

## Think You Have an American chestnut – Want to Check it's Pedigree?

The Canadian Chestnut Council has made arrangements with Dr. Brian Husband at the University of Guelph to extract, sequence and analyze the DNA from submitted leaf samples.

To recover the cost of lab materials and sequencing, a fee of \$25 per sample will be charged.

If you have a sample that you want to check please contact Heather Dover at [hj.dover@hotmail.com](mailto:hj.dover@hotmail.com). She will advise where the sample can be sent or make arrangements for it to be picked up.

### American chestnut - DNA Sampling Procedure - Simplified

#### 1. Select an appropriate leaf.

Sample a fresh, mature leaf only, preferably not from a branch with blight. Do **Not** sample leaves that are withered, yellowing, diseased, or insect damaged. A leaf that is in good health, fully intact, and recently completed opening is the best candidate for sampling for DNA testing. Only one leaf per tree is required for DNA testing but if the leaves are of a lower quality 2 or maybe 3 can be sampled.

#### 2. Prepare The Envelope

Use a paper envelope that will fit in the envelope that you will use to submit the sample/samples (a brown coin envelope or equivalent is a good choice). Label the sample envelope with an ID for the tree and include in the envelope a completed "Reporting a Chestnut in the Wild Form" that can be found on the Canadian Chestnut Council's website [www.canadianchestnutcouncil.ca](http://www.canadianchestnutcouncil.ca) under the Found a Chestnut/Report a Chestnut link. Please be sure to include the date taken and the contact information of the person submitting the sample/samples (name, address, e-mail and phone number). If multiple samples come from the same tree, be sure to mark this clearly on the envelopes.

#### 3. Take the sample.

Using a clean pruner (sterilized is best, see below), carefully remove the selected leaf from the tree. It is fine to cut the stem of the leaf or even to cut off a small portion of the leaf closest to the branch, as long as the sample leaf is mostly intact. If you are sampling multiple Chestnut trees with the same pruner, you should sterilize the pruner between trees by washing it with a diluted alcohol or bleach solution. This helps to prevent any chance of chestnut blight being spread from tree to tree. CAUTION: Chestnut blight is a wound pathogen, which means it infects trees through cuts and scrapes in the tree's bark. Take extra care not to scrape or damage the tree's trunk or branches when taking a sample!

#### 4. Prepare the sample for submission

Place the sampled leaf in the prepared envelope (if multiple leaves were sampled from the same tree, put each leaf in a separate sample envelope). It is okay to fold the leaf neatly, if that is needed to fit the leaf inside of an envelope, but try to limit the amount of folding of each leaf. Folding once is fine, but more than that will affect the drying process not allowing them to dry out properly, as will balling up or scrunching up the leaf. Multiple leaves in the same envelope will also hinder drying. If you have some, immediately add 20-25 ml (e.g. 2 tablespoons) of silica gel into the paper envelope with the leaf sample. Do not seal the envelopes. Leaving them open helps with air circulation and allows for checking the samples and changing out the silica. If you do not have silica it will be put in when the samples are received.

#### 5. Submit the Sample/Samples

E-mail Heather Dover at [hj.dover@hotmail.com](mailto:hj.dover@hotmail.com) for information as to where to send your sample. Multiple sample envelopes can be submitted in the same envelope/package. Dr. Brian Husband's lab will look after the drying and preparation of the samples for DNA extraction.

THANK YOU for your interest in the Preservation and Restoration of the American chestnut.