thermoscientific

A targeted next generation sequencing assay to analyze and characterize the gut microbiome for health research

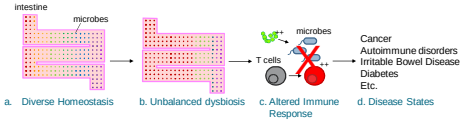
Anna McGeachy, Shrutii Sarda, David Merrill, Birgit Drews, Wing Lee, Heesun Shin, Ying Lian, Janice Au-Young, Rajesh Kumar Gottimukkala, Aren Ewing, Fiona M Hyland, Thermo Fisher Scientific, Oyster Point Boulevard, South San Francisco, California, United States of America, 94080

ABSTRACT

The gut microbiome has emerged as an important biomarker to research the potential efficacy of immune-modulatory drugs. Using the Ion AmpliSeq™ technology, we have created a highly sensitive and specific assay for robust characterization of microbiota. This highly multiplexed PCR approach enables an efficient and affordable means for conducting extensive analyses of the human microbiome and has applications in the study of phenotypic variability as it relates to disease, emergent resistance mechanisms, and future therapies.

INTRODUCTION

The balance of trillions of bacteria, fungi, and other microbes that comprise the human gut microbiota plays an important role in the regulation of human health. A healthy human gut has a variety of commensal or mutualistic bacteria living in relative homeostasis. When a microbial imbalance or maladaptation occurs, changing the makeup of the normal flora of bacteria, the gut enters a state of dysbiosis. Dysbiosis typically causes inflammation of the intestinal cell wall, disrupting the mucus barrier, epithelial barrier, and immunosensitive cells that line the gastrointestinal tract.



Disruption of microbial diversity in the human gut can lead to disease

(a) The healthy human gut is home to a wide diversity of microbial tenants. When this diversity is disrupted (b) the gut microbiome enters a state of dysbiosis, the composition of which has been associated with (c) altered immune response such as an increase in production of antimicrobial peptides (in green) or stimulation of T cells (grey to red) which in turn can lead to a number of (d) disease states.

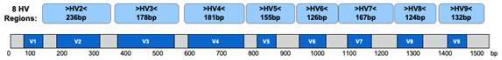
Targeted Next Generation Sequencing (NGS) of the 16S rRNA gene provides a cost-effective approach for globally characterizing microbial diversity in the gut microbiome. We have developed a targeted NGS panel that assays multiple hypervariable regions in the 16s rRNA gene (16s Pool) as well as a set of 73 species-specific primers (Target Species Pool) using Ion AmpliSeq™ technology. This new assay comprehensively characterizes the composition of the human gut microbiome providing increased resolution and specificity of species-level detection for key organisms associated with gut microbiome-related health disorders.

MATERIALS AND METHODS

Our assay includes customized primers to multiple hypervariable regions of the 16S rRNA gene. However, 16S alone can still be insufficient in distinguishing highly homologous organisms such as *Bacteroides* and *Lactobacillus*. We have designed species-specific primers that provide high strain coverage and high specificity performance. We have tested primers for 73 relevant species from genera such as *Bifidobacterium*, *Clostridium*, *Ruminococcus* and *Bacteroides*[1,2,3]. With this approach, we can analyze reads generated from sequencing to report taxonomic classifications and relative abundance for organisms in the sample with high specificity and sensitivity.

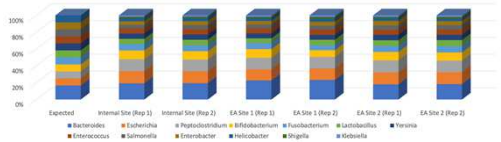
RESULTS

Figure 1. Targeted 16S Amplicons for pan-bacterial identification



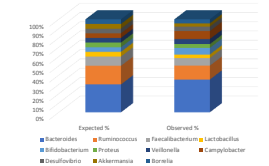
The bacterial 16S rRNA gene is a genomic region that shows extensive conservation (in grey) and variability (in blue), making it a common genotyping locus. We use targeted sequencing to cover 8 of the 9 hypervariable regions (HV1-9) using amplicons (in light blue, HV2-9) from 124-236 base pairs (bp) in length, making it compatible with a number of Ion Torrent sequencing technologies.

Figure 2. Reproducibility of 16S Pool on ATCC Standard Gut Microbiome Genomic Mix



We demonstrated highly-reproducible genus level identification using the 16S Pool with the commercially available ATCC microbial standard, Gut Microbiome Genomic Mix (MSA-1006). Here we show 2 representative replicates (8 total) from 3 sites including 2 external Early Access (EA) labs.

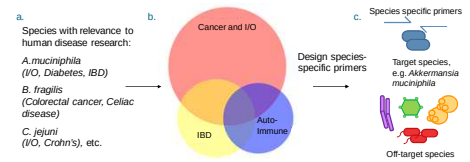
Figure 3. Accurate identification of genera in a microbial mixture sample using 16S Pool



#ID	Expected %	CN Normed Observed %
Bacteroides	30	35
Ruminococcus	20	15
Faecalibacterium	10	8
Lactobacillus	5	4
Bifidobacterium	5	7
Proteus	5	4
Veillonella	5	5
Campylobacter	5	9
Desulfovibrio	5	5
Akkermansia	5	4
Borrelia	5	4

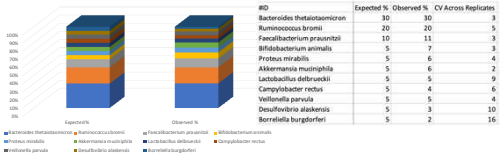
We generated 30 mixes using genomic DNA from commercial sources (ATCC and DSMZ) to better represent the diversity of disease-relevant species. Here we show the expected versus observed abundance for one of these mixes using the 16S Pool. We show the Copy Number (CN) normalized abundances across 8 replicates representing different library preps, operators, and sequencing chips. Furthermore, we can detect these species with 95-100% specificity and sensitivity at the genus level.

