Dispensing C. elegans to 96 well tracking plate using Integra VIAFILL

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We use this protocol and it's working

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ABSTRACT

Protocol for dispensing *C. elegans* into 96 well plates using the Interga VIAFILL dispenser. Bleach synchronized *C. elegans* should be prepared in advance. The X,Y,Z positions for dispensing can be adjusted according to the multiwell plates being used.

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

Configure the Integra VIAFILL

1 Insert a small cassette into the machine.

2 Configure X, Y, and Z settings for the multiwell plate by clicking on tool symbol -> stage alignment.

2.1 Put the plate into the stage and then press 'Move' so that the plate moves so that it is under the dispensing cassette.

2.2 Use the up and down arrows to move the dispensing tubes so that they hover just over the plate and make note of the height (this will be entered into the dispensing program).

2.3 Use the X, Y arrows to move the plate so that the dispensing tubes are in the correct position.

2.4 Save these settings.

Preparing *C. elegans*

3

1. Centrifuge larvae for 2 mins at 2500rpm (RCF:590, ascending 9; descending 7).

4 Remove supernatant with plastic Pasteur pipette.

5 Add 15 ml M9.

6 Gently mix the worm suspension and pipette 10 ul to a microscope slide or cover glass and count the number of worms present.

7 Dilute the worm suspension to achieve your desired worm density (e.g. 3 worms per 10 µl), by diluting in M9 buffer.
For example:
If you have 30 worms in one droplet, dilute your suspension 1 in 10 to obtain 3 worms per 10 ul

Note

Keep your worm solution in a sterile glass flask with a volume several times larger than the volume of solution to allow mixing of the solution by gentle swirling.

8 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. Calculate the average number of worms per droplet. If correct use the suspension as is, if not repeat steps 7 and 8 until you obtain a suspension of the correct average worm density.

Dispensing worms

9 Select the VIAFILL program.

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

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

Note

From this point onwards make sure to gently swirl the flask of worms to prevent the worms settling to the bottom.

12 Press "Prine" to prime the tubing.

13 Place a 96 well plate (containing agar and desired bacterial lawns/compound conditions) in the

stage.

14 Press run to dispense worms to each well of the plate, making sure to swirl the flask of worms.

15 Repeat steps 13 and 14 until all plates have been dispensed.

Cleaning the cassette

16 Recover the worm solution into the flask.

17 Place the tubing ends into hot water.

18 Prime several times so that the water runs through .

19 Recover the water and remove the cassette from the machine.

20 Place the cassette into an empty tip box for autoclaving, and autoclave.