Bacterial Cryopreservation

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Abstract

Please contact Dr. Steven Wilhelm (wilhelm@utk.edu) for additional information regarding this protocol.

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Protocol

Preparation

Step 1.

Take a dense culture and transfer a volume of cells (usually 20 mL) into a sterile centrifuge tube

Step 2.

Spin down the culture at 4,500 rpm, 10 min

Step 3.

Decant supernatant into bacterial waste

Step 4.

Resuspend pellet into 2 mL of the appropriate growth medium

Step 5.

Vortex 10 sec

Step 6.

Split the 2 mL into two cryogenic vials by adding 1 mL to each vial

Step 7.

Invert to mix several times

Step 8.

Immediately place into -80ºC freezer

Transferring Cells

Step 9.

Remove cryogenic vials one at a time from the freezer

Step 10.

Using a sterile pipette tip, scrape a small amount of frozen cells and place into 20 mL sterile growth medium. Slowly move the cells to the appopriate growth conditions.

AMOUNT
20 ml Additional info:

Step 11.

Place cryogenic vial immediately back into the freezer