Overview

This protocol describes the procedure for extracting bacterial DNA from the soil sample using NucleoSpin® Soil Kit. After the extraction, the DNA can be sent to the 16S sequencing to determine the composition of the bacterial microbiome.

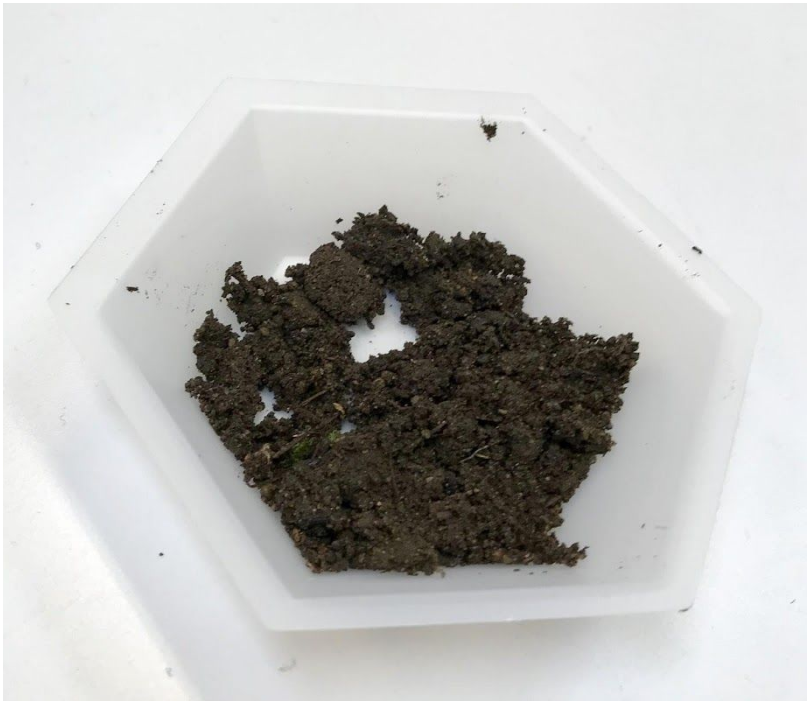
The max number of the samples that can be simultaniously run through this protocol is limited by the capacity of the centrifuge. Because for some steps the tubes have to be opened, a centrifuge can fit only ⅓ of tubes. For a 24 tube centrifuge, it can only fit 6 for this protocol.

Collecting soil samples

- ***Scrape the soil to reach ~2 inches below the surface. Mushy, aerated soil is better than dry/sandy/rocky soil.***
  The top layers of soil usually have a low concentration of live bacteria. In addition, it has a lot of non-bacterial organic material, like leaves, dog feces, etc.
- ***Collect a bunch of soil inside a 50 mL falcon, or a clean zip lock bag.***

