Promega

Product Application

Automated Purification of DNA from the Oral Microbiome Using Mouthwash Samples

Purify DNA from the human oral microbiome using the Maxwell® RSC Fecal Microbiome DNA Kit on the Maxwell® RSC Instrument.

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

Analyses: 16S and 18S rRNA gene sequencing

Sample Type(s): Fresh or frozen mouthwash collected using Scope®, Listerine®, and water

Input: 2ml

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:

- Maxwell® RSC Fecal Microbiome DNA Kit (Cat.# AS1700)
- Maxwell® RSC Instrument (Cat.# AS4500)
- PBS
- Heat block set to 95°C and 56°C
- Vortex Genie 2, Scientific Industries
- Vortex horizontal multitube adapter, MoBio (Cat.# 13000-V1)
- ZR BashingBead Lysis Tubes, Zymo Research (Cat.# S6012-50)
- Microcentrifuge

Protocol:

1. Centrifuge 2ml mouthwash sample at 10,000 x g for 5 minutes.
2. Remove supernatant.
3. Add 1ml of 1X PBS and vortex to resuspend the pellet.
4. Transfer 1ml of sample into a 1.5ml tube.
5. Centrifuge at 2,000 x *g* for 2 minutes.
6. Remove supernatant.
7. Add 1ml of Lysis Buffer (LB) and 40µl of Proteinase K Solution (PK) to the pellet in the tube and resuspend.
8. Transfer the sample to a ZR BashingBead Lysis Tube. Place bead beating tube containing sample, LB and PK on the vortex adapter. Bead beat sample at ~3,000rpm for 30 minutes.
9. Incubate sample at 95°C for 5 minutes.
10. Cool sample for 2 minutes on benchtop.
11. Vortex sample for 1 minute.
12. Incubate sample at 56°C for 5 minutes.
13. Pellet the solids/bead beating matrix by centrifuging at maximum speed for 5 minutes.
14. Transfer 300µl of supernatant into well #1 of the Maxwell cartridge and add 300µl of Binding Buffer. Add 20µl of RNase A Solution to well #3 of each cartridge. Elute samples in 100µl of Elution Buffer. Process samples with the Fecal Microbiome protocol on the Maxwell® RSC Instrument.

Results:

