SUNDAY-EEB-LB-4

A More Accurate, Cheaper, Faster Microbiome Preparation Method: Application to Study the Microbiome in Cocaine Addiction

Bo-young Hong[1], Mark Driscoll[2], Thomas Jarvie[2], Thi Dong Binh Tran[1], George M. Weinstock[1]

[1] The Jackson Laboratory for Genomic Medicine, Farmington, CT, [2] Shoreline Biome, Farmington, CT

Jackson Laboratory
917-951-7601
bo-young.hong@jax.org

INTRODUCTION

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

ID	Protocols	Sample used in this study	time n=96
K	KOH 'Rapid' protocol	Mock & Human & Mouse Stool	44 min
B	Bead pasting protocol	Mock	80 min
E	MasterPure Complete DNA and RNA Purification Kit	Mock	400 min
HMP	MoBio Power Soil DNA Isolation Kit	Mock & Human Stool	400 min
Q	QIAamp DNA Stool Kit	Mock	320 min
Z	ZymoBIOMICS™ DNA/RNA Mini Kit	Mock	280 min

RESULTS *BACTERIAL MOCK COMMUNITY*

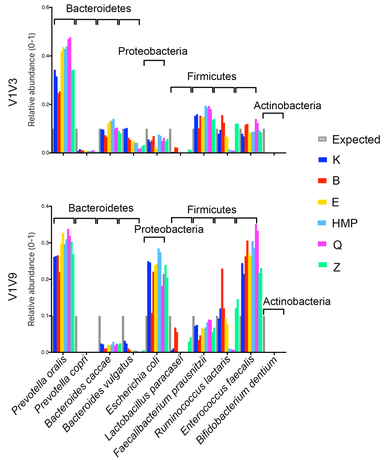


Figure 1. The custom mock microbiome results demonstrated that the novel K and B methods compare favorably to some of the most widely used methods (E, HMP, Q, Z), from both V1V3 and V1V9 amplicons via Illumina and PacBio sequencing, respectively.

RESULTS *HUMAN FECAL SAMPLES*

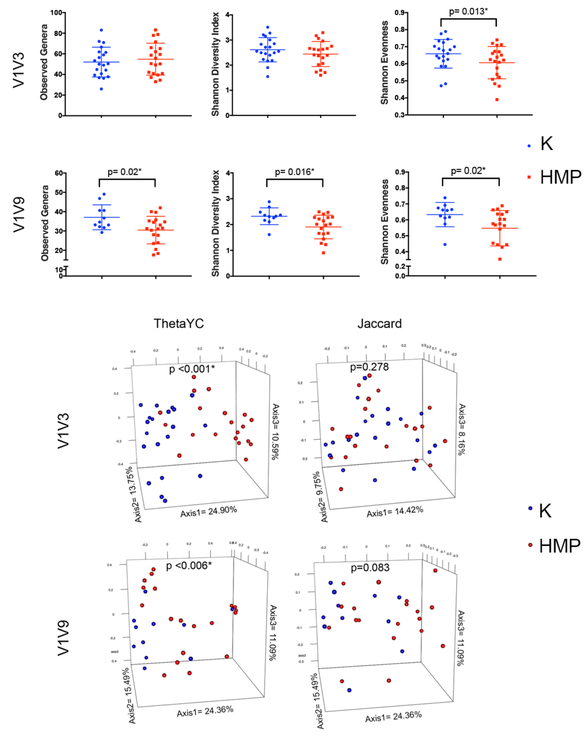


Figure 2. V1V3 sequencing demonstrated significantly increased evenness using the K method, while V1V9 sequencing demonstrated significant increases in observed genera richness, Shannon Diversity and Evenness of bacterial communities using K method. Significant bacterial community structure differences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

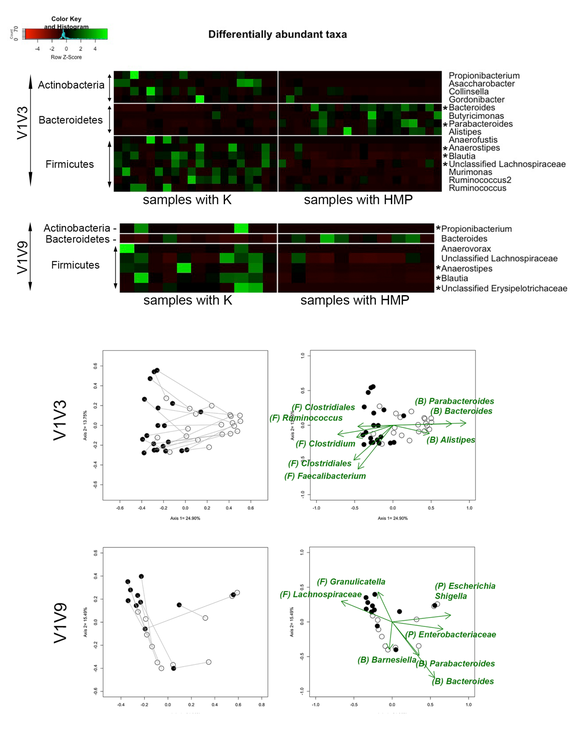
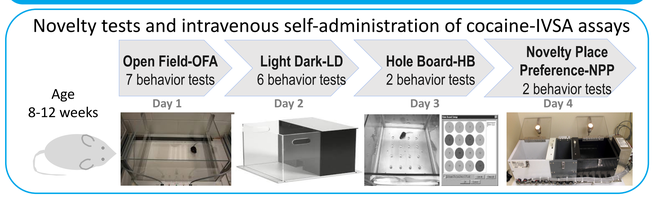


Figure 3. Firmicutes and Actinobacteria species were significantly increased for samples lysed with the K protocol, while Bacteroidetes species were significantly increased in samples lysed with the HMP protocol. Pair-wise analysis showed that Firmicutes species were driver taxa separating K protocol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

RESULTS *MOUSE FECAL SAMPLES*



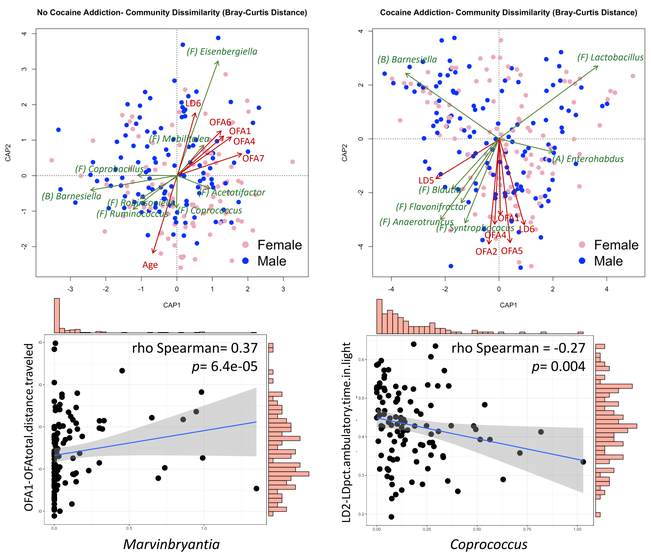


Figure 4. Behavioral phenotyping initiates at eight weeks of age with novelty tests. Fecal samples were collected before and after IVSA. The constrained ordination shows different bacteria and novelty behaviors co-drive the microbial community with and without cocaine addiction. Interestingly, in Addicted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

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 20 x for 96 sample preparations.

This work was supported by NIH R43 da 043947-01