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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.

Troubleshooting

Prep

1 Grow cell culture to an OD750 of 0.15 to 0.4

2 Centrifuge at 400g for 5 minutes at room temperature
00:05:00

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

4 Add the following:

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

5 Mix with a pipette

Vortex

6 Vortex at max speed for 15 seconds
00:00:15

Plating & Selection

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

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

9 Allow the liquid to dry while avoiding light

10 Seal the plates with parafilm

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

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

13 Incubate for 6 hours on an orbital shaker at 70rpm
06:00:00

14 Add antibiotic to an appropriate concentration

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

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