Freezing and unfreezing unicellular protists using Mr Frosty container

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Abstract

Citation: Maria Rubio-Brotons Freezing and unfreezing unicellular protists using Mr Frosty container. **protocols.io** dx.doi.org/10.17504/protocols.io.ggybtxw
Published: 22 Nov 2016

Protocol

Collect your culture in 15 mL falcon tube and centrifuge at 1000 g for 5 minutes
Step 1.

Discard medium and resuspend in 10 % DMSO growing medium
Step 2.

Use growing medium according to your organism, in our case is marine broth.

We usually make 10 mL final volume (1mL DMSO into 9mL growing medium).

Aliquot 1 mL of your culture into criogenic vials
Step 3.

Nalgene® cryogenic vials

Place your criovials into Mr Frosty containing 100% isopropanol
Step 4.

Nalgene® Mr. Frosty® Cryo 1°C Freezing Containers

Store Mr Frosty container at -80ºC for at least 4 h
Step 5.

We usually keep it for 24 hours

Move your criovials into a long-term storage container at -80ºC
Step 6.

Unfreezing your culture
Step 7.

Take your criovials out of -80ºC storage and place them into 35ºC water bath for 2-3 minutes

Step 8.

When you see they start thawing take them out of the water bath, usually is after 2-3 minutes

Transfer into fresh growing medium

Step 9.

We usually have 1mL frozen culture, transfer this 1 mL into 7 mL fresh growing medium flask

Monitor surival rates within the next 24h

Step 10.

After 2-3 days unfreezing, pellet your cells and resuspend with fresh growing medium

Step 11.

Centrifuge 1000 g for 5 minutes