

DNA extraction lab: will take place Wednesday Oct. 1

To determine the clonal identity of each of your trees, we will genotype them using microsatellites. In order to do this, we must first isolate and purify the DNA obtained from the leaf tissue samples that you collected. This is done through a process called DNA extraction. In this class, we will be using a DNA extraction kit from Qiagen. This kit is specifically designed for use with plant DNA.

Basic steps of DNA extraction:

1. Break open cells to expose DNA (often referred to as cell disruption or cell lysis)
2. Dissolve membrane lipids and proteins by adding a detergent
3. Digest RNA by adding an RNase enzyme
4. Purify DNA away from cell debris, other molecules and detergent using spin columns

For more information about the specific protocol, there is a link to Qiagen's plant DNA purification handbook on the course website.

Lab hygiene:

- Wash hands frequently or wear gloves (gloves should be switched out periodically)
- Be careful not to touch inside the caps of tubes when opening and closing them
- Dispose of used pipette tips in appropriate place.
- Don't allow pipette tips to touch your hands or other surfaces.
- Never reuse a pipette tip once it has come into contact with your DNA solution or other contaminants.

Documentation:

You should always take careful and detailed notes any time you perform an experiment. For this extraction lab, make notes on how the experiment goes, including any deviations from the protocol, in your lab notebook. Make sure to always write legibly and to title and date your notes at the top of the page.

It is also important to clearly label your tubes according to the tree that they came from. A mix up in tubes could result in trees being misidentified by clone. To ensure that no mix-ups occur, tubes should be labeled twice: 1) on the cap, and 2) on the side of the tube. Cap labels will be made using white, circular stickers (called tough spots) that can be written on and then adhered to the top of the tube. Side labels will be made by writing directly onto the tube with a sharpie. These two labeling methods are demonstrated below:



Pipetting:

The micropipettor is used to transfer small amounts (< 1 ml) of liquids. The scales on micropipettors are in microliters ($1000\mu\text{l} = 1$ ml). They are used in conjunction with disposable plastic tips. Micropipettors come in a range of sizes which are capable of pipetting different ranges of volumes. We will use three different sizes of pipettors in this lab: P20 = 0.5- 20 μl , P200 = 20-200 μl , and P1000 = 200-1000 μl . The desired volume is set by turning the centrally located rings clockwise to increase volume or counterclockwise to decrease volume, as shown here for one brand (Gilson). The other brands (e.g. Eppendorf) work in similar ways.



Amount = 12.5 μ l



Amount = 120 μ l



Amount = 750 μ l

The plunger will stop at two different positions when it is depressed. The first of these stopping points is the point of initial resistance and is the level of depression that will result in the desired volume of solution being transferred. The second stopping point can be found when the plunger is depressed beyond the initial resistance until it is in contact with the body of the pipettor. This second stopping point is used for the complete discharging of solutions from the plastic tip. You should not reach this second stop when drawing liquid into the pipettor, only when expelling the last drop.

Depress the plunger until you feel the initial resistance and insert tip into the solution, just barely below the surface of the liquid and not as deep as possible. Carefully and slowly release plunger. Discharge the solution into the appropriate container by depressing plunger. This time, depress the plunger to the point of initial resistance, wait one second, and then continue pressing the plunger as far as it will go in order to discharge the entire volume of solution. Completely remove the pipette tip from the solution before slowly releasing the plunger. Remove tip by pressing down on the tip ejector button. (See back page for diagram.)

Notes:

- When withdrawing liquids with the pipettor (“aspirating”), always release the plunger *slowly*.
- Be sure you use the proper size tip for each pipettor.
- Always use a new tip for each different liquid.
- Use the correct pipettor for the volume that is to be dispensed.

Grinding:

To break apart the leaf cells (step 1 in the basic steps of DNA extraction), you will grind the leaves using a small blue pestle that is designed to fit into a 1mL centrifuge tube. First you will dry-grind the leaves into a fine powder. Then you will add a buffer and continue grinding until no large clumps of leaf tissue are visible. It is important that you thoroughly grind up the leaves to ensure you get a high DNA yield at the end of the lab.



Vortexer:

A vortexer is a device used to mix small tube of liquid. As the motor runs, the rubber top piece oscillates rapidly in a circular motion. When a tube is pressed into the rubber cap, the motion is transmitted to the liquid and a vortex is created. There is a knob on the front to adjust the speed. The vortexer can be set to run continuously or to run only when pressure is applied to the rubber piece.



Centrifuge:

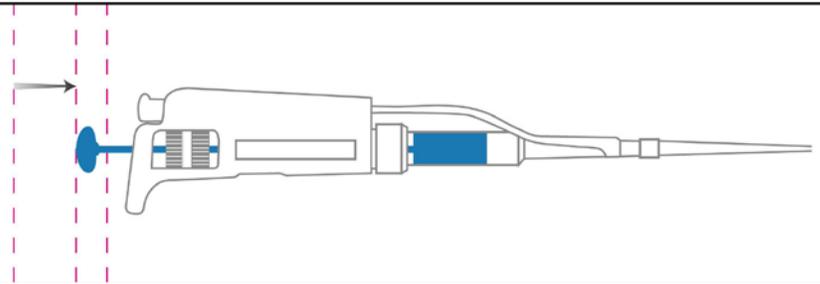
A centrifuge is a device that rotates tubes at a high speed to generate enormous centripetal forces that can separate liquids by their density.



