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🌐 Pasteuria farm protocol V.2

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This is a protocol to infect and maintain *Daphnia* with *Pasteuria ramosa* for use in laboratory experiments.

Meghan Duffy, Katherine Hunsberger, Rebecca Bilich 2022. Pasteuria farm protocol. **protocols.io**
<https://protocols.io/view/pasteuria-farm-protocol-b7g8rjzw>
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This is a protocol to infect and maintain *Daphnia* with *Pasteuria ramosa* for use in laboratory experiments.

Pasteuria farm protocol:

Written by Katherine Hunsberger

Additions made by Rebecca Bilich on 3/2/2021

Set up: start a farm with ~75 Mid 37 animals (young animals, if possible) and a dose of 2000 sp/mL of *Pasteuria* G/18

Use a 1000 mL Erlenmeyer flask and add *Daphnia* from a beaker of 75 by running them down the side of the flask, then rinsing the inner sides of the beaker and flask to dislodge any stuck animals. Small juveniles 0-2 days old are the most likely to be infected although adding variety of ages will help the

farm last longer.

Add water to bring the final volume of the farm up to 900mL.

Select Mid 37s already infected with *Pasteuria* G-18 to create the spore slurry.

Estimate the number of animals needed by supposing that each infected animal contains 60,000 *Pasteuria* spores.

Grind the animals up in microcentrifuge tubes using an electric pestle from 60 seconds, making sure to make a homogenous mixture with no obvious *Daphnia* parts. Then remove the slurry to a small (5 mL) beaker. Then rinse each tube with about 0.5 mL of filtered lake water, vortex the tubes for a few seconds, and add this water to the slurry as well.

Mix the slurry well with a pipet for 60 seconds and then add 10 mL to four wells in a Neubauer Hemocytometer. Do 4 cell counts, excluding spores that do not appear viable (degraded or immature spores.) If the grid is very dense, you can estimate by counting the 5 diagonal squares and multiplying the total by 5.

Choose 3 or more counts that are within 85% of each other, take the average of this number, and multiply it by 10,000 to get the concentration of the slurry in spores/mL.

Use the equation $M1 \cdot V1 = M2 \cdot V2$ to find the volume of slurry to add to each farm. Add this amount with a pipet and then feed each farm only 10 mL of food (after adding the spores, to mix them around.) Tap the flask with a small amount of cetyl alcohol. Also feed only 10 mL on the next feeding day to encourage *Daphnia* to consume *Pasteuria* spores. On subsequent feeding days feed 20 mL.

Maintenance: check farms for first time after ~30 days; then check every 7-10 days

- Follow similar rules as Metsch farm maintenance for adding more animals and/or spores or taking down the farms
- If you start checking a farm and the animals look pretty lightly infected, I would hold off another week or so before checking them to let the spores grow up

Storage: store in freezer indefinitely, but ramp up for experimental needs as far in advance as possible