

Feb 09, 2023

Version 2

Dispensing *C. elegans* to 96 well tracking plate using Integra VIAFILL V.2

DOI

<https://dx.doi.org/10.17504/protocols.io.261ge396yl47/v2>

e.warren¹

¹London Institute of Medical Sciences

Behavioural Genomics



e.warren Warren

LMS

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.261ge396yl47/v2>

Protocol Citation: e.warren 2023. Dispensing *C. elegans* to 96 well tracking plate using Integra VIAFILL. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.261ge396yl47/v2> Version created by **e.warren Warren**

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited



Protocol status: Working

We use this protocol and it's working

Created: February 09, 2023

Last Modified: February 09, 2023

Protocol Integer ID: 76717

Keywords: interga viafill dispenser, multiwell plate, using interga viafill protocol, interga viafill protocol, tracking plate, well plate, plate

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 Create a new program (alternatively open a perviously saved program "WORMS ELLIE")
 - 2.1 Select
Mode: Repeat Dispense
Tubing: 8 channel small
Plate: 96
Volume: 10 μ l
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
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 repeat steps 3 to 5.
- 7 Transfer the worm solution to a sterile glass bottle.

**Note**

Keep your worm solution in a sterile glass bottle 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 and pipette 10 μ l to a microscope slide or cover glass and count the number of worms present.
- 9 Dilute the worm suspension to achieve your desired worm density (e.g. 3 worms per 10 μ l), by diluting in M9 buffer.

Note

For example:
If you have 30 worms in one droplet, dilute your suspension 1 in 10 to obtain 3 worms per 10 μ l.

- 10 Gently mix the worm suspension by swirling the flask, and pipette ten 10 μ l 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 8 and 9 until you obtain a suspension of the correct average worm density.

Dispensing worms

- 11 Select the VIAFILL program.
- 12 Select the volume you wish to dispense (10 μ l).
- 13 Place the end of the tubing from the cassette into the worm solution .

Note

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

- 14 Press "Prime" to prime the tubing.



- 15 Place a 96 well tracking plate (containing NGM agar and desired bacterial lawns/compound conditions) in the stage.
- 16 Press "start" to dispense worms to each well of the plate, making sure to swirl the bottle of worms.
- 17 Repeat steps 15 and 16 until all plates have been dispensed.
- 18 Allow the plates to dry with the lids off inside a biosafety cabinet for 1 hour.

Note

Drying time may vary depending on hydration of tracking plates.

Cleaning the cassette

- 19 Press "recover" to recover the worm solution into the bottle.

Note

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

- 20 Place the tubing ends into hot water.
- 21 Prime several times so that the water runs through .
- 22 Recover the water and remove the cassette from the machine.
- 23 Place the cassette into an empty tip box for autoclaving, and autoclave.