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We use this protocol and it's working

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Eastern Hemlock Tissue Collection for DNA

Forked from Eastern Hemlock Tissue Collection for DNA

Karl Fetter[1]

[1]University of Connecticut, Department of Ecology and Evolutionary Biology

Karl Fetter

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ABSTRACT

Steps for collecting tissue from Eastern Hemlocks for DNA analysis. The Plant Computational Genomics lab is conducting a landscape genomics study of climate adaptation in the Eastern Hemlock. This protocol is a step-by-step guide to collect a tissue sample. The protocol records metadata in TreeSnap (https://treesnap.org) and is designed to allow revisiting a collection site to collect more data by future researchers.

Introduction

1 This protocol is intended for field collection of leaf tissue for the Eastern Hemlock conservation genomics project sponsored by the Plant Computation Genomics lab at the University of Connecticut. The goal of the project is to identify climate adapted genomic variation for seed banking and potential use in future breeding programs or for assisted migration. As such, we aim to maximize climatic variation within regions and managed areas and the associated genetic variation.

A complete data collection will include the sample, metadata of the tree, a photograph, and a tagged tree. In some managed lands, tagging the tree is not possible and this may be considered optional. However if possible, we ask collectors to tag their trees to allow new data collection from the tree, increasing the value of the DNA sequence data. A single collection 'site' has one individual tree, each each collection site should be spaced at least 2 km distant from other sites. Clumped collections with multiple individuals per site limits the utility of the climate data, which has a spatial resolution of 1 km.

Collectors will receive a collection kit in the mail containing coin envelopes, a plastic bag with silica gel, and a postage-marked return envelope. Metadata will be entered into TreeSnap. Samples can be mailed to:

Dr. Karl Fetter
UConn, EEB (Unit 3043)
75 N. Eagleville Road
Storrs, CT 06269, USA

Collection Materials

2 You will receive in the mail a collection kit with:

- Reusable mailer
- Coin envelopes
- Plastic ziploc bag to hold all samples
- silica gel in a coffee filter
- Tree tags
- Nails

Cutting shears or knife, a smart phone, hammer, and GPS, and return postage are required, but not supplied. If return postage is needed, please contact Karl Fetter.

Download and install TreeSnap onto your smartphone. TreeSnap is a light weight app for collecting metadata for tree studies.

Going into the field

3 A few things to remember when you are ready to collect.

1. Always collect with permission. For managed lands, a permit or permission from the land manager is required. For collecting on private lands, always ask the land owner before collecting. Most people love hemlocks and will give permission to make a small collection.

2. Bring your collecting kit, cutting shears, GPS, smart phone and hammer.

3. Bring a buddy and have fun collecting!

Finding a site

4 Road side botany is great fun, but be sure you aren't collecting a planted tree. Trees of wild provenance will only be used in downstream analyses.

Choose a site based on these characteristics:
- You have permission to collect
- Sites must be at least 2 km (1.24 miles) distant from each other
- Site is generally accessible by car or a short walk/hike
- The site will make a good TreeSnap entry

Good photographs for TreeSnap will include landmarks like telephone poles, signs, or other features that aid revisiting the site. When you can't tag a tree, be sure there are good landmarks in the photo, or point to the tree in the photo!

Identifying a tree to collect

5 - Eastern Hemlock trees should be adults or established juveniles (>4" DBH).
- Saplings or regen should be sampled only if no adult trees can be found.
- Trees should ideally be accessible for tissue collection by hand or with a pole pruner near a trail head or road.
- Collect trees of wild provenance. In a rural or urban setting, iIt's impossible to know if a tree near a house was planted. Avoid collecting in someone's yard.

There is no minimum sample size for a collector, but 10 to 20 samples from a collector is appreciated.

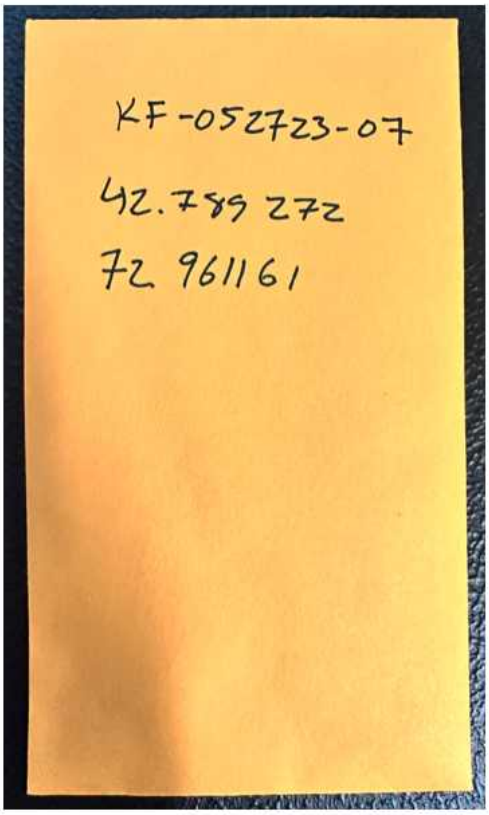
If the Carolina Hemlock occurs in your area, be sure you know how to differentiate the species.

