**SOP# 02.301.01**

**DNA Extraction from Bacterial Isolates - Triton**

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**Purpose**

This SOP describes a procedure used to extract and purify DNA from bacterial isolates 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**

* PCR – polymerase chain reaction
* M – molar, moles/liter
* DI water - Deionized water
* L – liter
* mL – milliliter
* uL – microliter
* um - micrometer

**Related Documents**

**NA**

NA

**Required Equipment and Materials / Reagents**

* Sterile, filtered pipettor tips (double check cat#)
* Pipetman (double check cat#)
* Eppendorf PCR thermal block, any brand PCR thermal block can be used
* In house vacuum line, if facility does not have an in house vacuum line a stand alone vacuum pump can be used
* NaCl (5M, Sigma Aldrich, S5150), any cell culture grade sodium chloride water solution can be used
* Tris (double check cat#)
* Triton X-100 (1M, Sigma Aldrich, X100), any nonionic lab grade solution can be used
* Deionized water, provided using in house water system (look up name)
* 0.2um Filter bottle (catalog #)
* 96 well PCR plate (VWR, catalog # 82006-704), any PCR clean 96 well plates may be used
* 8 tube PCR strip tubes (VWR, catalog # 93001-120), any PCR clean strip tubes may be used
* PCR sealing film (VWR, catalog # 82018-846), any sterile PCR sealing film may be used
* Bench top vortexer (catalog #)
* Bench top centrifuge (catalog #)

**Precautions**

* Personal protection equipment including gloves, lab glasses, and lab coat must be worn when executing this procedure
* Triton X-100 is a hazardous material that has acute toxicity, can cause eye and skin irritation. This chemical is also hazardous to the aquatic environment and must be disposed of as hazardous waste according to MIT Environmental Health and Safety regulations. Do we have an SOP for this? Or can we refer to an EHS document?

**Procedure**

1. Prepare lysis buffer in a \_\_\_\_\_\_\_\_\_\_ (what kind of container?)

|  |  |
| --- | --- |
| Reagent | Amount per L final buffer (mL) |
| NaCL (5M) | 30 |
| Triton X-100 | 100 |
| Tris (1M, pH 8) | 50 |
| DI water | 820 |

1. Filter sterilize buffer using 0.2um filter bottle and vacuum line
2. Aliquot 20uL lysis buffer into each well/tube of a 96 well PCR plate or PCR strip tube (dependent upon number of samples)
3. Add 5uL of bacterial isolate culture to wells/tubes, one isolate per well
4. Mix by pipetting
5. Seal with PCR film, or close caps if using PCR strip tubes
6. Load plate/tubes onto PCR thermal block
7. Run the following program on the thermal block
   1. 95°C – 10 minutes
   2. 80°C – 10 minutes
   3. Repeat steps A-B a total of three times
   4. Cool block to 4°C
8. Remove PCR plate/tubes from thermal block when program is complete
9. Gently vortex and spin down plate/tubes
10. Labeling?
11. DNA is now ready for storage in -20°C freezer or further PCR reactions for genetic analysis

**Version History**

**NA.** This is version 1 of this SOP.

**Worksheets**

**NA**

**Appendix**

NA