

# Making Lead Plates

---

## Introduction

This protocol will show the steps in which the WPI iGEM team used to make lead plates in which they force evolved both *L.rhamnosus* and *B.subtilis*. These lead plates had varying concentrations, please see our notebook for the math performed to arrive to the amount of lead to add. This amount is based off of the stock of lead water that you are using and both the ingredients in your media.

## Materials

- › Media of choice Broth w/ Agar
- › Scale
- › Heated Mixing Stand
- › DI Water
- › 1000 mL and 500mL graduated cylinder
- › Tin foil
- › Autoclave tape
- › Autoclave
- › Water bath
- › Weights for flaks
- › Lead stock (We used 100,000 ppm)
- › Petri Dishes
- › 2L flask
- › 4 500mL flasks (amount needed for 4 different concentrations (250mL of each))
- › Metal inoculation loop
- › Bunsun Burner
- ›

## Procedure

### Mix Agar Requirements

1. Measure out g requirement for your media of choice on a scale (Said on the side of the bottle)
2. Measure out 1L of DI Water in a graduated cylinder
3. In a 2L flask, mix media broth agar along with 1L of water

Repeat for a different media following that media's specific gram measurement

4. Add a stir bar into the 2L flask and place on a heated mixing stand to mixing while bring your media to a boil

## Split Media into Smaller Flasks

5. After done mixing, line up your 4 500mL flasks
6. In a 500mL graduated cylinder measure 250mL of mixed media and pour into one 500mL flask

Repeat for the next 3 flasks

## Autoclave

7. Cover each 500mL flask containing 250mL of media with tin foil and secure with a strip of autoclave tape
8. Place all 4 flasks into the autoclave
9. Autoclave for 1 hour
10. Set the waterbath to 50 degrees Celsius
11. After the autoclave is complete and the pressure and temperature are down to a safe environment, remove the flasks
12. Place weights on the flask and put them in the water bath for 1 hour

## Adding Lead & Pouring Plates

13. With 10 minutes left of the media in the water bath set up your lab space with your plates, and a pipette set to the first volume amount of lead for the first concentration
14. Grab the media from the water bath one flask at a time
15. Uncover the flask, and quickly add the stock lead
16. Swirl the flask rapidly, with the bottom of the flask over the flame
17. Flame with lip of the flask, and then pour plates
18. Continue this with the different concentrations

## Letting the Plates Set

19. Let the plates set over the course of 2 hours at room temperature, and then you can relocate them to the 4 degree celsius fridge.