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Ward lab alkaline bleaching protocol

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

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Abstract

Current protocol for how Ward lab synchronizes *C. elegans* by alkaline bleaching.

Materials

M9+gelatin

Na2HPO4 5.8 g
KH2PO4 3.0 g
NaCl 0.5 g
NH4Cl 1.0 g
gelatin 0.5 g
dH20 to 1l

1 Add M9+0.05% gelatin to nematode plates containing gravid adults, swirl the plate, recover the liquid with the worms and transfer to centrifuge tubes. For 10 cm plates, pour enough M9+0.05% gelatin to cover the surface of the plate and transfer to a sterile 15 ml conical tube.

2 Let the worms sink to the bottom of the tube and aspirate the liquid leaving 0.5 ml. Add M9+0.05% gelatin to 10 ml, and either let settle by gravity or spin at 1000g for 1 minute. Aspirate to 2 ml.

3 Prepare bleaching solution. Per bleaching reaction:

	A	B
	10 M NaOH	200 µl
	bleach	500 µl
	dH2O	1300 µl
	Total	2000

Scale accordingly for the number of bleaching reactions. We only bleach six strains at a time to avoid over-bleaching.

4 Add 2 ml bleaching solution to the 2 ml solution of worms. Either vortex or vigorously shake for 5 minutes, checking worms every minute. Once bodies are dissolved add M9 to 14 ml, centrifuge at 1000 g for 60 sec and aspirate liquid. Note: after the first centrifugation a film can be seen over the eggs. We usually aspirate less liquid that time, leaving 1 ml, and reduce the volume left for the following centrifugations.

5 Repeat for a total of 5 washes. For each wash, cap the tube and shake briefly before centrifuging at 1000 g for 60 sec and aspirating the liquid to 500 µl. For the first two washes bring volume to 10 ml with M9 , for the last three washes use 5 ml of M9.

6 After the final wash, centrifuge at 1000 g for 60 sec and aspirate the liquid to 500 µl. Bring volume to 7 ml with M9+5 µg/ml cholesterol. Rotate tube overnight at 20°C.

7 The next day count the number of worms in a 5 µl drop to determine the number of L1s per ml. If density is low, increase the volume of the drop. If density is high, decrease the volume of the drop. Centrifuge at 1000 g for 60 sec and aspirate the liquid to 500 µl. Add 1 ml of M9+0.05% gelatin. Transfer to a 1.5 ml tube. Spin 700 x g for 1 minute in a microfuge. Aspirate to 100 µl.

8 Resuspend pellet but pipetting and plate on desired number and type of plates