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We use this protocol and it's working

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Transferring C. elegans to S-basal to grow them in liquid culture

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ABSTRACT

This protocol describes day 2 of our workflow to grow worms in liquid culture to induce the production of natural products (in this case, 1-HP derivatives). In this protocol, we get an approximate count for the number of life-stage synchronized worms we have, ensure to concentrate them to 30,000 worms/ml, and provide *E.coli* OP50 for them to be able to progress

1 Remove flasks with L1-arrested worms from the shaker

2 Swirl vigorously for at least 30 seconds, then collect a 1 uL aliquot and place onto a glass slide for inspection. Use a 0.5 uL aliquot if there are more than 100 worms in the 1 uL aliquot.

3 Count using a tally counter. Repeat for at least 3 trials (may need to do more if you don't shake enough initially and end up empty).

4 Once all the aliquots are collected this way, pour each of the flasks volume into a 15 mL flip top tube. Measure the volume. Determine the total population from these two data, as shown below.

5 Total population = (total volume in mL) X (concentration of worms/uL) X 1000 uL/mL

6 Centrifuge the flip top tubes at 1200 rpm for 3 minutes.

7 Aspirate out supernatant, leaving about 0.5 mL behind.

8 Dilute in enough S-basal to achieve a concentration of 30,000 worms/mL.

9 The minimum volume should be 0.5 mL if there are less than 5,000 worms total.

10 Shake vigorously to ensure worms are transferred to solution. Pour into the same flask as before (to minimize transfer loss).

11 Add enough bacteria for a concentration of 2% E. Coli. Make sure the final worm concentration is 30,000 worms/mL (essentially, add less S-basal based on how much bacteria is being added).

12 Repeat steps 1-10 for controls with worms (one for DMSO and one for bacteria only).

13 If using negative controls, repeat steps 9 and 10 (no worms).

14 Essentially, as negative controls, the flasks will have the same total volume of solution (identical S-basal and bacteria) as the earlier flask. They will be exposed to the same conditions just without any worms. The negative controls will later be separated into bacteria-only, bacteria + DMSO, and S-basal + 1-HP in Day 4.

15 Put the flask back in the shaker