Cryopreservation of labyrinthulomycetes in trehalose

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Abstract

This method adapted from instructions provided by Daiske Honda, Konan University.

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Protocol

Prepare cryopreservation solution

Step 1.

mix

20% glycerol

10% trehalose

in 35ppt artifical seawater

Autoclave

Grow up cells

Step 2.

For Aurantiochytrium and Schizochytrium, we've grown cultures in 790By+ (or 1/2 790By+) to late-log or early stationary phase.

For Oblongichytrium, we've grown cells in GPY to late-log or early stationary phase.

NOTES
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We have not experimented with how growth phases affects cryopreservation success with these strains.

We are still testing Aplanochytrium and Labyrinthula.

Mix cells with cryopreservant

Step 3.

Mix gently 1 volume of cell culture with 1 volume cryopreservant solution

(final concentration 10% glycerol, 5% trehalose)

We usually mix 0.5 ml culture with 0.5 cryo solution in 2 ml cryovials

Slowly freeze cells

Step 4.

Place cyrovials in Mr Frosty (filled with isopropanol as instructed)

Place Mr Frosty in -80 freezer overnight

Long-term storage

Step 5.

Transfer cryovials to a -80 freezer for storage

To revive cells

Step 6.

Thaw at 30C for 3-5 min, until just melted

Pour into 10 ml growth medium (such as 790)

Return to standard growth conditions

NOTES

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Sometimes cultures growth is evident the next day; sometimes it takes nearly a week.