SOP


# 10.3.2 Subculturing for culture purity

## Objectives and scope

This SOP describes the various steps to follow to obtain pure cultures for downstream analyses such as nucleic acid extractions and sequencing. It follows the steps of subculturing bacterial strains preserved in cryotubes stored at either -20°C or -80°C.

This SOP is intended for Mini-Lab laboratory technicians.

## Principle

Reviving dormant bacterial strains in a cryobead BHIG (brain heart infusion broth with glycerol) storage medium on a chocolate agar InTray and ensure that culture is representative of one single organism.

It is important to work with monomicrobial cultures to avoid the risk of contamination of downstream analyses.

It is also important to have sufficient microbial culture available for nucleic acid extraction and sequencing.

**Note:**
In order to avoid contamination during the process of seeding InTrays, whenever the agar tray is open, you must keep it away from your mouth and avoid air flow.

## Safety and environment

- Wear your PPE for the duration of this technique: lab coat, gloves;
- All biological samples should be considered as potentially infectious and handled with the usual precautions;
- Refer to the document "6.8 Internal waste management", if you have questions about how to handle any waste product.

## Sample

- Type of material:
  - Bacterial cultures stored on microbeads in cryotubes
  - Bacterial cultures on InTray chocolate

## Equipment

| *Common Name* | *Associated SOP* |
|---|---|
| **Freezer -20°C** | SOP-7.7-REFCON |
| **Freezer -80°C** | SOP-7.7-REFCON |
| **Incubator INCUDIGIT-SV 30 L GG JP selecta** | SOP-7.2-INCUB |
| **INCUBATOR 56L (VWR IL56)** | SOP-7.2-INCUB |

## Consumables

| *Common Name** | *Storage conditions* |
|---|---|
| **Gloves** | *NA* |
| **1 µL inoculation loop** | *NA* |
| **10-µL inoculation loop** | N/A |
| **Transfer pipette** | *NA* |
| **InTray chocolate agar** | *2-8°C* |
| **InTray colorex agar** | *2-8°C* |
| **Water tube for inoculum 3 mL** | *15-30°C* |
| **Cryosystem BALL TUBE** | ***2–8°C*** |

## Procedure

***Preparation of InTray cassettes prior to handling: removal of excess water from InTray cassettes, if necessary***

1. Prepare the required number of chocolate agar InTrays, based on the number of strains to be analysed, one chocolate agar InTray per strain.

On how to prepare the InTray cassettes see SOP "10.3 Subculture: Seeding"

### Subculture of strains from frozen culture

#### First subculture

2. Label the InTrays with the corresponding sample number, microorganism name and today's date.
3. Remove the cryotubes from the freezer –80 °C one by one, to prevent thawing.


4. Using a 10uL loop, take a cryobead from the cryotube and place it on the edge of the chocolate agar InTray.
5. Keep the loop and use it to spread the cryobead over the chocolate agar InTray in a single quadrant. Then discard the loop and take a new one to spread it over three quadrants.

6. Discard the loop in the red benchtop bin.

7. Return the cryotube to the freezer.

8. Perform the same steps for each strain.
9. Incubate the chocolate agar InTrays at 35°C for 16–24 hours.

### Second subculture

After incubation, check the growth on the agar:

#### *a) If the bacterial culture is growing uniformly on the chocolate agar InTray (you can observe just one colony type)*

10. Take an InTray Chocolate cassette and prepare it as in 1 and 2.
11. Take an inoculation loop and collect an isolated colony from this primary InTray cassette.
12. Deposit the colony on the agar, then make a streak and continue with quadrant streaking, starting with quadrant 1, as shown in **Diagram 1**.
13. Incubate the chocolate agar InTrays at 35°C for 16–24 hours.
14. Discard the inoculation loop in the red benchtop bin.

#### *b) If the bacterial culture is not growing uniformly on the chocolate agar InTray (you can observe more than one colony type)*

15. Take an inoculation loop and collect any isolated colony type you can recognize from this primary InTray cassette
16. Spread each colony type on a chocolate agar InTray and in parallel on a Colorex InTray following the steps 10-13 for each colony type.
17. Incubate the chocolate agar InTrays at 35°C for 16–24 hours.
18. After incubation, if on the Colorex agar colonies appear different in colour and morphology, take the respective colonies on InTray cassettes and perform an ID test on each one
19. Once the ID of the organism is verified, restart from step 10

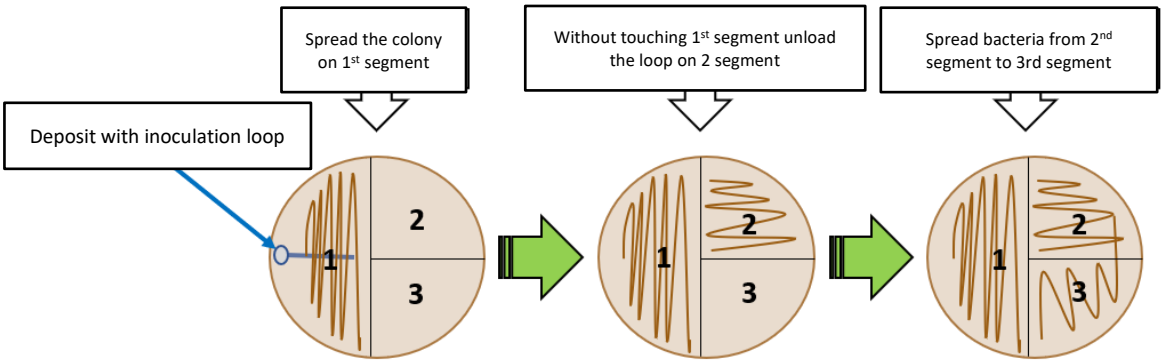
#### *c) If there is NO GROWTH on the InTray*

20. Return the InTray to 35°C for 16–24 hours
21. At the same time, repeat the subculture from the same cryotube repeating the steps 1-9
22. Incubate all the freshly inoculated chocolate agar InTrays at 35°C for 16–24 hours.

***Note: When the colonies are too few or too small after 24 hours or 48 hours of incubation, streaking will need to be repeated starting from a pure culture (second subculture) on several InTray Chocolate cassettes to obtain a sufficient number of colonies. This may occur for some types of fastidious bacteria such as some Streptococcus or Haemophilus.***

See SOP 10.3.1 Subcultures: Techniques for subcultures

- *Diagram 1: Subculture of a colony collected using a loop*




## Related documents

- SOP-10.3-SCENSE: 10.3 Subculture: Seeding
- SOP-10.3.1: TECHREP
- SOP-11.3-SOUENV
- DOC-6.8-DECHINT: 6.8 Internal waste management
- POS-8.1-CQI: 8.1 Internal Quality Control
- FM-9-SCENSE: 9 Subculture: Seeding
- IFU InTray Chocolate and Colorex Cassettes