ("Sacred Land" in Cree) in Whitemud Park at 14141 Fox Drive NW provides an urban space for Indigenous peoples, groups and communities in the region to host spiritual ceremonies, sweat lodges, cultural camps and talking circles, grow medicinal herbs and facilitate intergenerational learning in an appropriately designed outdoor learning space. The kihcihkaw askî site, located at 14141 Fox Drive NW, is historically and culturally significant and has served as a ceremonial site in the past. Long before becoming farmland, the kihcihkaw askî site was used for many centuries by the Indigenous people foraging for medicines for healing purposes. Ochre, a rare mineral, is also found close to the site and was used in spiritual and traditional ceremonies. Due to this significance, the Fox Farms site was chosen for the kihcihkaw askî project. (Source: City of Edmonton)

Whitemud Creek and the Whitemud Park South Trail are accessible beyond the fence of kihcihkaw askî. The creek flows into the North Saskatchewan River, which takes its name from the Plains Cree name kisiskâciwanisîpiy meaning swift flowing river.

Our visiting this land, and once again exercising relationships with its soil (to learn from its microbiome composition and health) is a powerful form of self-determination that is embodied in the #LandBack movement. This is also a stepping stone towards developing independent research capacity within SING Canada, which in future years will contribute to co-produced genomic research questions with Indigenous communities. We thank you for participating with us on this journey of growth.

Web Resources:

https://www.ikwc.org/kihciy-askiy/

https://www.edmonton.ca/projects_plans/parks_recreation/kihcihkaw-aski-development

https://www.cbc.ca/news/canada/edmonton/kihcihkaw-ask%C3%AE-edmonton-urban-cultural-site-healing-prayer-1.6794291

Field Site: kihcihkaw askî

RECOMMENDED FIELD ATTIRE/SUPPLIES

For the second day of the workshop when we will collect and analyze soil samples in the field, the following attire and supplies are recommended but not required.

- Hat
- Long sleeves and pants
- Long socks and closed toed shoes
- Bug spray/tick repellent (spray and lotion provided)
- Sunblock

FIELD LAB

Participants will be grouped into teams of 2 or 3. Each person will collect **three soil samples** and evaluate two of them, with the third replicate being completed by the faculty team.

Each lab team will have a **field kit** with many of the necessary consumables and reagents needed for soil collection, extraction and quantitation of soil DNA, quantitation of DNA extracts, genome library construction, and Nanopore sequencing on the MinION Mk1C. We will have a central mobile sequencing **field lab** with all the major lab equipment necessary to complete this work. Major equipment you can find at the central field lab includes pipettors and tips, scales, bento labs (minifuge and PCR/incubator), Qubit fluorometer, and MinION Mk1C sequencers. To assess the potential of mobile sequencing technologies to disrupt the conventional lab, much of the lab process will be conducted on location in the field.

RESEARCH COMPUTING AND ANALYSIS

We will not use computers for the field components of this workshop. The MinION Mk1C is an all-in-one instrument complete with a touch-screen user interface, flow cell chamber, and onboard computational capacities for sequencing, base calling, and basic sequence analysis.

This year's workshop will include lectures on metagenomics and an overview of basic metagenomic analysis of MinION sequencing data from SING Canada faculty members. For data analysis during the workshop, we will use Oxford Nanopore's EPI2ME environment, a user-friendly, web-based platform that includes a suite of bioinformatic tools for routine metagenomic analysis. The EPI2ME analysis we do during the workshop will give us a first look at the microbes that might be present in the soil. After the workshop, we will use more advanced bioinformatic tools to analyze the metagenomic data.

SOIL COLLECTION

Protocol Adapted from Nelson's Field Sampling Protocol for Soil. Effective July 13, 2023.

This protocol describes the collection of soils for metagenomic analysis. In this protocol each team member will collect several grams of soil from Nisku Prairie Reserve for subsequent extraction and analysis of microbial DNA.

CONSUMABLES NEEDED

Your lab team toolbox should contain a labeled bag with all the items listed below. Any other items and equipment needed in this protocol will be located on the field lab stations. If you are missing anything or need to replace an item please see a faculty member at the field lab station.