1

Preparation

Hold the instrument in a nearly vertical position. Depress the plunger smoothly to the first stop position.



rest position

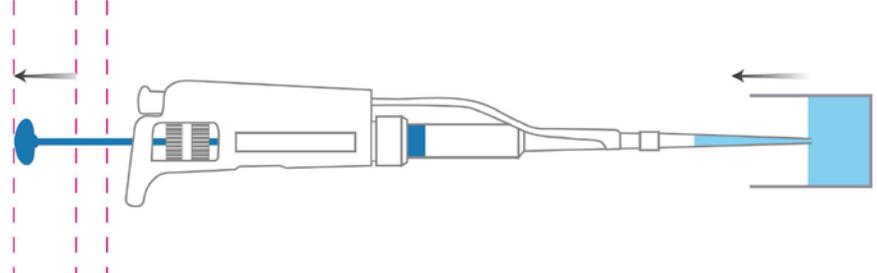
first stop

second stop or purge

2

Aspiration

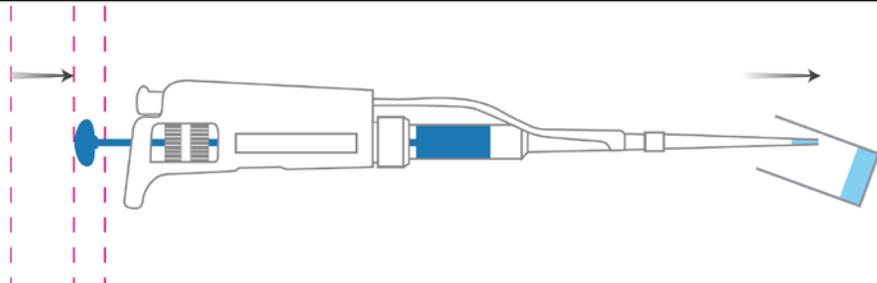
Immerse the pipette tip in the liquid*. Allow the plunger to move up smoothly to the rest position. Wait one second so that all the liquid has time to move up into the tip.



3

Distribution

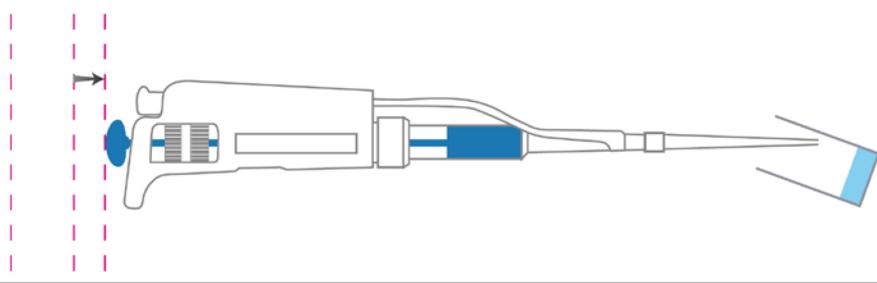
Place the pipette tip at an angle (10 to 45°) against the inside wall of the receiving vessel. Depress the plunger smoothly to the first stop position.



4

Purge

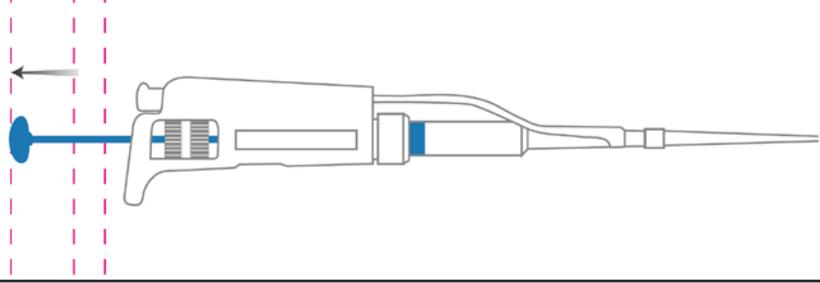
Wait one second, then depress the plunger to the second stop position. This "blow-out" stroke removes any remaining sample from the tip. Remove pipette tip end from sidewall by sliding it up the wall.



5

Home

Allow the plunger to move up to the rest position.



*The immersion depth of your tip can have a significant effect on your results. If the tip is immersed too deeply, droplets will form on the outside of the tip and they will be deposited along with your sample. If the tip is not immersed deeply enough, vortexing will occur and your pipette will not aspirate the selected volume.

volume μ l	immersion depth mm
0.1 - 1	1
1 - 100	2-3
101 - 1000	2-4
1001 μ l - 10 ml	3-6