**SOP# 02.303.01**

**DNA Extraction from clinical feces**

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**Purpose**

This SOP describes the procedure used to extract and purify DNA from human fecal material 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

PBS – phosphate buffered saline solution

**Related Documents**

SOP XXX – Sample collection

SOP# 03.003.01 – Biosafety cabinet operation and maintenance

SOP# 02.354.01 – 16S Illumina library prep

SOP# 02.041.01 – Bleach solution preparation

SOP# 03.102.01 – Hazardous liquid waste disposal

**Required Equipment and Materials / Reagents**

* MoBio PowerSoil DNA Isolation kit (MoBio, Catalog# 12888), 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
* Benchtop scale capable of weighting mgs. For example Mettler Toledo AB54-S
* Microcentrifuge capable of spinning at a speed of greater than or equal to 10,000g, for example Eppendorf 5427R
* Pre-sterilized, filtered pipettor tips, any sterilized, filtered pipettor tips may be substituted
* Pipetteman, for example VWR model# 89079
* ProteinaseK (Qiagen, Cat# 19133), this product is used as provided by vendor. Any ProteinaseK that is >600mAU/mL and sterile may be used
* Wet ice
* Ultra pure grade PBS, for example Amresco K812
* Class II Type A2 Biosafety cabinet (Labconco), any manufactured biosafety cabinet may be used as long as it is Class II or higher
* Disposable spatula sterilized via autoclaving, any autoclavable disposable spatula may be used (example - VWR, small spatulas - cat# 80081-194, large spatulas – cat# 8008-190)
* Hot water bath capable of reaching 65**°**C and 95**°**C , for example Echotherm IC20
* Solutions C1 C2 C3 C4 C5 C6 are provided in 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 human fecal matter must be done within a BL2 area inside of a Class II biosafety cabinet
* All waste material generated from this protocol, must be treated with 10% (volume/volume) bleach solution for a total of 30 minutes of contact time. After bleach treatment it may be disposed of down the laboratory sink.
* All work surfaces must be treated with 20% bleach (volume/volume) for a total of twenty minutes of contact time before and after (SOP# 02.041.01 – Bleach solution preparation). 20% bleach mixture must be no more than 7 days old. For the treatment of solid surfaces Wescodyne, Cidex OPA, or Sporicidin maybe be used as alternative disinfectants.
* Solution C5 of the MoBio PowerSoil kit contains ethanol and is flammable
* Solution C4 of the MoBio Powersoil kit contains Guanidine hydrochloride and cannot be mixed with bleach.
* All waste generated during this procedure must be disgarded as hazardous waste, SOP# 03.102.01 – Hazardous liquid waste disposal

**Procedure**

**The work flow for this procedure is presented in the figure below (Fig 1). Follow the step by step instructions to ensure consistency. Record sample labels and results in lab notebook.**

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1. Clean Biosafety cabinet (SOP# 03.003.01 – Biosafety cabinet operation and maintenance)
2. Remove clinical specimen containers containing fecal samples from -80°C freezer
3. Samples in RNAlater can be thawed at room temperature on the bench top. Samples without RNAlater should be thawed in a 4°C refrigerator for no more than 30 minutes.
4. Label all tubes in sequence and write identifying sample name as well as corresponding tube number in lab notebook.
5. Once thawed, pellet sample by centrifuging at 2934 rcf for 10 minutes
6. Remove supernatant via pipetting without disrupting pellet
7. Add 3mL of PBS to wash the pellet, do not resuspend
8. Pellet sample by centrifuging at 4,000rpm for 10 minutes
9. Remove supernatant via pipetting without disrupting pellet
10. Add 3 mL of to wash the pellet, do not resuspend
11. Pellet sample by centrifuging at 4,000rpm for 10 minutes
12. Remove supernatant via pipetting without disrupting pellet
13. Use disposable spatula to weigh the addition of 250mgs of sample to the MoBio PowerBead tube.
14. Check Solution C1. If Solution C1 is precipitated, heat solution to 60°C using a hot water bath until dissolved before use.
15. Vortex samples for three seconds.
16. Add 60 ul of Solution C1 and invert three times or vortex for one second.
17. Add 20uLs of ProteinaseK solution and invert three times or vortex for one second.
18. Incubate samples at 65°C for 10 minutes using hot water bath, invert the tubes to mix a two times during this incubation
19. Secure tubes horizontally using the MO BIO 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. Incubate samples at 95°C for 10 minutes using hot water bath, invert the tubes to mix a few times during this incubation
2. Centrifuge tubes at 10,000 x *g* for 30 seconds at room temperature. CAUTION: Be sure not to exceed 10,000 x *g* or tubes may break.
3. Transfer the supernatant to a clean 2 ml Collection Tube (provided).

Note: Expect between 400 to 500 ul of supernatant. Supernatant may still contain some particles.

1. Add 250 ul of Solution C2 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. Add 200 ul of Solution C3 and invert to mix. Incubate at 4°C for 5 minutes.
5. Centrifuge the tubes at room temperature for 2 minutes at 10,000 x g.
6. Avoiding the pellet, transfer up to, but no more than, 750 ul of supernatant into a clean 2 ml Collection Tube (provided).
7. Shake to mix Solution C4 before use. Add 1200 ul (2 x 600uL) of Solution C4 to the supernatant and vortex briefly or invert to mix.
8. Load approximately 675 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.
9. Repeat step 29 a total of three times until all sample/C4 liquid has been applied to the
10. Add 500uL of Solution C5 and centrifuge at room temperature for 30 seconds at 10,000 x *g*.

Note – Solution C5 contains ethanol and is flammable

1. Discard the flow-through but do not discard Spin filter.
2. Centrifuge again at room temperature for 1 minute at 10,000 x *g*.
3. Carefully place spin filter in a clean 2 ml Collection Tube (provided in kit). Avoid splashing any Solution C5 onto the Spin Filter.
4. Add 100 ul of Solution C6 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.
5. Let tubes sit at room temperature for 5 minutes
6. Centrifuge at room temperature for 30 seconds at 10,000 x *g*.
7. Discard the Spin Filter. The DNA in the tube is now ready for any downstream application. No further steps are required. We recommend storing DNA frozen at -20°.

**Version History**

This is the first version of this document

**Worksheets**

APerrotta\_labNotebook pages 54, 56, 57

**Appendix**

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