Promega

Product Application

Purification of Microbiome DNA from Skin Swabs in OMNIgene®•SKIN Collection Devices

Purify microbiome DNA from skin swabs collected in OMNIgene®•SKIN 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: Skin swabs collected in OMNIgene®•SKIN collection devices

Input: 900µl

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®•SKIN (DNA Genotek™, Cat.# OMR-140)
- 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 skin swabs in a DNA Genotek™ OMNIgene®•SKIN collection device, as instructed by the manufacturer.
2. Add 300µl of Lysis Buffer and 40µl Proteinase K to the tube containing the collected 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, ~ 1300µl) to a ZR BashingBead™ Lysis Tube (0.1 & 0.5mm).
5. Place the bead beating tube in the horizontal 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 900µl of the liquid into well #1 of the reagent cartridge.

Results:

Skin samples were collected from three individuals from three physiological locations (dry, sebaceous, wet) on the human body using OMNIgene®•SKIN collection devices. DNA was purified as described in the methods above. Purified DNA was used in 16S V3/V4 metagenomic sequencing, and the resulting microbial profiles are shown in Figure 1.

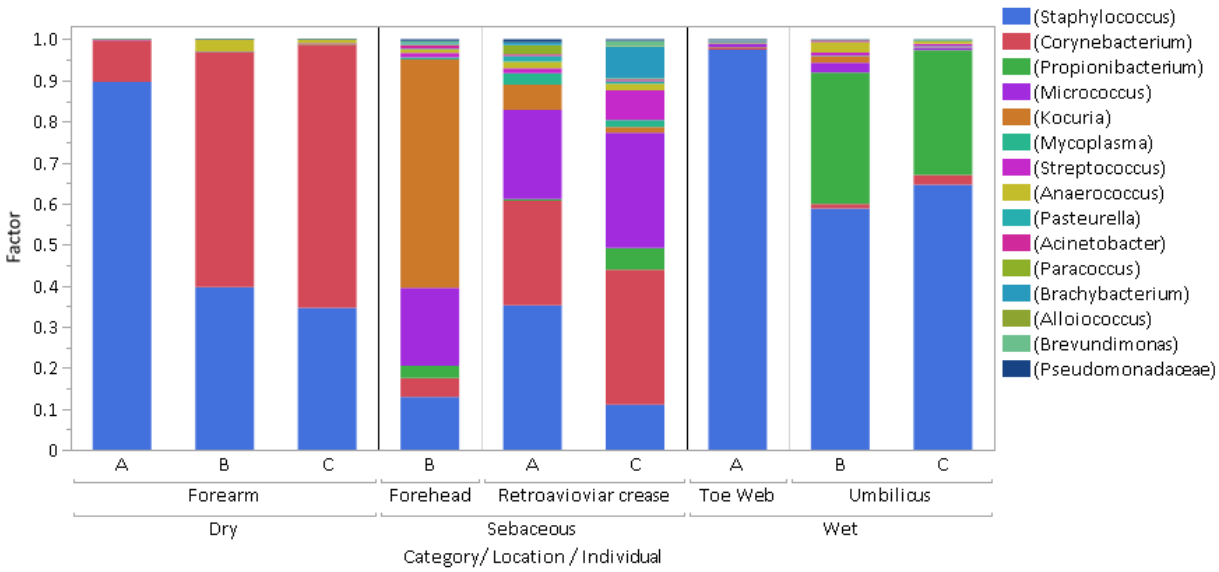

Figure 1. Skin microbiome taxonomic distributions from 16S V3/V4 metagenomic sequencing of DNA from skin swabs collected in OMNIgene®•SKIN collection devices and purified using the Maxwell® RSC Fecal Microbiome DNA Kit with bead-beating. DNA was purified according to the methods above. Microbial DNA 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 increased to 20µl of each eluate in a 50µl PCR reaction with 2 additional PCR cycles; 2µl or 8µl of purified amplicon was input into Index PCR based on amplicon PCR yield; 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 15 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.