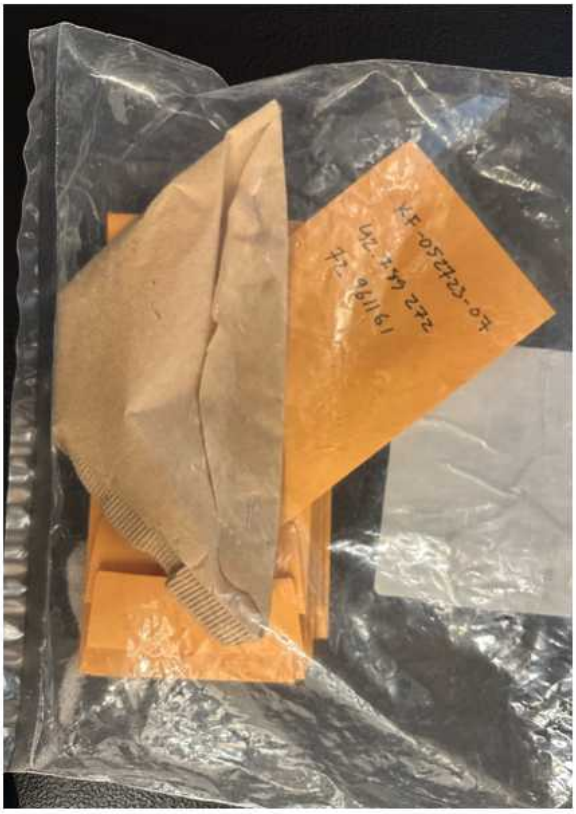
Collecting tissue

6 Collect approximately 1 to 2 grams of leaf tissue for each sample. 1 gram is about 5 segments of terminal branches (see images below). Needles will be extracted and very woody samples should be avoided if possible. Freshly expanded green leaves or buds are great to include.

Make the collection by cutting the stem with a knife, shears, or snapping the branch.

- Deposit the collection into the coin envelope. Label the envelope with "your intials"-"MonthDateYear"-"CollectionNumber". For example, my first collection for today (July 19th, 2023) would be KF-071923-01.
- Write the latitude and longitude of the tree on the coin envelope.
-Collect the DBH





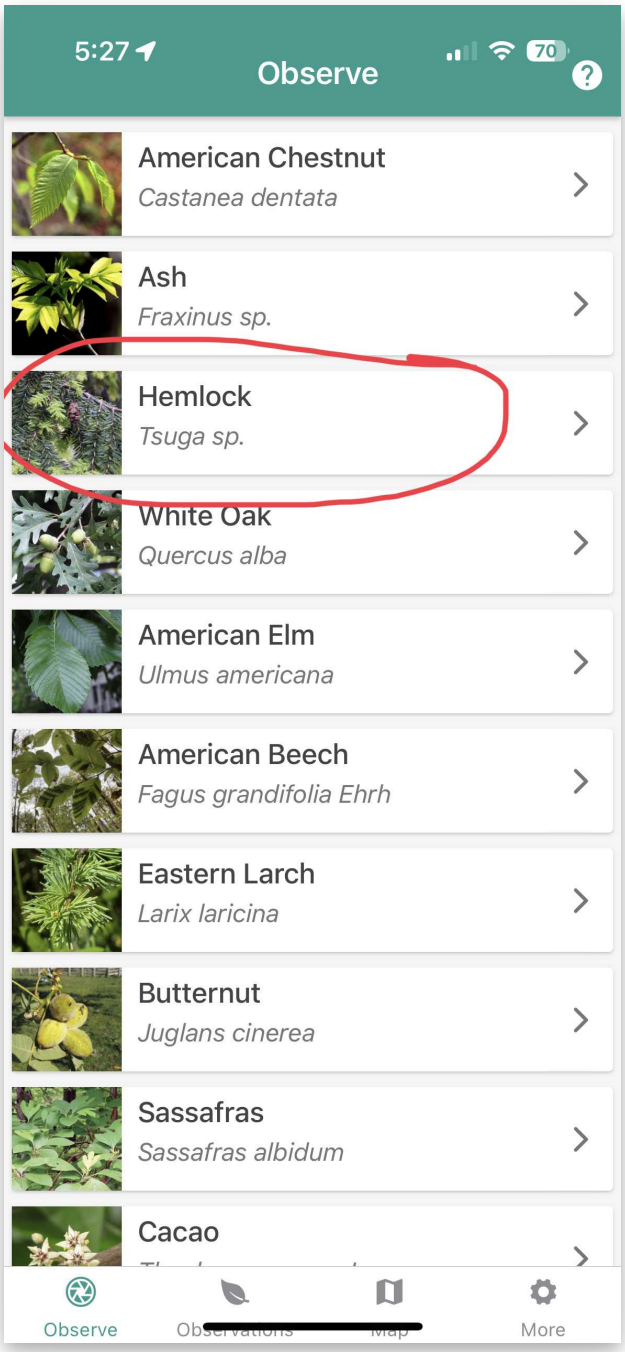
Coin envelopes should be labelled with the sample ID and lat-longs of the tree. Place the sample int the plastic bag with the silica gel.

