Thawing CeNDR Mapping Sets

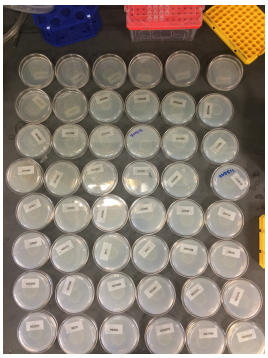
May 2017

1. When you receive your strains, immediately remove the plate from the dry ice and place it in a rack to support 96-well plates.

2. Allow the material to thaw completely.

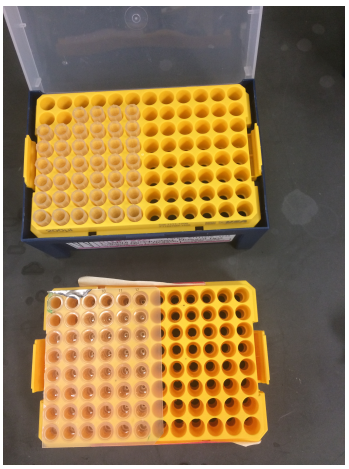
3. If possible, briefly spin down the plate to remove any liquid from the foil cover.

4. While the strains are thawing, label 48 6 cm plates with the provided labels on the bottom of the plate.
    - Array plates in a 6 x 8 grid to match the 48-well plate in which the strains arrived
    - Note that the labels are arrayed in (mostly) alphanumeric order. **Keep the plates in the above order as the strains are frozen in the plate in this order.** Wild strains all look identical to each other. Strain contamination is easy and all precautions to avoid mixing strains should be taken.
    - Place the plates with the bottom, labeled-side up.



5. Once the material is thawed, ***carefully*** remove the foil cover. Make sure that you do not jostle the tubes, because you do not want a strain from one well to contaminate a neighboring well.
    - To prevent strain contamination, it may help to first score the foil between the rows. Then, only peel off one row at a time. Alternatively, you can remove the entire foil cover and then tape over a row after you have pipetted out the worms from that row.

6. Starting with the upper-left, colored well, pipet 150 μl from the first well on to the first plate. To keep track of which wells you have pipetted from and which plates you have pipetted on to, use pipette tips in the same pattern as the 6 x 8 grid of the 48-well plate. When a plate has a strain on it, it will be lid-side up.
    - Pipet the material on an area of the plate that does not contain bacteria.
    - Keep the plate with the lid-side up until all the liquid is absorbed.



7. Pipet the material from the well to the right of the first well onto the next plate. Continue pipetting from each neighboring well on to the next plate in your alphanumeric-labeled array of plates. You can double-check the name of the strain with the well by consulting the Mapping Set grid provided.

8. You should see worms moving around ~10 minutes after all the liquid is absorbed.

9. Once the plates are dry, store the plates parafilmed and lid-side down at 20ºC.

10. After 3-4 days at 20ºC there should be gravid adults. We recommend cleaning the strain by the following method:
    - Pipet 15 μl of bleach solution (see recipe below) on the side of a 6 cm plate.
    - Place 10-20 gravid adults into the bleach spot.
    - Once the bleach is absorbed, incubate the plate at 20ºC.
    - The next day, pick 15-20 L1s from the bleach plate to a clean 6 cm plate.

11. Once you have a clean population, use animals from this plate to make your own freezer stock..

Bleach Solution

Reagent	Amount Needed for 10 ml	Amount Needed for 200 ml
NaOCl (from Fisher, cat #SS290-1)	2 ml	40 ml
NaOH Pellets*	0.2 g	4 g
dH_2O	up to 10 ml	up to 200 ml

* *NOTE: If using a 10 M NaOH solution, add 0.5 ml to 10 ml Total Bleach Solution.*

• Store at 4°C