COPAS *BIOSORT* Control Protocol CB-03
Batch Sorting of Mixed *C. elegans* Population

Scope

This protocol instructs COPAS operators in batch sorting of a preferred population of *C. elegans* contained in a mixed population. Enrichment of the preferred population occurs.

This protocol should be used for: All procedures requiring high precision of specific size organisms.

Materials

M9 Buffer (See Sample Preparation Protocol SP-06)
COPAS GP Sheath (Union Biometrica P/N 300-5070-000)
COPAS Cleaning Reagent (Union Biometrica P/N 300-5072-000)
Microtiter plates
Microscope slides
15 ml conical tube

Procedure

Worm Preparation:

Choose two (or more) plates containing clean, healthy, mixed *C. elegans* populations.

Add 3ml of M9 buffer with 0.01% Triton X-100 to each plate and mix slightly.

Decant worms into conical tube and pellet by centrifugation (at low speed) or settling.

Discard supernatant leaving 3 ml of fluid on worm pellet.

Perform Sucrose Sedimentation according to Sample Preparation Protocol SP-04) for Sucrose Sedimentation Technique with the harvested worms if the plates are excessively contaminated.

Instrument Setup:

Set up the COPAS instrument as directed in the Operator's Manual.

Fill the sheath bottle with appropriate COPAS sheath reagent.

Pour 40ml of COPAS cleaning reagent into the sample cup.

Using the COPAS software, adjust the pressures to read the following:

Sheath	5.4 psi
Sample	7.1 psi
Sorter	3 to 5 psi

Process the COPAS cleaning reagent for a minimum of 2 minutes.

Release pressure from sample cup. Aspirate the remaining COPAS cleaning reagent from the sample cup. Rinse the cup two times with distilled water.

Add 40 ml of distilled water into the sample cup and process the distilled water for a minimum of 2 minutes to clean the cleaning solution from the sample valve.

Turn off sample valve. Open the sample cup. Aspirate and discard the unused distilled water.

Add 40 ml of the prepared worm prep to the sample cup. Cap firmly.

NOTE: Adjust the sample pressure until animal flow begins. This will assure a narrow sample stream.

Optimize the instrument gains for sorting and select a region for sorting. Turn COINCIDENCE CHECK ON.

Set the sort width of the instrument to 3. Using the FILL PLATE MODE, sort 1 worm per well into 12 wells of a microtiter plate or lid. Review the plate to determine if the sort delay is set correctly. Repeat if necessary, changing the delay, until at least 11 of 12 single worms are collected. The sort width can now be made higher if necessary.

Using the MANUAL SORT MODE, sort 5 worms per well onto a microscope slide. Review the slide to determine is this is the population of choice.

If you have not selected the population of choice, adjust the sort region and repeat until the region is determined.

Place a 15ml conical tube or other receptacle under the sort window of the waste tray.

Adjust the PER WELL number to the amount needed. The maximum number allowed is 9999.

Click the MANUAL SORT button for sorting to begin. Observe the first few drops to make sure the collection tube is properly aligned.

NOTE: At this point, the instrument may be left unattended however, when large sort numbers are selected, the instrument should be checked periodically.

If a problem occurs during sorting, press STOP to escape the MANUAL SORT MODE.

Questions?

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