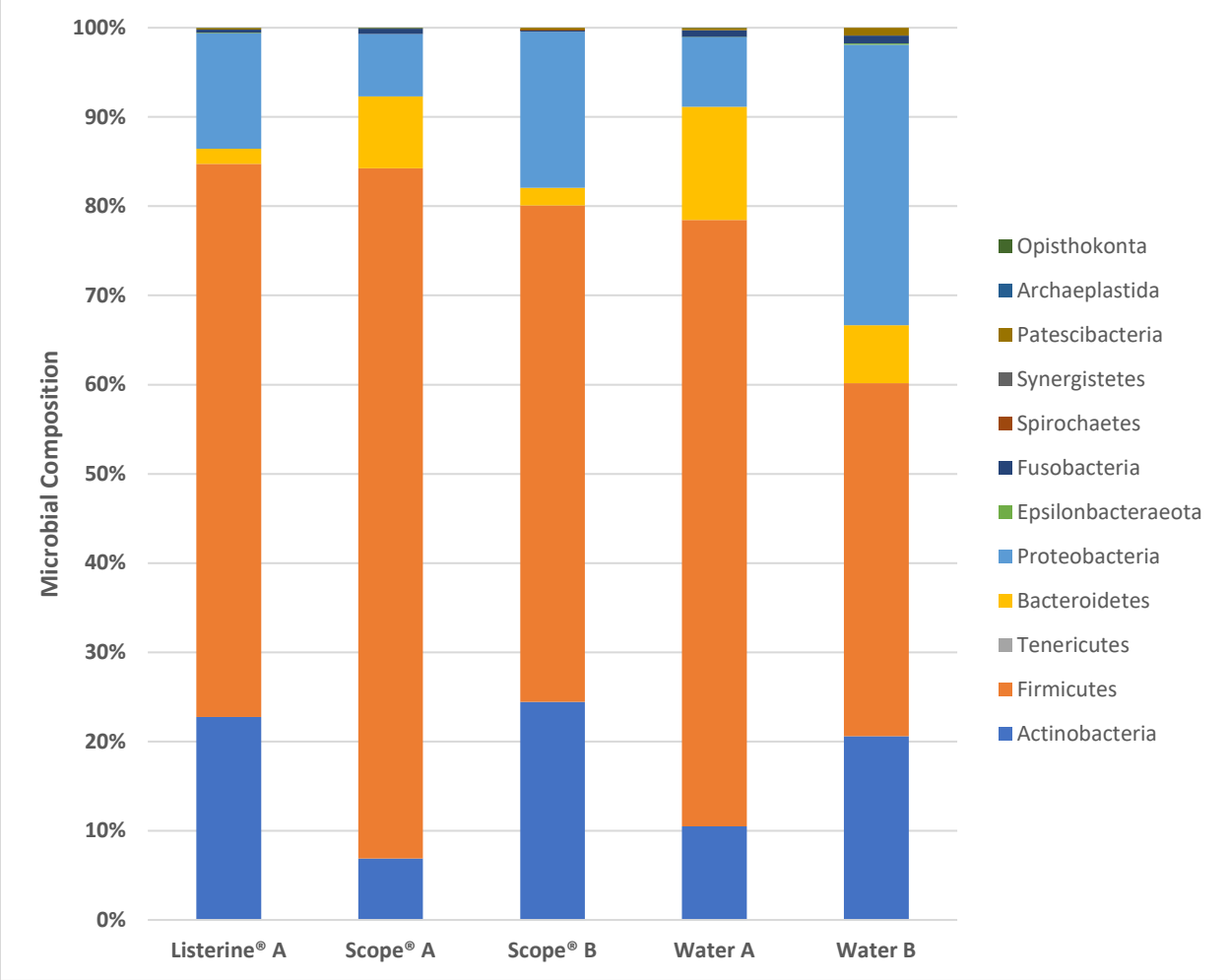
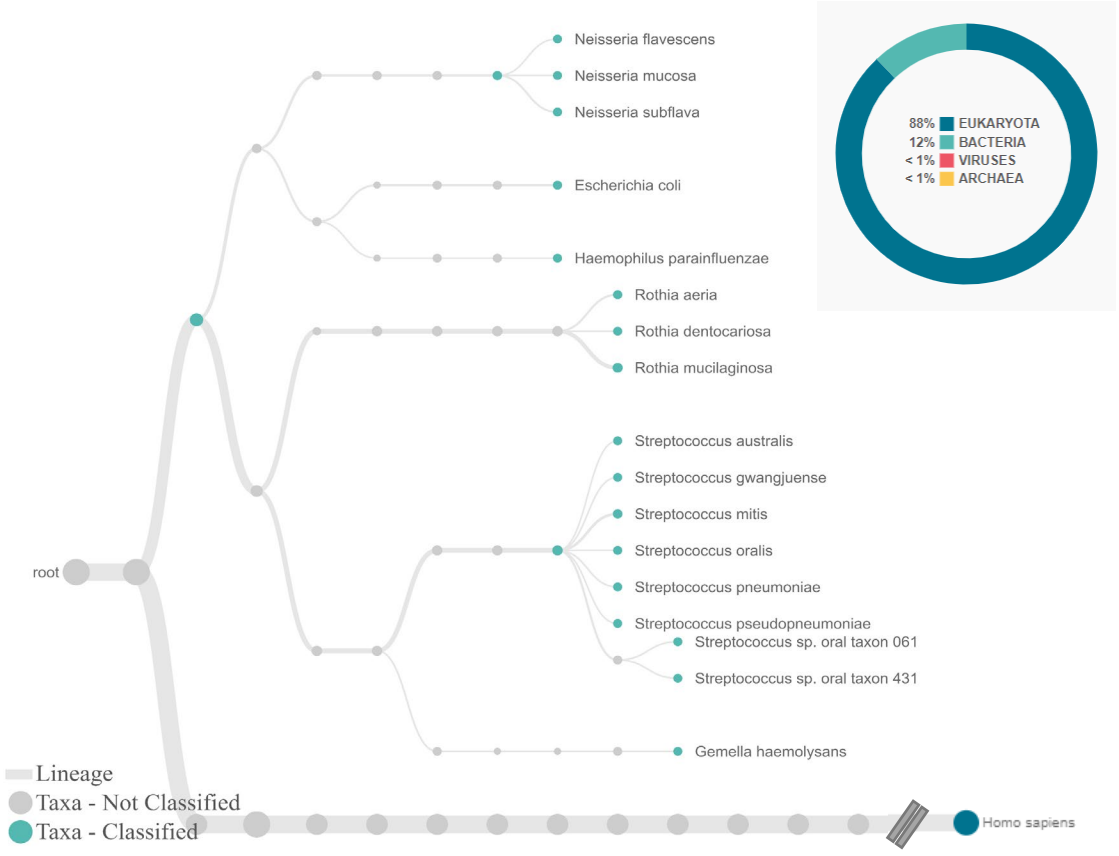


