

## Processing Potato Tubers for RNA Extraction

- ❑ Wash tubers gently with water and dry, then spray with 70% ethanol and dry again. Record the fresh weight of each tuber.

Choose tubers of nearly equal size! The volume of the tuber does effect the yield of RNAs expressed in various parts of the tuber. If you are interested in liquid nitrogen.

- ❑ Slice the tuber in half longitudinally (stem to bud).
- ❑ For unwounded samples, immediately prepare a slice ~5 mm thick (we use a kitchen mandoline for uniformity). The slice (or entire half) is cut into 5 mm cubes and frozen in liquid nitrogen (LN<sub>2</sub>). Store at -80°C until needed.
- ❑ For wounded samples, store slices in the dark at room temperature in a Petri dish containing damp filter paper. At the end of the wound period, blot the slice dry and chop into 5 mm cubes. Freeze in LN<sub>2</sub>. (Samples may become discolored or slimy during the incubation period.)

If fresh samples are to be examined by histochemical staining, you must remove suberized tissue to expose fresh underlying tissue at the end of the induction period e.g. the suberized layer can mask expression.

- ❑ Samples are prepared for extraction by grinding in an Omni Mixer (Omni Corporation International). Samples are kept frozen with LN<sub>2</sub> during the entire grinding step. All containers and tools are kept at LN<sub>2</sub> temperatures. Samples can be ground in a mortar and pestle.
- ❑ The processed samples are transferred to BlueMax tubes (Falcon 2098) and stored at -80°C or below until extractions begin.
- ❑ Extract RNA as described elsewhere.