Induction protocol by Jessie 4-24-08.
Notes about amount of tissue to collect:

- A 1.5mL microfuge tube only holds about 100mg of tissue. This is a pretty tight fit and I would recommend storing only 50-75mg of tissue per 1.5mL tube so that you are not jamming tissue in which will be hard to retrieve later. 2mL tubes are available for purchase and they probably would hold 100mg of tissue better. If collecting more than this, you will have to find bigger vials/containers or use more than one tube.
- Protein extraction only requires 10-20mg of tissue for a yield of 75-150ug of protein, but you should collect enough for a few extractions just in case.
- For RNA extractions, 100mg of tissue is required for a yield of 30ug of total RNA. Again you should collect enough for a few extractions just in case
- For either RNA or protein work, two or three individual plants should be chosen as standards, and a VERY large sample should be taken from this plant to use as a standard. This sample sample should be used in every Western blot or real-time PCR plate, so collect the appropriate amt of material for the experimental design.

Protocol:

- Set incubator to 38°C
- Label 2 petri dishes per plant, one for the induced and one for the non-induced treatment
- For each plant/leaf cut out (2) 2.5cm x 4cm squares of filter paper (I used Whatman 3MMCHR, cat # 3030633 paper, it is a roll of 2.5cm paper)
- Soak the filter paper in diwater.
- With forceps, take one soaking piece of filter paper, shake off any excess water, and place in the non-induced Petri dish..
- Place a leaf from the first plant directly onto the filter paper.
- With the forceps, take another piece of filter paper, shake off excess water, and place on top of leaf. I did not force the filter paper flat if the leaf was not flat.
- Repeat with another leaf for the same plant but place it in the non-induced Petri dish.
- Seal all Petri dishes with parafilm
- Place the induced Petri dishes in the incubator for 1 hour*

  *For using the incubator/drying oven in A137:
  - It takes 3 to 5 minutes for the incubator to get back up to 38°C after having the door open so add 5 minutes to the total incubation time.
  - Use the bottom shelves and place all Petri dishes on the rear portion of the shelves. These areas are the least variable in temperature. In my test, the rear bottom shelf maintained a temperature of between 38.2 and 38.6 when set to 38°C. The second shelf down in the center of the shelf, in contrast, varied between 37.4 and 38.7 °C.
  -DO NOT OPEN INCUBATOR DURING THIS HOUR. OPENING WILL CAUSE THE TEMP TO DROP VERY QUICKLY AND YOU MUST MAINTAIN AN EVEN 38°C. TAPE THE DOOR CLOSED

AND PUT A SIGN ON THE INCUBATOR SO THAT NO ONE ELSE OPENS THE DOOR.

- Leave the non-induced Petri dishes at Room temperature (22°C)
- After induction period, allow samples to rest for 2 hours. NOTE: IN THE FUTURE, IF THE TISSUE IS TO BE USED IN RNA QUANTITATION EXPERIMENTS, THE TISSUE SHOULD PROBABLY BE FROZEN IMMEDIATELY AFTER INDUCTION. Volkov et al 2003 found that for a different heat shock gene, the half-life of the mRNA transcript was about one hour at room temperature following induction for one hour.
- Then freeze all induced and non-induced samples at –80 or begin extractions immediately. If protein or RNA degradation is a problem, you might try snap-freezing samples in liquid nitrogen.