1 Face masks (from field station)
2 Nitrile gloves (from field station)
3 DNA Away and Kim Wipes (from field station)
4 Disposable scalpels
5 15 mL Falcon tubes
6 Ziplock bags
7 Foil
8 Markers

RECOMMENDED FIELD PROTOCOL

Each lab group will collect 6-9 soil samples total in this protocol. Specifically, each person will sample one location three times, with a distance of 1 m between replicates.

Notes: Protocol should be carried out while wearing a face mask and gloves. Change gloves between each numbered sample site and DNA-Away and dry scalpel/spade between sample collections. Please be careful not to spill any DNA Away.

1. Near the soil collection site, place down a fresh piece of foil and securely place the collection bag and collection tube down on top of it. This should be a place where these items will be within reach but securely out of the way of any possible contamination from top sediments. **Take a photo of your sampling site.**

2. Using a sterile, disposable scalpel or spade if needed, gently remove the surface sediment and any plant material (1-2cm) from the sampling location.

   Note: Remember respectful sampling. Do take what is needed, but without excess. Replace any removed surface sediments and soil when sampling is complete.

3. Following removal of surface layer as outlined above, open one 15 ml tube for soil collection and place lid on the foil. At the surface of the sampling site, push the opened falcon tube down into the sampling site, wiggling a bit to allow the tube depth in the soil to increase until you

have collected approximately 3-5 grams (1 gram being roughly equivalent to the 1 ml line of the 15mL tube for most soil types).

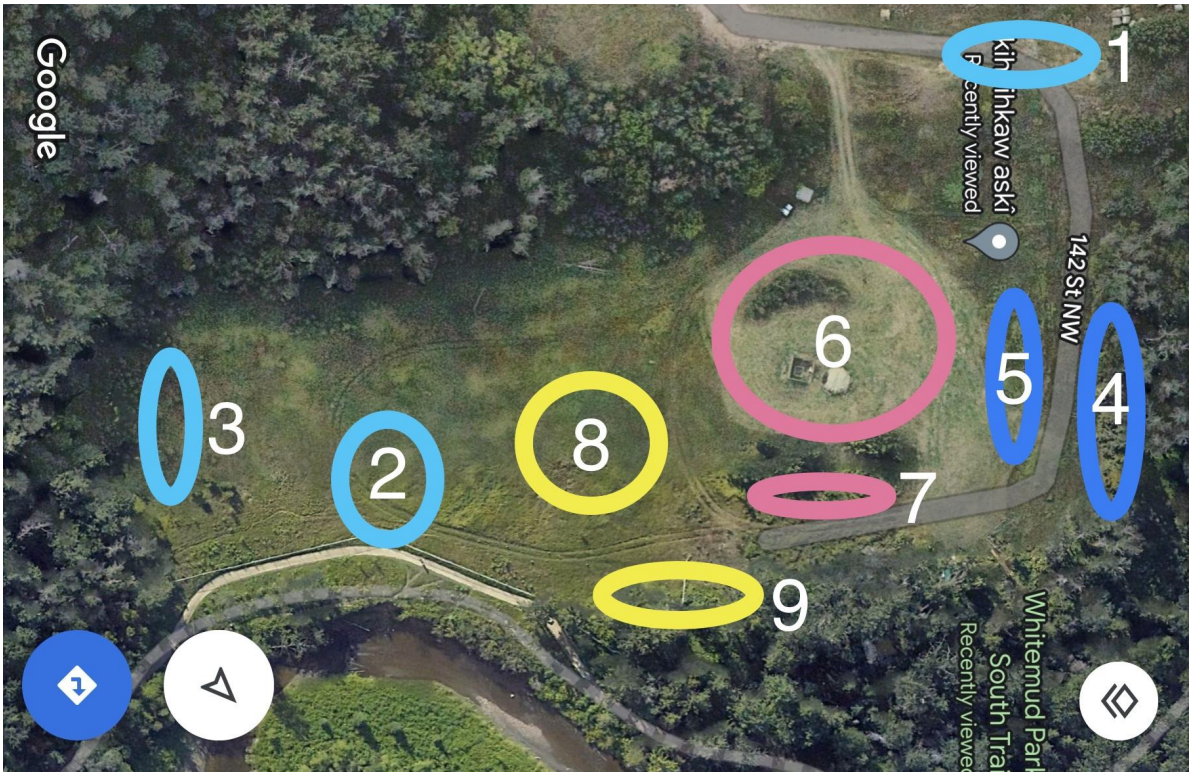
4. Some soil will stay in the tube as you gently lift the tube from the collection site. Some soils are too loose for this and may begin to pour out. If this happens it is recommended to use the scalpel/spoon to drive under the tube to the opening and offer some support for the soil to stay in the tube until you can turn the tube upward for the soil to securely fall to the base of the tube.

