

Science Institute Final Paper (DRAFT VERSION)

- Submit for feedback deadline: TBD – likely June 12th
- Final submission deadline: TBD – likely week of June 22nd
- (Calibri or Times New Roman 11pt font; single-spaced)

Timeline

- **Week of June 11:** Start thinking of a site to collect plants. Barcoding at home materials should get to you this week.
- **Week of June 18:** Collect 5 plant samples and process perform the DNA extraction. You will need at half a day least a day for samples to dry on the filter paper. Mail back samples by **Monday May 25**
- **Week of June 1:** You can start to write up the abstract and methods parts of your paper. Ideally DNA sequencing results will get to you this week.
- **Week of June 8:** You can send in a draft of your paper for feedback and revision.

Experimental Description

In your experiment you will attempt to identify non-native plants in your area (plant's not native to the northeastern United States).

To do so you will need to choose plants (from your yard, a park, etc.) and identify them using the iNaturalist Seek app (https://www.inaturalist.org/pages/seek_app) and DNA Barcoding.

You will collect the plants (more info below), and get an identification from the app. In some cases, the app may be able to give you a genus and a species for the plant (but sometimes it may not be able to identify it, or perhaps may give you more limited information such as what family it is in). With what information you have, you should look for information online about the plant. I also suggest using a plant identification key (such as <https://gobotany.nativeplanttrust.org/simple/>)

With a sample of the plant collected, you will do a DNA extraction with the provided materials. You will generate some DNA extract which you will sample using a piece of filter paper. Once the paper has dried, you will send it back to me where I can do PCR, electrophoresis, and sequencing. With the DNA Sequencing, you will analyze the data on DNA Subway.

Collecting plants

You will need to choose plants that are accessible and avoid plants that may be dangerous (e.g. Poison Ivy/Oak, thorns, etc. – “leaves of three leave it be” – see this guide: <https://www.cdc.gov/niosh/topics/plants/identification.html> and <https://www.almanac.com/content/poison-oak-identification-and-treatment>). In public parks, you will also need to avoid significantly damaging plants, and since we normally need only a leaf this is quite possible.

How to pick plants

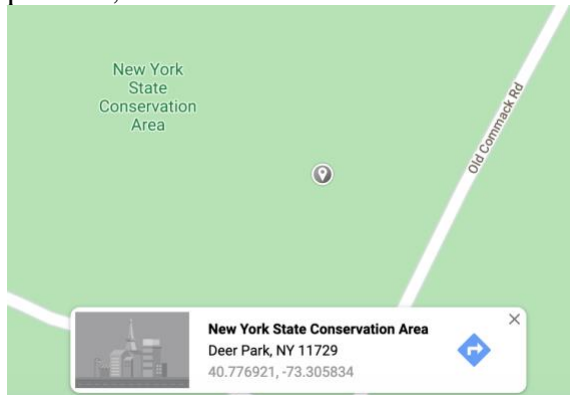
For your experiment you will need **5 plants**. You could choose plants that are familiar (e.g. an Oak tree), but some of the most interesting plants may be weeds, vines, or even patches of grass. If you are looking for plants that are non-native, where might you be most likely to find them? Many but not all cultivated plants (e.g. potted plants) will be non-native. Some parks make an effort to only plant native species. Many species of plants are also invasive (what is an invasive plant? See: <https://www.fs.fed.us/wildflowers/invasives/>). Try to get an identification with the Seek App, but if you cannot ID the plant, it may be even more interesting to barcode. Remember, if you are unsure if the plant is dangerous, do not touch it.

Sampling the plant

We only need a small amount of the plant to get DNA. However, we do need to take a lot of notes when collecting it. (Here are some professional instructions on plant collecting, a little too much for what we are doing now, but interesting to read: <https://www.brit.org/plant-collecting-how/data-record-and-photography>).

