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DNA EVERYWHERE: A Guide for Simplified Environmental Genomic DNA Extraction Suitable for Use in Remote Areas

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Earth and Environmental Sciences Area

Executive Summary

Collecting field samples from remote or geographically distant areas can be a financially and logistically challenging. With participation of a local organization where the samples are originated from, gDNA samples can be extracted from the field and shipped to a research institution for further processing and analysis. The ability to set up gDNA extraction capabilities in the field can drastically reduce cost and time when running long-term microbial studies with a large sample set. The method outlined here has developed a compact and affordable method for setting up a "laboratory" and extracting and shipping gDNA samples from anywhere in the world. This white paper explains the process of setting up the "laboratory", choosing and training individuals with no prior scientific experience how to perform gDNA extractions and safe methods for shipping extracts to any research institution. All methods have been validated by the Andersen group at Lawrence Berkeley National Laboratory using the Berkeley Lab PhyloChip.

Equipment & Supplies

Equipment-
VWR Centrifuge Galaxy MiniStar
Vortex-Genie 2 Mixer
    Horizontal 15mL Tube Holder
    Horizontal Microtube Holder
Postal Scale
1000µL Pipette
PPE-
Safety Glasses
Lab Coats
Gloves
Microcentrifuge Tube rack
Supplies-
Zymo Fecal DNA MiniPrep (50 prep)
2mL Tubes
15mL Falcon Tubes
Steel BBs
Pipette Tips
Sterile Forceps
Sterile Alcohol wipes
Bleach
Wash Bottles
Aluminum Foil
Plastic Bags
WhirlPak Sample Bags
3M Temperature Indicators
PolarTech Insulated Shipping Containers
PolarTech Freeze Packs
Sharpies
Pens
Waste Containers
Garbage Bags
Bleach Tub
Food Coloring
Wax Paper
Timer
Hand Trowel
Dishwashing Brush

Methods

Field DNA extractions are performed by substituting equipment commonly used at Lawrence Berkeley National Laboratory (LBNL) (and other research institutions) under sterile conditions and ample power supply with smaller, more durable replacements and setting up a makeshift "laboratory" in a tent or office space. Sterile conditions are achieved as best as possible, but by no means compare with laboratories such as LBNL where these methods were developed. Emphasis is given on maintaining a sterile workspace. Blank samples have been routinely tested to measure contaminant levels and have been consistently insignificant.

Field extraction equipment was thoroughly tested against LBNL extraction equipment in the Andersen Lab at LBNL. 15mL centrifuge tubes were filled with sterilized steel BBs and affixed to a Vortex Genie 2 in place of a tissue lyser for homogenization. A 14,000 x g centrifuge was replaced by one with a max speed of 2,000 x g and the protocol was modified to extend spin times for all centrifugation steps. Samples are extracted immediately after collection, eliminating the need for -80^{O}C freezers. Extracts are shipped with six-PolarTech Freeze packs in a 12"x12"x12" Styrofoam shipping container. 3M Temperature Indicators ensure that samples are not exposed to temperatures above ambient for a prolonged period of time. Extracts should be shipped overnight mail and processed, or stored at 4^{O}C, immediately upon receipt.

All variations from standard operating procedures in handling and processing were studied and verified using Qubit fluorometric quantification, Agilent bioanalysis and amplification/gel electrophoresis.

Training

The participating organization will select individuals as Sample Technicians and will be required to complete a three-day training course, led by a Course Instructor. The three days are divided into three lessons: Basic Laboratory Techniques, Guided DNA Extractions, and Independent DNA Extractions. If at any time, the Course Instructor feels the selected individuals need more time in one of these sections, they may repeat a section. Course Instructors can be the Principle Investigator, a research associate familiar with the following techniques, or a Sample Technician that has received further training and practice and has proven capable in leading the course.

Day 1: Basic Laboratory Techniques

Part I. The most important laboratory techniques that are emphasized in this section are safety and sterilization.

Some main safety points that should be made are:

1. Always wear eye protection

2. Always wear gloves and wash hands before entering and after leaving the "laboratory"
3. Do NOT eat or drink in the "laboratory"
4. Wear skin protection: long sleeves, long pants, closed toe shoes at all times

The instructor will also review the safety concerns with the chemicals used and instruct the students what they should do in the case of a chemical spill. It is important that the instructor understands that many students have little to no scientific and laboratory background so it is crucial to go slow, encourage a lot questions, and repetitiously explain everything in meticulous detail, no matter how simple it seems.

Maintaining sterile conditions in the field and in a "laboratory" that is set up in a tent or office building is one of the most challenging aspects, and one of the most emphasized techniques taught in this course. Especially in "laboratories" that do not have proper ventilation, air filtration, closed windows etc. it is crucial to keep samples, reagent bottles and pipette tips covered at all times. Other points that need to be emphasized are:

Avoid:

1. Touching the inside of any reagent bottle or sample tube
2. Touching your face, eyes, mouth etc. while wearing gloves
3. Sneezing or coughing into a reagent bottle or sample tube
4. Touching the bottom of a spin column
5. Touching the top or inside of a spin column

Always:

1. Use a new pipette tip when going into a reagent bottle
2. Use a new pipette tip if you are unsure if you need to
3. When in doubt, sterilize!
4. Close reagent bottles after use
5. Close sample tube caps after use

For more details on teaching sterilization techniques, see the Course Handbook (attached).

