

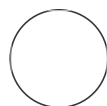
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🌐 Bleach/Sync Large Scale Culture Plates (LSCP)

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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.

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We use this protocol and it's working

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- 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.