   Note: If the above recommended sampling strategy is not possible due to soil density or texture, use a scalpel to scoop the soil into the tube.

5. Immediately following the collection of the soil into the tube, dust off any soil clinging to the outside of the tube and firmly screw on the tube cap. Label the side of the tube with sampling site information: your initials, sampling site code (e.g., "1-Light Blue"), date, etc. Immediately place tube inside a clean Ziplock bag and label the bag with the same information.

   E.g., Sample Site Code
   Collector Initials, Replicate #
   Geocoordinates
   2023-Jul-18

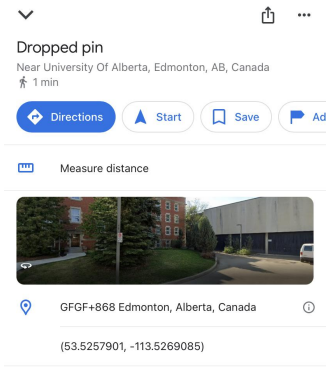
No.	Description	Map Colour	Sample Site Code (for tube)
1	Adjacent to horse pasture	Light Blue	1 - Light Blue
2	Future medicine garden	Light Blue	2 - Light Blue
3	Forest encroaching old pasture	Light Blue	3 - Light Blue
4	Old forest adjacent to gravel path	Dark Blue	4 - Dark Blue
5	New trees adjacent to gravel path	Dark Blue	5 - Dark Blue
6	Gathering grounds (former horse pasture)	Pink	6 - Pink
7	New water drainage pools	Pink	7 - Pink
8	Grass around the sweat lodge	Yellow	8 - Yellow
9	Forest adjacent to Whitemud Creek Trail	Yellow	9 - Yellow



6. Using a Kim Wipe with a little DNA Away on it, clean your scalpel/spade. Change your gloves. then collect the second soil sample by repeating steps 1-5.

7. Make sure you have not left any waste behind in the field.

8. Using the Google Maps App, drop a pin in the exact position of your soil sample. Scroll down to the dropped pin details panel to access the geo-coordinates (see screenshot below). Record coordinates on the sample tube. Please record to all decimal places given in Google Maps.



9. Proceed immediately to the lab station to complete **DNA EXTRACTION FROM SOILS.**

Photos of our collection sites across kihcihkaw askî. Together with IKWC we co-developed a sampling design that would allow us to produce an initial baseline survey of the microbial composition of the soils, which is one part of the bio-cultural relationality and ecological well being of the site (e.g., for collecting medicines in adjacent old growth forest and in the future site of their medicinal garden).

1 - Light Blue

2- Light Blue

3 - Light Blue

4 - Dark Blue

5 - Dark Blue

6 - Pink

7 - Pink

8 - Yellow

9 - Yellow

DNA EXTRACTION FROM SOILS

Protocol adapted from DNeasy® PowerLyzer® PowerSoil® Kit. Effective July 13, 2023.

Each participant will extract DNA from 2 soil samples for a total of 4-6 per group (depending on the number of lab team members). Each participant should process one of each soil sample (e.g., the sample they collected and one their partner collected).

