16s rRNA sequences derived from single cell Multiple Displacement Amplification (MDA) of HMP fecal, oral and skin samples
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1 Abstract

2 Introduction

This SOP describes the 16s rRNA gene sequences derived from single bacterial cell multiple displacement amplification (MDA). The bacteria were obtained from HMP approved fecal, oral and skin samples. A microbial cell fraction was purified from ethanol (EtOH) fixed oral and skin samples. Human fecal samples were further purified by Nycodenz gradient density centrifugation. Phase contrast and fluorescent microscopy with SYBR Green I staining confirmed that the fraction included DNA-containing bacteria with a variety of morphologies. Sample processing and flow cytometry was performed under BSL2 conditions to meet biosafety requirements for handling and high speed sorting of unfixed human-derived materials (Schmid et. al., 2007). Single green fluorescent cells were sorted into 384-well microtiter plates by flow cytometry using SYBR Green fluorescence and a forward scatter PMT for detection. Sorted single cells were lysed, DNA amplified by MDA, and 16s rRNA gene sequences were obtained by PCR from an aliquot of the MDA reactions as described previously (Chitsaz et. al., 2011). We are providing forward and reverse 16s rRNA sequences of single cell fecal, oral and skin organisms. The 16s sequences are used to taxonomically classify the MDA reactions.

3 Requirements

4 Procedure

The files provided are multi-fasta files containing forward and reverse 16s rRNA sequences that have been trimmed using CLC Genomics Workbench using the default settings and removed sequences that were < 200 bp. Individual single cell reactions can be identified by the “WGACA” unique identifier with “F” or “R” denoting the forward or reverse sequence. Please note, not all single cell MDA reactions will have both forward and reverse sequences.

Below are examples of fasta headers and how to track them back to the single cell MDA reaction using our unique "WGACA" identifier.

The forward and reverse reads can be identified from the ‘WGACA01T1[F|R]#####’. An example is shown below.

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Forward read:
>1042139-140-141-142_A04_TIGR_WGACA01T1F15058_1042140_1122243671925_016_1122232824624
Reverse read:
> 1042143-144-145-146_A04_TIGR_WGACA01T1R15058_1042144_1122244669009_016_1122232827120

Another format is that the forward and reverse reads can be identified from the 'WGACA#####_[F|R]'. An example is shown below.
Forward read:
>B906294_G07_JTC_WGACA51778_F_Exp82_1064147643749_1064147643789_026_1127963858876
Reverse read:>B906293_G07_JTC_WGACA51778_R_Exp82_1064147644137_1064147644177_026_1127963860718

5 Implementation

6 Discussion

7 Related Documents & References

Schmid I, Lambert C, Ambrozak D, Marti GE, Moss DM, Perfetto SP; International Society of Analytical Cytology. International Society for Analytical Cytology biosafety standard for sorting of unfixed cells. Cytometry A. 2007 Jun;71(6):414-37.

Chitsaz H, Yee-Greenbaum JL, Tesler G, Lombardo MJ, Dupont CL, Badger JH, Novotny M, Rusch DB, Fraser LJ, Gormley NA, Schulz-Trieglaff O, Smith GP, Evers DJ, Pevzner PA, Lasken RS. Efficient de novo assembly of single-cell bacterial genomes from short-read data sets. Nat Biotechnol. 2011 Sep 18;29(10):915-21.

8 Revision History

This is an HMP_specific requirement, not included in the SIGS submission. Please be sure to update this when any changes as made, to help the DACC organize SOPs.

Version	Author/Reviewer	Date	Change Made

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1.00	Joyclyn Yee-Greenbaum	11/28/2011	Establish SOP