

Procedure for collecting leaf material for western blots

1. Preparing labels

- a. use the 1/2" x 1-3/4" self-adhesive labels in the cupboard above the printer
 - b. Cut the labels in half so that they are 1/2 x 7/8", leaving them in long strips on the page.
 - c. Write the following on each label:
 - H-D xx/xx/20xx** (H-D is the approved abbreviation for this experiment; use the appropriate abbreviation if you are involved in a different experiment. Xx/xx/20xx is the date of the collection)
 - plant id** (This is whatever is used as a label on the pot)
 - INDUCED/CONTROL** (whichever is appropriate)
 - d. Attach the labels to 1.5 ml microcentrifuge tubes. This next step is crucial: **WRAP TRANSPARENT TAPE COMPLETELY AROUND THE LABEL.**
2. Place the tubes in order in a plastic tube box with a lid. Do not use Styrofoam; we want the hot air of the oven to circulate around the tubes. Leave the box lid open.
 3. Preparing moist filter paper "dots"
 - a. Obtain a box of filter papers from the wet lab.
 - b. With a paper punch from the "Office Supplies" drawer, punch disks of filter paper into a Petri dish filled with distilled water.
 - c. with a forceps, place one disk in each labeled microcentrifuge tube.
 4. Clip 1 to 1.5 cm² of leaf material from the plant and place it in the tube. Ideally this would be the Most Recently Fully Expanded Leaf (Mr Fel). If Mr Fel is not available, take the healthiest, greenest material you can find. Close the tube.

IF INDUCTION IS INTENDED:

1. Prior to collecting leaves, make sure the big grey oven in A137 is turned on. Set the temperature (on the right hand side at the top of the oven) to 38C. Make sure the temperature has reached 38C before using.
2. Place the set of collected leaves in the oven for 90 minutes.
3. After 90 minutes, remove and place on the counter top in a secure area for 2 hours.
4. Label a Styrofoam microcentrifuge tube storage box with same information you put on the tubes, but indicate the range of tube numbers that are included in the box.
4. After 2 hours, place tubes in the styro storage box, tape it shut and place it in the -80C freezer in the wet lab.