Part II. The second part of Day 1, the Instructor will thoroughly explain the equipment, troubleshooting options and routine maintenance. The Student Handbook contains specific points about each piece of equipment, but focuses strongly on proper pipette use. Explain how each of the following pieces of equipment works, proper use, maintenance (cleaning) and troubleshooting:

- Timers
- Scale
- Vortex Genie
    - How to operate and how to switch out top plates
- Centrifuge
    - Emphasize the importance of balancing, and making "dummy" tubes if necessary to achieve balance

- Pipettes

Part III. Teaching proper pipette use is one of the most challenging and most important sections of the course. This technique is crucial for sample extractions and should not be rushed through. Many students will learn proper pipetting technique at different speeds, but even the ones that pick it up quickly need practice so do not hesitate to have them repeat pipette practice lessons and improvise while the others catch up. The Student Handbook goes through the general guidelines for using a pipette, it is helpful for the instructor to demonstrate each of these as he/she goes through the explanation. It can also be helpful for students to have a pipette while the instructor is explaining the guidelines so they can follow along and mimic what the instructor is doing.

Once the instructor feels that each student comprehends the guidelines for pipette use, he/she can move on to the pipetting practice exercises. These exercises can be adapted or changed at the Principle Investigator and Instructors discretion. If some students are catching on quicker than others, other practice exercises may be added. Pipetting exercises can be found in Appendix A of the Course Handbook. If the instructor feels confident in each student's ability to properly and accurately use a pipette, then this section of the course is completed.

Day 2: Guided DNA Extractions

The instructor will identify and introduce the various columns used during the DNA Extraction and discuss the sample collection techniques (this may vary due to material extracting). Each student will then sterilize their workspace by bleaching (10% solution) their table top, centrifuge, vortex, pipette, pipette boxes etc. The first extraction will be of only a blank sample. This helps students become familiar with each column and the protocol without having to face any potential issues, such as filters clogging, lack of visibility when pipetting after homogenization etc. The instructor will perform a blank extraction as well, and explain and demonstrate each step prior to the students following. Encourage questions and explain what is going on- for example, explain to them what is happening when you are lysing cells: the reagent and the beads are breaking open cells to pull the DNA into solution, so you can run it through a filter where it will stick and then clean off all the extra junk that gets stuck to it. Instructors should encourage students to take notes on the protocol for future use.

If the instructor is satisfied with the student's blank sample extraction, he or she will then demonstrate the proper method for sample collection. In the case of compost, the technician will dig ~50cm into the pile at the selected location with a shovel and then scoop out compost with a sterilized hand trowel. Sterile forceps will transfer the compost into a labeled 15mL centrifuge tube until full, but not compacted. Each student will perform his or her own sample collection under the guidance of the Instructor.

The instructor will again demonstrate each step of the extraction process and then allow each student to perform the same step on his or her sample and a blank sample under guidance and observation of the instructor. This is a great opportunity to again discuss what issues could arise at each step and how to troubleshoot them. If extracting compost or soil samples, the instructor may follow along with the DNA Extraction Protocol (attached). If different types of samples are being extracted, and/or there is a different kit being used, the protocol can be adjusted easily to incorporate the equipment available (ie. adjust centrifuge time to achieve the same force as the kit protocol suggests, altering homogenization techniques, extracting filters etc.).

Depending on the aptitude of the students, the Instructor may opt to repeat extractions two to three or four times depending on the time available. A minimum of two guided sample extractions is strongly recommended.

Day 3: Independent DNA Extractions
Students will perform sample and blank extractions from start to finish without the guidance of the instructor. They are welcome to work together and ask questions of the instructor, but are not walked through each step. The instructor is encouraged to observe each student and encourage questions. At the instructor's discretion, students will repeat extractions a few times pending the time available, but a minimum of two extractions is strongly encouraged. Once the instructor feels confident in the extracted samples and blanks, they will ship the samples to their collaborating laboratory for further processing and analysis. PCR amplification and gel electrophoresis is an adequate way to evaluate whether DNA was extracted and there is an absence of DNA in the blank samples. Upon confirmation of these two parameters, the student has completed the training course.

Further training and practice is encouraged after the course prior to acquiring samples for a data set. It is valuable to have the students perform a series of extractions and have them shipped for evaluation prior to data set acquisition.

Shipping

Samples can be shipped using cold packs in a Styrofoam container. No permits or special shipping requirements are needed to ship gDNA, as it is a biologically inactive material. Overnight or 2-day shipping is suggested. Temperature monitors are also strongly suggested to ensure the samples are not exposed to high temperatures at any point during the shipping process. Samples can be stored at $4^{O}C$ until shipped, and can be stored under the same conditions upon receipt.

Conclusion

Upon completion of the Training Course and validation of sample quality, a sampling plan can be deployed with the participating organization. The organization is then

qualified to collect samples, perform the extractions and ship the samples to the research institution of choice. This provides an affordable alternative to routine traveling to remote areas to collect samples, and transporting biologically active, and often hazardous samples across borders. Remote gDNA extraction allows organizations around the world, in developing countries and in remote areas to have the ability to partner with research institutions and have access to sophisticated scientific research.

Please direct any questions or comments to Dr. Gary Andersen, glandersen@lbl.gov or Gabrielle Pecora, gnpecora@ucdavis.edu.

COURSE HANDBOOK

LABORATORY BASICS & EQUIPMENT INSTRUCTIONS

Sterilization

Prior to beginning any extractions, wipe down all surfaces with a 10% bleach solution including: bench top, vortex, centrifuge, tube rack, pipettes, pipette boxes, gloves, timers and scale.