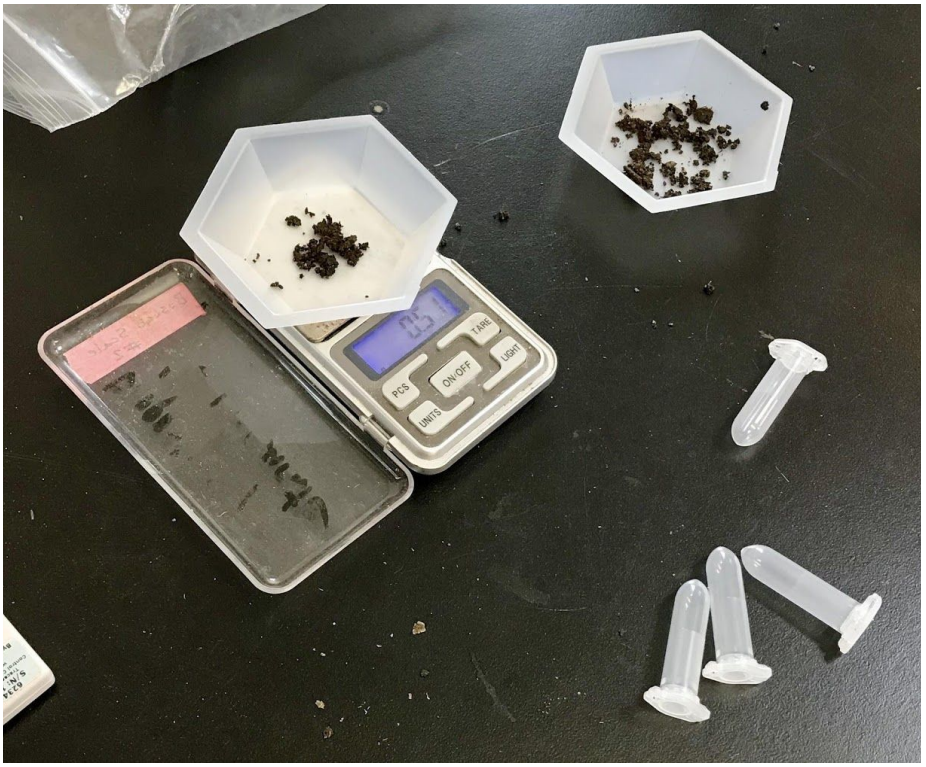
Preparing samples for the extraction

- ***Spread the soil in a weigh boat.***



- ***Remove pieces of twig, rocks, and other bulky material.***
- ***Weight ~500 mg of soil and transfer to a bead beating tube (included in the kit).***
  Make sure to avoid any big particles.

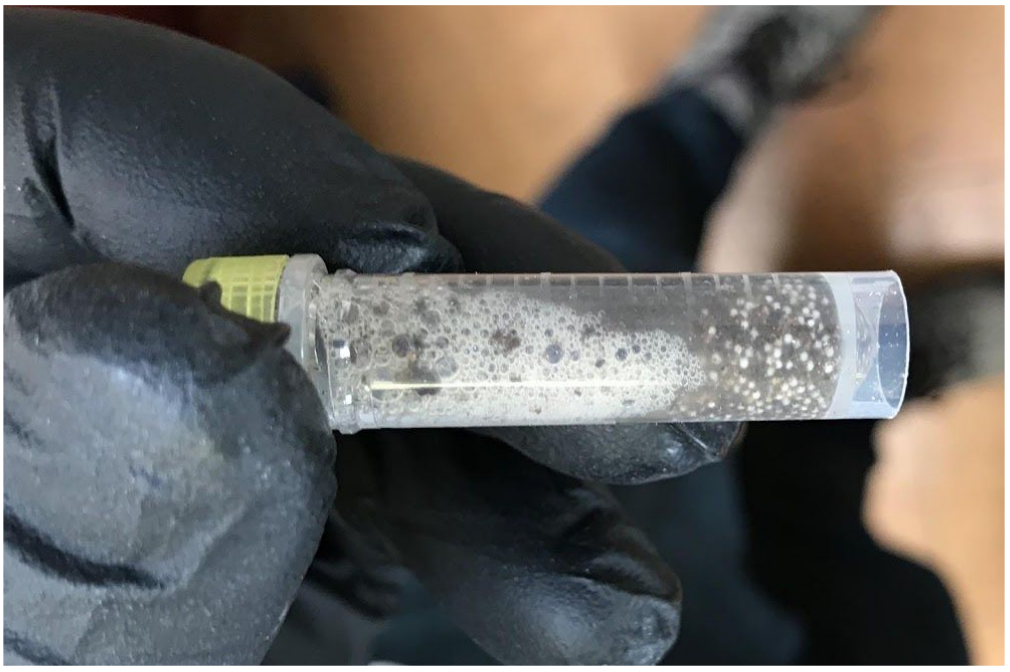
When loading the soil into the tube, it could not fit in the tube if it is too fluffy. Tap the tube to compact it.
500 mg of soil is a pinch and looks very small on a weigh boat. That is normal.