Here are the minimum collections notes

1. You must have a note on where the sample was collected. One way to do this is to get GPS coordinates. You can do this by going to <http://maps.google.com> , finding the site you collected at, and placing a “pin” down as close to the collection site as possible,



2. Write a description of the plant including your estimate of its height (in cm or m). What color is it? Does it have bark? How are the leaves shaped (see: this guide <https://www.thoughtco.com/id-trees-using-leaf-shape-venation-1343511>)? Are there flowers or buds?
3. Take a few good photos of the plant.
4. Finally, take sample of a few leaves. The most concentrated DNA are in the smallest leaves/buds usually at the tip of the plant this time of year.

Barcoding at Home Protocol

I've mailed out barcoding kits that you can use at home. Set up an area like a small desk and table where you have room. Have some paper towel in case there are spills. You can also take and include some photos of your home lab setup to include in the paper!

We will be following a modified version of this protocol:

Rapid DNA Protocol

<https://dnabarcoding101.org/lab/protocol-2.html#standard>

Your barcoding kit should include

- 1.5 ml tubes (~15)
- Plastic bulb dropper pipettes (~3)
- A recycled Styrofoam tube rack
- A tube of Lysis buffer (Guanidine Hydrochloride) – This is an irritant (like soap) so avoid spills and wash your hands before and after work
- TE wash buffer

Inside the yellow envelope there is another envelope which will contain

- Plastic pestles (~5)
- Two discs of filter paper. These are designed to be torn into strips. You can write in pencil on each strip, and you will dip the ends into your DNA isolation solution.

The Yellow Envelope has an address sticker. You will return this to me by mail including

- **The dried DNA filter paper strips** (Make sure each strip is labeled with your name and sample number)
- **Pestles**

All the other tubes and materials can be disposed of in the garbage.

Protocol

1. Obtain plant tissue ~10 mg or 1/8- to 1/4-inch diameter by removing a piece of the tissue with a clean tweezers or scissors. If you are working with more than one sample, be careful not to cross-contaminate specimens.
2. Place tissue in a clean 1.5-mL tube labeled with a sample identification number (initials and the # 1, 2, 3, 4, or 5).
3. Add 50 µL of lysis solution to each tube.
4. Twist a clean plastic pestle against the inner surface of 1.5-mL tube to *forcefully* grind the tissue for at least 2 minutes. Use a clean pestle for each sample. Ensure the sample is ground into fine particles.
5. Tear off a strip of filter paper (~ 1 inch long, 1/2 inch wide) and using pencil label the strip.
6. Next, add 200 µL of wash buffer to a clean 1.5-mL tube labeled with the sample identification number.
7. For each labeled filter paper strip, dip the end of the strip into the tube with the ground plant sample. You don't need to wet the whole sample, just the tip.
8. In the wash buffer tubes, dip the filter paper in wash buffer and leave for 1 minute.

9. Remove the trip and allow it to air dry (being careful not to cross contaminate). I have included bank absorbent paper. Let it dry undisturbed for several hours.
10. Tape all of the labeled strips to a piece of paper and the return in the envelope.

Writing the paper

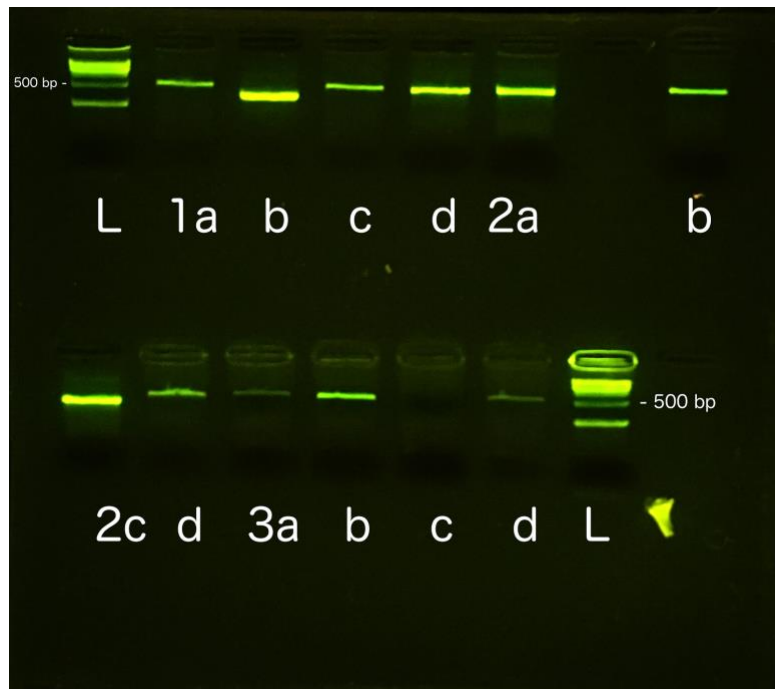
Your paper will have a layout with the following sections:

