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Paramecium cultures for RNAi

In 1 collection

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Abstract

Filtering and preparing *P. bursaria* cultures for downstream RNAi

Choosing culture

1 Use an old culture of *Paramecium bursaria* (between one and six months old).

This is to ensure that cultures start from a vegetative growth stage

Filtering

2 In a sterile flow hood, filter 100mL of *P. bursaria* culture through a 40um cell strainer (3x). Keep the flow-through.

This removes large clumps and debris from the culture
Remain in hood for the remainder of the protocol

3 Transfer *P. bursaria* to a fresh culture flask and leave for 1-2 hours.

This allows cells to digest any residual food present in the food vacuole

4 Aliquot 5uL onto a slide and count cells (x10).

This is to ensure the correct volume and cell number (~50 cells per well) is added

5 Pour *P. bursaria* into a sterile square petri dish.

Place the lid of the petri dish under one side so that the petri dish is at an angle

6 Using a multi-channel pipette, aliquot the calculated volume of *P. bursaria* into each well.

The final cell number should be ~50 cell per well
Shake the petri dish gently with your hand (two to three times), and then aliquot as quickly as possible
Try not to pipette back and forth in the petri dish, as this can push the cells to the edge and create an uneven distribution of cells across the wells

7 Top up to 250uL per well with NCL.

Autoclaved NCL with antibiotics added in the flow hood under sterile conditions:
Ampicillin ([M] 0.1 mg/mL), IPTG ([M] 0.4 millimolar (mM)), and β-sitosterol ([M] 0.0008 mg/mL)

8 Add a lid to the plate and place at 23 °C

Or whatever condition required for the experiment
Under standard conditions, ensure the plate is exposed to adequate light