Promega

Product Application

DNA Purification from OMNIgene®•VAGINAL microbiome collection devices

Purify bacterial DNA from OMNIgene®•VAGINAL microbiome collection devices using the ReliaPrep™ Blood gDNA MiniPrep System.

Kit: ReliaPrep™ Blood gDNA MiniPrep System (Cat. #A5081)

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

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

Input: 300µl

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 TM330, 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)

Protocol:

1. Add 40µl of Proteinase K and 20µl of RNase (optional) to swab and stabilization liquid. Invert tube 5-10 times to mix.
2. Incubate 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 MP FastPrep-24™ 5G).
6. Add 300µl of lysate to a clean 1.5mL tube.
7. Add 600µl of 100% Isopropanol and gently invert to mix.
8. Add 300µl of Cell Lysis Buffer to lysate. Gently mix by inversion.
9. Load 600µl of sample into a ReliaPrep™ Binding Column placed in a collection tube, spin for 1 minute at maximum speed. Discard flow through.
10. Load the rest of the sample on to the ReliaPrep™ Binding Column, and spin for 1 minute at maximum speed. Place Binding Column into a new collection tube.
11. Add 500µl of Column Wash Buffer (CWD).
12. Spin 2 minutes at maximum speed and then discard flow through.
13. Repeat steps 11 and 12 twice, for a total of three washes.
14. Place Binding Column in a labeled elution tube. Add 100µl of pre-warmed (50°C) Elution Buffer to Binding Column. Spin 1 minute at maximum speed.

Results:

Samples were collected from six individuals with an OMNIgene®•VAGINAL microbiome collection device. DNA was purified from 300µl of pre-processed lysate with the ReliaPrep™ Blood gDNA MiniPrep System as described above. NanoDrop™ A260/A280 purity ratios were ≥ 1.7 while A260/A230 ratios were near 1.0 (Figure 1). Samples yielded an average of $9.4x10^7$ 16S rRNA gene copies/µl (Figure 2). Little to no PCR inhibition was observed with 16S rRNA gene qPCR amplifications (data not shown).

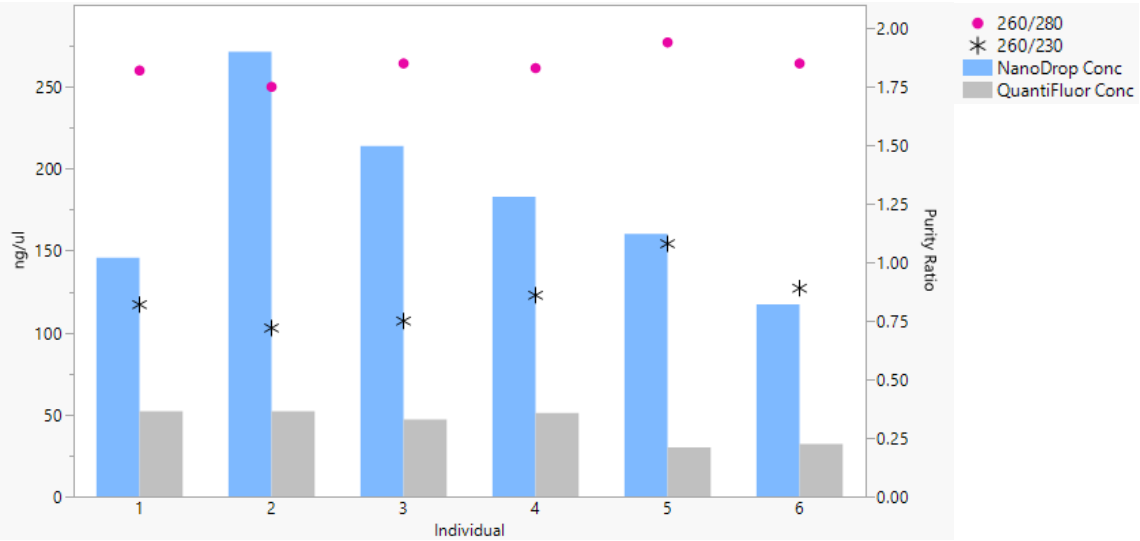

Figure 1. NanoDrop™ and QuantiFluor® ONE dsDNA concentrations with NanoDrop™ purity ratios. The left Y-axis is DNA concentration in ng/µl and the right Y-axis is the NanoDrop™ purity ratio.