Making a 10% bleach solution

1. Add 25mL (line marked on bottle) bleach to empty spray bottle labeled "10% BLEACH"
2. Fill bottle up to fill line (225mL)

STERILIZATION DO'S & DON'TS

Never:

- Touch the inside of any reagent bottle or sample tube
- Touch your face, eyes, mouth etc. while wearing gloves
- Sneeze or cough into a reagent bottle or sample tube
- Touch the bottom of a spin column
- Touch the top or inside of a spin column

Always

- Use a new pipette tip when going into a reagent bottle
- Use a new pipette tip if you are unsure if you need to
- When in doubt, sterilize!
- Close reagent bottles after use
- Close sample tube caps after use

Equipment

"TAYLOR" TIMERS

The digital timers are used for timing vortex and centrifuge runs. They take AAA batteries. Instructions for use:

1. Press M button to set minutes.
2. Press S button to set seconds.
3. Press START/STOP to start and stop the times.

4. To pause the timer, press START/STOP, and again to resume time.
5. When alarm beeps, press START/STOP to turn off alarm. The timer will recall the last set time.
6. Press M and S at the same time to reset to zero.

CABELA'S DIGITAL SCALE

The digital scale must warm up and be calibrated prior to weighing out samples. To calibrate, plug in the scale and press the "ON" button. Allow the scale to warm up for 3-4 minutes. Once the scale has warmed up, calibrate by following the instructions below:

1. With an empty weigh pan, press and hold the TARE button until "CAL-0" is shown.
2. Press the TARE Button again. The 0 in CAL-0 will begin flashing.
3. When CAL-F is shown, stack the 2-50g weights on the center of the pan and press the TARE button. The F in CAL-F will flash.
4. When CAL-0 is shown again, remove the weights and press the TARE button. The 0 in CAL-0 will flash.
5. When the calibration process is finished, the scale will reset to zero.
6. If the unit does not reset to 0.00, repeat calibration from step 2.

This scale is used only for weighing samples into the Bead Bashing tubes for the lysis step of the extraction process. In order to do this, place a small piece of Weigh Paper onto the scale and press the TARE button. The scale should read 0.00, at which point you may begin weighing samples. Once you have measured 0.20g of compost, transfer the compost into the Bead Bashing tube.

VORTEX GENIE 2

When using the vortex, it is good practice to space samples out evenly and in a balanced manner. Do not touch the vortex when it is running.

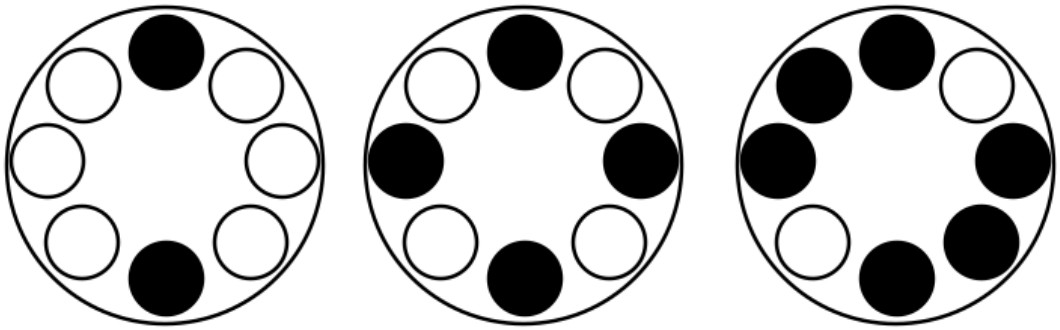
Changing the vortex tube holder attachments

1. Make sure the Vortex is powered off.
2. Gently pull the attachment off, careful not to crack or break the attachment.
3. Line up the flat part of the attachment to the flat part of the vortex and push attachment down until you hear a click.

GALAXY MINI-STAR CENTRIFUGE

The Galaxy Mini-Star Centrifuge has a top speed of 2,000 x g. The centrifuge is designed to operate when the rotors are balanced, spinning the centrifuge with an unbalanced rotor can cause mechanical damage and potentially personal injury. **Before spinning, the rotor MUST be balanced.** If the centrifuge is run unbalanced, it will "walk" (move around on the bench top). If this happens, immediately stop the run and balance the centrifuge. Dummy tubes can me made to balance an uneven number of samples by adding water to a 2mL microcentrifuge tube. Be sure that all tubes are closed prior to loading into the centrifuge.

Examples of balanced rotors are:



RAININ PIPETTES-CLASSIC

The Ranin pipettes are carefully calibrated and delicate. It is important to NEVER adjust the pipet volume below or above the pipette's minimum and maximum range. Doing so will effect accuracy of the pipette, disturb calibration and damage gears rendering the pipette unusable. (See Pipetting below)

