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CEM (Circular Estimate Method) – Step-by-step Protocol V.2

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

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Abstract

This protocol is part of the entitled paper '**Circular estimate method (CEM) - A simple method to estimate *Caenorhabditis elegans* culture densities in liquid medium.**' DOI: 10.1186/s12575-018-0089-2, published at Biological Procedures Online (2019), and describes the step-by-step procedures for the achievement of accurate and reliable estimates with the extremely close relationship to the real nematode counting. It can be used on different nematode species and adapted to different laboratory conditions.

Attachments

DOCX

CEM_Step by step pro...

19KB

Materials

MATERIALS

Rose Bengal, sodium salt **Bio Basic Inc. Catalog #**RB0809.SIZE.50g

Troubleshooting

Before start

Prior to the procedure, make sure you have these equipments available.

- One pipette 1000 µL capacity.
- Pipette tips
- One 24 cellular culture well plate.
- Scissor (only in case nematodes are larger than 500 µm).
- Deionized water (only in case of the estimate aliquot of nematode suspension will be performed on a smaller volume than 500 µL).
- Dye (Rose Bengal - only used when necessary, to enhance the nematode visualization).
- A light microscope with coupled
- Software for image edition.

1 Stock the nematode suspension inside a falcon tube according to its total volume (15 or 50 mL falcon tube).

2 Prime the pipette tip with the same liquid/medium of the nematode suspension, or with the nematode suspension itself, make five times up and down movement to avoid animal clogging.

3 Gently vortex the falcon tube and carefully transfer an aliquot ranging between 250 – 500 µL to a well of the 24 cellular culture well plate.Note:(a) Always take an aliquot from the middle of the falcon tube content, to perform more accurate samplings.(b) For nematodes bigger than 500 µm is advisable to do a little cut on the tip of the pipette tip to avoid animal clogging.

4 After transferred, if the aliquot volume pipetted was less than 500 µL is necessary to fill up the established volume (500 µL) with deionized water.Note:(a) Optionally, the pipetted aliquot can be stained to enhanced visualization when necessary.(b) The suspension can be stained with 5 % Rose Bengal (300 mg l-1) and incubated for 30 minutes at 80 °C. This process kills every worm and gets them stretched shape.

5 Then, the sample is homogenized with the same pipette, with five times up and down movements, with the tip located right in the center of the well (This process spread evenly the nematodes inside of the well).

6 Now it is necessary to wait until the animals reach the bottom of the well and the movement from homogenization get stopped (approximately 40 - 60 seconds).

7 Finally, the photo(s) can be taken using a light microscope with a coupled camera.Note: (a) If the zoom is not sufficient to cover the total area of the well, then it is necessary to take two or more photos, to be joined posteriorly and thus covering the entire well area. (b) The photos must be taken with a scale bar for reference that will help at the moment of the edition of these pictures.(c) These photos will allow the researcher to count how many animals there are inside of a circle relative to 1/10 of the total area/volume of the well. For this reason, it is necessary to know the area where the nematode suspension will be estimated.(d) According to the total well area informed by the manufacturer, calculate the area of a circle 1/10 of the size of an entire well.(e) The formula to calculate a circular area is:$A=\pi r^2$(f) If necessary, re-write this formula as follows:$r= \sqrt{(A/\pi)}$(g) After calculating the radius of this 1/10 circle, and as consequence, its diameter now it is possible to draw a circle that can represent a 1/10 of the total area with the usage of an image editing software.Edition and Estimate.OBS: The following steps must be performed with the use of image editing software. In our case, we choose to use the open-source software ImageJ (Fiji version) to edit the pictures and all the commands are referred to this software (Schindelin et al., 2012). However, it is possible to adapt the following steps to any image edition software.

8 Calibrate the software with help of the reference scale bar of the photo using the tool “Straight” (or similar) of the toolbar. This tool is used to measure the length of the scale bar belong to the photo. A relationship between how many pixels there are in a unit of length (1 mm) has to be made.

9 In the toolbar there is a flap called “Analyze” (or similar), by selecting this option, will open up a new toolbar, then just pick-up the “Set scale” tool and set the “known distance” as the numeric value equal to scale bar and then set the “Unit of length” as mm.

10 After calibrating, select the “Oval” tool (or similar), click and drag over the picture to form a circular shape. After, click on one of those points over the circle and drag until reaching the value of the calculated 1/10 diameter both on height as in width (the value will be different for each brand).

11 Now, just drag the circle to the board wall of the well and press the key “Ctrl” plus “B” and the circle will be fixed. Click again over the circle, drag and repeat randomly the process three times more to draw the areas where the animals will be counted.

12 After that, start to counting animals inside the circle, for this, zoom the picture to better view the animals and select the “Multi-point” (or similar) tool on the toolbar, then just click over the animal to starts the counting.Note:(a) Only count the animals that are entirely inside the circle (do not count the animals that crossed the circle).

13 After all animals inside the first circle had been counted, fix again the edition pressing the key “Ctrl” plus “B” and made the same process to the other 3 circles.

14 Now you just need to extrapolate the average obtained in those 4 circles to the entire well volume or to the entire nematode suspension volume.OBS: Reference of the image editing software:J. Schindelin, I. Arganda-Carreras, E. Frise, V. Kaynig, M. Longair, T. Pietzsch, S. Preibisch, C. Rueden, S. Saalfeld, B. Schmid, J.Y. Tinevez, D.J. White, V. Hartenstein, K. Eliceiri, P. Tomancak and A. Cardona, Fiji: an open-source platform for biological-image analysis, Nat. Methods, 2012, 9(7), 676-682.