Small-scale silencing experiment in vegetative Euplotes crassus (provisional) Version 3

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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 overnight at 37°C.

Step 2.

Prepare a 1:100 dilution of the bacterial culture, and grow it at 37ºC until it reaches an OD_{600} of 0.4.

Step 3.

Add 0.4 mM IPTG and induce RNA transcription from the L4440 plasmid in the bacteria overnight.

Step 4.

Collect the bacteria by centrifugation at max speed for 10 minutes and wash them twice with ddH_2O.

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 24ºC and monitor daily the change in phenotype.