

Small-scale silencing experiment in vegetative Euplotes crassus (provisional) Version 4

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Abstract

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Protocol

Step 1.

Grow RNAse III deficient E.coli strain HT115 in LB with antibiotic selection o/n at 37°C.

Step 2.

Prepare a 1:100 dilution of the bacterial culture, and grow it at 37°C until it reaches an OD₆₀₀ of 0.4.

Step 3.

Add 0.4 mM IPTG, and induce RNA transcription from the L4440 plasmid in the bacteria o/n at 37°C.

Step 4.

Collect the bacteria by centrifugation at max speed for 10 minutes, and wash them once with ddH₂O.

Step 5.

Isolate at least 30 well-starved Euplotes crassus cells, and feed them twice a day with feeding bacteria.

Step 6.

Leave the Euplotes crassus cells at 24°C, and monitor daily the change in phenotype.