This protocol describes the extraction of DNA from soils collected in the previous steps. In this protocol you will isolate DNA from the soil you collected and reduce the amount of inhibitors such as metal ions that can interfere with DNA sequencing. This protocol will yield DNA from many of the microbes present in the soil, and may include bacteria, fungi, viruses, and protozoans, etc. You may also isolate plant and animal DNA present in the soil, such as insects and nematodes. To maximize microbial DNA yield, participants should be careful not to transfer obvious animal or plant parts such as small insects, worms, or roots into the extraction tubes.

REAGENTS AND CONSUMABLES NEEDED

Your lab team toolbox should contain the items listed below. There are three Ziplock bags labeled "DNA EXTRACTION FROM SOILS'' containing the tubes, columns, and solutions needed for the next steps. However, please note that the weigh trays, microspatula/scoop/plastic spoon, and markers can be found in the Ziplock bag labeled "SOIL COLLECTION". All other items and equipment needed in this protocol will be located on the field lab stations.

1. Soil samples from sample site (participant A)
2. Soil samples from sample site (participant B)
3. Weigh trays and microspatula or scoop or plastic spoon
4. PowerBead tubes
5. 15 mL Falcon tube labeled "Power Bead" for PowerBead Solution
6. 1.5 mL tube labeled "C1" for Solution C1
7. 15 mL Falcon tube labeled "C2" for Solution C2
8. 15 mL Falcon tube labeled "C3" for Solution C3
9. 15 mL Falcon tube labeled "C4" for Solution C4
10. 15 mL Falcon tube labeled "C5" for Solution C5
11. 1.5 mL tube labeled "E" for Elution with nuclease-free molecular grade water (H_2O)
12. Silica Spin Columns
13. 1.5 mL collection tubes with lids
14. 2 mL collection tubes without lids
15. 2 mL screw capped elution tubes
16. Markers

NOTES

1. There are enough 2 mL lidded collection tubes and aliquoted reagents to conduct 8 extractions (each team is conducting 4 extractions, and teams of three will have two extraction kits). If you need additional reagents or consumables at any point, please ask a faculty member.

2. All reagents are stable at ambient temperature until their expiry date.
3. If solution C1 has precipitated, incubate at 60°C in heat block/plate until precipitate dissolves.
4. Mix each solution before use by making sure the lid is securely fastened and inverting several times. This is especially important for C4.

PROTOCOL

1. Using individual weigh trays, microspatula, and the scale, weigh out 0.2-0.3 g of each soil sample (2 from each local site). As much as possible, make sure that only soil makes it into the weigh trays. **Warning:** Avoid transferring obvious plant or animal parts into the weigh tray, such as roots or small insects that may be present as the plant tissue can disrupt the success of the DNA recovery and sequencing.

2. Add 0.2-0.3 g of each soil sample to the PowerBead tubes provided. Label the top and side with sample details.
   Tip: bend two opposite sides of the weigh tray towards each other and pinch together to form a funnel.

3. Add 750 µL of PowerBead Solution to the PowerBead Tubes containing soil, dispose of pipet tip in the waste container and use a fresh tip for each sample.

4. Add 60 µL of Solution C1 to each sample and invert several times or vortex briefly to mix. Use a fresh tip for each sample.

5. Place PowerBead tubes in the vortex tube holder and vortex at maximum speed for at least 20 min.

6. While the vortexer is going, this is a good time to eat a quick lunch.

7. Making sure that the centrifuge is balanced, centrifuge Power Bead Tubes in the Bento Lab at 8 kG for 60 s.
   Note: Centrifuge for 3 min at 8 kG for clay soils or if your soil is not completely pelleted after 60 s.

8. Transfer the supernatants to clean 2 mL lidded collection tubes without disturbing the pellet. Label the tubes. Make sure you get a similar amount from each tube.
   Note: expect 400-500 µL. Supernatant may still contain some soil particles at this step.

9. Add 250 µL of Solution C2 to each tube and vortex for 5 s.

10. Centrifuge the tubes for 60 s at 8 kG. Avoiding the pellet, transfer up to 600 µL of supernatant to clean 2 mL lidded collection tubes. Label the tubes.