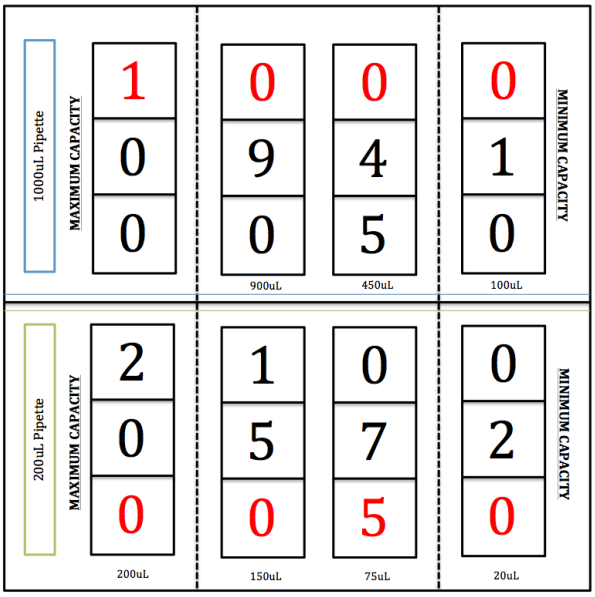
Pipetting

GENERAL RULES

1. Set pipette volume ONLY within the range specified for that micropipette. Do not attempt to set a volume beyond the pipette's minimum or maximum values. This will cause damage to the pipette.

Pipette	Volume Range
P1000 (Blue Tips)	100-1000uL
P200 (Green Tips)	20-200uL

Examples of what the volume windows will look like on each pipette are depicted below.



2. Always use a pipette tip when pipetting. Failure to do so will contaminate the pipette.
3. Always keep the pipette in a vertical position when there is fluid in the tip. Tilting the pipet horizontally can cause liquid to travel into the pipette piston cause contamination.
4. Use your thumb to control the speed at which the plunger rises after taking up or ejecting fluid. Letting the plunger snap back can damage the pipette.

Using the Pipette

LOADING/CHANGING TIPS

To load pipette tips, open the box of sterile pipette tips, firmly place the end of pipette piston into one pipette tip and slowly remove it from the box. Never pick up the tip and put it on the pipette, this can cause contamination of samples, pipette tip boxes and pipettes. Remove the tip slowly to avoid spilling the box of pipette tips. Each box of pipettes are pre-sterilized, in the case where a box of tips are spilled or a tip is dropped, dispose the effected tips, as they are no longer sterile.

Whenever liquid is being drawn up from a reagent bottle or sample, change the pipette tip for every collection to avoid contaminating the reagent bottle or sample. To be safe, change tips after every use (every collection/expulsion).

To expel the pipette tip, hover the pipette over a “TIP WASTE” bin and push firmly down on the tip eject button.

LOADING REAGENTS

With a sterile pipette tip, push the plunger to the first stop, lower the pipette tip into the reagent or sample fluid. It is very important that **ONLY THE PIPETTE TIP IS ENTERED INTO A REAGENT BOTTLE OR SAMPLE TUBE**. The pipette tip is sterile, where the pipette piston has a higher chance of having contaminates on its surface.

If necessary, tilt the reagent bottle so that ONLY the pipette tip needs to be entered into the bottle. It is also important, that **ONLY THE PIPETTE TIP IS SUBMERGED INTO THE REAGENT**, if the pipette tip and piston are submerged, the accuracy of the volume measured will be effected and cause reagent contamination.

EXPELLING REAGENTS

While keeping the pipette in a vertical position AT ALL TIMES, slowly lower the pipette tip into the receiving tube at a 20^0 angle, touching the tip to the inside of the tube, slowly push the plunger to the second stop-keeping the plunger pushed down to the second stop remove the pipette from the tube (this avoids drawing up fluid from the receiving tube) and release the plunger. Expel the pipette tip.

TECHNIQUE

Prior to picking up the pipette, uncap the tube or bottle in which you are taking fluid.

1. Load a new pipette tip onto the pipette.
2. Once the reagent bottle is uncapped, pick up the pipette in one hand and hold it vertically. Hold the tube in the other hand. Both the pipette and the tube should be close to eye level.
3. Press the plunger with your thumb to the first stop, holding this position, submerge the pipette tip into the fluid to be pipetted.
4. Slowly release the plunger while keeping the pipette tip submerged in the fluid.
5. Once the plunger is fully released, keeping the pipette vertical, transfer the tip to the collection tube, touching the side of the tube slightly with the pipette tip.
6. Uncap the receiving tube. Lower the pipette tip into the tube at a 20^0 angle, touching the pipette tip to the side of the tube, slowly press the plunger to eject the fluid to the second stop.
7. Keeping the plunger pressed to the second stop, remove the pipette from the tube and slowly release the plunger and expel the tip in the waste container.

PIPETTE MIXING

There are several steps in the DNA Everywhere DNA extraction protocols that require pipette mixing.

1. After a reagent is drawn into the pipette tip, lower the tip into the receiving tube.
2. Expel the reagent ONLY TO THE FIRST STOP
3. Slowly release and press the plunger, ONLY TO THE FIRST STOP, drawing up and expelling the liquid in the receiving tube. (Watch the tube, often times the mixing can be observed when using reagents and samples with different colors, viscosities or chemicals)
4. Once the reagent tube is mixed, or the solution has been pipette mixed 4-6 times, press the plunger to the second stop-HOLD, remove the pipette from the tube and expel the tip into the proper container.

