Stable transfection protocol of unicellular relative to animals Corallochytrium limacisporum

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Abstract

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Protocol

Perform transfection following transient transfection protocol
Step 1.

Plasmid used contains PAC gene (puromycin resistance), fused to mcherry protein under endogenous promoter (actin).

See:

https://www.protocols.io/view/transient-transfection-of-unicellular-relative-to-hmwb47e

After 3 days electroporation add puromycin as a selective agent
Step 2.

concentration 300ug/mL

After 7 days electroporation, centrifuge cells and resuspend with fresh medium and puromycin
Step 3.

Centrifuge at 1500 g for 5 minutes

Increase final volume by 1:2 dilution

Keep doing 1:2 dilutions every 3-4 days
Step 4.

Transfer into appropiate flask whilst increasing final volume of fresh medium.

Culture should increase number of transformants with time and cells that didn't integrate the plasmid should die.

Approximately after 20 days electroporation the culture should be mainly composed of transformants

Step 5.

Plate 100 uL of the transformed culture into marine agar plates with puromycin for single colony isolation

Step 6.

Let them grow for 3-4 days

Grow single colonies in fresh medium without puromycin

Step 7.

Grow single colonies separately, label accordingly.

In principle this would mean that the culture is stably transformed -- Need to perform other assays to verify plasmid integration, this would include:

-cycles of freezing/thawing

-southern blot

-qPCR

-flow cytometry