Protocol

Extraction

1. Thaw the standard on ice.

2. Vortex the tube for at least 30 seconds[1]. Pulse centrifuge briefly.

3. Prior to DNA extraction, add the ZymoBIOMICS® Spike-in Control I to your sample of interest at a final concentration of 0.1-10%[2], then mix thoroughly by vortexing. When working with feces, we recommend spiking-in 20 µl of the product into 10 mg of feces. Refer to Appendix A about how to prepare 10 mg feces aliquots with DNA/RNA Shield™.

Calculations

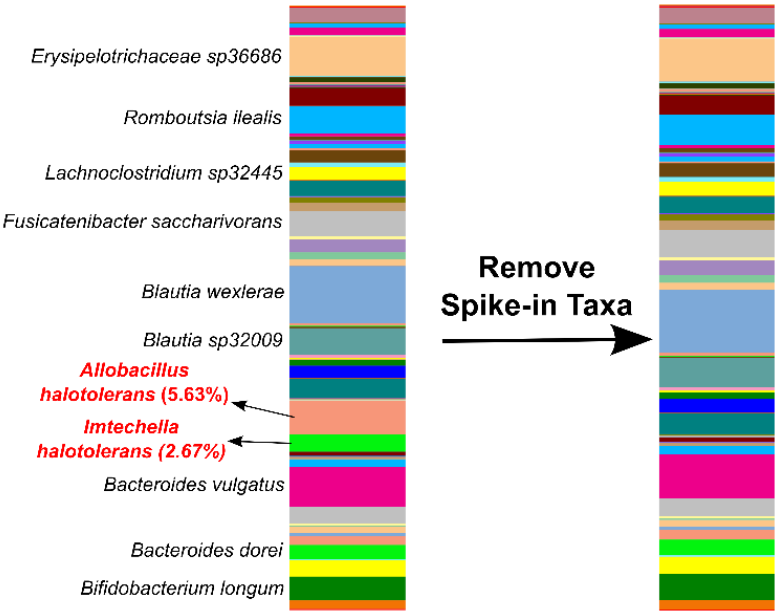

Figure 1. Quantify the total cell number in 10 mg of feces using the ZymoBIOMICS® Spike-in Control I. 50 µl of ZymoBIOMICS® Spike-in Control I was spiked into 100 µl of a fecal suspension, 10% (w/v) feces suspended in the solution of DNA/RNA Shield™. DNA extraction was performed with the ZymoBIOMICS® DNA Miniprep Kit using 5 minutes of bead beating time on MP Fastprep®-24. 16S library was prepared with Quick-16S™ NGS Library Prep with primers targeting 16S V3-V4 region. Sequencing was performed on MiSeq™ using 600 cycles. Species-level taxonomy profiling was performed with the bioinformatics pipeline used by the ZymoBIOMICS® 16S Service.

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1 Cells may aggregate due to freeze-thaw cycling; therefore, it is critical to mix thoroughly before use.
2 20 µl of the standard contains 4 x 10^7 cells. For sample types with unknown cell concentration, the end-user might need to optimize the amount of the product to be added.

Note: All example calculations are performed using data taken from Figure 1 above.

To calculate total cell number from a sample, choose the correct theoretical value from Table 1 (pg. 1), according to sequencing and analysis methods used.

For shotgun sequencing analysis based on whole genome references (e.g. Centrifuge), use "Total DNA (ng)"; for shotgun sequencing analysis based on marker gene references (e.g. mOTU, MetaPhlAn2), use "Cell Number". Finally, for targeted 16S sequencing, use "16S Copies". Refer to Appendix A: Sequencing Data Analysis for in-depth explanation of analysis reporting methods.

16S Targeted Sequencing

1. Complete the following equation by filling in the relevant data.

$$\frac{\text{SpikeIn Volume Used (µl)}}{20\ \text{µl}} \times \text{Theoretical Value (Table 1)} \times \left(\frac{1}{\%\ \text{Relative Abundance}} - 1\right)$$

$$\downarrow$$

$$\frac{50\ \text{µl}}{20\ \text{µl}} \times 6 \text{ x } 10^7\ \textit{I. halotolerans}\ 16S \text{ copies per prep} \times \left(\frac{1}{2.67\%\ \textit{I. halotolerans}} - 1\right)$$

2. Simplify the left side of the equation to scale the theoretical data relative to the input volume used.

$$1.5 \text{ x } 10^8\ \textit{I. halotolerans}\ 16S \text{ copies per prep} \times \left(\frac{1}{2.67\%\ \textit{I. halotolerans}} - 1\right)$$

3. Simplify the right side of the equation to determine the absolute abundance scaling factor.

$$1.5 \text{ x } 10^8\ \textit{I. halotolerans}\ 16S \text{ copies per prep} \times 36.5$$

4. Simplify the remaining equation to determine total gene copies.

$$5.5 \text{ x } 10^9\ 16S \text{ copies}$$

(Continued on next page)