APPENDIX A

Pipette Practice A (ORANGE)

Part I-Practice Pipetting and Pipette Mixing with Viscous Solutions Part II-Precision Pipetting Practice

Part I- Practice Pipetting and Pipette Mixing with Viscous Solutions

1. Using the sterile technique, remove 6-2mL microcentrifuge tubes.

2. Label the tubes 1-6

3. Using the 1000uL pipette, pipette 500uL of Solution A into tubes 1-6.

4. Next, you are going to add 500uL of Solution X to Tube 1. Prior to doing so, read through Step 4 and Step 5. ***When adding Solution X, watch as you dispense the solution into Solution A. Because Solution A and Solution X vary in viscosities, you should notice "swirls" when you eject Solution X into Solution A.**

5. Pipette mix by slowly ejecting Solution X to the first stop on the pipette. Slowly release the plunger so a mixture of X and A are aspired into the pipette tip, slowly eject to the first stop and aspire the mixture again. Repeat 4-5 times or until you no longer see "swirls" when ejecting.

6. Repeat Step 4 & 5 in Tubes 2-6.

Part II-Pipetting Precision Practice

1. Cut a small piece of wax paper and tape it to your tabletop.

2. Using the sterile technique, remove 6-2mL microcentrifuge tubes and label them 1-5 and "DYE".

3. Using the 1000uL pipette, pipette: **500uL of YELLOW** onto the wax paper.

4. Add **100uL of RED** to the center of the YELLOW.
   *Be careful not to poke a hole in your wax paper the pipette tip.

5. Set a 1000uL pipette to the total volume expected to be on the wax paper (600uL).

6. Aspire the liquid from the wax paper.

Is there any of the mixture remaining on the wax paper?_____

Are there any bubbles or gaps in the pipette tip? _____

7. Place the 600uL into the clean 2mL microcentrifuge tube labeled "DYE"
8. In each of the numbered tubes, pipette 1,000uL of water.
9. In tube 1, add 20uL of DYE to the water. Pipette Mix.
10. In tube 2, add 50uL of DYE to the water. Pipette Mix.
11. In tube 3, add 75uL of DYE to the water. Pipette Mix.
12. In tube 4, add 100uL of DYE to the water. Pipette Mix.
13. In tube 5, add 200uL of DYE to the water. Pipette Mix.
14. Briefly vortex all tubes and spin down.

Tubes 1-5 should visually depict a gradient dilution, meaning Tube 1 should have a lighter shade of the mixture than Tube 2 and so on.

Pipette Practice B (PURPLE)

Part I-Practice Pipetting and Pipette Mixing with Viscous Solutions Part II-Precision Pipetting Practice

Part I- Practice Pipetting and Pipette Mixing with Viscous Solutions

7. Using the sterile technique, remove 6-2mL microcentrifuge tubes.
8. Label the tubes 1-6
9. Using the 1000uL pipette, pipette 500uL of Solution A into tubes 1-6.
10. Next, you are going to add 500uL of Solution X to Tube 1. Prior to doing so, read through Step 4 and Step 5. ***When adding Solution X, watch as you dispense the solution into Solution A. Because Solution A and Solution X vary in viscosities, you should notice "swirls" when you eject Solution X into Solution A.**
11. Pipette mix by slowly ejecting Solution X to the first stop on the pipette. Slowly release the plunger so a mixture of X and A are aspired into the pipette tip, slowly eject to the first stop and aspire the mixture again. Repeat 4-5 times or until you no longer see "swirls" when ejecting.

12. Repeat Step 4 & 5 in Tubes 2-6.

Part II-Pipetting Precision Practice

15. Cut a small piece of wax paper and tape it to your tabletop.

16. Using the sterile technique, remove 6-2mL microcentrifuge tubes and label them 1-5 and "D".

17. Using the 1000uL pipette, pipette: **600uL of RED** onto the wax paper.

18. Add **400uL of BLUE** to the center of the RED.
    *Be careful not to poke a hole in your wax paper the pipette tip.

19. Set a 1000uL pipette to the total volume expected to be on the wax paper (1000uL).

20. Aspire the liquid from the wax paper.

Is there any of the mixture remaining on the wax paper?_____

Are there any bubbles or gaps in the pipette tip? _____

21. Place the 1,000uL into the clean 2mL microcentrifuge tube labeled "DYE"

22. In each of the numbered tubes, pipette 1,000uL of water.

23. In tube 1, add 20uL of DYE to the water. Pipette Mix.

24. In tube 2, add 50uL of DYE to the water. Pipette Mix.

25. In tube 3, add 75uL of DYE to the water. Pipette Mix.

26. In tube 4, add 100uL of DYE to the water. Pipette Mix.

27. In tube 5, add 200uL of DYE to the water. Pipette Mix.

28. Briefly vortex all tubes and spin down.

Tubes 1-5 should visually depict a gradient dilution, meaning Tube 1 should have a lighter shade of the mixture than Tube 2 and so on.

Pipette Practice C (TEAL)

Part I-Practice Pipetting and Pipette Mixing with Viscous Solutions
Part II-Precision Pipetting Practice

Part I- Practice Pipetting and Pipette Mixing with Viscous Solutions

13. Using the sterile technique, remove 6-2mL microcentrifuge tubes.

14. Label the tubes 1-6

15. Using the 1000uL pipette, pipette 500uL of Solution A into tubes 1-6.

16. Next, you are going to add 500uL of Solution X to Tube 1. Prior to doing so, read through Step 4 and Step 5. ***When adding Solution X, watch as you dispense the solution into Solution A. Because Solution A and Solution X vary in viscosities, you should notice "swirls" when you eject Solution X into Solution A.**

17. Pipette mix by slowly ejecting Solution X to the first stop on the pipette. Slowly release the plunger so a mixture of X and A are aspired into the pipette tip, slowly eject to the first stop and aspire the mixture again. Repeat 4-5 times or until you no longer see "swirls" when ejecting.

