Sequence-based profiling of microbiomes requires comprehensive microbial lysis. A DNA extraction method that is stringent enough to lyse all cells while not damaging DNA is needed, especially for long-read applications. We report results of a non-bead beating, non-enzymatic, novel ‘Rapid’ microbiome DNA extraction procedure suitable for 16S rRNA gene based microbiome profiling applications.

## METHODS

<table>
<thead>
<tr>
<th>ID</th>
<th>Protocols</th>
<th>Sample used in this study</th>
<th>Time n=96</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>KOH ‘Rapid’ protocol</td>
<td>Mock &amp; Human &amp; Mouse Stool</td>
<td>44 min</td>
</tr>
<tr>
<td>B</td>
<td>Bead pasting protocol</td>
<td>Mock</td>
<td>80 min</td>
</tr>
<tr>
<td>E</td>
<td>MasterPure Complete DNA and RNA Purification Kit</td>
<td>Mock</td>
<td>400 min</td>
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<tr>
<td>HMP</td>
<td>MoBio Power Soil DNA Isolation Kit</td>
<td>Mock &amp; Human Stool</td>
<td>400 min</td>
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<tr>
<td>Q</td>
<td>QIAamp DNA Stool Kit</td>
<td>Mock</td>
<td>320 min</td>
</tr>
<tr>
<td>Z</td>
<td>ZymoBIOMICS DNA/RNA Mini Kit</td>
<td>Mock</td>
<td>280 min</td>
</tr>
</tbody>
</table>

## RESULTS

### HUMAN FECAL SAMPLES

![Figure 2. V1V3 sequencing demonstrated significantly increased evenness using the K method, while V1V9 sequencing demonstrated significant increases in observed genera richness, Shannon Diversity and Evenness of bacterial communities using K method. Significant bacterial community structure differences were observe for the samples prepared between K and HMP methods.](image)

![Figure 3. Firmicutes and Actinobacteria species were significantly increased for samples lysed with the K protocol, while Bacteroidetes species were significantly increased in samples lysed with the HMP protocol. Pair-wise analysis showed that Firmicutes species were driver taxa separating K protocol from HMP. This trend was consistent between Illumina and PacBio sequencing approaches.](image)

### MOUSE FECAL SAMPLES

![Figure 4. Behavioral phenotyping initiates at eight weeks of age with novelty tests. Fecal samples were collected before and after IVSA. The constrained ordination shows different bacteria and novelty behaviors co-drive the microbial community with and without cocaine addiction. Interestingly, in Addicted mice, the distance traveled-OFA1 significantly increases with *Marvinbryantia* while the time spent in light-LD2 is negatively correlated with *Coprococcus*.](image)

## CONCLUSIONS

In summary, we benchmark a novel K DNA extraction protocol that avoids bead beating and enzymatic treatments, while at the same time demonstrating improved performance compared to commonly used DNA lysis and purification methods for the accurate representation of mock communities and human fecal gut microbiome samples. We conclude that the novel ‘K’ DNA extraction protocol offers a reliable alternative for preparing fecal specimens for 16S rRNA gene amplicon sequencing that maintains representation of microbial populations in a sample, with the added benefits that the K method reduces hands-on time by up to 20x for 96 sample preparations.

This work was supported by NIH R43 da 043947-01