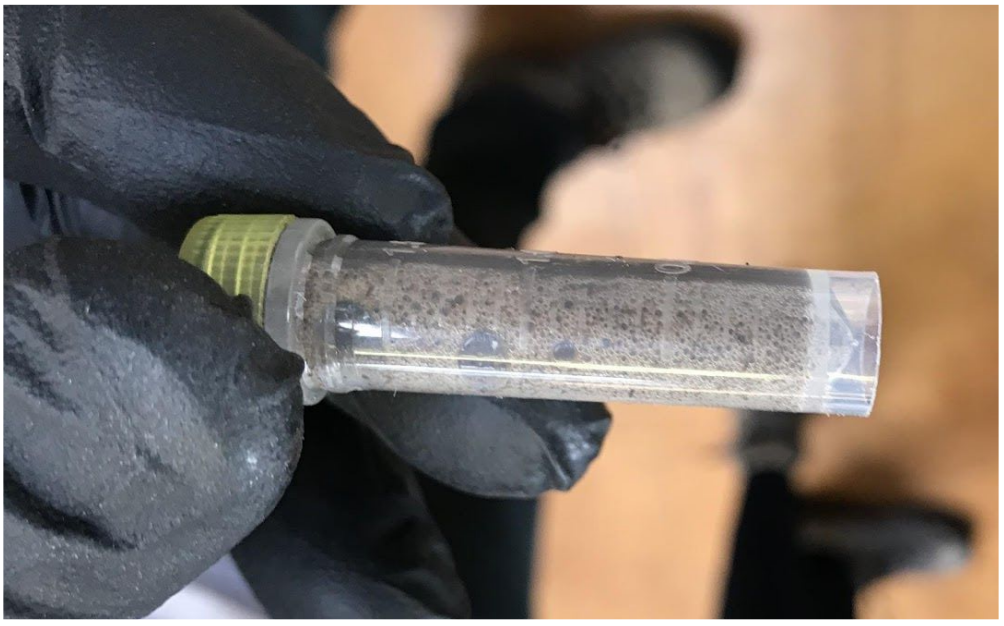
- ***Make 2 or 3 individual extractions from one collected soil sample.***
  Biological replicates are essential for the downstream microbiome data analysis.

Extraction (using NucleoSpin® Soil Kit)

- ***Add 700 µL of SL2 buffer.***
  SL2 works better for the samples from the flowerbeds because they usually contain a lot of organic material.
  SL1 should be used for soils which have a lot of minerals. In other words, more sandy samples.
  Don't use SX unless you cannot detect enough DNA in the final product. Otherwise, it can result in contaminating the final product with humic acids which can inhibit PCR and therefore lower the quality of sequencing.
- ***Mix the tubes by inverting them up and down until the beads are not sitting in the bottom anymore.***
  If it does not mix easily, tab the tube upside down.