Figure 4. Target Species Pool for species relevant to human disease



(a) To increase assay sensitivity and specificity to key species in human health, we selected 73 species from the literature[1,2,3] pertinent to research areas including; (b) Cancer, Immuno-Oncology (I/O), Irritable Bowel Disease (IBD), and Auto-Immune disorders (Venn diagram above[4]). (c) We generated our Target Species Pool using proprietary software to identify unique genomic targets and primers for the relevant species, resulting in a highly specific panel.

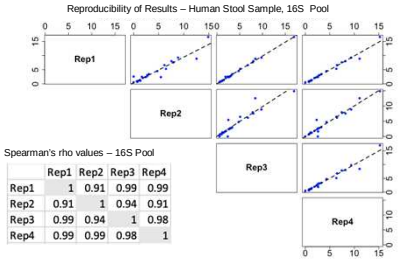
Figure 5. Species level identification in microbial mixture sample using Target Species Pool



#ID	Expected %	Observed %	CV Across Replicates
Bacteroides thetaiotaomicron	30	30	3
Ruminococcus bromii	20	20	5
Faecalibacterium prausnitzii	10	11	3
Bifidobacterium animalis	5	7	3
Proteus mirabilis	5	6	4
Akkermansia muciniphila	5	6	2
Lactobacillus delbrueckii	5	5	9
Campylobacter rectus	5	4	6
Veillonella parvula	5	5	4
Desulfovibrio alaskensis	5	3	10
Borreliella burgdorferi	5	2	16

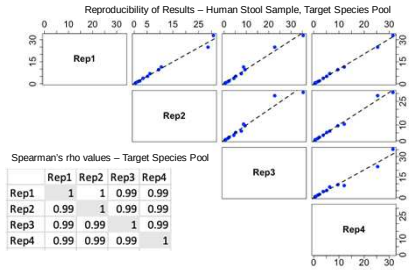
We tested species identification using our Target Species Pool against the same microbial mixture sample demonstrated in Figure 3. We display the average and coefficient of variation (CV) across 8 replicates representing different library preps, operators, and sequencing chips, with robust quantitative detection of high and low abundance species. Overall species detection is at 100% specificity and sensitivity.

Figure 6. Reproducibility of species identification in a healthy stool sample



Spearman's rho values – 16S Pool

	Rep1	Rep2	Rep3	Rep4
Rep1	1	0.91	0.99	0.99
Rep2	0.91	1	0.94	0.91
Rep3	0.99	0.94	1	0.98
Rep4	0.99	0.99	0.98	1

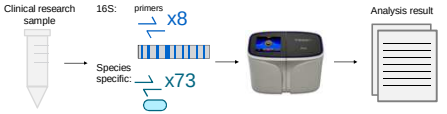


Spearman's rho values – Target Species Pool

	Rep1	Rep2	Rep3	Rep4
Rep1	1	1	0.99	0.99
Rep2	0.99	1	0.99	0.99
Rep3	0.99	0.99	1	0.99
Rep4	0.99	0.99	0.99	1

We tested our 16S and Target Species Pools against healthy stool samples. The images above display the intra-sample abundance correlations for a single representative sample. In the 16S Pool, Spearman's rho values for Genus level identification (inset) are all above 0.90. In the Target Species Pool, we see an improvement to Spearman's rho values (inset) all at or above 0.99 for species level identification.

Figure 7. Complete solution from sample to result for microbiome health research



We describe the molecular biology and design underlying our new best-in-class sample to analysis microbial community profiling. Briefly, the use of specific primers covering 8 of the 9 16S hypervariable regions and specific primers for 73 species can be used with Ion Torrent technology to provide robust microbial community profiling. We demonstrate exceptional sensitivity and specificity with the assay, making our solution a reliable measure for researching human disease.

CONCLUSIONS

Using the Ion AmpliSeq™ technology, we have created a highly sensitive and specific assay for robust characterization of microbiota, identifying species present at the genus-level with 95% sensitivity 100% specificity using the 16S Pool. By adding the Targeted species pool, we can additionally identify microbes present at the species-level for 73 bacterial species with 100% specificity and sensitivity.

We have demonstrated that this assay provides a highly accurate measure of the relative abundance of different microbes, is highly reproducible across multiple sites using ATCC microbiome standards and highly reproducible when tested on stool samples from healthy individuals.

Finally, this new targeted NGS assay using best-in-class AmpliSeq™ technology provides an efficient and robust workflow for conducting cost-effective analyses of the human gut microbiome.

REFERENCES

1. Routy, Bertrand, et al. "Gut Microbiome Influences Efficacy of PD-1–Based Immunotherapy against Epithelial Tumors." Science, American Association for the Advancement of Science, 5 Jan. 2018.
2. Matson, Vyara, et al. "The Commensal Microbiome Is Associated with Anti–PD-1 Efficacy in Metastatic Melanoma Patients." Science, American Association for the Advancement of Science, 5 Jan. 2018
3. Gopalakrishnan, V., et al. "Gut Microbiome Modulates Response to Anti–PD-1 Immunotherapy in Melanoma Patients." Science, American Association for the Advancement of Science, 5 Jan. 2018
4. BioVenn - a web application for the comparison and visualization of biological lists using area-proportional Venn diagrams T. Hulsen, J. de Vlieg and W. Alkema, BMC Genomics 2008, 9 (1): 488

ion torrent ThermoFisher SCIENTIFIC