Glass Bead Transformation of Chlamydomonas

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Abstract

A short and relatively cheap method for non-homologous nuclear transformation of Chlamydomonas reinhardtii. Works best with linearized DNA. Requires 500 micron glass beads for DNA transformation.

Citation: Josh Timmons Glass Bead Transformation of Chlamydomonas. **protocols.io** dx.doi.org/10.17504/protocols.io.dtn6md
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Protocol

Prep

Step 1.
Grow cell culture to an OD750 of 0.15 to 0.4

Prep

Step 2.
Centrifuge at 400g for 5 minutes at room temperature

DURATION
00:05:00

Prep

Step 3.
Resuspend in 1/100th the original volume of TAP

Prep

Step 4.
Add the following:

8000PEG to 5% final conc.
3 ug of DNA
0.3g of 500micron glass beads
0.4mL Chlamy cell suspension

Prep

Step 5.
Mix with a pipette

Vortex

Step 6.
Vortex at max speed for 15 seconds

DURATION
00:00:15

Plating & Selection

Step 7.

Take 25uL of the cell suspension and add to 100uL of TAP with an appropriate antibiotic

Plating & Selection

Step 8.

Spread on a TAP or YA plate, with an appropriate antibiotic, using large glass beads

Plating & Selection

Step 9.

Allow the liquid to dry while avoiding light

Plating & Selection

Step 10.

Seal the plates with parafilm

Plating & Selection

Step 11.

Allow the colonies to grow (colonies will appear in 1-3 weeks)

Plating & Selection

Step 12.

Transfer the remaining cell/vortex culture to a 125mL flask with 20mL of TAP (w/o antibiotic

Plating & Selection

Step 13.

Incubate for 6 hours on an orbital shaker at 70rpm

DURATION

06:00:00

Plating & Selection

Step 14.

Add antibiotic to an appropriate concentration

Plating & Selection

Step 15.

Take 50uL of the cell suspension and spread on a TAP or YA plate with an appropriate antibiotic with large glass beads

Plating & Selection

Step 16.