Synchronization via Bleaching (Egg Prep)

Reagents Needed:

M9
20% alkaline hypochlorite solution

Procedure:

1. Chunk worms onto a seeded 10cm NGM plate. Allow the worms to grow 2-3 days so that there are lots of eggs and gravid adults on the plate.
2. Once you have plenty of eggs/adults, pour 5mL of M9 onto the plate and gently swirl it to dislodge the worms.
    a. "Power washing" the bacteria off the plate will bring along the already-laid eggs. This could be a benefit or a drawback depending on the goals of your experiment.
3. Using a glass pipette, transfer the worms to a 15mL conical (red-capped) tube.
4. Centrifuge for about 1 minute at 1000 rpm (173 x g) to pellet the worms.
5. Aspirate most of the M9 without disturbing the worm pellet.
6. Add about 12 mL of the 20% alkaline hypochlorite solution to the tube.
7. Mix the tube by inverting gently for approximately 5 minutes. Check under the microscope regularly and stop bleaching as soon as you see that the few remaining adult bodies are partially dissolved or split in half. It varies by strain but rarely do you need to bleach beyond 6 minutes. Avoid over-bleaching because this will kill the embryos.
8. Once most of the bodies have dissolved, centrifuge at 1000 rpm (173 x g) for 1 minute.
9. Aspirate most of the 20% alkaline hypochlorite solution without disturbing the worm pellet.
10. Add about 15mL of M9 to the tube and mix well.
11. Centrifuge again at 1000 rpm (173 x g) for 1 min.
12. Aspirate most of the M9 without disturbing the worm pellet.
13. Repeat steps 10-12 *at least* one more time.
14. Add about 7mL of fresh M9 and agitate to resuspend the pellet.
15. Let the eggs hatch overnight with gentle rocking. Since there is no food the larvae should be halted at the L1 stage. Distribute the liquid onto seeded plates or into liquid culture.

* NOTE: This protocol also is used to remove bacterial and yeast contamination from a worm strain.

Recipes:

<u>M9</u> (1L)
* Common lab stock in worm room.
5.8g $Na_2HPO_4 \cdot 7H_2O$
3.0g KH_2PO_4
5.0g NaCl
0.25g $MgSO_4 \cdot 7H_2O$
ddH_2O to 1L
• Filter (0.22μm) and bottle.

<u>20% Alkaline Hypochlorite Solution</u> (15mL)
* Should be made up fresh each time.
8.25mL ddH_2O
3.75mL 1M NaOH
3.0mL Bleach (do not use germicidal bleach)

Reference:

Sulston, J. & Hodgkin, J. (1988) Methods. *in:* Wood, W.B. (ed.) *The Nematode* Caenorhabditis elegans. Cold Spring Harbor Laboratory, Cold Spring Harbor: 587-606.

Synchronization via Bleaching (Egg Prep)- Small Scale

This is a quick and dirty variation of the previous protocol. If you only need a small number of semi-synchronized worms or if you simply need to remove yeast or bacterial contaminants, this protocol is easier and less time-consuming than the full-scale egg prep. This procedure is also called 'spot bleaching'.

Reagents Needed:

20% alkaline hypochlorite solution

Procedure:

1. Pipette ~10μL of the 20% alkaline hypochlorite solution onto the unseeded portion of a new plate.
2. Pick approximately 10 *gravid* adults off of the contaminated plate and place into the 20% alkaline hypochlorite solution on the clean plate. Try to avoid bringing too much bacteria with the worms.
3. After several minutes, the worms should break open and release the bleach-resistant eggs. The drop will dry up fairly quickly, so continue adding small drops of 20% alkaline hypochlorite solution every couple of minutes until most of the bacteria and adult worms have dissolved.
4. Leave the plate right side up overnight.
5. The next day, check to see that L1s have hatched and moved onto the bacteria field. If so, chunk out the dried-up bleach/NaOH spot (to prevent contamination by any bacteria that may have survived).

Recipes:

20% Alkaline Hypochlorite Solution (500μL)
* Should be made up fresh each time.
275μL ddH_2O
125μL 1M NaOH
100μL bleach (do not use germicidal bleach)

Reference:

Cold Spring Harbor Laboratories *C. elegans* Course 2007