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Standard Operating Procedure: Microbank

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2 Works for me dx.doi.org/10.17504/protocols.io.bqbjmskn

Protocols Bark Beetle Mycobiome

Bark Beetle Mycobiome Research Coordination Network

ABSTRACT

This protocol describes how to store and retrieve bacterial and fungal cultures with Microbank™.

This protocol is part of the Bark Beetle Mycobiome (BBM) Research Coordination Network. For more information on the BBM international network: Hulcr J, Barnes I, De Beer ZW, Duong TA, Gazis R, Johnson AJ, Jusino MA, Kasson MT, Li Y, Lynch S, Mayers C, Musvuugwa T, Roets F, Seltmann KC, Six D, Vanderpool D, & Villari C. 2020. Bark beetle mycobiome: collaboratively defined research priorities on a widespread insect-fungus symbiosis. Symbiosis 81: 101–113 https://doi.org/10.1007/s13199-020-00686-9.

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Microbank™ is a ready-to-use system designed to simplify the storage and retrieval of bacterial and fungal cultures. It is comprised of a unique cryovial system incorporating treated beads and a special cryopreservative solution. Microbank™ provides a more reliable means of maintaining important cultures than repetitive subculture, which can result in contaminated cultures, lost organisms or changed characteristics. The specially formulated preservative ensures longer survival of fastidious cultures and higher quantitative recoveries (Pro-Lab Diagnostics).

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Materials and Methods:

1. Prepare the hood for use (wipe down with ethanol, UV 10 minutes)
2. Put on new sterile gloves and spray alcohol on the gloves before placing hands in the hood.
3. Use a sterile scalpel to scrape the top layer of agar from a PURE culture.
    a. Use area with high sporulation when possible or areas of new growth.
    b. If there is liquid in the petri dishes, be cautious for cross contamination. Wipe down the area with ethanol between wet petri dishes.
4. Close the cap and label the vial with the full sample number.
5. If culturing fungus, vortex the Microbank vial for ~20s. If culturing bacteria, invert the tube 4 times.
6. Allow the Microbank vial to sit at room temperature for ~1 hour.
    a. This allows the organism to attach to the microbeads in the vials.
7. Under the hood, using sterile tweezers, remove the agar pieces.
8. Remove ~380ul of the cryopreservative from the Microbank vial.
9. Close the vials – finger tight – and store in the -80 freezer.
    a. From this point on, the vials must be kept on a freezer block if removed from the -80 freezer and placed back in the freezer as soon as possible.

Microbank Recovery:

1. Remove Microbank vial from -80 freezer and place on a freezer block.
2. Prepare the hood and put on new sterile gloves. Spray alcohol on hands before placing them in the hood.
3. Open the microbank vial and use a sterile loop to recover the culture from the vial. Alternatively, use sterile tweezers to remove a bead from the vial.
4. Streak the inoculation loop or bead onto PDA or NA. The Microbank bead may also be inserted into the appropriate media. Do not replace a bead in the microbank vial.
5. Close the Microbank vial and replace into -80 freezer immediately.
6. Record the placement of each microbank vial on the physical copy (in pencil) and the electronic copy which can be accessed on the server under GAZIS-Inventory-Microbank.

Personal Protective Equipment: Laboratory Coat, Gloves; sterile

Equipment Needed: Laminar flow hood; sterilizer

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