Purification of Microbiome DNA from Oral Swabs in OMNIgene®•ORAL Collection Devices

Purify microbiome DNA from oral swabs collected in OMNIgene®•ORAL collection devices using bead beating and the Maxwell® RSC Fecal Microbiome DNA Kit on the Maxwell® RSC Instrument.

Kit: Maxwell® RSC Fecal Microbiome DNA Kit (Cat.# AS1700)

Analyses: Next-generation sequencing (NGS)

Sample Type(s): Oral swabs collected in OMNIgene®•ORAL collection devices

Input: 600µl

> This protocol was developed by Promega Applications Scientists and is intended for research use only.
>
> Users are responsible for determining suitability of the protocol for their application.
>
> For further information, see Technical Manual TM640, available at:
> **www.promega.com/protocols**
>
> or contact Technical Services at:
> **techserv@promega.com**

Materials Required:

- OMNIgene®•ORAL (DNA Genotek™, Cat.# OMR-110)
- CaseWork Spin Baskets (Cat.# AS8101)
- CaseWork Microcentrifuge Tubes (Cat.# AS8201)
- Vortex
- Heat Blocks set to 95°C and 56°C
- ZR BashingBead™ Lysis Tubes (0.1 & 0.5mm) (Zymo Research, Cat.# S6012-50)
- Horizontal Vortex Adapter for 1.5/2.0ml Tubes (e.g. QIAGEN, Cat.#13000-V1-24) or similar
- Maxwell® RSC Instrument (Cat.# AS4500)
- Maxwell® RSC Fecal Microbiome DNA Kit (Cat.# AS1700)

Protocol:

1. Collect oral swabs in a DNA Genotek™ OMNIgene®•ORAL collection device, as instructed by the manufacturer.

2. Add 300µl of Lysis Buffer and 40µl Proteinase K to the swab and stabilization liquid. Invert tube 5-10 times to mix.

3. Transfer swab into a Casework Spin Basket in a Casework Microcentrifuge Tube and centrifuge at maximum speed for 2 minutes to remove all liquid from the swab.

4. Transfer all of the stabilization liquid (Casework Microcentrifuge Tube flow-through and remaining liquid in the collection device) to a ZR BashingBead™ Lysis Tube (0.1 & 0.5mm).

5. Place the bead beating tube in the horitzontal vortex adapter and vortex tubes at maximum speed (~3,000rpm) for 30 minutes.

6. Continue with Step 3 in Section 4.B. of the Maxwell® RSC Fecal Microbiome DNA Kit Technical Manual (TM640). For Step 9, transfer 600µl of lysate into well #1 of the reagent cartridge.

Results:

DNA was purified from oral swabs collected in OMNIgene®•ORAL collection devices as described above. Purified DNA was used in 16S V3/V4 metagenomic sequencing, and the resulting microbial profiles are shown in Figure 1.
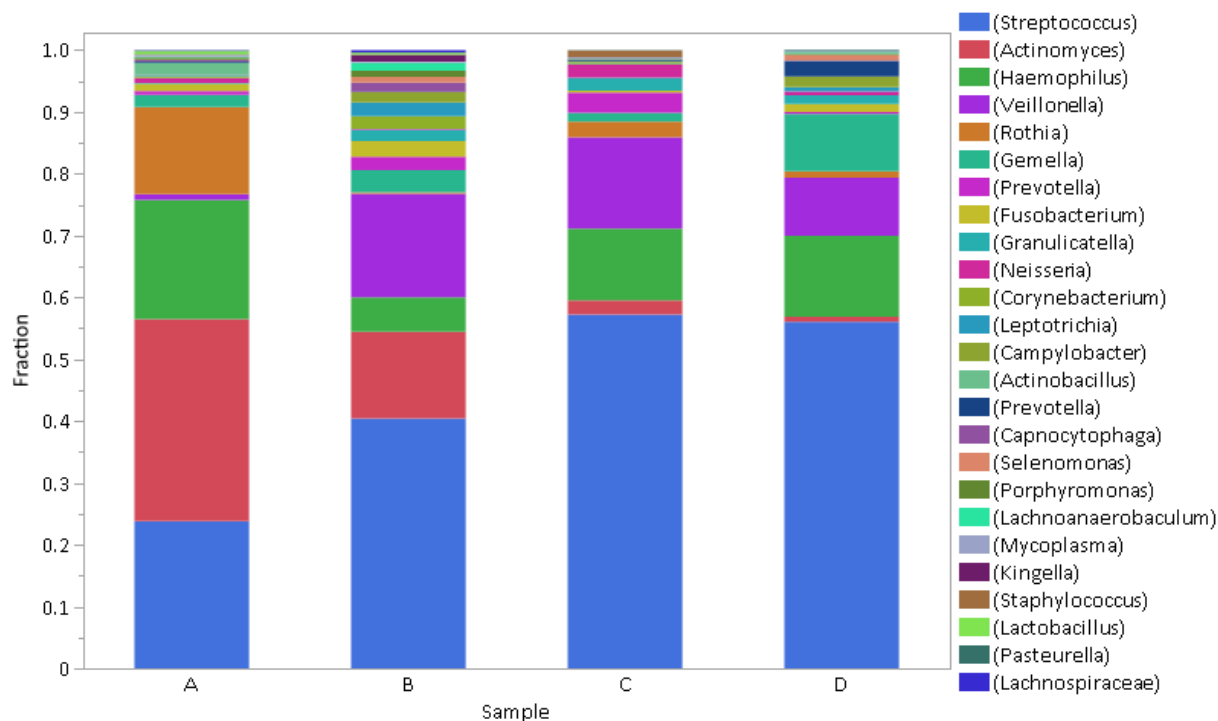

Figure 1. Oral microbiome taxonomic distributions from 16S V3/V4 metagenomic sequencing of DNA from oral swabs collected in OMNIgene®•ORAL collection devices and purified using the Maxwell® RSC Fecal Microbiome DNA Kit with bead-beating. DNA was purified according to the methods above. Microbial DNA was sequenced over the V3 and V4 variable regions of the 16S gene following the Illumina 16S Metagenomic Sequencing Library Preparation Guide[1] with the following differences: DNA input for amplicon PCR was reduced to 2.5ng with 2 additional PCR cycles; GoTaq® Long PCR Master Mix (Cat.# M4021) was used for all amplification steps; and the ProNex® Size-Selective Purification System (Cat.# NG2001) was used for all purification steps. Libraries were normalized and pooled based on quantification with the ProNex® Library Quant Kit (Cat.# NG1201) and were sequenced on an Illumina MiSeq Instrument with a v3 600-cycle reagent kit. Sequencing data was analyzed at the genus level using a pipeline based on the *mothur* open source software package (v1.43.0)[2]. Percent abundance of the top 25 OTUs are shown.

References:

1. Illumina. 16S Metagenomic Sequencing Library Preparation – Preparing 16S Ribosomal RNA Gene Amplicons for the Illumina MiSeq System. https://support.illumina.com/content/dam/illuminasupport/documents/documentation/chemistry_documentation/16s/16s-metagenomic-libraryprep-guide-15044223-b.pdf. Accessed 04/2021.
2. Schloss P.D., Westcott S.L., Ryabin T., Hall J.R., Hartmann M., Hollister E.B., Lesniewski R.A., Oakley B.B., Parks D.H., Robinson C.J., Sahl J.W., Stres B., Thallinger G.G., Van Horn D.J., Weber C.F. (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol. 75: 7537-41.