Promega

Product Application

Automated Purification of Urine DNA using the Maxwell® Fecal Microbiome Kit

Purify DNA suitable for next-generation sequencing (NGS) of microbial populations from human urine 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: NGS sequencing of the V3/V4 region

Sample Type(s): Clean-catch urine

Input: 1ml to 10ml

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) or Maxwell® RSC 48 Instrument (Cat.# AS8500)
- Bead lysing tubes (e.g. ZR Bashing Bead Lysing Tubes, 0.1 & 0.5mm, Zymo Research Cat.# S6012-50, or similar)
- Vortex Genie 2 Digital (Scientific Industries, Cat.# SI-A236, or similar)
- Horizontal Vortex Adapter for 1.5/2.0ml Tubes (Qiagen, Cat.# 13000-V1-24, or similar)
- Heat block suitable for 2.0ml microcentrifuge tubes

Protocol:

1. Mix urine sample well before pelleting cells from 1ml to 10ml aliquots via centrifugation at 4°C at maximum speed.
2. Carefully decant the supernatant. A small amount of urine can be left behind to avoid disturbing the pellet.
3. Resuspend cell pellet(s) in 400µl Lysis Buffer and 40µl Proteinase K.
4. Transfer cell pellet suspension to bead lysing tubes, place on a horizontal vortex adapter, and vortex at maximum speed (3000rpm) for 30 minutes.
5. Continue with Step 3 in Section 4.B. of the Maxwell® RSC Fecal Microbiome Kit Technical Manual (TM640).

Results:

DNA was successfully purified from 1ml and 10ml volumes of urine using the Maxwell® RSC Fecal Microbiome Kit with bead beating. DNA was used for 16S V3/V4 metagenomic sequencing and the taxonomic distributions of urine samples from four individuals are shown at the genus level (Figure 1). Because the DNA yield from most urine microbiome samples was very low (<320fg/µl), a PCR enrichment step was included during the library preparations. PCR replicates were included to check for skew introduced during the enrichment step, and no skew was identified (Figure 1). The genera detected in all urine sample (except for *Pasteurella)* are expected to be present in the healthy urine microbiome[1]. Plotting the Bray-Curtis dissimilarity of each purification replicate indicated that between sample volumes, the taxonomic distribution was highly reproducible in microbial purification and library preparation using this method (Figure 2). Bray-Curtis dissimilarity was greater between 1ml and 10ml sample volumes for some individuals, which may be explained by background present in the 1ml samples (Figures 1 and 2, Female #2 and Male #1), though the 1ml and 10ml samples from each individual still clustered together (Figure 2).

