Cryopreservation of *Chlamydomonas reinhardtii*

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Freezing :

1. Cells are grown in a liquid TAP medium (1-6 x 10^6 cells / mL)
2. Mix 4.85 mL of culture and 150 mL of methanol
   (final conc. 3%) in a test tube
3. Transfer 500 mL of the mixture to a Falcon cryotube
4. Place the Falcon tube onto a “Mr. Frosty” (ambient temp.)
5. Place the “Mr. Frosty” in a deep freezer (-80˚C) for 90 min
6. Cool down the Falcon tube to -196˚C in a liquid N_2 bath
7. Store the frozen Falcon tubes in a liquid N_2 container (Cryosystem 4000, MVE)

Thawing :

8. Thaw a frozen stock at 35 ˚C
9. Transfer the thawed cell suspension into a TAP liquid medium (10mL) in a test tube
10. Incubate at 25 ˚C over night under the dim light
11. Spread 800 mL of the culture on a TAP agar plate
12. Incubate at 25 ˚C for 1-2 weeks
13. Typically 2000-4000 colonies appear within 1-2 weeks