

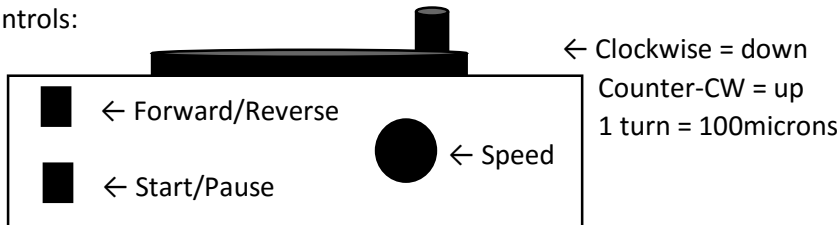
## Vibratome Protocol

Prep – 5% Agarose (Sea-Plaque) in PBS in large flask

Microwave to dissolve agarose:

- Use paper towel to stuff the top of flask to minimize evaporation
- Be sure not to overflow (check repeatedly)
- Microwave until solution is clear and is difficult to boil over
- Estimated time: 10 min for 100mL
- Can be stored in RT until fungus grows or longer in 4C

1. Prepare samples in metal tray in correct orientation
  - a. Dry the sample before with Kimwipe if possible
2. Pour cooled agarose into tray (maintain agarose temperature with 37C water bath)
  - a. May need to use forceps to reorient sample
  - b. Do not have sample at the very bottom of the tray, should be somewhat floating
  - c. Wait until agarose is solidified (place in ice to speed up process)
3. When agarose is solid, remove from metal tray
4. Use super glue to attached agarose/sample to vibratome tray
  - a. Use Kimwipe to dry flat side of agarose first = allows better attachment
  - b. Hold down agarose block firmly for 30 secs, let sit for 1 min
  - c. Attach block in front of marked line (line indicates where the blade reaches)
5. Turn on vibratome
  - a. Ensure that drainage tube leads to container
  - b. Add ice around tray area
6. Secure vibratome tray, with sample (tray can move if bumped)
7. Fill vibratome tray with PBS and some ice
8. Use lever to open space and insert blade (may need to use forceps)
9. Controls:



- a. Watch blade to ensure high quality cutting
  - b. When cutting sample, speed should be set to 3-4
  - c. When blade reaches the end, will automatically go backwards, make sure to **PAUSE** before it hits the back of the tray or it will move the tray
10. Scoop out sample after sectioning into PBS
    - a. Can pop out sample from agarose afterwards