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Dispensing (non-L1) C. elegans to 96 well imaging plates using Integra VIAFILL

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Behavioural Genomics

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Protocol status: Working
We use this protocol and it's working

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Abstract

Protocol for dispensing later larval stage *C. elegans* into 96 well plates using the Interga VIAFILL dispenser. A bleach synchronised *C. elegans* population should be "re-fed" onto agar plates in advance.

Materials

M9 Buffer [3 g KH_2PO_4, 6 g Na_2HPO_4, 5 g NaCl, 1 ml 1 M $MgSO_4$, H_2O to 1 litre. Sterilize by autoclaving.]

Integra viafill

Integra Viafill Cassette: 8 Channel Small Bore. Part number: 5722

Troubleshooting

Configure the Integra VIAFILL

1 Insert a small cassette into the machine.

2 Create a new program (or use existing program) with the following settings:

2.1 Mode: Repeat Dispense
Tubing: 8 channel small
Plate: 96
Volume: 10 µl
Map: Full
Speed: Fast

2.2 To adust the height, put a plate into the stage and press the "Height" button on the display. The plate clearance, dispense and pre-dispense heights can all be easily adjusted using the arrows. Click the back arrow to return to the program screen.

2.3 Save these settings.

Preparing *C. elegans*

3 Wash worms off plates with several ml of M9 solution and transfer to 15ml falcon tubes.

4 Centrifuge for 2 minutes at 1500 rpm (RCF:210, ascending 9; descending 7) – program 1

5 Remove supernatant with plastic Pasteur pipette.

6 Add 15 ml M9.

7 Repeat steps 3 to 6.

8 Transfer the worm solution to a sterile conical flask.

9 Gently mix the worm suspension by swirling the flask, and pipette ten 10 µl droplets to a microscope slide or cover glass. Count the number of worms present in each droplet.

10 Dilute the worm suspension to achieve your desired worm density (30 to 60 worms per 100 µl) by adding M9 buffer.

Dispense *C. elegans* with the VIAFILL

11 Add a sterile stir bar in the conical flask, and place on a magnetic stirrer.

12 Turn the magnetic stirrer onto the lowest setting to agitate the worm suspension.

13 Select the VIAFILL program.

14 Select the volume you wish to dispense (10 µl).

15 Place the end of the tubing from the cassette into the worm solution.

16 Press "Prime" to prime the tubing. Check all tips of the cassette are dispensing correctly.

17 **Test dispense** 96 droplets onto a plate lid.

18 Count the number of worms in 10 random droplets. Dilute worm solution with M9 to the correct desired density again if necessary.

19 Press "Recover" and re-prime the tubing before dispensing onto imaging plates

20 Place a 96-well imaging plate in the stage and press "Start".

21 Once all imaging plates have been filled, dry with the lids off under laminar flow for 1 to 2 hours.

Cleaning the cassette

22 Press "Recover" to recover the worm solution into the bottle.

Note

Dispose of excess worms by adding Rely+On™ *Virkon* to 1%.

23 Place the tubing ends into water.

24 "Prime" the tubing several times so that the water runs through.

25 Remove the end of the tubing from the water and "Prime" with air to remove any remaining liquid.

26 Remove the cassette from the machine, place in an empty tip box, and autoclave.