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In devel.

RNAi by feeding in *Euplotes focardii* (povisional)

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Protist Research to Optimize Tools in Genetics (PROT-G)

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PROTOCOL STATUS

In development

We are still developing and optimizing this protocol

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

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

3 Add 0.4 mM IPTG, and induce RNA transcription from the L4440 plasmid, in which it is included the gene of interest to be silenced, in the bacteria at 37°C, until they reach a OD600 of 1.
Bacteria with L4440 plasmid, empty vector (EV), were grown as well to be used as control.

4 Collect the bacteria by centrifugation at 7000 rpm for 8 minutes, wash them twice with ddH_2O, then resuspend in 1.5 ml of sea water (at 4°C) to an OD600 of 4 to feed 100 ml of *Euplotes focardii* culture with a cell density of ~3000 cells/ml.

5 Feed *Euplotes focardii* culture every 3 days, at least for 10 days and checked the phenotype.