- 1) **Abstract:** This should be a 50-100-word summary of what your project was about, and your results.
- 2) **Introduction:** (*0.25-0.5 page*) Introduce what DNA Barcoding is and tell about the idea for your project. This should be interesting to someone who does not know much (or anything) about DNA, barcoding, etc. It should clearly convey why you did this project with the samples you chose. State your hypothesis about what you thought you might find.
- 3) **Material and methods:** (1-1.5 pages) Step-by-step instructions on the following:
 - Sample collection
 - DNA extraction
 - PCR and electrophoresis
 - DNA Sequencing
 - DNA Subway

Some parts of this I will simply provide info for (i.e. PCR settings and information about the DNA sequencing). Much of the DNA extraction, PCR, and DNA subway section can be written now from the manual and from your notes; you can edit these parts when we have finished our work.

4) Data: (Varies) This section should include sample information for each sample and sequence information. Be sure to use charts and other labeled figures where appropriate. In this section you will have photos and tables. Include any photo(s) of your collected samples. **Each photo or table must have a legend.** I have provided examples in the templates, be sure that you modify these appropriately for your data. You must include a photo of your PHYLIP-NJ tree.

You should provide your gel photo **with labeled lanes**. Use a photo editing software to add a number to each lane. You will be provided with a gel photo for the PCR I do for you.



Gel 1: Lane (L) is a 1KB ladder. (1a) is... (b)... (c)...

You should provide a table indicating what samples you generated and information on what species they were and if DNA Sequencing succeeded. You can modify the one below:

Sample Name	Sample identity (if known) and notes	Collection site	DNA Sequencing results
sm-1	Potato leaf (Solanum tuberosum ?)	Supermarket	No quality warning: 600bp
sm-2	Tomato leaf (Solanum lycopersicum?)	Supermarket	Low quality warning: 200bp

Table 1: Enter a title for your table

You should provide a table describing the BLAST results for your samples. You can modify the one below:

Sample Name	BLAST	Top BLAST hit	Mismatches
sm-1	Yes	Solanum tuberosum	0
sm-2	No - quality too low	N/A	No pellet visible