5. Note that the copy number above still includes gene copies belonging to the other Spike-In organism. In order to remove these copies, multiply the value provided in Table 1 by the same sample input proportion used above (Step 2), and subtract from the total.

$$5.5 \text{ x } 10^9 \text{ 16S copies} - \left(\frac{50 \text{ µl}}{20 \text{ µl}} \times 1.4 \text{ x } 10^8 \textit{ A. halotolerans } \text{16S copies}\right)$$

$$\downarrow$$

$$5.1 \text{ x } 10^9 \text{ total 16S copies}$$

6. In order to estimate total cell number from total 16S copy number, assume an average of approximately three (3) 16S gene copies per bacterial genome. As bacteria are monoploid, genome copy number and cell number can be considered equivalent.

$$\frac{5.1 \text{ x } 10^9 \text{ total 16S copies}}{3 \text{ 16S copies per genome}} = 1.7 \text{ x } 10^9 \text{ total cells}$$

Shotgun Metagenomic Sequencing (Centrifuge)

1. Complete the following equation by filling in the relevant data.

$$\frac{\text{Spike-In Volume Used (µl)}}{20 \text{ µl}} \times \text{Theoretical Value (Table 1)} \times \left(\frac{1}{\text{\% Relative Abundance}} - 1\right)$$

$$\downarrow$$

$$\frac{50 \text{ µl}}{20 \text{ µl}} \times 67.2 \text{ ng } \textit{I. halotolerans } \text{gDNA} \times \left(\frac{1}{2.67\% \textit{ I. halotolerans}} - 1\right)$$

2. Simplify the left side of the equation to scale the theoretical data relative to the input volume used.

$$168.0 \text{ ng } \textit{I. halotolerans } \text{gDNA} \times \left(\frac{1}{2.67\% \textit{ I. halotolerans}} - 1\right)$$

3. Simplify the right side of the equation to determine the absolute abundance scaling factor.

$$168.0 \text{ ng } \textit{I. halotolerans } \text{gDNA} \times 36.5$$

4. Simplify the remaining equation to determine total genomic DNA.

$$6132.0 \text{ ng total gDNA}$$

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5. Note that the above quantity still includes genomic DNA belonging to the other Spike-In organism. In order to remove these copies, multiply the value provided in Table 1 by the same sample input proportion used above (Step 2), and subtract from the total.

$$6132.0 \text{ ng total gDNA} - \left(\frac{50\ \mu l}{20\ \mu l} \times 58.2 \text{ ng } A.\,halotolerans \text{ gDNA}\right)$$

$$\downarrow$$

$$5986.5 \text{ ng total gDNA}$$

6. In order to estimate total cell number from total genomic DNA, assume an average size of approximately 5 Mb per bacterial genome. As bacteria are monoploid, genome copy number and cell number can be considered equivalent.

$$\frac{\text{Total gDNA x DNA Conversion Constant}}{\text{Average Bacterial Genome Size}}$$

$$\downarrow$$

$$\frac{5986.5 \text{ ng total gDNA} \times 1.079 \text{ x } 10^{12} \text{ bp/ng}}{5 \text{ Mb (or } 5 \text{ x } 10^{6} \text{ bp)}}$$

$$\downarrow$$

$$1.3 \text{ x } 10^{9} \text{ total cells}$$

Shotgun Metagenomic Sequencing (mOTU, MetaPhlAn2)

1. Complete the following equation by filling in the relevant data.

$$\frac{\text{Spike-In Volume Used (µl)}}{20\ \mu l} \times \text{Theoretical Value (Table 1)} \times \left(\frac{1}{\%\text{ Relative Abundance}} - 1\right)$$

$$\downarrow$$

$$\frac{50\ \mu l}{20\ \mu l} \times 2.0 \text{ x } 10^{7}\ I.\,halotolerans \text{ cells} \times \left(\frac{1}{2.67\%\ I.\,halotolerans} - 1\right)$$

2. Simplify the left side of the equation to scale the theoretical data relative to the input volume used.

$$5.0 \text{ x } 10^{7}\ I.\,halotolerans \text{ cells} \times \left(\frac{1}{2.67\%\ I.\,halotolerans} - 1\right)$$

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3. Simplify the right side of the equation to determine the absolute abundance scaling factor.

$$5.0 \text{ x } 10^7 \ \textit{I. halotolerans} \text{ cells} \times 36.5$$

4. Simplify the remaining equation to determine total cell number.

$$1.83 \text{ x } 10^9 \text{ total cells}$$

5. Note that the above quantity still includes cells belonging to the other Spike-In organism. In order to remove these cells, multiply the value provided in Table 1 by the same sample input proportion used above (Step 2), and subtract from the total.

$$1.83 \text{ x } 10^9 \text{ total cells} - \left(\frac{50 \text{ µl}}{20 \text{ µl}} \times 2.0 \text{ x } 10^7 \textit{ A. halotolerans} \text{ cells}\right)$$

$$\downarrow$$

$$1.78 \text{ x } 10^9 \text{ total cells}$$