**SOP# 02.302.01**

**DNA extraction from bacterial isolates – cell pellets**

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**Purpose**

This SOP describes the procedure used to extract and purify DNA from a liquid culture of a bacterial isolate for downstream genetic analysis.

**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**

RCF – relative centrifugal force

rpm – revolutions per minute

x *g* - times gravitational force

uL – microliter

**Related Documents**

SOP# 03.001.01 – Anaerobic chamber operation and maintenance

SOP# 03.003.01 – Biosafety cabinet operation and maintenance

SOP# 03.102.01 – Hazardous liquid waste disposal

SOP# 02.041.01 – Bleach solution preparation

**Required Equipment and Materials / Reagents**

* MoBio Ultra Clean Microbial DNA Isolation kit (MoBio, Catalog# 12224), no substitutions may be made for this item
* MoBio vortex adapter (MoBio Cat# 13000-V1), no substitutions may be made for this item
* Bench top vortexer (VWR, Cat# 14005-824), any benchtop vortexer that can fit the vortex adapter above can be used
* Microcentrifuge capable of spinning at 10,000 x g, for example Eppendorf 5427R
* Pre-sterilized, filtered pipettor tips; any sterilized, filtered pipettor tips may be substituted
* Pipetman, for example VWR model# 89079
* Wet ice
* Class II Type A2 Biosafety cabinet (Labconco), any manufactured biosafety cabinet may be used as long as it is Class II or higher.
* Solutions MD1 MD2 MD3 MD4 MD5 and Microbead solution provided in the MoBio DNA isolation kit

**Precautions**

* Personal protection equipment including gloves, lab glasses, and lab coat must be worn when executing this procedure
* All handling of bacterial isolates must be done within a BL2 area inside of a Class II biosafety cabinet or anaerobic chamber
* See SOP# 02.041.01 – Bleach solution preparation.
* All liquid biological waste material generated from this protocol, must be disposed of hazardous waste as per SOP# 03.102.01 –Hazardous liquid waste disposal.
* Solution MD4 of the Ultra Clean Microbial DNA Isolation kit contains ethanol and is flammable

**Procedure**

1. Remove the desired liquid culture from the incubator.
2. Ensure that the culture is fully closed.
3. Vortex liquid bacterial culture for one second using a benchtop vortex.
4. Transport culture to a BL2 area within the Class II biosafety cabinet or anaerobic chamber, as appropriate for the culture. (See SOP# 03.001.01 – Anaerobic chamber operation and maintenance, and SOP# 03.003.01 – Biosafety cabinet operation and maintenance.)
5. Aliquot 1.8mL of liquid culture into the MoBio-provided 2mL collection tube
6. Centrifuge at 10,000 x g for 1 minute at room temperature
7. Decant the supernatant into a \_\_\_\_\_\_\_\_\_, being careful not to lose the cell pellet
8. Centrifuge at 10,000 x g for 1 minute at room temperature
9. Remove all remaining media supernatant via pipetting, again be careful not to disturb the cell pellet.
10. Re-suspend pellet in 300uL of MoBio MicroBead Solution and vortex for 1 second to mix
11. Transfer all of suspended cells to the MicroBead Tube (provided by MoBio) and close it tightly.
12. Check Solution MD1. If Solution MD1 is precipitated, heat solution to 60°C using a hot water bath until dissolved before use.
13. Add 50 ul of Solution MD1 and invert three times or vortex for one second to mix.
14. Secure tubes horizontally using the MoBio Vortex Adapter tube holder for the vortex. Vortex at maximum speed (3000rpm or setting 10) for 10 minutes.

Note: If you are using the 24-place Vortex Adapter for more than 12 preps, increase the vortex time by 5-10 minutes.

1. Centrifuge tubes at 10,000 x *g* for 30 seconds at room temperature. **CAUTION**: Do not exceed 10,000 x *g* or tubes may break.
2. Transfer the supernatant to a clean 2 ml Collection Tube (provided in kit).

Note: Expect between 300 to 350ul of supernatant. Supernatant may still contain some particles.

1. Add 100 ul of Solution MD2 and vortex for 5 seconds. Incubate at 4°C for 5 minutes.
2. Centrifuge the tubes at room temperature for 1 minute at 10,000 x g.
3. Avoiding the pellet, transfer up to, but no more than, 600 ul of supernatant to a clean 2 ml Collection Tube (provided in kit).
4. Shake to mix solution MD3 before use
5. Add 900 ul of Solution MD3 and vortex for 5 seconds
6. Centrifuge the tubes at room temperature for 2 minutes at 10,000 x *g.*
7. Load approximately 700 ul onto a Spin Filter and centrifuge at 10,000 x *g* for 1 minute at room temperature. Discard the flow through but do not discard Spin filter.
8. Repeat step 20 a total of three times until all sample/MD4 liquid has been applied to the
9. Add 300 ul of Solution MD4 and centrifuge at room temperature for 30 seconds at 10,000 x *g*.
10. Discard the flow through but do not discard Spin filter.
11. Centrifuge again at room temperature for 1 minute at 10,000 x *g*.
12. Carefully place spin filter in a clean 2 ml Collection Tube (provided in kit). Avoid splashing any Solution MD4 onto the Spin Filter.
13. Add 50 ul of Solution MD5 to the center of the white filter membrane. Alternatively, sterile DNA-Free PCR Grade Water or elution buffer may be used for elution at this step.
14. Let tubes sit at room temperature for 1 minute
15. Centrifuge at room temperature for 30 seconds at 10,000 x *g*.
16. Discard the Spin Filter. The DNA in the tube is now ready for any downstream application. No further steps are required.
17. We recommend storing DNA frozen at -20°C.

**Version History –** NA

**Worksheets** - NA

**Appendix** - NA