

## Seeding/Spotting NGMA Plates with Bacteria

1. First locate your supplies:

- 6 cm or 10 cm Plates in the Clean Hood or 4°C room - (pre-dried 48 hours).
- OP50 Culture (or other bacteria, if needed) for Seeding - in a 2 L flask stored in the 4°C room.
- A 50 ml sterile disposable conical tube for 6 cm plates only OR a sterile 100 ml glass bottle for 10 cm plates (needed if using the 25 ml tips).
- Repeater pipet handle (Drawer #2-B5)
- Repeater pipet sterile, wrapped, disposable tips (Shelf #1-E4)
  - Use the 5 ml tips to seed 6 cm plates
  - Use the 25 ml tips to seed 10 cm plates

***NOTE: you will need the orange adapter piece for 25 ml tip!***

2. Transfer the OP50 from the Stock Flask to the tube or bottle.

- ONLY POUR AS MUCH AS YOU NEED FOR YOUR NUMBER OF PLATES (Once dispensed, extra bacteria should *not* be used later, but bleached and disposed of).
- ***NOTE: Use a flame for sterile technique - sterilize the Glass Flask edge before and after dispensing into the bottle. But DON'T Flame the plastic tube!***

3. Work in the clean hood. Set up the plates in stacks that are not too high, about 10 plates high.

4. Load the pipet tip in the repeater pipet handle (using sterile technique to prevent contamination of the tip).

5. Set the pipet to dispense 100 ul for 6 cm plates and 1000 ul for 10 cm plates.

6. Seed 6 cm plates by dispensing one spot to the middle of the plate. Seed 10 cm plates by dispensing one spot to the middle of the plate, then after seeding the stack, shake the plates slightly (left to right, then up to down) to spread the bacteria (ask for a DEMO!).

- ***NOTE: Take care not to spatter the bacteria onto the edges of the plate when dispensing and spreading the bacteria.***

7. Leave plates stacked lid side up for at least 24 hours for bacteria to soak in and grow. Make sure plates grow at least 2 days at room temp before storing in the 4 °C room.

- ***NOTE: Seeded plates should not be dried overnight with the Clean Hood on, since this will over-dry the bacteria.***

8. Finally, put the plates in pre-bleached plastic bins, and store them at 4 °C. Put away bacteria and supplies!!