11. Add 200 uL of Solution C3 and vortex briefly to mix.

12. Centrifuge for 60 s at 8 kG. Avoiding the pellet, transfer up to 750 µL of supernatant into a clean 2 mL lidded collection tube. Label the tubes.

13. Add 1200 µL of Solution C4 to the supernatant and vortex for 5 s. Note: The tubes will be very full, close the lids carefully to avoid any splashing, sample loss, and potential cross contamination.

14. Load 675 µL of the supernatant onto a Silica Spin Column and centrifuge at 8 kG for 60 s. Discard the flow through in the waste container and place the silica tube back in the collection tube.. Repeat 675 µL transfer, spin, and flow through discards until all of the supernatant has been passed through the spin column (should take 3 loads total).

15. Add 500 µL of Solution C5 and centrifuge for 30 s at 8 kG. Discard the flow through.

16. Place the spin columns in a fresh **unlidded** collection tube. Dry spin by centrifuging again for 60 s at 8 kG.

17. Carefully place the spin filters in clean 2 mL unlidded collection tubes. Avoid splashing any Solution C5 onto the spin columns.

18. Add 25 µL of tube E (Elution with nuclease-free molecular grade water, H_2O) to the center of the white filter without touching the white silica membrane. Make sure the elution buffer does not end up on the sides of the tube but makes full contact with the filter. If it does end up on the side, tap the tube on the table gently to bring the liquid down. Incubate the tubes at ambient temperature for 60 s.

19. Centrifuge for 30 s at 8 kG. Discard the spin columns.

20. Transfer complete eluates to separate clean screw cap 2 mL tubes and label with your Sample IDs and Date.

21. The DNA is now ready for downstream applications. Proceed immediately to **DNA QUANTITATION.**

DNA QUANTITATION

Protocol adapted from Qubit® dsDNA Broad Range Assay Kit User Manual. Effective July 13, 2023.

This protocol describes the quantitation of DNA isolated in the previous step. In this protocol you will use a Qubit fluorometer to precisely determine the concentration of DNA present in your isolations in units of ng/uL. This concentration will determine what volume of your isolated DNA needs to be included in genome library construction and nanopore sequencing. You will need a total of 400 ng/uL for subsequent steps, although as little as 200 ng/uL may generate usable sequencing data.

REAGENTS AND CONSUMABLES NEEDED
Your lab team toolbox should contain the items listed below. All other items and equipment needed in this protocol will be located on the field lab stations.

1. Soil DNA Extractions from previous steps
2. Qubit™ Flex 500 µL Tubes

Notes:
Handle Qubit tubes by the top and do not touch the bottoms
Standards and working solutions are located at main lab stations
dsDNA working solution is potentially mutagenic

PROTOCOL

1 In a tube rack, set up the required number of assay tubes for your samples. You will need 4-6 tubes, one for each soil DNA extraction your group performed.

2 Label the tube lids with your sample IDs.
Note: Do not label the side of the tube as this could interfere with the sample read.

3 Add 198 µL Qubit™ 1X BR working solution to each tube. This solution will be pre-prepared for you by a faculty member.

4 Add 2 µL of each DNA extraction to the corresponding tube.

5 Mix each sample vigorously by vortexing on high speed for 3–5 seconds. Briefly spin down.

6 Allow all tubes to incubate at ambient temperature for 2 minutes, then place them in the Qubit fluorometer to read.

7 On the Qubit Homescreen, press the dsDNA icon.

8 Press the 1X dsDNA Broad Range (BR) icon. Press “run samples” to proceed.

9 When prompted, load the tubes containing your diluted DNA extracts one at a time. Select the units for the output sample concentration, then select Next.

10 In the Sample volume screen, enter the sample volume added to the assay tube (2 µL). Enter the volume directly in the sample volume text box, use the + or – buttons or adjust the sample volume wheel to select the sample volume added to the assay tube.

11 Insert a sample tube into the sample chamber, close the lid, then press Run samples. When the reading is complete (~3 seconds), remove the sample tube.

12 Samples are displayed on a graph with the results in a list below. The value listed is the concentration of the original sample. You will use this value to determine the volume of DNA to include in library prep. Note this value in your lab notebook and give these numbers to Rick for use in future steps.

