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RNAi feeding

In 1 collection

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Abstract

Daily resuspension and feeding of *P. bursaria* cultures

Remove supernatant

10m

1 Spin down *P. bursaria* plate at 800 x g, 4°C, 00:10:00 10m

Ensure that centrifuge is loaded with balance plate.

2 Transfer *P. bursaria* plate to OpenTrons OT-2 Liquid Handler.

Ensure that: *P. bursaria* plate is in position 7; Fresh waste plate is in position 10; and Pipette rack is refilled with P200 tips in position 1
Take care when loading the *P. bursaria* plate into position 7
Tips can be removed from the outer edges of the Pipette rack beforehand, to save on wastage

3 Run OT-2 protocol "96-well (1 at at time)" using the OpenTrons App.

This removes 150 µL of supernatant
You may have to manually remove ~ 75 µL supernatant from Row F once the protocol has finished (multichannel pipette, in the hood)

Repeat

4 Return to step 1 and repeat for each plate.

Feeding

5 In a sterile hood, prepare 60 mL NCL containing 120 µL Ampicillin, 240 µL IPTG, and 12 µL β-sitosterol.

Ampcillin ([M] 0.1 mg/mL), IPTG ([M] 0.4 millimolar (mM)), and β-sitosterol ([M] 0.0008 mg/mL)
Conduct the remainder of the experiment under sterile conditions

6 Add 150 µL supplemented NCL to each well.

Use a sterile square petri dish and multi-channel pipette

7 Add 8.3 µL *E. coli* stock (OD 3) to each well.

Use frozen stock plate and multi-channel pipette
Ensure layout of frozen stock plate matches the *P. bursaria* plate
You can use the same pipette tips for each REPLICATE (so for everything taken from the same row of the stock plate)
Make sure to pipette stock plate up and down ~20 times (10x in the middle, 10x moving clockwise around the edge) before removing first aliquots from the stock plate
Pipette up and down gently 3 times before removing aliquot from stock plate, and after adding to *P. bursaria* plate

8 Pipette *P. bursaria* plate gently up and down ~20 times to mix cells

10x in the middle, 10x moving clockwise around the outside of the well to prevent cells clumping at the edges
This prevents biofilm formation

9 Return *P. bursaria* plate to 23 °C culture room.

Repeat

10 Return to step 6 and repeat for each plate.