Transient transfection of unicellular relative to animals Corallochytrium limacisporum with electroporation using Neon SystemVersion 1

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Abstract

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Protocol

Centrifuge 5 mL of culture at 1500g 5min
Step 1.

Old culture: over 7 days passage

Resuspend cells in 5mL Sorbitol 1M
Step 2.

Count cells
Step 3.

Need around 1-5 x 10^5 cells

Mix cells with plasmid DNA (between 3 to 7 ug) and incubate for 10 min at room temperature
Step 4.

Neon system only allows 10 uL final volume in the tip-- adjust your cell and DNA dilutions accordingly for a final volume 10 uL

Electroporate cells with plasmid in Neon tip
Step 5.

Settings:

Apply twice in the same tip:

2000V

20ms

2pulses

Transfer cells into chamber with growth medium for recovery overnight

Step 6.

Medium of Corallochytrium is Marine Broth, each well: 500 uL medium

Observe transformants on the next day after electroporation

Step 7.

Efficiency varies from 1 to 20 transformants per 1-5x10^5 cells