It is essential to write the sample ID and the Lat.-Longs on the envelope. If TreeSnap fails, the written data is the backup. Put the sample in the bag with the silica gel. Reseal the bag to dry the sample. Moist samples will mold and the DNA will be degraded.

Metadata

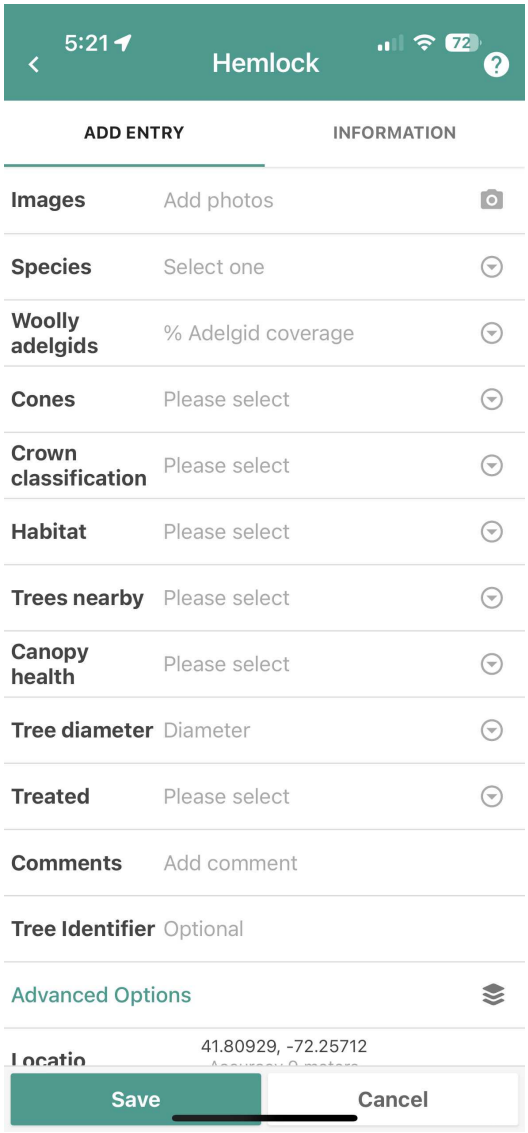
7 Metadata should be collected which allows 1) revisiting the tree, 2) general health characteristics, and 3) a general description of the site. A photograph should be taken of each tree. Use TreeSnap to collect these data. LeafSnap can be downloaded from the App Store or Google Play Store. After installing the app and registering, click on the Hemlock button. If you are without cell service in the field, when you connect to service again, open the app and click the notification to upload data.

Open TreeSnap and click on the hemlock button.



Landing page of TreeSnap.

Enter information into each field as applicable.



- In the 'Tree Identifier' field, name each collection with "your initials"-"MonthDateYear"-"CollectionNumber". For example, my first collection for today (7/19/23) would be KF-071923-01.
- Collect or estimate the diameter at breast height (DBH) using inches.
- In the comments field, add a short description of the site. For example, "Tree located at the Happy Logger trail head", or "Near intersection of Pond St and VT 116".

The lat-longs of the tree are automatically saved when you create the entry (not when you save it!). So be sure to create the entry next to the tree. If you have a GPS that is more precise than your phone and you wish to submit those points instead, enter them in the comments field.

Don't worry if you don't have cell phone reception in the field. The data is temporarily saved on your phone. After you connect to cellular service or wifi again, open the app and select the yellow pop-up box to upload data to TreeSnap.

Save the entry and move on the next tree.

Tree Tagging (Optional)

8 A tagged tree is incredibly valuable, as it allows for a confirmed recollection of tissue or germplasm. When tagging your tree, angle the galvinized nail at a 30 - 45 degree angle and allow the tag to freely dangle. Hammer the nail approximately an inch into the tree.

If tagging a tree is not an option, then take descriptive photographs that have landmarks.

After you've collected

9 After you've made your collections. Be sure your samples are in the resealable plastic bag with the silica gel. Put the bag containing samples in the mailer and reseal it. Using the provided postage, mail the samples to:

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