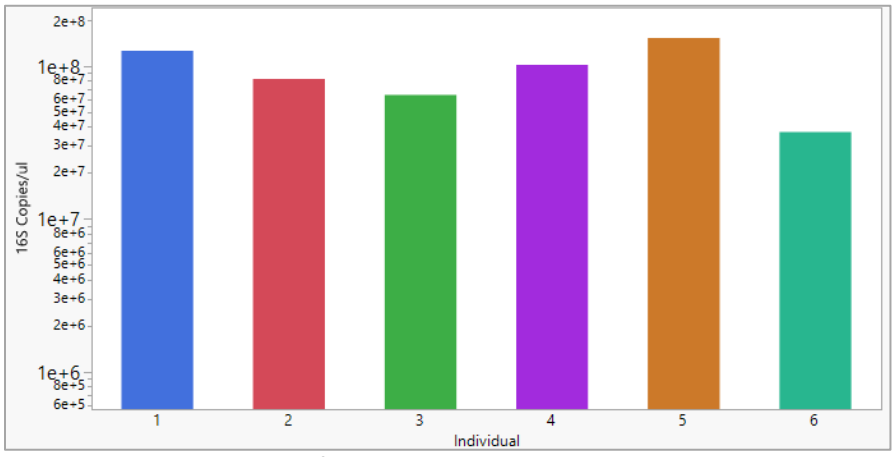

Figure 2. Average 16S rRNA gene copies/µl of undiluted eluates. N=2 amplification replicates using 2µl of undiluted eluate. Y-axis is log scale.

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 (≥ 93%); data not shown. Figure 3 shows the relative abundance of genera other than *Lactobacillus*. With *Lactobacillus* excluded, donor specific difference are more apparent in each sample.

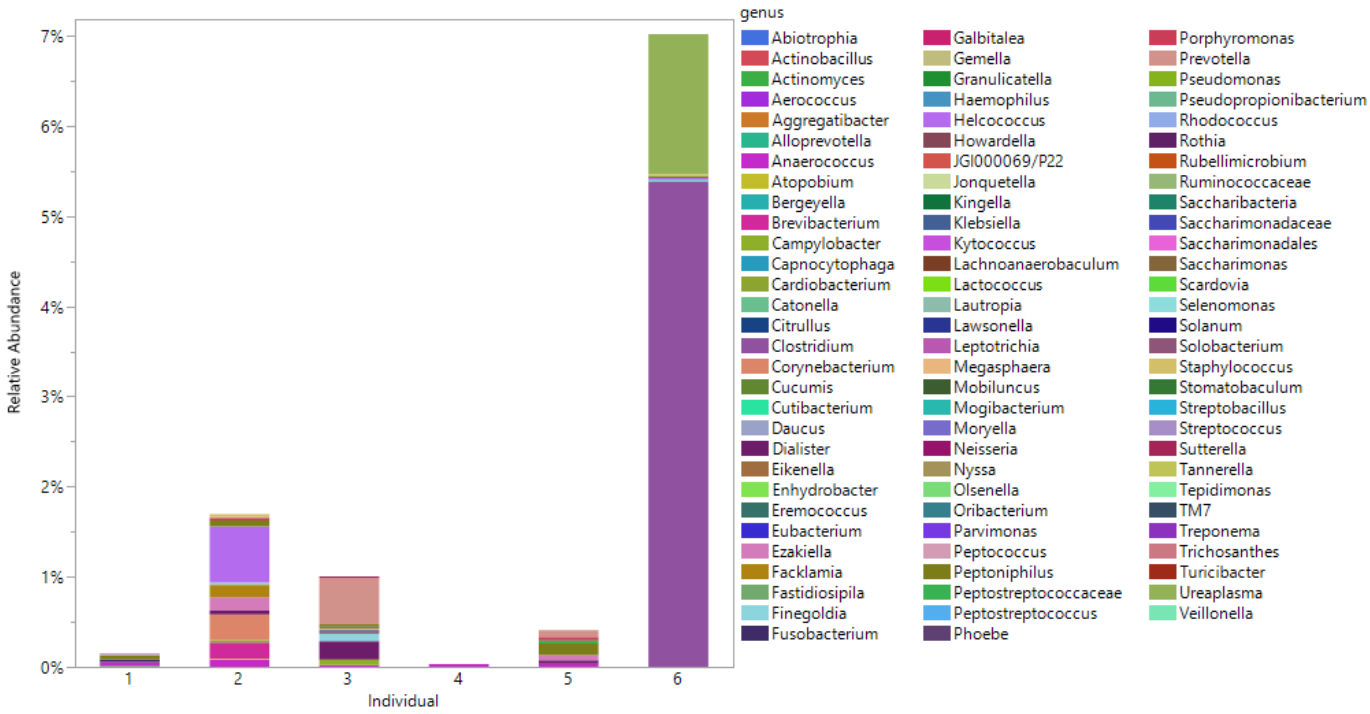

Figure 3. Genus level comparison of the bacterial profiles purified from vaginal swabs of 6 donors as determined by LoopSeq™ 16S Long Read Kit with the genus *Lactobacillus* excluded.

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