From protocol by Dr Luke Mackinder
Edited by Marta Rudzite, Helen Underwood
University of York 2017

# *Chlamydomonas* transformation

## 1. Preparing *Chlamydomonas* batch culture for transformation

Inoculation to produce a 4L culture:

i. Inoculate 50 mL of TAP with a single, fresh colony from a TAP streak plate.

*Use a 250mL conical flask for efficient aeration and mixing while shaking.*

i. Grow, while continuously shaking, for 5 days at 50 – 100 µE until culture density reaches ~ 1 x 10^7 cells/mL.
ii. Transfer 50 mL into 400 mL of fresh TAP.
iii. Grow while shaking for another 2 days at 50 – 100 µE until culture density reaches ~ 8-9 X 10^6 cells/mL.
iv. Inoculate 4L of fresh TAP with all 450 mL *Chlamydomonas*, grow while bubble air and continuously stirring for ~30 hrs at 100 µE until culture density reaches 2-4 X 10^6 cells/mL.

*Ensure the final inoculation uses an exponential-phase culture.*
*For smaller volumes (up to 1L), this step can be done in a conical flask with continuous shaking.*
*The high volume is in case the culture does not reach the desired concentration, a larger volume of cells can be spun down for transformation.*

## 2. Transformation

*Work with open cultures should be performed in an appropriate flow hood to decrease contamination risk.*
*Use 50 mL Chlamydomonas, at 2-4 x 10^6 cells/mL, per transformation.*
*Each transformation requires 5 x 10^7 cells.*

**Materials to prepare**

i. TAP Sucrose, 40mM sucrose -- 15mL per transformation
ii. Selection plates

*Plate preferences depend on type of screening that will be performed*
*We use rectangular plates (250 mm x 250 mm)*
*1.5% TAP agar with appropriate antibiotic*
*Label on the side to avoid interference with the colony picker camera during selection*

iii. DNA insert cassettes digested with appropriate enzyme
iv. Electroporation cuvettes
v. Centrifuge tubes
vi. Glass L-Shaped spreaders
vii. Pipette tips and pipettes

From protocol by Dr Luke Mackinder
Edited by Marta Rudzite, Helen Underwood
University of York 2017

**Harvesting**

1. Aliquot *Chlamydomonas* culture into centrifuge tubes
2. Pellet *Chlamydomonas* culture using centrifuge (4 min at 1000x g)
3. Discard supernatant and avoid disturbing cell pellet

**Electroporation**

1. Use TAP 40 mM sucrose to dilute cell pellet to 2x10^8 cells/mL.
2. Add 250µL of *Chlamydomonas* culture per electroporation cuvette.
4. Incubate at 16°C for 5 mins.
5. Prepare recovery tubes (15 mL falcon tubes) with 8mL TAP sucrose.

   *Steps 6-8 should be performed as quickly as is possible, especially step 8.*
   *Before electroporation, dry the cuvette.*
6. Add transformation cassette DNA (14.5ng/kb cassette per 250µL) and mix briefly.

   *Do not mix by repeatedly pipetting, use pipette tip to mix by stirring.*
7. Electroporator should be set to 800V, 25µF.
8. Administer pulse and immediately add the mixture from the cuvette to the recovery tube.

**Plating and growth**

1. After all electroporations are complete, transfer recovery tubes to a shaker, and grow for 6 hours in the dark.
2. Centrifuge the recovery tubes for 4 min at 1000x g.
3. Discard most of the supernatant, leaving about 500µL.
4. Resuspend the pellet in the residual supernatant and spread on a transformation plate using a glass spreader.
5. After all plates are adequately dry, place them upside-down in stacks in plastic bags, and return to growth room.
6. Incubate transformation plates at 2-10 µE for 1-2 days.
9. Increase the light intensity to 25-50 µE for 8-12 days

   *This should yield >2mm colonies that can be used for selection*