Cryopreservation of *Chlamydomonas* used at Fernández & Galván´s Lab (University of Cordoba, Spain)

This protocol is based on those already presented at the *Chlamydomonas Genetics Center* by Richard Sayre and that published by Crutchfield et al., 1999 (Eur. J. Phycol 34: 43-52). Some modifications have been introduced to make the viability reproducible and acceptable.

0. Grow cells in TAP medium to a cell density of about 1-5 millions per ml
1. Mix cells with methanol to reach a final concentration of 3%(v/v) (Like Richard Sayre we also found that a 3% methanol gives the best results).
2. Incubate cells in the methanol solution at 4ºC in the dark during 1 to 14 h. Our best results were found 4-5 h.
3. Transfer 1,8 ml of the mixture to 2 ml cryopreservation tubes and place them in a 1ºC Freezing Container (Nalgene, Cat. no. 5100-0001) that will be incubated at -80ºC during 4h. (We recommend instructions of the Freezing Container manufacturer)
4. Then, place the tubes directly in liquid nitrogen.

5. For thawing, the tubes are transferred directly from liquid nitrogen to a thermostatized bath at 35ºC during 3 min. It is important to make sure that the inner level of cell in the tubes is a little below the water level in the bath, otherwise 3min thawing would not be enough. Even so, sometimes it appears that the tube is not thawed in 3min (it might be when cell concentration is high); then the time can be increased in extra 30s to 1 min with no apparent decrease in efficiency.
6. Take 0.5-1 ml from the tube to inoculate 200 ml of TAP medium. Incubate the cells in the dark for 12 h, then 2 days at dim light, and finally grow at normal light. These steps are critical since to cultivate cell directly at intense light after thawing decreases strongly cell viability. This viability was estimated in each step after dilutions from the cultures and plating in TAP medium
7. Though in some cases cell recovery can reach values of 30-40%, routinely an acceptable recovery of 2-10% is obtained.