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[Contact]

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**Maze Pouring Protocol**

**Rating:**

**Yellow** (no supervision required after training)

[Aseptic technique](https://docs.google.com/document/d/1qZs2GS0wH85JVMnXy2t4CrEO7a2GxikeEwcOgO6lw5A/edit?usp=sharing) training required, hot liquids

### **Introduction:**

This protocol will guide you through the process of pouring nutrient media into mazes. If this step isn’t done, the slime molds have no media to move on and the oat would have nothing to rest on so the results of the experiment would no doubt be unexpected at best and nonexistent at worst.

Estimate time: 20 min of work, 30 min of unsupervised cooling.

### **Safety Information:**

The lab has little to no safety issues. Just use common sense and refrain from cutting or burning yourself. Call 911 if there is an emergency or reference our [list of emergency contacts](https://docs.google.com/document/d/1yHtU0-1RkkxgRz55dj0mR-y-fYJJy3vjRIxNxnydBKg/edit#) [link pending]

#### Required PPE:

* Lab coat
* Nitrile Gloves (multiple layers may help with handling hot liquids, paper towels can also be used)

### **Technical Requirements:**

Proper aseptic technique is required for this lab. For more information please reference [this document](https://docs.google.com/document/d/1yHtU0-1RkkxgRz55dj0mR-y-fYJJy3vjRIxNxnydBKg/edit#). [Link Pending]

### **Materials:**

* BSC fan hood (or other sterile environment)
* Spray bottle of 70% ethanol
* Latex or Nitrile Gloves
* Media
* Petri dishes
* Falcon Tube
* 3D Printed Mazes

### **Procedure:**

*This procedure is time sensitive. Read the entire procedure before beginning.*

1. Remove all extraneous material (such as stringing plastic) from the mazes with a file or razor.
2. Align the door of the BSC with the arrows on the sides. Turn on the fan and light for the biological safety cabinet (BSC). Unless otherwise specified, the remaining steps in this procedure is done inside the BSC and assumes that a sterile environment is maintained. Ensure that all items going in and out of the BSC have been sterilized with 70% ethanol, gloves are worn, and a proper sterilization techniques are maintained.
3. Spray down the mazes and respective plates **heavily** with 70% ethanol and lay them out to dry. Petri dishes and mazes should be laid out next to each other, and not nested in each other.
4. Allow ethanol to evaporate for around 10-15 min.
5. [Outside of BSC] Heat up the liquid with either an oven or a microwave (in wet lab) until liquid. You are now on a timer, do the rest of the stuff quick before the media solidifies.
6. [Back in the BSC] Place the mazes into their respective plates. Lay out the plates in a grid with the lids off.
7. Using a falcon tube, eyeball the proper amount of media, (around 35 ml), and pour into a plate. Take extra care to ensure that all areas of the maze are filled. Repeat until all plates in the grid are full.
8. Lay the lids on each plate in a way that air is still allowed to circulate. This will give you space to lay out more plates in the BSC.
9. Air out the plates for 30 minutes to an hour to ensure that most of the water vapor escapes. This will prevent fogging on the petri dishes, allowing for better imaging.
10. For best results, place plates in fridge overnight to remove any remaining traces of water vapor. If that is not desired (which it usually isn’t), you are now ready to use these plates to [transfer slime molds](https://docs.google.com/document/d/1yHtU0-1RkkxgRz55dj0mR-y-fYJJy3vjRIxNxnydBKg/edit#) or do other things with.

**Storage, Disposal and Clean up:**

It really isn’t recommended to store the plates that come out of this protocol so if at all possible put slime molds into the mazes right away. If storage is really necessary, wrap each plate in parafilm and store in the fridge.