

Small-scale silencing experiment in vegetative Euplotes crassus (provisional) Version 2

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Abstract

Citation: Estienne Swart Small-scale silencing experiment in vegetative Euplotes crassus (provisional). protocols.io

dx.doi.org/10.17504/protocols.io.hiib4ce

Published: 01 Apr 2017

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 o/n bacterial culture.

Step 3.

Grow it at 37°C until it reaches an OD₆₀₀ of 0.4.

Step 4.

Add 0.4 mM IPTG and induce the bacteria o/n.

Step 5.

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

NOTES

Rachele Cesaroni 31 Mar 2017

Recipe for complete sea water (1 L):

36 g Reef Crystals 1 ml Walne's solution 1 ml of 10 µg/ml FeSO₄

0.2 ml of 2 mg/ml thiamine (light sensîtive; store at 4 $^{\circ}$ C) Add distilled water up to 1 L

Step 6.

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

Step 7.

Leave the Euplotes crassus cells at 24ºC and monitor daily the change in phenotype.