Note: You will need approximately 40 ng/uL for optimal sequencing results, although as little as 20 ng/uL will produce usable results.

13 Bring your extractions to a faculty member to store for library construction and sequencing later in the workshop.

14 After you have turned in your extractions, see Betsy, Cheyenne, Rick, and/or Warren for a flow cell demo.

PREPARE MinION Mk1C AND FLOW CELL

Protocol adapted from Nanopore Community Protocols. Effective July 13, 2023.

This protocol describes how to run diagnostics on the MinION system and flow cells. In this protocol you will prepare the Minion Mk1C and SpotOn Flow Cell for use in sequencing genomic DNA present in your soil DNA.

REAGENTS AND CONSUMABLES NEEDED

Your lab team toolbox should contain the items listed below. MinION Sequencers are located in the field lab station.

1 Nanopore SpotOn Flow Cell

PROTOCOL

1 Plug in your MinION Mk1C device and press the power button on the right hand side. You should hear a fan when the instrument boots up. Once the instrument is booted up, have one of the faculty members enter their credentials to log on.

2 The Mk1C is a touch screen interface. Press the  icon on the left hand side of the screen. You should see four options. “Start Sequencing”, “Analysis”, “Flow Cell Check”, and “Hardware Check”.

3 Open the flow cell panel located under the touchscreen and confirm that the Configuration Test Cell is in place. Close the flow cell panel again.

4 With the Configuration Test Cell in place, select “Hardware Check” on your MinION Mk1C to run a system diagnostic and confirm hardware is in good working order.

5 If the hardware check is successful, remove Configuration Test Cell by lifting it gently on the left hand side, and pulling it out to the left.

6 Insert a fresh MinION Flow Cell and press down firmly to ensure correct thermal and electrical contact.

7 Close the flow cell panel, and perform a diagnostic check by clicking “Flow Cell Check”. Confirm that there are at least 800 viable pores for sequencing. If there are less pores than this, insert a different MinION Flow Cell and try again.

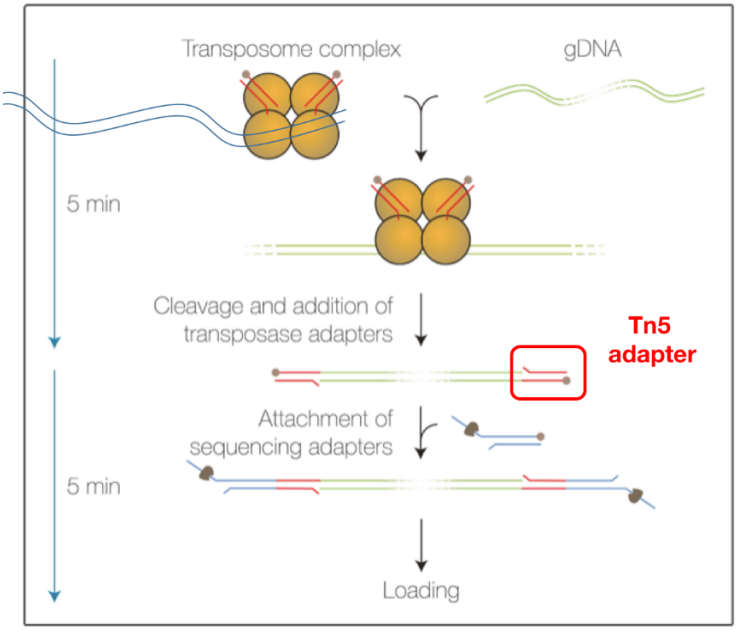
8 Proceed immediately to **NANOPORE LIBRARY CONSTRUCTION**

NANOPORE LIBRARY CONSTRUCTION

Protocol adapted from Nanopore Rapid Sequencing gDNA Field Sequencing Kit. Effective July 13, 2023.

This protocol describes the step-by-step instructions to build genome libraries using the Nanopore Field Sequencing Kit. In this protocol you will tagment (break up and tag) the DNA and add Nanopore sequencing adapters to the ends of the DNA. This protocol has been designed for use in field contexts without refrigeration, and all reagents are stable for up to 1 month at 30ºC.