Table 2: *Enter a title for your table*

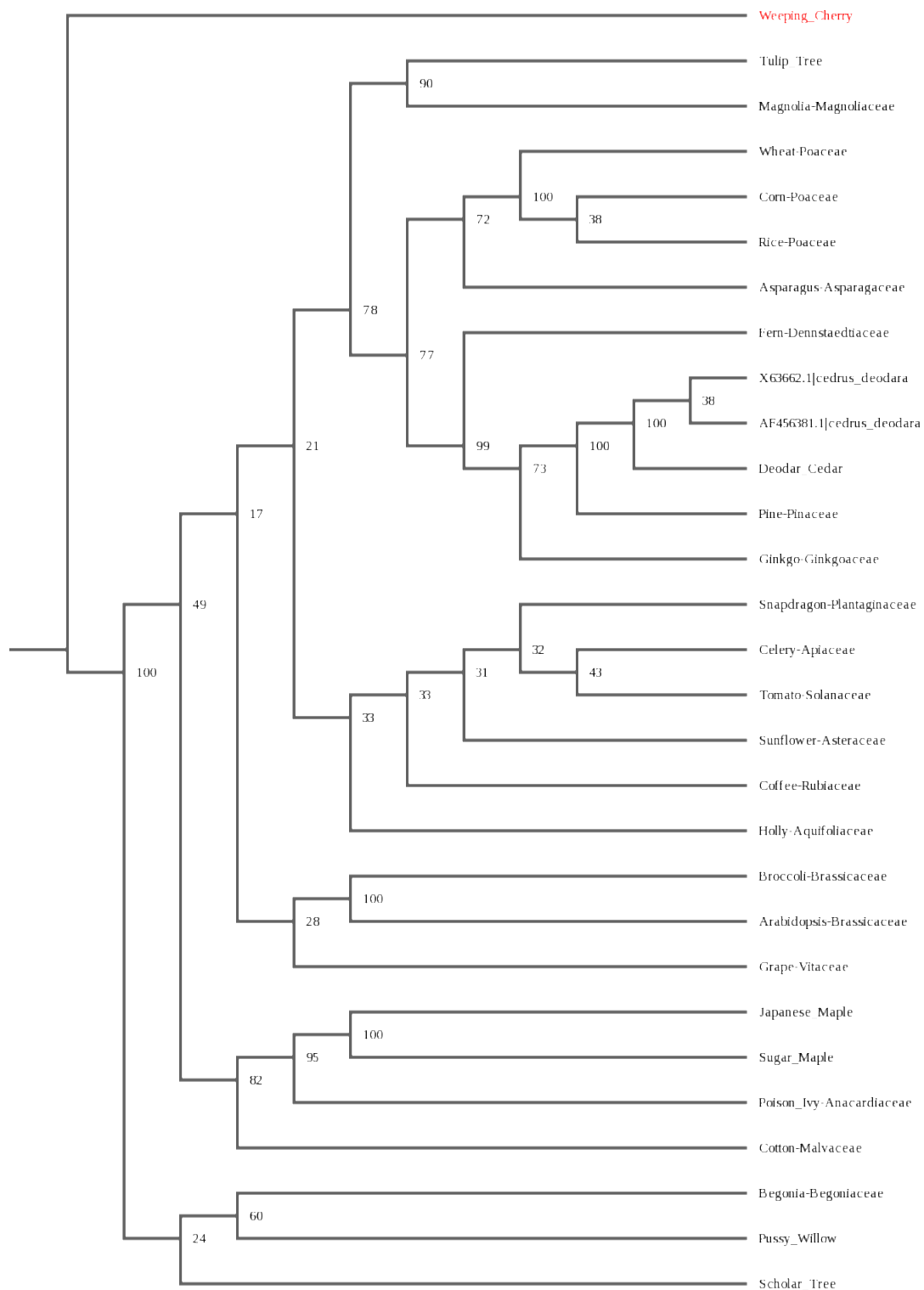


Figure 1. Neighbor-joining phylogenetic tree. Make sure to add a caption describing what the tree is showing (e.g. are any of your samples closely related? Do your BLAST hits have close

relationships with your samples). On DNA Subway there is a link to download the image on the lower left of the tree displayed.

5) Conclusion and Discussion: (length varies) This section “tells the story” and should include actual analyses such as information on how many mutations you found between different samples. Can you comment on how diverse ($\sim \infty$ to how many mutations) a family is based on your data? Are there other corroborating data on this? Your phylogeny (NJ and ML trees) should be included here. You should comment on how your Seek App identification matched (or failed to match) your DNA Barcoding results.

6) Citations: (length varies) Cited works (included websites) in the following form:
http://en.wikipedia.org/wiki/Vancouver_system

Plant Identification resources

Encyclopedia of life: <http://eol.org/>

Leafsnap (an app for iphone/ipad – website is also very useful!) <http://leafsnap.com/>

iNaturalist (a free app): <https://www.inaturalist.org/>

iNaturalist seek (a free app): https://www.inaturalist.org/pages/seek_app

OSU online woody plant identification: http://oregonstate.edu/dept/ldplants/plant_ident/

Plant leaf types: <http://www.botanical-online.com/hojastiposangles.htm>

Plants of NY atlas: <http://newyork.plantatlas.usf.edu/>