

DNA extraction

Genetics 362 Lab

We are now beginning a project that will stretch over several weeks. Over this time, you will extract DNA from an organism, use PCR to amplify many copies of a particular gene, sequence it, and compare the sequence to a large public database to determine what species your organism is most closely related to.

Today in lab, you will work in pairs to extract DNA from your organism of choice. The technique is very similar to the last DNA extraction that you did, except this should produce DNA that is of higher quality and purity, so should be more suitable for PCR.

Materials needed:

plants
1.5ml microcentrifuge tubes (2 per student pair)
pipettors and tips (1000 μ l)
blue pestle for grinding tissue
5-prime DNA extraction kit
100% isopropanol (300 μ l per student pair)
70% ethanol (300 μ l per student pair)
paper towels

Equipment:

65C heat block
centrifuge (set to 13,000 – 16,000 x g) **For big epis only!**

Pipetting:

Accurate pipetting is essential to successful implementation of molecular biology techniques. Many techniques fail in the hands of undergraduates because of inaccurate pipetting. These techniques are extremely sensitive to having exactly the right amount of each enzyme and chemical.

Look on the disk at the top of each pipettor to see the maximum volume that the pipettor will hold. If it says 1000 μ l, we call this a 1000 μ l pipettor. We also have 200 μ l, 100 μ l and 10 μ l pipettors. 10 μ l isn't very much liquid. Go to the side of the room and get a pipettor of each size – you and your lab partner can share.

All of the pipettors should have 3 digits that change as you rotate the disk on the top of the pipettor. These digits tell you the volume that you will be picking up. But the digits have different meanings on the different-sized pipettors, so it can be a bit confusing. When some of the 1000 μ l pipettors read 100, this means you will pick up 1000 μ l, and when it reads 020, you will pick up 200 μ l. The 1000 μ l pipettor does not go below this volume. However, on the 100 μ l and 200 μ l pipettors, 100 means that you will pick up 100 μ l. On the 10 μ l pipettors, 100 means 10 μ l. Is that confusing or what? Always check the top of the pipettor to see the maximum volume. Use this information to help you interpret the digits on the side.

Some pipetting rules:

Never rotate volume adjustor beyond the upper or lower range.
Never use without a disposable tip in place.
Never lay pipettor down with filled tip-fluid could run back into the piston.
Never let plunger snap back after withdrawing or ejecting fluid. This could damage the piston.
Always use a plastic tip before picking up a sample.
Use a fresh tip for every sample and every solution to avoid contamination.
Always use the smallest-volume pipettor that will hold the desired volume.

The plunger on the pipettor has two stops – one gentle stop (first stop), and then one that you can't pass (second stop). The first stop is for picking up liquid and the second is for ejecting the liquid. The second stop ejects a bit of air after the liquid to make sure all of the liquid comes out.

When picking up sample, depress plunger to **first stop**, place tip into tube, suck up liquid slowly. Always watch the liquid as you suck it into the pipettor. Make sure you are actually picking up liquid. If you pick up air bubbles, expunge the liquid and start over. Check to see that the volume looks correct.

When ejecting sample, touch tip to side of tube or place tip into liquid already in tube, push plunger down slowly to second stop. Eject tip into trash so you don't accidentally reuse it.

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DNA extraction

Methods:

- 1) **Collect tissue:** I have placed a number of plants in the lab for you to choose from. When you make your choice, write down the plant ID information from the tag

Plant ID: _____ Leaf hairs or no hairs? _____

Also write down the plant ID on the paper your TA put near the plants.

It is essential that you keep track of what plant your sample came from and whether it had leaf hairs! Otherwise, your sequence data at the end of the semester will be useless.

Collect a piece of leaf tissue (a piece of leaf tissue about the size of a pencil eraser), and place it in a 1.5ml microcentrifuge tube. **Label** the tube with your name. **Do not use too much tissue or the kit will not properly purify the DNA.**

- 2) **Grind tissue:** You need to grind up the tissue as best you can with the blue pestle.
- 3) **Lyse cells:** Add 300µl of the **cell lysis solution** to the leaf tissue. If your tissue isn't completely ground up at this point, grind some more with the pestle. This solution contains SDS (detergent) to dissolve the cell membranes, as well as salt and buffer to stabilize the pH. Remember what detergent does to cell membranes? _____
- 4) **Incubate** cell lysate (the lysed cells from 3) at 65C for 15-60 minutes (15 min is fine).
- 5) **Invert** tube several times to mix and **cool** to room temperature. (5 min)
- 6) **Protein precipitation:** Add 100µl **protein precipitation solution** to your cell lysate, and **mix** well by inverting tube continuously for about 2 minutes (150 times). This step causes proteins to become insoluble in water, but the DNA is still soluble, so it stays in solution. This allows us to separate the DNA and proteins and obtain pure DNA.
- 7) **Centrifuge** at 13,000-16,000 x g for 3 minutes (g stands for gravity on earth). This causes the proteins and intact tissue to form a tight pellet at the bottom of the tube. This is the stuff you want to get rid of. Your DNA is still dissolved in the

liquid.

8) Transfer the liquid into a clean microcentrifuge tube without disturbing the pellet at the bottom of the tube. You can either pour the liquid or use a pipettor. Don't contaminate your new tube with junk from the pellet – better to leave some liquid behind.

9) DNA precipitation:

DNA is not soluble in alcohol, so when you add alcohol the DNA will come out of solution. In this way we can separate the DNA from any cellular junk and salts still in the solution.

Add 300µl 100% isopropanol

Mix sample by inverting gently 50 times

10) Centrifuge at 13,000-16,000 x g for 1 minute

Now you should see a pellet of DNA at the bottom of the tube (it won't be really obvious)

Pour off supernatant (the liquid) into sink or beaker. Be careful not to let your DNA pellet escape from the tube.

Place tube upside down on paper towel briefly (about 1 minute) to drain.

11) Add 300µl 70% ethanol, and invert several times to clean pellet.

12) Centrifuge at 13,000-16,000 x g for 1 minute

Your DNA is still in the pellet at the bottom of the tube, but **the pellet may not be stuck** to the tube as well as before.

Carefully pour off supernatant without allowing the pellet to escape. You can use the pipettor to withdraw some of the liquid if that helps.

Place tube upside down on a paper towel to drain liquid and to air-dry for 15 minutes.

13) Hydrate DNA:

Add 50ul DNA hydration solution to your dried DNA extraction.

The DNA will dissolve in the liquid. DNA hydration solution contains a buffer to stabilize the pH and help preserve the DNA.

Your TA will collect the tubes so **make sure your tube is well labeled with your name and the plant ID.**

Next week, you will begin the PCR reaction.

14) Due next week: 1-2 paragraphs materials and methods. You turn in a lab report on this project at the end of the semester when it is finished. You should write up the materials and methods for this portion of the project now so you don't forget what you did and so that you can get feedback.

You should **describe the plants your whole class sampled** (crosses used to generate these plants, phenotypes of the plants). Also, include **your plant ID** and the **phenotype** of the plant.

You should also **describe the DNA extraction procedure**. What company did the chemicals come from? Describe each step and the **purpose of each step?** This should not be a list of bullet points, but should be in sentence format.