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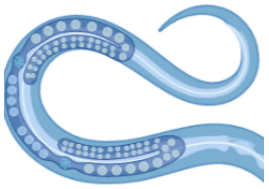
Bleach/Sync Large Scale Culture Plates (LSCP)



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

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Abstract

This abstract describes how to bleach/synchronize a large mixed-stage population of C. *elegans* grown on Large Scale Culture Plates (LSCP). For more information on LSCPs, refer to Shaver et al., 2021.

1 Wash LSCP with 50mL M9, Centrifuge and aspirate the supernatant from the wash 15 mL at a time (it should take about 3 centrifuges since some of the buffer will soak into the agar or be irrecoverable).

2 Repeat the 50 mL washes 2 more times for a total of 3 washes. (Shaver et al., 2021)

3 Centrifuge the flip top tubes at 515 RCF for 1 minute. Aspirate out supernatant.

4 Add 2.5 mL of bleach solution (7:1:2 ratio of ddH2O, 5M NaOH, and bleach) to the flip top tube and swirl around the solution. Flip the tubes to ensure the pellet completely dissociates from the bottom and mix vigorously. Vortex if needed.

5 Perform bleaching for only up to 3 tubes at a time to ensure quality.

6 Check the effect of bleaching every 30 seconds by aliquoting a 1 uL sample onto a glass slide to see how the worms are responding.

7 Once most of the adult worms are splitting apart and releasing their eggs, stop the bleach by diluting the tube all the way to 15 mL with M9.

8 Centrifuge immediately at 525 RCF for 1 minute. Aspirate out the bleach solution quickly.

9 Wash egg pellet with 10 mL of M9 once, centrifuging at 309 RCF for 3 minutes. Aspirate out the buffer after each centrifuge. Shake the tubes vigorously after adding the M9 to ensure the pellet dissociates.

10 Add a final volume of 3 mL M9 to the tube and shake just enough to free the pellet from the bottom but without letting the eggs collect on the upper sides of the tube.

11 Transfer to a 50 mL autoclaved flask covered in aluminum foil to minimize cross contamination, and put in a shaker at 1 RCF and 20 C.

12 Allow to grow to L1 arrest overnight.