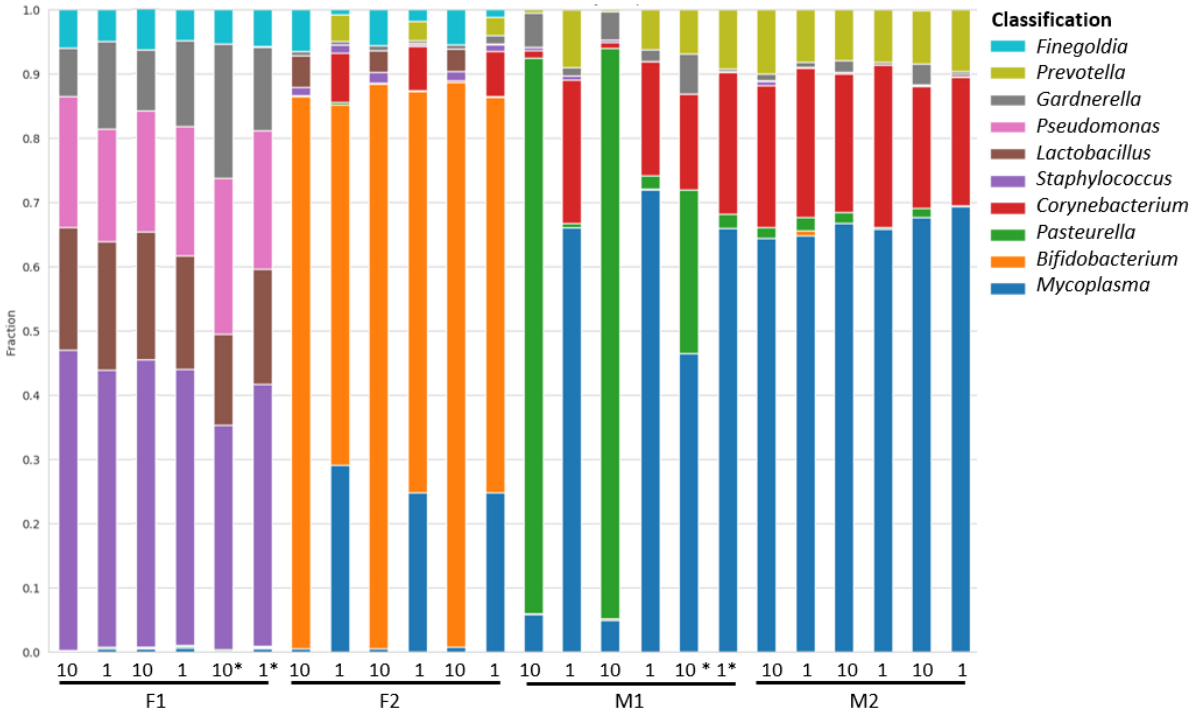

Figure 1. Taxonomic distributions of the urine microbiome of four individuals comparing 1ml to 10ml samples. DNA was purified from 1ml or 10ml urine samples from two males (M1 and M2) and two females (F1 and F2) in technical triplicate with the Maxwell® Fecal Microbiome kit with bead beating. Microbial DNA was sequenced over the V3 and V4 variable regions of the 16S rRNA gene following the Illumina 16S Metagenomic Sequencing Library Preparation Guide[2] with the following differences: for all eluates with DNA concentrations <320fg/µl, 20µl of DNA input was used for Amplicon PCR with 3 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. PCR replicates (*) were performed during Amplicon PCR to assess for skew due to the amount of template input and additional cycles. Libraries were normalized and pooled based on quantification with the ProNex® NGS 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)[3] and visualized with MultiQC[4] v1.11. Percent abundance of all OTUs ≥0.1% are shown.

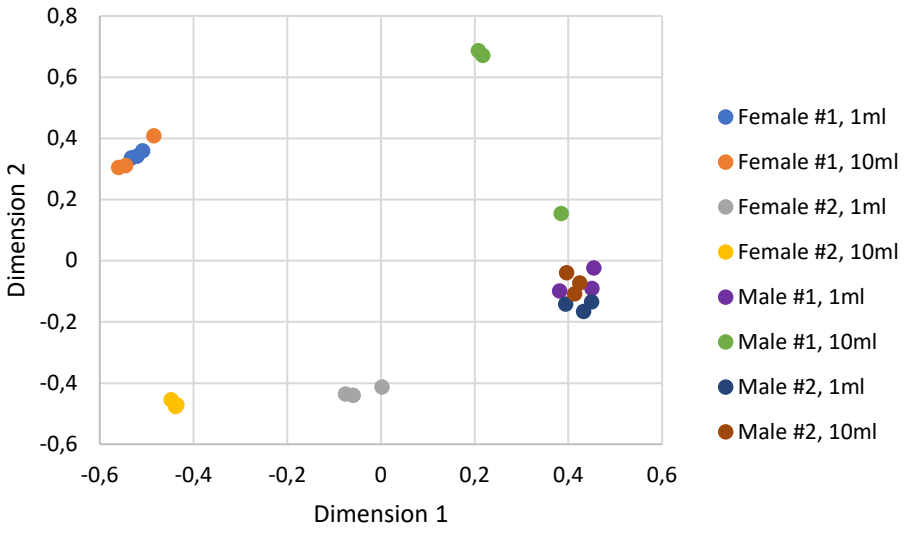

Figure 2. Two-dimensional Non-Metric Multidimensional Scaling (NMDS) plots on Bray Curtis Dissimilarity of 1ml and 10ml urine microbiome samples. Bray Curtis Dissimilarity for taxonomic distribution between purification replicates of female and male 1ml and 10ml urine microbiome samples is plotted here. Each point is a replicate. Sample points that are closer together are more similar than those farther apart.

References:

1. Perez-Carrasco, V., Soriano-Lerma, A., Soriano, M., Gutiérrez-Fernández, J., & Garcia-Salcedo, J. A. (2021). Urinary Microbiome: Yin and Yang of the Urinary Tract. Frontiers in Cellular and Infection Microbiology, 11, 421.
2. 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.
3. 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.
4. Ewels, P., Magnusson, M., Lundin, S., & Käller, M. (2016). MultiQC: summarize analysis results for multiple tools and samples in a single report. *Bioinformatics*, *32*(19), 3047-3048.