Promega

Product Application

DNA Purification from OMNIgene®•VAGINAL microbiome collection devices

Purify bacterial DNA from OMNIgene®•VAGINAL microbiome collection devices using the Maxwell® RSC PureFood GMO and Authentication Kit.

Kit: Maxwell® RSC PureFood GMO and Authentication Kit (Cat.# AS1600)

Analyses: NanoDrop™, QuantiFluor® ONE dsDNA, qPCR, NGS

Sample Type(s): OMNIgene®•VAGINAL microbiome collection devices (Cat. #OMR-130 from DNA Genotek[1])

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 TM473, available at: **www.promega.com/protocols**

or contact Technical Services at: **techserv@promega.com**

Materials Required:

- Heat block
- Centrifuge
- Casework Spin Basket (Cat. #AS8101)
- Casework Microfuge Tube (Cat. #AS8201)
- Bead-beating tube (ex: MP Biomedicals Lysing Matrix SS)
- Bead-beater (ex: MP Biomedicals FastPrep-24™ 5G Instrument)
- Maxwell® RSC Instrument (Cat.# AS4500)

Protocol:

1. Add 40µl of Proteinase K and 20µl of RNase to swab and stabilization liquid. Invert tube 5-10 times to mix.
2. Incubate lysate at 50°C for 1 hour.
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 to a 2ml Matrix SS tube (~1mL).
5. Bead-beat (Ex: 60 seconds using a QuickPrep Adapter on MP FastPrep-24™ 5G).
6. Add 600µl of lysate to the Maxwell® PureFood GMO and Authentication Kit cartridge and follow TM473 *Section 5 Purifying DNA on the Maxwell® Instruments*.
    a. Add 300µl of Lysis Buffer to well #1 of each cartridge.
    b. Place a Maxwell® RSC Plunger into well #8 of each cartridge.
    c. Add 100µl of Elution Buffer to each Elution Tube.

Results:

Samples were collected from six individuals with an OMNIgene®•VAGINAL microbiome collection device. DNA was purified from 600µl of pre-processed lysate with the Maxwell® RSC PureFood GMO and Authentication Kit as described above. NanoDrop™ purity ratios ≥ 1.7 suggest DNA eluates are high quality DNA (Figure 1) and 5 out of 6 samples yielded ≥ $1x10^8$ 16S rRNA gene copies/µl (Figure 2). Little to no PCR inhibition was observed with 16S rRNA gene qPCR amplifications (data not shown).

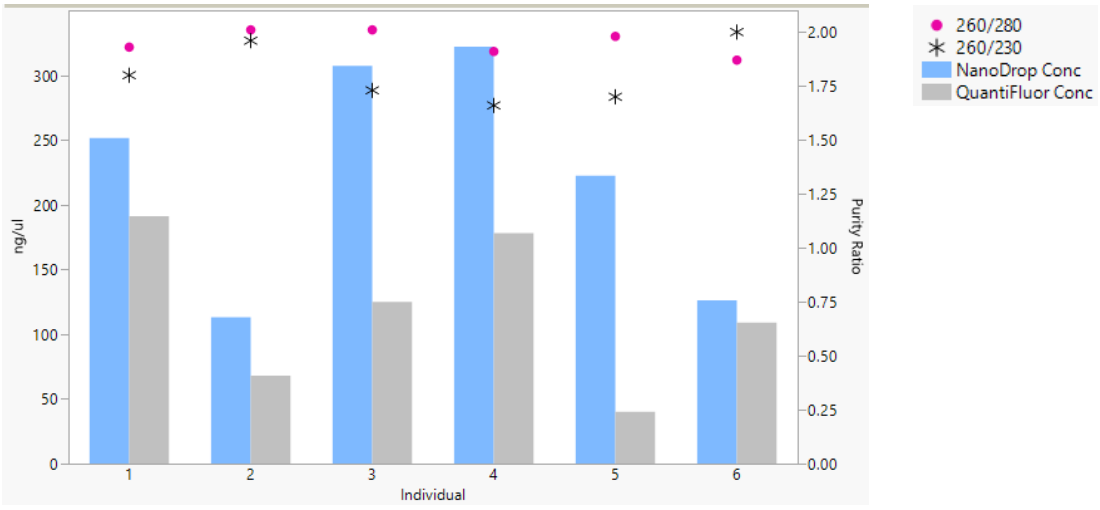

Figure 1. NanoDrop™ and QuantiFluor® ONE dsDNA concentrations with NanoDrop™ purity ratios. The left Y-axis is DNA concentration in ng/µl and the right Y-axis is the NanoDrop™ purity ratio.