18. Repeat Step 4 & 5 in Tubes 2-6.

Part II-Pipetting Precision Practice

29. Cut a small piece of wax paper and tape it to your tabletop.

30. Using the sterile technique, remove 6-2mL microcentrifuge tubes and label them 1-5 and "DYE".

31. Using the 1000uL pipette, pipette: **300uL of BLUE** onto the wax paper.

32. Add **300uL of GREEN** to the center of the BLUE.
*Be careful not to poke a hole in your wax paper the pipette tip.

33. Set a 1000uL pipette to the total volume expected to be on the wax paper (600uL).

34. Aspire the liquid from the wax paper.

Is there any of the mixture remaining on the wax paper?_____

Are there any bubbles or gaps in the pipette tip? _____

35. Place the 500uL into the clean 2mL microcentrifuge tube labeled "DYE"

36. In each of the numbered tubes, pipette 1,000uL of water.

37. In tube 1, add 20uL of DYE to the water. Pipette Mix.

38. In tube 2, add 50uL of DYE to the water. Pipette Mix.

39. In tube 3, add 75uL of DYE to the water. Pipette Mix.

40. In tube 4, add 100uL of DYE to the water. Pipette Mix.

41. In tube 5, add 200uL of DYE to the water. Pipette Mix.

42. Briefly vortex all tubes and spin down.

Tubes 1-5 should visually depict a gradient dilution, meaning Tube 1 should have a lighter shade of the mixture than Tube 2 and so on.

Solutions

Instructor will prepare the following prior to beginning pipette practice exercises.

Solution “A”: Water
Solution “X”: Rubbing Alcohol
Colored “solutions”: fill a 15mL centrifuge tube with water and add one drop of the respective food coloring. Shake and aliquot into micro centrifuge tubes and label

DNA Extraction Protocol

Compost

1. Sampling and Homogenization
2. Extraction Preparation
3. DNA Extraction

*Protocols designed to be used with Zymo™ Fecal DNA MiniPrep kit

Personal Protective Equipment (PPE)

Personal Protective Equipment (PPE) Prior to beginning, put on lab coat, gloves and goggles.

Sampling and Homogenization Protocol

Sampling

1. Wearing gloves and boots, dig about 50cm (1.5-2 feet) into the compost pile near the temperature indicators.
2. Once you have dug to the desired depth, sterilize a hand trowel by dipping a brush into a 10% bleach solution and scrubbing the trowel. After the trowel is clean, rinse with water.
3. Scoop a trowel-full of compost out of the hole.
4. Using sterile forceps, transfer the compost on the hand trowel into a 15mL Bead Tube until loosely full. You can push the compost down further in the tube but do not pack the tube tightly; the beads need to be able to move up and down through the compost.

Homogenization

1. Place Bead Tube (loosely full with compost sample) in the 15mL tube attachment on the Vortex Genie.
2. Turn the Vortex to maximum speed and run for 5 minutes.

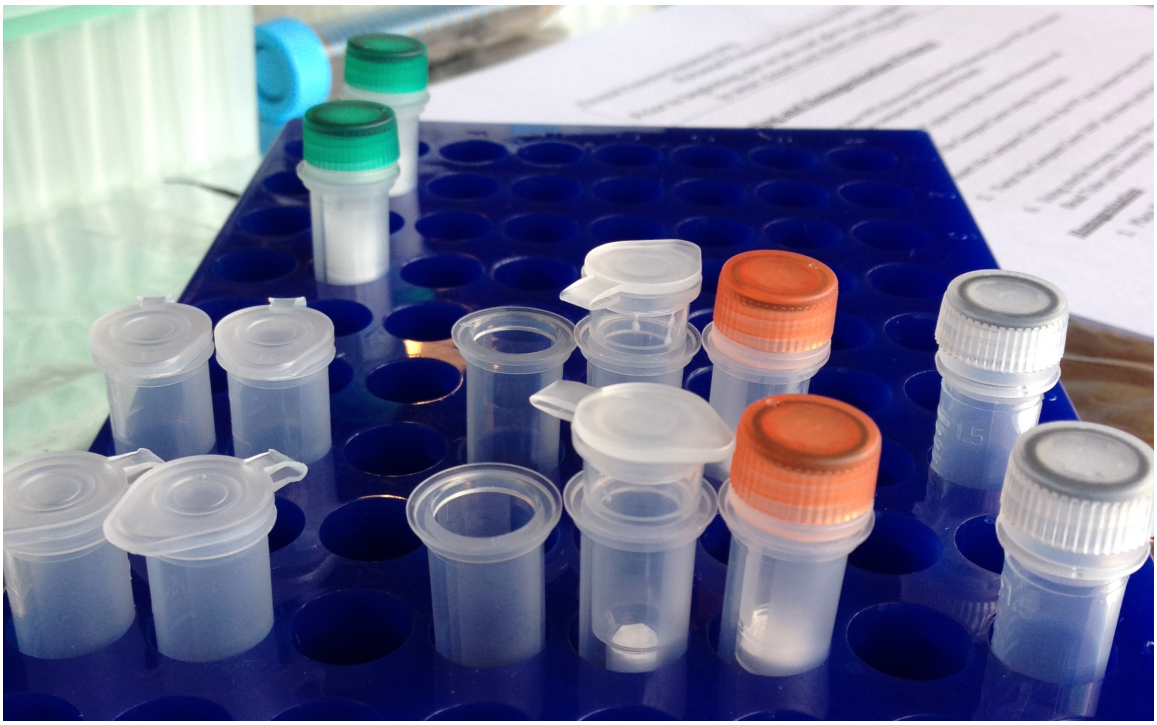
3. Remove tube from Vortex and empty a small portion of the homogenized sample onto a plate or weigh boat.

