Transformation *Chlamydomonas reinhardtii* (Nepagene)

Things to prepare:

- sterile Eppendorf tubes (15 ml)
- sterile 2M sucrose solution
- sterile maximum efficiency medium (ME, Thermo)

1. grow cells to a densitiy of $4*10^6$ - $8*10^6$
2. Enzymatically linearize 1 µg DNA (enough for two transformation):
    1. 1 µg DNA, 0.5 µl enzyme, 1 µl enzyme buffer, fill up to 10 µl with MQ
    2. incubate 30 min at 37°C
3. For five transformations (can be up-/ downscaled), harvest $2,6*10^7$ cells in a sterile 50 ml Falcon (Keep the negative control in mind!)
4. centrifuge cells for 2 min, 4000 rpm at room temperature (RT)
5. Decant the supernatant
6. Resuspend cell pellet in 200 µl TAP-NH_4-sucrose (40 mM)/ ME-sucrose (40 mM)
7. Transfer 40 µl of the cells to a sterile 1.5 ml reaction tube and add 5 µl of linearized DNA
8. Prepare one sterile 15 ml Falcon per Transformation
9. Transfer to 2 mm gapped electroporation cuvette (green or blue) and put the cuvette into the electroporation slot
10. Electroporate with suitable electroporation protocol
11. Immediately add 750 µl TAP-NH_4-Sucrose (40 mM) to the electroporated cells in the electroporation cuvette, subsequently transfer cells to the sterile 15 ml Falcon
12. Let cells regenerate overnight under agitation of 120 rpm and light intensity of 60-80 µE at RT
13. After regeneration, spin down cell for 2 min, 4000 rpm at RT, decant supernatant
14. Resuspend cells in the remaining supernatant and plate out on TAP-NH_4-agar plates with antibiotic