



Maternal Lines Protocol V.2

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This a protocol to set up and maintain Daphnia maternal lines for clones that will be used in experiments.

Meghan Duffy, Rebecca Bilich 2022. Maternal Lines Protocol. **protocols.io**

<https://protocols.io/view/maternal-lines-protocol-b7hbrj2n>

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This a protocol to set up and maintain Daphnia maternal lines for clones that will be used in experiments.

Maternal Lines Protocol

By Rebecca Bilich 3/2/21

Set up adult and/or visibly pregnant Daphnia of the desired clone individually in 50 mL beakers in 30-40 mL of filtered lake water. Tap with cetyl alcohol and feed 1 mL Ankistrodesmus falcatus at 1,000,000 c/mL, 3-4 times a week.

Every other day, perform a water change:

Dump the contents of the beaker into a watch glass, rinse the beaker with filtered lake water, fill beaker with fresh filtered lake water and pipet the desired Daphnia back into the beaker.

Keep track of the number of clutches the adult female has. Remove the first two clutches of babies, keep the mother, and record the clutch on the outside of the beaker (for instance, by adding a yellow sticker after the mom's first clutch and a red sticker after the second clutch.)

Use the babies in the third clutch as the mothers of the next maternal generation. Set them up

individually and repeat the process.

If a lot of animals are dying, it's a good idea to keep the mothers of the previous generation around for a few weeks in case replacement babies are needed.

The third or later clutches born to the third generation of moms should be suitable for use in experiments, or the third clutch born to a later generation if desired.