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Cryopreservation of microalgal cultures

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1 Works for me This protocol is published without a DOI.

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ABSTRACT

Protocol for long-term .cryopreservation of microalgal cell cultures.

PROTOCOL CITATION

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Preparing the cryoprotectant 10m

1 Make a [M]**10 % (v/v)** solution of **DMSO** in the appropriate culture medium for the strains being preserved.

Eg. **45 mL culture medium** medium and **5 mL DMSO** to make **50 mL** cryoprotectant.

Work in the fume hood when using DMSO.

2 Filter sterilise the cryoprotectant using a **0.2 µM filter**.

Preparing the cultures 15m

3 Prepare the cryotubes with a 1:1 ratio of cryoprotectant:culture

Eg. **1 mL cryoprotectant** and **1 mL culture** for a 2 mL cryotube.

4 Once the cryoprotectant and culture have been mixed in the cryotubes, allow **00:10:00** for the cryoprotectant to penetrate. 10m

Progressive freezing 16h

5 Place the cryotubes into a Mr Frosty freezing container (filled to the line with isopropyl alcohol/isopropanol/propan-2-ol).

6 Place the freezing container in a **-80 °C** freezer **Overnight** .

7 Remove the tubes from the freezer and flash freeze them in liquid nitrogen.

8 Store the tubes either in liquid nitrogen or in a **-150 °C** freezer for as long as required.

Culture recovery 2w

9 To recover a cryopreserved culture, thaw the cryotubes in a water bath pre-heated to **40 °C** to prevent ice crystals from re-forming as they thaw.

10 Add the defrosted culture to **20 mL fresh culture medium** .

11 Place the culture at the appropriate temperature but **keep it in the dark** for **24:00:00** , for example by wrapping the culture flask in aluminium foil. 1d

12 After **24:00:00** , remove the aluminium foil. Monitor the culture to determine if recovery has been successful. 1d