

Scoring procedure:

July 14, 2024

Measuring *H. rosa* Growth Rate — 35C day / 24C night**Setup:**

Samples are in the growth chamber set to 35C. They're split into two racks. Try to minimize the time the rack is outside of the growth chamber---only work on one rack at a time.

A dry 48-well tissue culture plate should be on the bench next to the scope, stored upside down.

Use the first three rows for clones 1.2, 1.5, and 1.7 (reps 1-8 ea.), and the second three for 2.3, 2.4, and 2.5.

Transferring samples:

1. Load a tip onto the pipette
2. Invert tube 5 times
3. Flick cap to knock any droplets out of the treading
4. Open cap away from 48-well plate (to prevent dripping sample into the wrong well)
5. Add 150 uL of sample into the correct well
6. Close cap, dispose of tip

Counting:

Start at the left center of the well, and work your way around the perimeter counter clockwise until you reach the start, then move inward to the center.

Count moving rotifers only. Ignore eggs, dead rotifers, and anhydrous rotifers.

Note the presence of ciliates in the “contaminated” column. Ciliates are unicellular, but bigger than bacteria. Bacteria look like little sparkles and have much simpler movement than the ciliates, which will often interact more with their environment (i.e., turn when they bump into things). Both the bacteria and ciliates are easiest to see at the edges of the wells where the background is dark.

Cleanup:

Rinse the plate in the sink with the DI nozzle a good 3-4 times with decent pressure, to make sure any leftover rotifers get blasted away. Leave the plate to dry on the dish rack and move the dry plate that's already on the rack over to the bench. (these plates take more than 24 hours to fully dry)

Notes:

The clicker counter may have been moved by Kristen, so it might not be right next to the scope.

Make sure you switch tips between each transfer, not just between clonal lines.

Cover scope when done.