Figure 1: Overview of Nanopore library chemistry.
Figure adapted from Field Sequencing Kit Protocol.

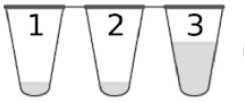


REAGENTS AND CONSUMABLES NEEDED

Your lab team toolbox should contain the items listed below. All other items and equipment needed in this protocol will be located on the field lab stations.

1 Approximately 400 ng high molecular weight input DNA extracted from soil.

2 Field Sequencing Kit
Note: Strip of three foil-covered 200 µL tubes with varying amounts of lyophilized reagents. 1: Tagmentation mix; 2: Rapid adapter mix; 3: Sequencing buffer.



3 Resuspension buffer (RTB)

PROTOCOL

1 You will select only 1 of your samples for library preparation. The others will be prepared later.

2 Using the concentration data from the previous step, transfer 400 ng of your isolated genomic DNA into a 2 mL lidded tube and adjust the volume to 10 µL with nuclease-free molecular grade water. See Rick for specific volumes needed. Mix by flicking gently and spin down with a microfuge.
Note: If your DNA yield is less than 40 ng/µL. Just add 10 µL of your isolated genomic DNA without adding water.

3 Tear off or cut off Tube 1 (left hand tube containing tagmentation mix, see figure above).

4 Using a clean, empty pipette tip, pierce the foil of Tube 1 without disturbing the pellet.

5 Add 10 µL of the input DNA to Tube 1.

6 Mix gently by pipetting up and down several times. Make sure all the liquid is collected at the bottom of the tube.
Tip: you can use the pipette tip to physically drag any drops on the side to the bottom, or gently tap the tube on a level surface to bring all the liquid to the bottom.

7 Incubate the tube at **ambient temperature** for 1 min and then at **80°C** for 1 min.

8 Using a clean, empty pipette tip, pierce the foil of Tube 2 without disturbing the pellet.

9 Transfer 10 µL of the tagmented DNA from Tube 1 to Tube 2.

10 Mix by gently pipetting up and down. Make sure all liquid is collected at the bottom of the tube.

11 Incubate the reaction for 5 min at **ambient temperature**. (move on to next step during incubation)

12 Using a clean empty pipette tip, pierce the foil of Tube 3 (right hand tube) without disturbing the pellet.
Note: Tube 3 is nearly full of dry sequencing buffer reagents. Be especially cautious when piercing the tube.

13 Add 65 µL Resuspension Buffer (RTB) into Tube 3. Mix by pipetting up and down. Make sure all the liquid is collected at the bottom of the tube.
Note: the reagents in this solution may take longer to dissolve, pipet up and down until the solution is completely clear of particulates.