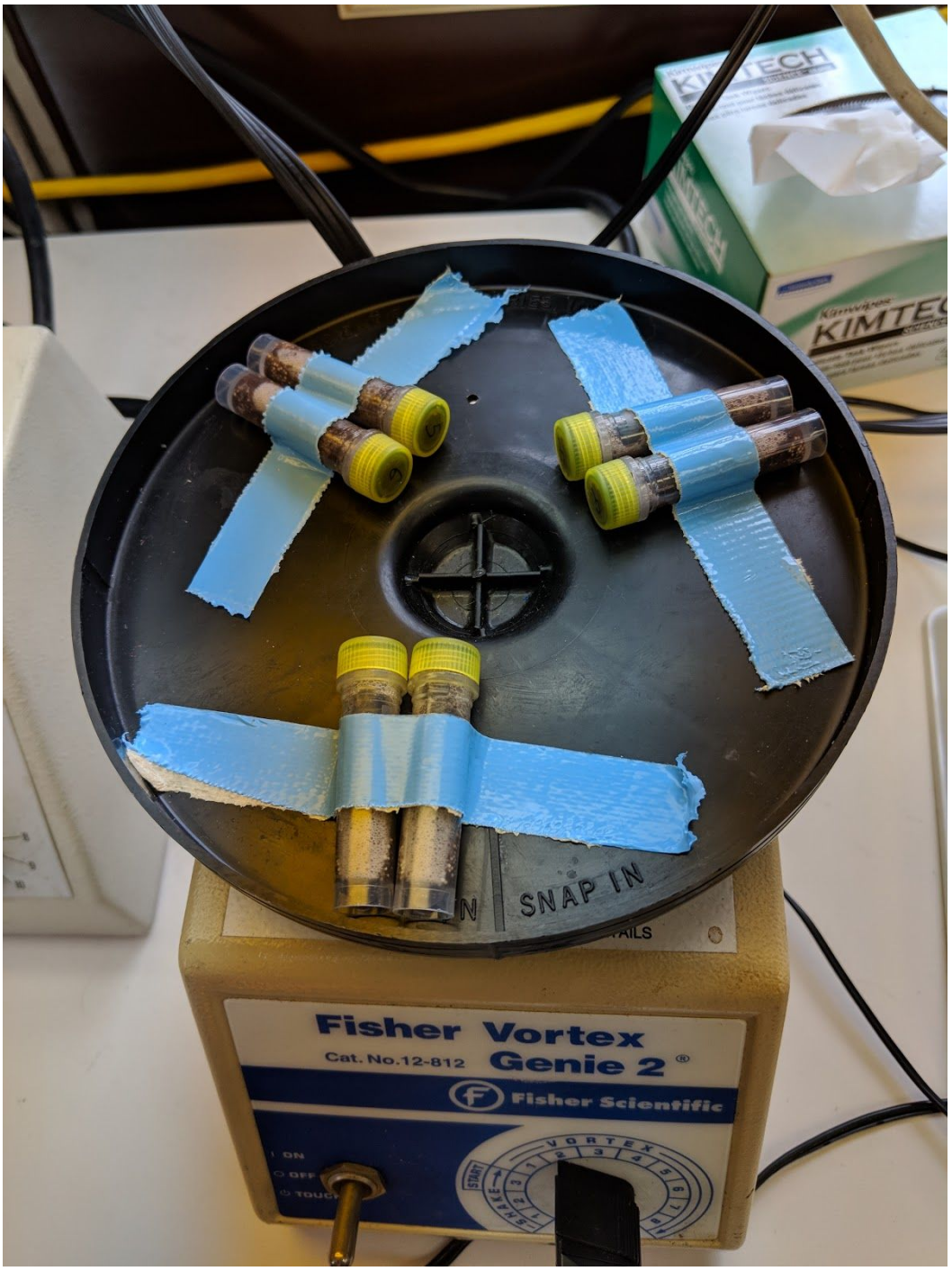


Not mixed



Mixed

- ***Tape the tubes to the vortexer with caps pointing inward. Make sure they a balanced.***
  Run vortexer at the max speed for 5 minutes.



- ***After vortexing place samples on ice or in -20C from 30 seconds to 1 minute, to cool them down to room temperature.***
  Vortexing warms them up a lot and heat can degrade DNA.

- ***Centrifuge for 2 min at 11,000 RPM.***
- ***Transfer clear supernatant to a new collection tube.***
  Be careful about not sucking in any pellet material or debris.
- ***Add 150 µL Buffer SL3 and vortex for 5 s.***
- ***Incubate for 5 min at 0–4 °C.***

- ***Centrifuge for 1 min at 11,000 RPM.***
- ***Place red ring removal column in a collection tube, 2 mL with a lid.***
- ***Load up to 700 µL clear supernatant from the previous step onto the filter.***
- ***Centrifuge for 1 min at 11,000 RPM***
  This step filters supernatant. So the supernatant is what preserved and the filter is what is discarded.
- ***If there is more than 700 µL of supernatant to filter then use a new collection tube and filter additional supernatant into it. Then combine the liquids into the first collection tube.***
- ***Discard the filter.***

- ***Add 250 µL Buffer SB and close the lid.***
- ***Vortex for 5 s.***

- ***Place a green ring column in a collection tube, 2 mL.***
- ***Load 550 µL sample onto the column.***
- ***Centrifuge for 1 min at 11,000 RPM.***
- ***Discard flow through and place the column back into the collection tube.***
  After discarding flow throw into a trash, tap the upside-down tube onto a paper towel. So more liquid is removed from it.
- ***Load the remaining sample onto the column.***
- ***Centrifuge for 1 min at 11,000 RPM.***
- ***Discard flow through and place the column back into the collection tube.***

- (This protocol skips the SB wash because skipping it does not affect the yield and shortens the protocol)

- ***Add 550 µL Buffer SW1 to the column.***
- ***Centrifuge for 30 s at 11,000 RPM.***
- ***Discard flow through and place the column back into the collection tube.***

- ***Add 700 µL Buffer SW2 to the column.***
- ***Close the lid and vortex for 2 s.***
- ***Centrifuge for 30 s at 11,000 RPM.***
- ***Discard flow through and place the column back into the collection tube.***

- (This protocol skips the second SW2 wash because skipping it does not affect the yield and shortens the protocol)

- ***Centrifuge for 2 min at 11,000 RPM***
  This step dries the sample.

- ***Place the column into a new 1.5 mL microcentrifuge tube.***

- ***Add 50 µL of SE buffer to the column.***
  Make sure that the SE buffer drips directly into the filter and don't splash all over the tube.
- ***Close the lid and Incubate for 2 min at 52 °C.***
- ***Centrifuge for 30 s at 11,000 RPM.***
- ***Discard the filter and close the tube.***
  The tube contains the extracted DNA!!!

- ***Quantify the amount of collected DNA using Qubit.***