Euplotes focardii RNA extraction

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Abstract

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Protocol

Step 1.

Filter and collect Euplotes focardii cells (ca.40000 cells) by centrifuging at 4°C for 5 min at 3000 rpm.

Step 2.

Dissolve the cell pellet in 750 µl Trizol reagent solution (3 volumes of Trizol to 1 volume of cells), vortex for 2 min.

Step 3.

Incubate 5 min at room temperature, add 200 µl of chloroform to the solution and vortex for 15 sec.

Step 4.

Incubate 15 min at room temperature then centrifuge the sample at 4°C for 20 min at 12000 rpm.

Step 5.

Move the supernatant containing RNA in a new tube and precipitate the RNA by adding isopropanol vol1:1.

Step 6.

Incubate 10 min at room temperature, collect the RNA by centrifugation at 4°C for 30 min at 13000 rpm and discard the supernatant.

Step 7.

Wash twice the RNA pellet with 1 ml of ethanol 75% (in DPEC water) by centrifuging 5 min at 8000 rpm and then let the pellet dry.

Step 8.

Resuspend the RNA pellet in 20-30 µl of DPEC water and incubate 10 min at 55-60°C.

Step 9.

Treat the RNA sample with DNAse I to remove genomic DNA.

Step 10.

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