14 Proceed immediately to **PRIMING AND LOADING THE FLOW CELL**

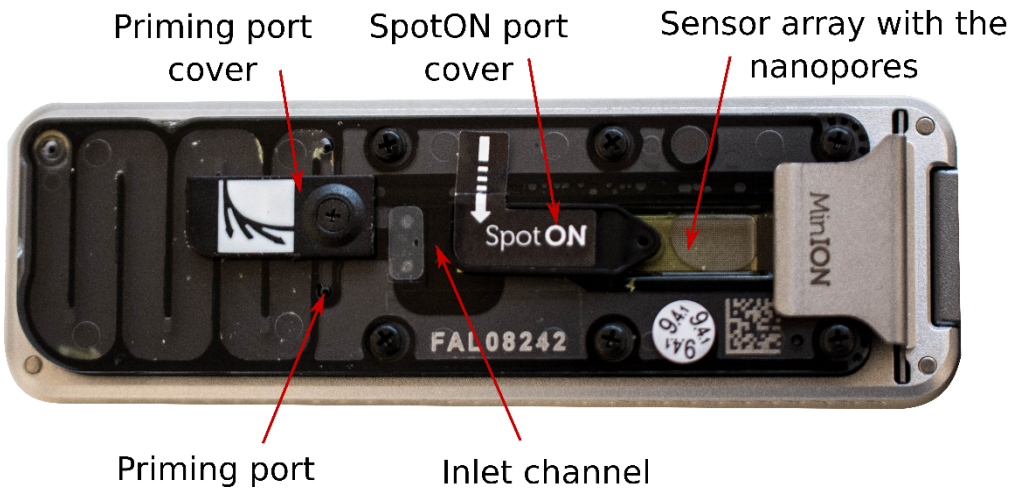
PRIMING AND LOADING THE FLOW CELL

This protocol describes the preparation of the MinION flow cell for sequencing and loading your prepared library into the flow cell for sequencing.

REAGENTS AND CONSUMABLES NEEDED

1 Flow Cell Priming Kit
FB: Flush Buffer; FLT: Flush Tether

Figure 2: Diagram of MinION SpotOn flow cell. Note positions of port covers and sensor array.



PROTOCOL

Note: Take care at every step not to introduce any air bubbles into the flow cell ports.

1 Slide the priming port cover clockwise to open the priming port.

2 After opening the priming port, check for any small air bubbles under the cover.

3 Using a P1000 set to 200 µL with a clean and empty tip, insert the tip of the pipet into the priming port cover and dial back to 220-230, or until you can see a small volume of the yellow storage buffer enter the pipet tip. Remove and discard the pipet tip with the storage buffer.

4 Visually check that there is continuous buffer from the priming port across the sensor array.

5 Individually mix the Flush Tether (FLT) and Flush Buffer (FLB) tubes by pipetting and spin down.

6 Prepare the priming mix by adding 30 µL of Flush Tether to the tube of the Flush Buffer, and mix well by pipetting up and down. Cap the tube and then invert the tube 3 times.

7 Avoiding the introduction of air bubbles, load 800 µL of the priming mix into the flow cell via the priming port. Wait for 5 minutes. During this time, move to the next step to prepare the DNA library for loading.

8 To prepare the library for loading, add 65 µL of the resuspended Tube 3 material into Tube 2 and mix by pipetting up and down several times.

9 Complete the flow cell priming by gently lifting the SpotOn cover to make the sample port accessible.

10 With both the priming port and SpotOn ports open, SLOWLY load 200 µL more of the priming mix into the flow cell via the PRIMING PORT, not the sample port. Avoid the introduction of any bubbles.
Note: You should see a small volume of liquid come out of the sample port, which will expel any bubbles present there.
Note: Immediately load your DNA library after this step.

11 Hovering the pipet tip over the sample port without making contact, add 75 µL of the DNA library (mix from Tube 2 and Tube 3) to the flow cell via the SpotOn sample port in a drop wise fashion. Visually confirm that each drop flows into the port before adding the next drop.

12 Gently replace the SpotOn sample port cover, making sure the bung enters the SpotOn port. Close the priming port and close the flow cell panel. Immediately proceed to Nanopore Sequencing.

NANOPORE SEQUENCING

1 After loading your DNA library, begin the sequencing run by tapping the icon on the home screen and tapping the green “Start Sequencing” box.

2 Enter your experiment name, which should be your sample ID, extraction replicate number, and run number (the run number will be 1). Press “ok”.
(i.e., 1-LightBlue_SC23-1_JK-1_Run-1_23-08-19)

3 Press “continue to kit selection”

4 Under “sample type” press DNA. Under PCR-free tap “PCR-free”. Under Multiplexing tap “No”.

5 Tap “Field Sequencing Kit” in the option list below.

6 Tap “Continue to run options”

7 Set run time to 24 hours. Leave Bias Voltage as the default.

8 Tap “Skip to Final Review”. Note: We will use the default settings for basecalling and outputs.

9 Click “Start” to begin the sequencing run.

10 Take a few minutes to observe the real time sequencing results of your run. IT’S INCREDIBLE.

While you wait check out these videos on how Nanopore Sequencing works:
https://www.youtube.co/watch?v=RcP85JHLmnI
https://www.youtube.com/watch?v=A1NLE0Jbvo8