**SOP Full Length 16S PCR Preparation 27F-1492R**

**Document Number: XX**

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**Purpose**

This SOP describes the procedure used for doing Full Length 16S PCR and obtain unpurified PCR product

**Scope**

**Regulatory References:** NA

**Responsibility of experimentalist:** understanding and performing this procedure as described; reporting any deviations or problems to area supervisor; adequately documenting the procedures and results.

**Responsibility of area manager or supervisor:** ensuring that the analyst performing this procedure is qualified; ensuring that the procedure is followed, and updating the procedure as necessary.

**Definitions/Abbreviations**

Polymerase chain reaction: PCR

Deoxynucleotide solution mix : dNTPs

**Related Documents:**

laminar flow hood SOP

**Required Equipment and Materials / Reagents**

* PCR plate (VWR; Cat# 82006-704)
* HF buffer (BioLabs, Cat# B0518S)
* HF Phusion DNA Polymerase (BioLabs, Cat# M0530L)
* dNTPs (Biolabs: Cat# N0447L)
* 16S primer 27 F (3uM) (AGAGTTTGATCMTGGCTCAG)
* 16S primer 1492 R (3uM) (GGTTACCTTGTTACGACTT)
* Ultra Pure Distilled Water (Invitrogen: Cat# 10977-015)
* Sterile Aluminum foil plate cover (VWR, Cat# 60941-076)
* PCR plate cover (VWR, Cat# 82018-846)

**Precautions**

Personal protection equipment including gloves and lab coat must be worn when executing this procedure.

PCR preparation and pipetting should be done in the laminar flow hood

**Procedure**

1. Prepare master mix for reactions

|  |  |  |
| --- | --- | --- |
| Reagent | 1x RXN (uL) | 106x RXN (uL) |
| H2O | 12.25 | 1,298.5 |
| 5x HF Buffer | 5 | 530 |
| 10mM dNTPs | 0.5 | 53 |
| 27F | 2.5 | 265 |
| 1492R | 2.5 | 265 |
| Phusion | 0.25 | 26.5 |

1. Aliquot 23uLs of master mix into a PCR plate or tubes
2. Aliquot 2uLs of extracted DNA (see above, do not mix by pipetting in extraction plate so you don’t disturb the invisible pellet)
3. Mix reaction plate and spin down (you can mix the reaction plate by pipetting while aliquoting the DNA)
4. Cycle plate using the full\_16S protocol on the thermal cycler:

1. 98 C 30 sec  
2. 98 C 10 sec  
3. 52 C 30 sec  
4. 72 C 60 sec  
5. Go to 2 30X  
6. 72 C 5 min

7. 10 C hold

1. When PCR cycling is complete you obtain unpurified PCR product

Note 1: Unpurified PCR product need then to be clean up using SPRI beads

**Version History: NA**

**Worksheets: NA**

**Appendix: NA**