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Cryo_preservation_Synechocystis_PCC_6803

In 1 collection

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ABSTRACT

Cryopreservation protocol used during the interlaboratory study published by Mager et al. 2023.

Precultures for Cryo conservation of Synechocystis PCC 680..

1 Starting from a BG11 agar plate with your strain of interest, inoculate a BG11 liquid culture

Note

For our BG11 recipe, please refer to our protocol "BG11_and_inducer_preparation"

1.1 Grow the strain of interest with your settings of choice.

Note

For the interlab study, Synechocystis PCC 6803 was grown in 100 mL Erlenmeyer glass flasks with cotton plugs in shaking incubators set at 100 rpm under 50 µmol photons · m-2 · s-1 constant white light illumination, ambient CO_2, and 30°C in BG11 medium. After reaching OD730 of 2, illumination was increased to 80 µmol photons · m-2 · s-1 to account for the high cell density in the flasks.

1.2 Grow the cells to an OD730 of 2.5-3

Cryo conservation of Synechocystis PCC 6803

2 Centrifuge cells at 12.000 g (fixed angle rotor) for 15 min and wash in the same volume of fresh BG11 medium

2.1 Centrifuge cells at 12.000 g (fixed angle rotor) for 15 min and resuspend in 10% of the initial volume with BG11 and 15% (v/v) final concentration glycerol

Note

For this, BG11 media was mixed with 50% (v/v) autoclaved glycerol to a 15% (v/v) final concentration glycerol in a ration of 70:30. This does mean that the BG11 is slightly diluted, however we did not observe any impact on the longevity of the conserved cells.

2.2 Aliquot cells in tubes to 350µl each

2.3 Optionally, freeze cells in liquid nitrogen. Store cells at -80°C.

> Note
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> For the interlab study, cells were frozen in liquid nitrogen and shipped to participating laboratories on dry ice to maintain cryoconservation.

Inoculating a culture from Cryo conserved Synechocystis PC..

3 Use 330 µL of prepared cryoconserved cells to inoculate a 100 ml flask containing 10 mL of BG11 medium

3.1 Cultivate for 48 hours at your settings of choice

> Note
>
> For the interlab study, this was done in glass flasks with cotton plugs in shaking incubators set at 100 rpm under 50 µmol photons · m-2 · s-1 constant white light illumination, ambient CO_2, and 30°C in BG11 medium.