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# Sample Preparation Protocol SP-05
# Obtaining synchronous cultures of *C. elegans* hatchlings

## Scope
This protocol describes a modified procedure of the standard alkaline bleaching protocol and results in highly synchronized *C. elegans* animals specially intended for drug screening assays performed with the COPAS instruments and *ReFLx Sampler* module.

## Materials
M9 buffer (See Sample Preparation Protocol SP-06)
0.33 N NaOH
6% Sodium-hypochloride solution (for example Chlorox Ultra®)
$dH_2O$
15 ml conical tubes
40 μm nylon cell strainer (Falcon®; Becton Dickinson catalog number 352340)
6 cm Petri dish
Centrifuge
Dissecting Microscope
Agar plates with *C. elegans* gravid adults

## Procedure
Wash animals off the agar medium using M9 buffer. Use approximately 5 ml of the diluent per plate (the exact volume is not critical). When more than one plate is needed, add the 5 ml from the 1st plate to the 2nd plate and so on until all of the plates have been washed, and transfer the remaining liquid in a 15 ml conical tube.

Repeat the procedure with an additional 5 ml of M9, reversing the plate order by starting with the last plate and going back to the first plate. Add the remaining liquid to the conical tube.

Pellet the worms by centrifugation (~1000xg) and aspirate off supernatant.

Add 5 ml of fresh NaOH-hypochloride solution (3 ml 0.33 N NaOH + 1.4 ml 6% Na-hypochlorite solution + 0.6 ml $dH_2O$) and incubate for ~2.5 min with vigorous shaking and occasionally vortexing.

Pellet worms by centrifugation (see above) and aspirate off the supernatant. It is important that the organisms do not remain in the bleaching solution over 5 minutes before washing occurs or the embryos will die. Therefore, be sure to include the centrifugation and aspiration steps in the 5 minutes incubation time (e.g. 2.5 minutes with shaking, 1 minute for centrifugation,and 1.5 minute for aspirating off the supernatant).

Re-suspend the released eggs in 5 ml of M9 buffer by vortexing (to further break carcasses), and spin down again (see above).

Repeat the washing step once, aspirate off the supernatant and add 1ml of M9.

Re-suspend the egg preparation and transfer the total volume into a 6 cm Petri dish. Wash the tube at least twice with 1 ml of M9 buffer and add it to the liquid in the petri dish. Add enough diluent to the Petri dish that its surface is completely covered with liquid (approximately 4 to 5 ml).

Incubate the Petri dish @ 20ºC for 20 hours.

The eggs are allowed to hatch in the absence of food and the resulting culture will consist of starved worms arrested in the L1 stage of development. Pass the hatchlings through a 40 μm nylon cell strainer to remove debris and larger clumps of unhatched eggs. Collect the hatchlings into a fresh 15 ml conical tube. Determine the concentration of hatchlings / ml using a microscope and adjust the concentration of to between 1000 and 2000 per ml, by adding M9 buffer.

NOTE: For most applications, two to three agar plates with moderately heavy growth of gravid adults are enough for approximately 20 ml of a hatchling preparation at a concentration of 2000 animals/ml.

NOTE: This protocol is optimized for obtaining a highly synchronized L1 population of wild type N2 worms. In cases where other strains are used, it is necessary to determine the amount of starter plates and the precise incubation time in the NaOH-hypochloride solution for each strain and to adjust both, if necessary, to achieve similar results.

In addition, the allowed time for the eggs to hatch can be extended to up to 24 hours in many cases without affecting the synchronization of the hatchlings and their capability to undergo regular development.

**Questions?**

**For further information, please contact Union Biometrica, Inc. directly at 617.591.1211 or email your questions to appsupport@unionbio.com**