Figure 1. Bacterial classifications identified by phylum from various mouthwash samples from Individuals A and B. Water, Scope® and Listerine® were used to collect mouthwash samples from two individuals. Individuals rinsed their mouths with 2 x 5ml of each type of mouthwash for 30 seconds in 50ml conical tubes. The samples from the same mouthwash product per individual were pooled and stored at -80°C after collection. Pooled mouthwash was thawed, vortexed, and aliquoted into 2ml samples before pre-processing as described. DNA was purified using the Maxwell® RSC Fecal Microbiome DNA Kit (Cat.# AS1700) on the Maxwell® RSC Instrument (Cat.# AS4500). 16S and 18S rRNA gene sequencing was performed using the LoopSeq™ 16S & 18S Long Read Kit (Loop Genomics, Cat.# LG004LR18C) with MiSeq Reagent Kit V3 600 Cycles chemistry (Illumina, Cat.# MS-102-3003) on the Illumina MiSeq® Instrument. Genera identified that were expected to be present in the oral samples, include gram (+) bacteria such as *Streptococcus* (Firmicutes), *Rothia* (Actinobacteria), *Gemella* (Firmicutes), *Granulicatella* (Firmicutes), as well as gram (-) bacteria such as *Neisseria* (Proteobacteria), *Prevotella* (Bacteroidetes), *Haemophilus* (Proteobacteria), *Veillonella* (Firmicutes), and *Porphyromonas* (Bacteroidetes).

Sample ID	**Scope® B**
Library Prep Kit	**SQK-LSK109**
Flow Cell Type (# Pores)	**Flongle (92)**
BaseCaller Model	**High Accuracy (Guppy v4.3.4)**
EPI2ME Analysis	**Fastq WIMP r2021.03.05**
Reads Analyzed	**685,402**
Total Yield	**676.1Mb**
Avg Quality Score	**11.72**
Classified	**632,915**
Unclassified	**52,487**

Figure 2. Oxford Nanopore Genomic DNA by Ligation Sequencing results from Scope® mouthwash sample from Individual B. 500ng of purified mouthwash microbiome DNA was sequenced according to manufacturer's protocol using the Ligation Sequencing Kit (SQK-LSK109)[1,2] using the Oxford Nanopore MinION with a Flongle adapter. Fast5 sequences were base called using Guppy and analyzed using EPI2ME v3.3.0 FASTQ WIMP workflow for taxonomic classification. Left Table: Sequencing run details. Below: Species level subtree representing the NCBI taxonomy associated to the most common assignments (minimum 0.1% abundance cutoff).



References:

1. Oxford Nanopore Genomic DNA by Ligation (SQK-LSK109) Protocol. Version GDE_9063_v109_revY_14Aug2019 Last update 21/04/2021.

2. ProNex® Chemistry-Based Clean-up in the Oxford Nanopore Ligation Sequencing Kit. PA411. 10/19.