Assembly of Measurement Standards for Metagenomic Analyses by Labcyte Echo 525

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Abstract

Many laboratories have developed control samples in the form of synthetic spiked controls, generous donor samples, or mock communities (MC); however, none have been universally adopted as the “gold standard” for all studies to be compared. In this study, we will focus on the assembly of both a DNA and cell-based MC. In addition to agreeing on the members of the MC and their respective proportions, assembly is a difficult task. Given the extreme sensitivity of DNA sequencing, minimal amount of material is required to form the MC. With this sensitivity comes the increased risk of contamination, resulting in noise in the final analysis. We employed Labcyt’s Echo 525, a unique technology capable of delivering extremely precise nanoliter volumes through acoustic liquid transfer requiring no physical contact, thus eliminating the potential for cross contamination often experienced with tip-based liquid handlers. 

Methods

**MC Bacterial Strains**

<table>
<thead>
<tr>
<th>Species</th>
<th>Media</th>
<th>Growth Conditions</th>
<th>16S rRNA Copy Number (nmol/l)</th>
<th>Genome Size (Mbp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acinetobacter sp.</td>
<td>LB</td>
<td>37.0°C Aerobic</td>
<td>6.7*</td>
<td>2.6</td>
</tr>
<tr>
<td>Corynebacterium jeikeium</td>
<td>Tryptic soy</td>
<td>37.0°C Aerobic</td>
<td>3*</td>
<td>2.8</td>
</tr>
<tr>
<td>Micrococcus luteus</td>
<td>Tryptic soy</td>
<td>30.0°C Aerobic</td>
<td>2</td>
<td>2.5</td>
</tr>
<tr>
<td>Propionibacterium avidum</td>
<td>Reinforced</td>
<td>37.0°C Aerobic</td>
<td>3*</td>
<td>3.4</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>LB</td>
<td>Anaerobic</td>
<td>4.6*</td>
<td>2.3</td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td>LB</td>
<td>37.0°C Aerobic</td>
<td>5.6*</td>
<td>2.6</td>
</tr>
</tbody>
</table>

**Echo 525**

Liquid transfers were performed using the Echo 525 Liquid Handler (Labcyte, Sunnyvale, CA) that employs acoustic energy to eject fluids. The Echo 525 Liquid Handler can transfer in 25 nL increments to allow miniaturization with accuracy and precision while moving sample from source to destination in a touchless manner.

**Quantification and Profiling**

Bacterial genomic DNA was quantified by Qubit 3.0 (Invitrogen), and analysis was performed on a Fragment Analyzer (FA) utilizing the High Sensitivity Genomic DNA kit (DNF-488-33). FA profiles were overlaid and compared using PRSsize (Advanced Analytical). Cell viability was determined with BacTiter-Glo (Promega).

**16S qPCR**

Quantitative real-time PCR (qPCR) 16S quantification used primers (1369F-1492R) targeting regions flanking V9 of the 16S rRNA gene. Standard curve used a serially diluted plasmid containing nt 1369 to 1492 of an E. coli 16S rRNA gene.

**Whole Genome Shotgun Sequencing**

Next Generation Sequencing (NGS) was performed on a MiSeq® utilizing the Nextera XT library preparation kit (Illumina) for MCs. Communities were run in triplicate including positive and negative controls. Computational tools Bowtie2 (v2.2.3) and MetaPhAn (v1.7.8) were utilized for analysis on the resulting sequencing data.

Goals and Significance

Create pools of bacteria and bacterial DNA to serve as mock communities for standardization of microbiome sequencing studies using the Echo 525

Transfer each type of media to determine the best fluid for each bacteria with Echo

Transfer 6 types of bacteria to determine:
- Accuracy of transfer
- Precise liquid delivery

Generate pools of 6 types of bacteria demonstrating creation of MC
- Equal bacterial numbers
- Variable bacterial numbers

Transfer of DNA to determine dependence of concentration transferred on:
- Genomic size
- Genotype counts

Generate pools of 6 types of bacterial DNA demonstrating creation of MC
- Equal input DNA
- Variable input DNA

Results

**DNA**

**Results**

**Cells**

**Conclusions**

- Echo transfers multiple media types
- Echo accurately transfers multiple bacterial strains

**Challenges**

- Differing opinions on 16S copies and genome size within each strain
  - Chose the 16S copy number by literature sources
- Undetectable concentrations of DNA post-cellular extraction
  - No PicoGreen nor Qubit quantification
- Low concentrations of DNA can degrade or absorb into plastic
  - Related to extreme dilutions from the 1st set of experiments

Acknowledgements

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