**OakSTeP: Sampling oaks for Sudden Oak Death**

You have to view training video at [www.oakstep.org](http://www.oakstep.org) before using written instructions: **NOT** to be used without viewing the training video. Can be printed out and taken to the field with you

**1- Getting ready: what you will need:**

* The set of plastic Petri dishes filled with *Phytophthora*-specific growth medium you requested from U.C. Berkeley
* The Data sheet accompanying the Petri dishes, where a range of codes is assigned to your plates, and sampling information must be filled in
* A pencil to use on the Data sheet, and a Sharpie to transcribe a sequential code on each Petri dish
* A sharp tool to debark the oak such as an hatchet (our favorite), and axe, or a chisel with mallet
* A sharp knife to use as a scalpel to excise underbark wood chips
* Tweezers to detach wood chips and place them in the Petri dish
* A spray bottle with 75% alcohol or a bottle of Lysol
* A pack or a roll of paper towels
* A roll of masking tape
* Working gloves
* A flashlight to help you identifying symptomatic portions of the stem
* These instructions and a mobile phone to watch the instructional video at [www.oakstep.org](http://www.oakstep.org)

**2- Selecting good candidate oaks for sampling:**

* Use the “Risk” function in the free App *“SODmap mobile*” to ensure the trees are in an area with High or Moderate infection risk. Simply stand next to the trees you want to sample and tap the “Risk” icon. Go to [www.sodmapmobile.org](http://www.sodmapmobile.org) to learn how to correctly use the App
* Make sure your tree is one of the following five species: California Coast Live Oak, California Black Oak, Shreve’s Oak, Canyon Live Oak, or Tanoak. Go to [www.calflora.org](http://www.calflora.org) if you need help identifying trees.
* Make sure the tree is still alive. In order to be a good candidate the tree must still **have some green foliage**, and **NO** frass (sawdust create by bark beetles) and **NO** charcoal spheres produced by the fungus *Anulohypoxylon thuorsianum* must be present on the main stem
* A California bay laurel, a toyon, a rhododendron, or a Pacific madrone must be within 30 feet from the tree you want to sample. Go to [www.calflora.org](http://www.calflora.org) if you need help identifying trees.
* Most SOD-infected oaks have so called “ bleeding” trunks, e.g. some viscous dark sap drips on the outer bark in the absence of any mechanical damage. Sap is thick, amber to black in color, has a good smell of wine fermentation (as opposed to watery foul smelling bacterial wetwood), and can be dripping on the outside bark as translucent droplets, or may be dried up and appears as a brown crust on top of the grey bark of oaks. Go to [www.suddenoakdeath.org](http://www.suddenoakdeath.org) for pictures of bleeding trunks

**3- Sampling oaks:**

* Spray alcohol or Lysol on the cutting part of the tool you will use for debarking, on your knife, and on the tweezers. Wipe dry with paper towel
* Go in front of the “bleeding” portion of the stem, and gently debark a 2” x 2” area located at the edge of the bleeding in an imaginary top left corner of the bleeding area. Do not go deep, simply remove the bark to expose the cambial layer and the outer wood. If the wood exposed shows a dark irregular line that separates the infected portion of the wood ( slightly brown, towards the bleeding portion of stem) from healthy tissue (lighter in color, sometimes pink-reddish, on the side of the line away from the bleeding) then stop debarking. If not debark another 2” x 2” section nearby until the line is visible.
* Use your sharp knife to peel a wood chip (1/8 of an inch by 1/8 of an inch) right across the line, make sure the chip is small but contains both the healthy side and the diseased side of the wood. Make sure chip is still attached to tree on one side. You will need 2 good chips for sampling
* Clean all tools with alcohol or Lysol as explained above and repeat the debarking and wood chipping on the imaginary top right corner of the bleeding area, so that two distinct parts of the stem will be sampled
* Get the clean tweezers and collect one of the chips. Open a Petri dish and gently place the wood chip inside the gel at the bottom of the dish. Try to submerge the chip in the gel as much as possible. Each chip is placed in the Petri dish at the corner of an imaginary square that fits comfortably inside the circular Petri dish. Close lid.
* Sterilize tweezers with alcohol or Lysol, dry with paper and repeat, until all four chips (two from two distinct parts of the bleeding portion of the stem) have been placed in the Petri dish. Close the Petri dish.
* If you want you can use multiple Petri dishes on the same oak.

**3- Data entry, storage, suggested donation and shipping:**

* Go to a comfortable place, make sure your Petri dish has four wood chips submerged in the media, place two pieces of masking tape across the lid and the bottom of the dish, so that the lid will not open
* Take the data sheet, the sharpie, and the pencil
* The data sheet is organized in rows, each row starts with a code (one letter and three numbers). Each Petri dish will have a specific code, do not use the same code for multiple dishes. Your first Petri dish will have the code in the first row, the second the code in the second row and so forth. Turn the Petri dish upside down and using the sharpie write on the plastic bottom the specific code for that Petri dish. Make sure it is legible, if not legible you can write it twice.
* Fill in all the information in the row associated with that code in the Data sheet. Do so every time you sample one oak, do not wait until the end of the day. Use a pencil. Data will include the following: date, tree species, bleeding (yes or no), health (0=still perfect health/1=10 % of crown declining/ 2= 30% of crown declining/ 3= 60% of crown declining- 4=75% or more of crown declining), dead oaks within 60 feet (yes/no), type of property (Private or Public), Latitude and Longitude (the App SOD map mobile provides Lat and Long when tapping the risk button)
* Plates (used or unused) must always be kept at a cool temperature (use a cooler and place in a fridge at the end of the day. Once used, plates and filled in data forms have to be shipped to U.C. Berkeley within 96 hours (four days) from the time the chips have been plated. You have to ship a minimum of 5 plates with the accompanying data sheet filled out (Data sheets contain each 5 rows) using only overnight service. There is a suggested donation of $50 for each plate mailed back (minimum donation has to be $250 for five plates). Donations are meant to support any general activity by the Garbelotto Lab. To make the donation, go to the link below:

<https://nature.berkeley.edu/matteolab/?p=4031>

In the “Special Instruction” Box at the bottom of the donation page, write Your Oakstep Registration ID simply followed by the word “diagnostics”. You will receive an email and a receipt in PDF format: transcribe the Confirmation Number in the box at the bottom of the Data Sheet

* Shipment has to leave Monday to Wednesday only using overnight service, and be addressed to:

**Oakstep program, c/o Doug Schmidt, 54 Mulford Hall, Department of ESPM, University of California, Berkeley, CA 94720. Tel (510) 643-4282.**

Please send an email to [diagnostics@oakstep.org](mailto:diagnostics@oakstep.org) the day you make the shipment, specifying your OakSTeP registration ID, the number of plates that were shipped, your donation confirmation number, and the day plates were shipped. Make sure the data sheet is included with shipment.

* Unused plates have to be shipped back **five weeks** after they were received. Please ship to same address above. Send an email to [disposal@oakstep.org](mailto:disposal@oakstep.org) the day the shipment leaves, specifying your OakSTeP registration ID and how many unused plates you are returning. No donation is suggested when sending back unused plates.