Only empty a small amount of homogenized sample onto plate and continue to empty sample as needed.

Extraction Preparation

1. Sterilize all surfaces with 10% Bleach Solution
    a. Bench Top
    b. Centrifuge
    c. Pipettes
    d. Tube Racks
    e. Pipette Tip Boxes
    f. Gloves

2. Set up tube rack as follows:



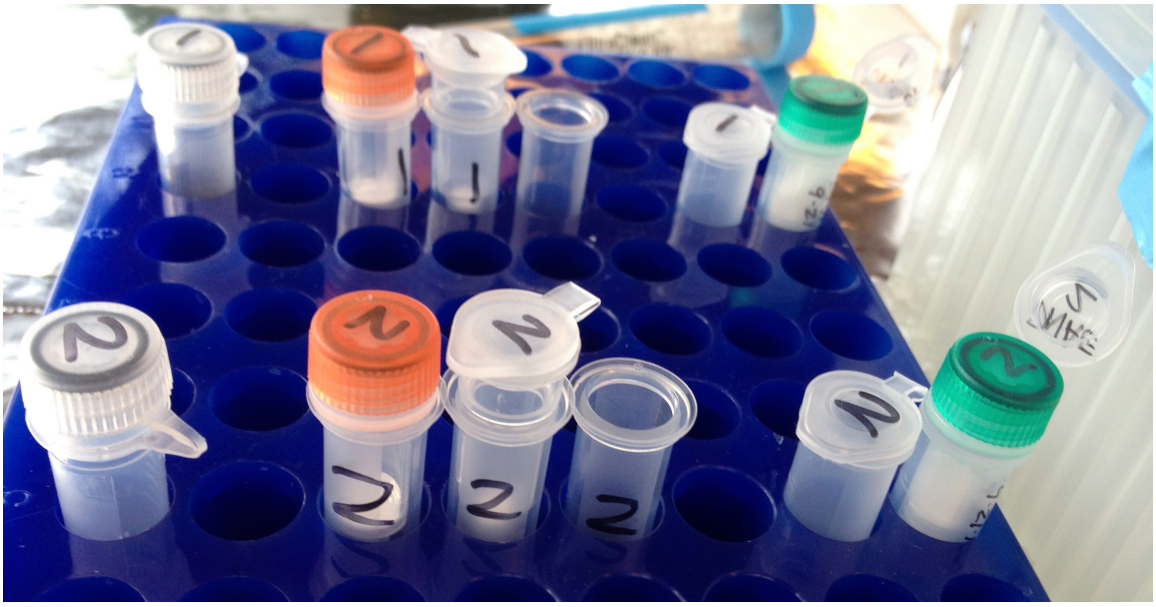
(Tubes per sample extraction: 4 Collection Tubes, 1 **BEAD** Tube, 1 **ORANGE** Filter, 1 **GREEN** Filter, 1 **WHITE** Filter, 2 Microcentrifuge Tubes)

3. Prepare **GREEN Filters** by snapping off the base, inserting column into a collection tube and spin in the Galaxy MiniStar centrifuge for 10 minutes. *(If*

the filter is dry, add 400-600uL water prior to spinning the filter) **Discard collection tube and place Filter in final 1.5mL tube.**

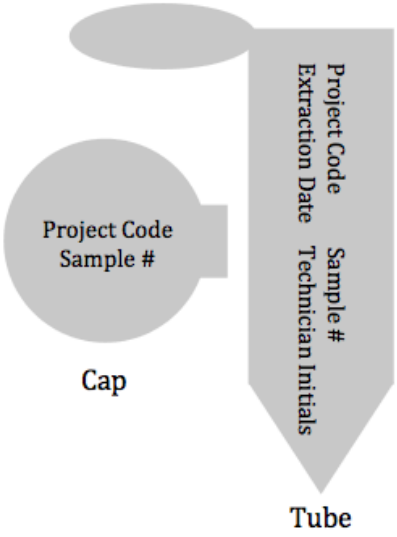
4. Label the tops of all tubes and columns according to the sample number. **Label the sides of ALL collection tubes with the sample number** (as depicted in the photo below)**.** The final tube labeling must contain the Project Code, Sample Number, Date Extracted, and Technician Initials, as depicted in the schematic below.

5. Snap off the base of the **ORANGE Filters.**

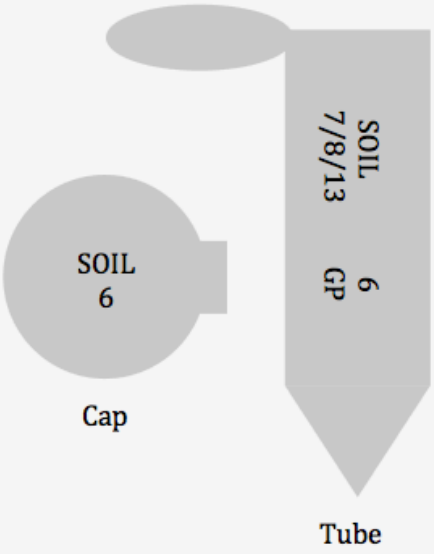


Final Tube Labeling

Project Code:
Sample #:
Extraction Date:
Technician Initials:



