**SOP# 02.281.01**

**Bacterial Isolate Revival**

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**Purpose**

This SOP describes the procedure used to revive bacterial isolates.

**Scope**

For exploratory purposes.

**Regulatory References**

NA

**Responsibility**

* Responsibility of experimentalist – understanding and performing this procedure as described; reporting any deviations or problems to area supervisor; adequately documenting the procedures and results
* Area manager or supervisor – ensuring that the analyst performing this procedure is qualified; ensuring that the procedure is followed and update the procedure as necessary

**Definitions/Abbreviations**

Primary isolate - isolates generated from isolation rounds using human fecal matter, stored on a 96 well plate

Culture – an actively growing cultivation of an isolate in liquid media

Revived freezer stock – A glycerol stock made from an aliquot of a culture, used as the stock of each particular isolate after revival to limit freeze/thaw and cover opening cycles on the primary isolation plate.

**Related Documents**

SOP# 02.041.01 Bleach solution preparation

SOP# 003.001.01 – Anaerobic chamber operation and maintenance

SOP# 03.003.01 - Biosafety cabinet operation and maintenance

SOP# 02.040.01 – Glycerol buffer preparation

SOP# 02.203.01 Bacterial isolation from feces - CGM SOP# 02.080.01 - Pre-reduced media and buffers - liquid sparging

**Required Equipment and Materials / Reagents**

* 96 well plate of previously isolated bacteria from any isolation procedure (e.g. SOP# 02.203.01 Bacterial isolation from feces – CGM)
* Aluminium foil plate covers – (VWR, catalog # 60941-076)
* Class II Type A2 Biosafety cabinet (Labconco), any manufactured biosafety cabinet may be used as long as it is Class II or higher.
* Centrifuge capable of holding 96 well plates, for example Eppendorf Model# 5811F
* Sterile inoculation loops (VWR, cat# 89126-870)
* 50% glycerol buffer (SOP# 02.040.01 – Glycerol buffer preparation)
* Cryogenic vials (VWR catalog# 89094-802)
* Freezer boxes, for example Argos catalog# R3027A
* -80°C freezer
* Sterile culture tubes, for example VWR catalog# 60818-576

**Precautions**

* Personal protection equipment including gloves, lab glasses, and lab coat must be worn when executing this procedure
* All handling of human derived isolates must be done within a BL2 area inside of a Class II biosafety cabinet or the anaerobic chamber
* All work surfaces must be treated with 20% bleach for twenty minutes before and after procedure (SOP# 02.041.01 Bleach solution preparation)

**Procedure**

1. Remove desired plate of primary isolates from -80°C freezer and allow to thaw on benchtop.
2. Briefly vortex plate and spin down for 12 seconds in centrifuge
3. Place plate back in to -80°C freezer for 5 minutes so it can re-freeze
4. Bring plate into the anaerobic chamber or biosafety cabinet as needed. See SOP# 03.003.01 - Biosafety cabinet operation and maintenance and SOP# 003.001.01 – Anaerobic chamber operation and maintenance.

Note: Steps 5 – 12 of this procedure must be performed in the anaerobic chamber.

1. Carefully remove the aluminum foil plate cover
   1. Note: this should be done while volume in wells is still frozen to avoid splashing. Dry ice may be used while carrying plate to work area to ensure it remains frozen
2. Use inoculation loop to sample plate wells corresponding to the isolates selected for revival, and streak out sample onto agar plates of desired media.
3. Cover plate with aluminum foil and replace in 96 well plate freezer racks in the -80°C freezer
4. Place agar plates into the incubator
5. Check growth of plates after 24 hours
6. If single distinct colonies have grown continue to step 11, otherwise wait another 24 hours or until single distinct colonies have grown

Use an inoculation loop to pick a single colony from the plate and inoculate 7mLs of desired reduced liquid media in a culture tube (SOP# 02.080.01 - Pre-reduced media and buffers - liquid sparging)

1. Once the culture is turbid, generally 24 - 48 hours later, gently vortex the culture for one second.
2. Combine 200uLs of culture and 200uLs of 50% glycerol buffer (SOP# 02.040.01 50% Glycerol buffer preparation) in a new, labeled cryotube. This is now a ‘revived freezer stock’.

Note: The label must include isolate identity, date of experiment, and experimentalist name. This information must match the information and date in the lab notebook of the experimentalist

1. The remaining volume of the culture may be used for various procedures
2. Remove the vial form the anaerobic chamber (SOP# 003.001.01 – Anaerobic chamber operation and maintenance)
3. Store the revived freezer stock in a freezer box within the -80°C freezer
4. After an isolate has been revived, use revived freezer stock for future uses. Do not return to the primary isolation 96 well plate unless necessary.

**Version History**

NA

**Worksheets**

NA

**Appendix**

NA