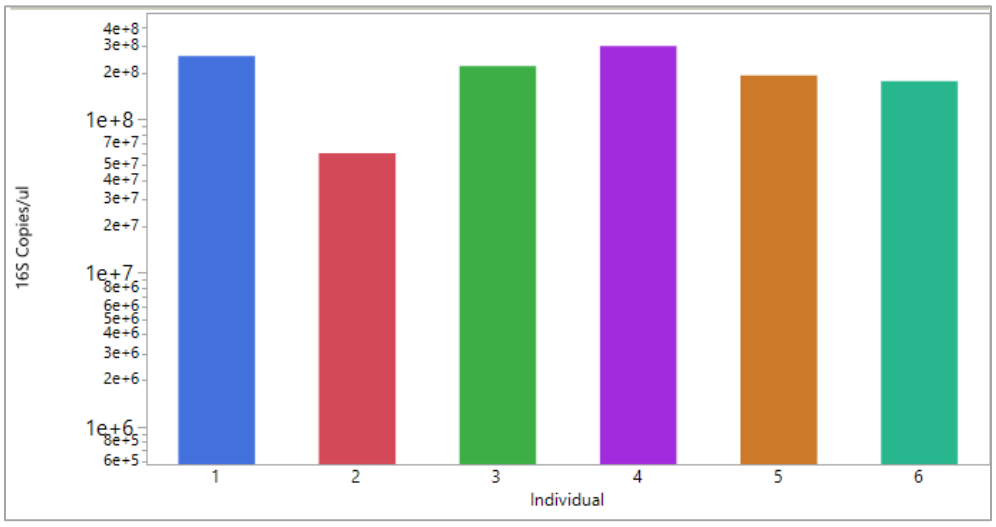

Figure 2. Average 16S rRNA gene copies/µl of undiluted eluates. N=2 amplification replicates using 2µl of undiluted eluate. Y-axis is log scale.

Eluates were analyzed with Loop Genomics LoopSeq™ 16S Long Read Kit to sequence full-length 16S rRNA genes. The library preparation was performed as indicated by the manufacturer, except for three modifications (1) sample concentration was measured by the QuantiFluor® ONE dsDNA System, (2) all clean-up steps in the workflow were performed with the ProNex® Size-Selective Purification System, and (3) library concentration was measured using the ProNex® NGS Library Quant Kit. Libraries were sequenced with 2x300 reads on an Illumina MiSeq instrument. Data was analyzed with the Loop Genomics bioinformatics workflow.

The organisms detected were grouped by taxonomy which were then used to calculate the relative abundance of bacterial taxa in the population. As expected with vaginal samples, the population is dominated by the *Lactobacillus* genus (≥ 96%); data not shown. Figure 3 shows the relative abundance of genera other than *Lactobacillus*. With *Lactobacillus* excluded, donor specific differences are more apparent in each sample.

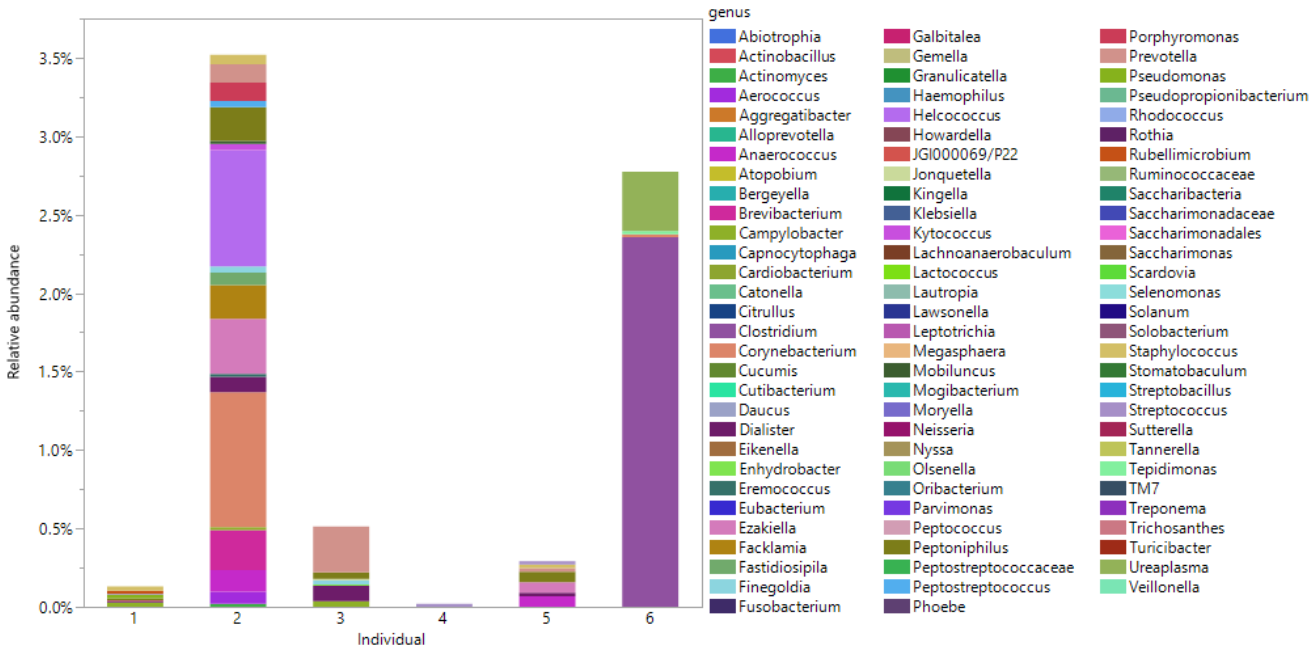

Figure 3. Genus level comparison of bacterial profiles purified from vaginal swabs of 6 donors as determined by LoopSeq™ 16S Long Read Kit with the genus *Lactobacillus* excluded.

[1] OMNIgene®•VAGINAL is For Research Use Only, not for use in diagnostic procedures.
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