Copying *C. elegans* N2 Library from 384-well plates to 96-well plates Using a BioTek MicroFlo

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Information about the labeling of the 384-well plates and the position of the wells: The database will give you an ID like **WRM0610aA01** where **WRM06**10aA01 is the name of the library, WRM06**10**aA01 is the number of the plate, WRM0610**a**A01 corresponds to the quadrant (in the order $_{cd}^{ab}$, as 96 well plates were transferred in 384s), and WRM0610a**A01** corresponds to the well position.

1. Remove two 384 well plates from -80°C freezer.
2. Spin down for 30 sec at 1200 rpm.
3. Use microplate dispenser to add 200 ul LB Miller + Chloramphenical (12.5 µg/ml) (see program notes below) to four 96-well plates. (Note: For each copy of the library you are making, you need to dispense medium into 4 times that number. e.g. - If you are making two copies of the library, you need eight plates; if you are making three copies, you need 12 plates, etc).
4. Label plates as they come off of the dispenser.
5. Lay out plates to mimic the four 384-well plate wells that each correspond to a single 96-well plate well.
6. Remove foil from 384-well plate.
7. Place a 96-pin replicator in the “a” position of the thawed 384-well plates. Move around to pick up cells.
8. Place the 96-pin replicator in the appropriately labeled 96-well plate.
9. Repeat steps 7 and 8 if you are making multiple copies of the library.
10. Repeat steps 7, 8 and 9 for the remaining positions (“b”, “c”, and “d”).
11. Put a new foil on the 384-well plate, using a roller to secure well. Place plate on ice.
12. For the first inoculated plate, gently move the 96-pin replicator around to dislodge the cells into the wells. Remove the 96-pin replicator.
13. Cover the plate with a Rayon Breathable Plate Cover (Fisher Catalog # 14-222-043) membrane but leave on the outer cover of the membrane until you are ready to move the plates to 37°C.
14. Repeat steps 12 and 13 for the remaining seven plates.
15. Repeat steps 3-14 for the second 384-well plate.
16. Return 384-well plates to the -80°C freezer.
17. Add medium to a “blank” plate.
18. When you have copied all the plates you want, bring the 96-well plates to a 37°C incubator or room.
19. Remove the outer cover of the Rayon Breathable Plate Cover membranes.
20. Incubate at 37°C for 24 hrs without shaking.
21. Remove plates from 37°C after 24 hrs.
22. One at a time, remove the Rayon Breathable Plate Cover cover and add 200 µl of 30% glycerol using the microplate dispenser (see program notes below).
23. Place foil on plate and secure using a roller.
24. Store at -80°C.
25. You can test the bacteria by streaking out samples from a few wells on LB+chloramphenicol plates.

Program Notes for the BioTek Microplate Dispenser-

Adding LB Miller + Chloramphenicol:

1. Plate Type: 96
2. Step Type: Peri-Pump and Dispense
3. Peri-Pump Selection: Sec (dependent on which pump has your cassette - the primary or secondary)
4. Dispense: 200 µl
5. Cassette: 10 µl
6. Column: as is (i.e. - hit “enter” to leave as is so that all columns are filled the same)
7. Flow Rate: High
8. Dispense X-Position: 0
9. Dispense Y-Position: 0
10. Dispense Height: 0336
11. Pre-dispense: Yes
12. Pre-dispense volume: 50 µl
13. Number of pre-dispense: 1

Adding 30% Glycerol:

Exactly the same as adding LB Miller + Chloramphenicol, except Flow Rate is LOW.