Example: Sample #6
SOIL-Haiti Project
Project Code: SOIL
Extracted on: July 8, 2013
Extracted by: Gabrielle Pecora



DNA Extraction Protocol

(Modified from the Zymo® Fecal DNA Extraction Kit Protocol)

1. Lysis [Steps 1-6]
2. Filtration [Steps 7-9]
3. Bind DNA to Column [Steps 10-15]
4. DNA Washing [Steps 16-19]
5. Eluting DNA [Steps 20-24]

Lysis

1. Turn on scale. Place a piece of weigh paper on the scale and press the "TARE" button until the weight reads 0.00g.

2. Add 0.20g of compost sample to the weigh paper. Transfer this into a **BEAD TUBE** (see Photo Glossary) using sterile forceps**.**

   > *Try not to get compost material on the scale, aluminum foil or in the threads of the tube.*

3. Re-sterilize bench top, scale, and gloves with 10% bleach.

4. Add 750uL **Lysis Solution** to the **BEAD TUBE**.

5. Secure tubes on a vortex fitted with a 2mL tube holder attachment and process at maximum speed for 5 minutes.

6. Centrifuge the **BEAD TUBE** in the Galaxy MiniStar centrifuge for 5 minutes.

   > *Make sure the centrifuge is balanced prior to starting. Note, that at times the samples might have different weights or volumes depending on sample tubes versus extraction blanks. If there are an uneven number of blank versus sample tubes, make a "dummy" blank to properly balance weights across from each other.*

Filtration

7. Transfer up to 400uL supernatant from the **BEAD TUBE** to a **ORANGE Filter** tube in a **Collection Tube** and centrifuge 3.5 minutes.

   > *It is possible that there is more than 400uL in the Bead Tube, ONLY transfer 400uL*
   >
   > *Be sure to label the collection tubes holding the orange filters as you will need to save the filtrate.*

8. Discard the **BEAD TUBE**.

9. Remove tubes from centrifuge and discard the **ORANGE Filter** tube saving the filtrate in the collection tube.

Bind DNA to Column

10. Add 1,200uL of **Fecal DNA Binding Buffer** to the filtrate in the **Collection Tubes** from Step 4.*

> *Fecal DNA Binding Buffer is viscous and bubbly, be aware of any excess Binding Buffer drops on the outside of the pipette tip. To prevent transferring bubbles, when expelling fluid do not push plunger all the way down, if a small bubble is left in the pipette tip, that is OK.*

> ** To streamline step 10 and 11, first add 400uL of **Fecal DNA Binding Buffer** to each tube. Then add 800uL of Binding Buffer to one sample, pipette mix in the collection tube and transfer 800uL of the sample-Buffer mix to a **White Filter Tube** in a new collection tube. Repeat the 800uL addition, pipette mixing and transfer with all samples*

11. Transfer 800uL of sample-buffer mixture to a **WHITE Filter** tube in a new collection tube. Keep the remaining 800uL in your rack.*

12. Centrifuge for 5 minutes.

13. Discard the flow through from the **Collection Tube,** saving the filter.

> *Avoid getting drops of the flow through on the sides of the Collection Tubes.*

14. Transfer the remaining 800uL of sample-buffer mixture to the white column and centrifuge again for 5 minutes.

> The DNA is now all bound to the matrix in the white column. You will perform a series of washes to purify the DNA before finally eluting.

15. Discard the flow through. Place the **WHITE Filter** tube into a new **Collection Tube.**

DNA Washing

16. Add 200uL **DNA Pre-Wash Buffer** to the filter and centrifuge for 5 minutes.

17. Discard flow through.

18. Add 500uL **Fecal DNA Wash Buffer** to the **WHITE Filter** tube and centrifuge for 5 minutes.

19. Discard flow through and spin again for 5 minutes.

This is a dry spin to ensure that all the ethanol has passed through the column. Be careful removing the columns at this step, do not let any ethanol drops touch any part of the column. Residual ethanol will disrupt downstream processes. This step can be repeated if necessary.

Eluting DNA (Removing DNA from Column)

20. Transfer the **WHITE Filter** tube to a clean 2mL microcentrifuge tube.
21. Add 100uL **DNA Elution Buffer** directly to the column matrix.
22. Centrifuge for 2.5 minutes to elute the DNA.
23. Before discarding the columns make sure all the **DNA Elution Buffer** passed through the column and is now in the microcentrifuge tube. If not then either add more elution buffer or re-centrifuge the tubes.
24. Transfer the eluted DNA from Step 11 (set pipette to 105-110uL) to a prepared **GREEN Filter** in a clean and labeled (see below) 2mL microcentrifuge tube and centrifuge for 4 minutes.
25. Store DNA in clearly labeled tubes in the freezer for shipment. The filtered DNA is now suitable for PCR and other downstream applications.

Shipping Instructions:

SOIL Haiti

1. Place DNA in Sample Bags labeled with "SOIL [Sample #]-[Sample #], Date, Technician Name".
2. Place Sample bags in Styrofoam shipper with 6 frozen PolarTech Freeze packs.
3. Stick a WarmMark® temperature indicator to the inside of the Styrofoam container. Close the lid immediately.
4. Fill out a "Chain of Custody" form. Sign the form and place it on top of the Styrofoam container and tape the cardboard box shut.
5. Ship the container overnight mail to:

_____

_____

_____

Acknowledgments

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