Characterization of gut microbiota and identification of key bacterial species associated with immune response using a new Ion Torrent next-generation sequencing assay

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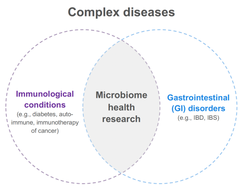
ABSTRACT

The gut microbiome affects immunity both locally and systemically; recent studies support a potential link between bacterial signatures in the gut and response to immune checkpoint blockade in cancer. A low-cost targeted solution to characterizing and profiling microbial diversity in the gut is sequencing of the 16S rRNA gene. However, 16S sequencing alone is often insufficient to gain species level resolution, due to the gene's high homology across different bacteria. **Here we describe a first-of-its-kind targeted sequencing solution based on Ion AmpliSeq™ technology that supplements 16S gene targets, with highly species-specific primers for a cohort of bacteria likely associated with cancer, response to cancer immunotherapy, as well as several gastrointestinal and auto-immune disorders.** This assay can be used to better understand the composition and diversity of the human gut microbiome in the context of these phenotypes, as compared to other currently existing solutions.

Our species-specific content provides comprehensive strain coverage with 100% sensitivity and 100% specificity for 73 different bacterial species including *H. pylori, B. vulgatus, B. adolescentis*, and several others from highly homologous genera like *Lactobacillus* and *Bacteroides* which are indistinguishable using just 16S targets. In addition, we have developed an approach to identify species-specific signatures/targets that easily extends to any new bacterial species that might become relevant to gut health in the future. **Furthermore, our new Ion Reporter™ solution provides an end-to-end sample to result workflow, that includes algorithms to automatically analyze reads from sequencing and generate a report with accurate taxonomic classifications, sample diversity metrics and relative abundance visualizations for organisms across multiple samples.**

INTRODUCTION

The healthy human gut is home to a wide diversity of microbial tenants. When this diversity is disrupted, the gut microbiome enters a state of dysbiosis, the composition of which has been associated with altered immune response such as an increase in production of antimicrobial peptides or stimulation of T cells, which in turn can lead to a number of disease states like cancer, diabetes, auto-immunity, IBD/IBS etc.



With the growing need for analysis solutions that allow users to accurately detect and quantify bacteria relevant to complex disease research, **we have launched a new Ion Torrent™ based simple-to-use, highly accurate and reproducible microbial analysis solution that enables going from sample to result with a short turn-around-time. The added species content vastly improves the accuracy of detection of certain bacteria compared to 16S, making it better suited for high resolution analysis of complex disease phenotypes.**

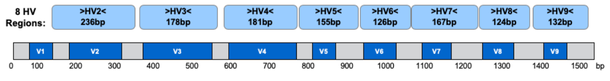
MATERIALS AND METHODS

Using the Ion GeneStudio S5™ system, we sequenced 2 ATCC community controls comprising of known bacteria mixed in equal proportions, and 30 custom mixed population samples of DNA from 73 target bacterial species at varying concentrations (1% to 12%).

With our more comprehensive 16S design covering 8 hyper-variable regions, we identified all the organisms at the genus level with 100% sensitivity and 95% - 100% specificity. Using our species-specific primers, we identified all the targeted species with 100% sensitivity and 100% specificity in both, the community controls and mixed population samples at the species level. Furthermore, both the 16S and Target Species pools of the assay were tested against healthy stool samples, and the abundances are highly reproducible with Spearman's rho of 0.90 to 0.99 across multiple replicates.

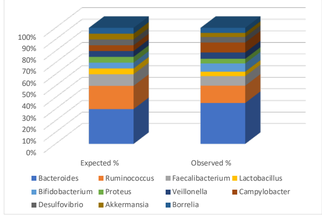
RESULTS

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.

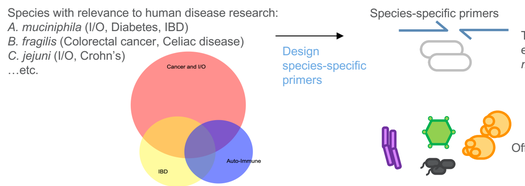
Figure 2. Accurate Genus Level Detection And Quantification With 16S Pool



#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

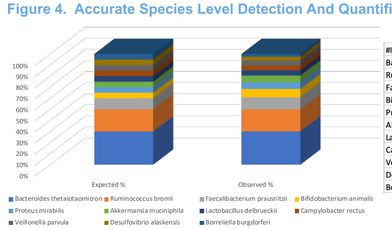
We generated 30 mixes using genomic DNA from commercial sources (ATCC and DSMZ) to better represent the diversity of disease-relevant species. Here we show the expected versus observed abundance for one of these mixes using the 16S pool.

Figure 3. Targeted Species Pool For Species Relevant To Complex Disease Research



16S-based identification is often limited to genus level resolution. To enhance assay performance to key species in human health, we selected 73 species from the literature[1,2,3] with pertinence to research in Cancer, Immuno-Oncology (I/O) response, Irritable Bowel Disease (IBD), and Auto-Immune disorders. Using internal software, we identify unique genomic targets and primers for each of these species to generate our target species pool.

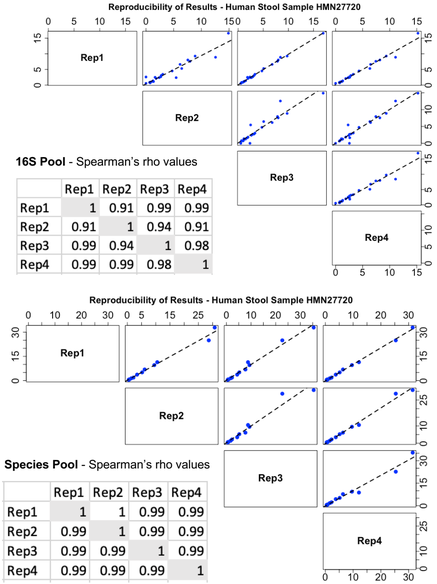
Figure 4. Accurate Species Level Detection And Quantification With Target Species Pool



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

We tested species identification using our targeted species pool against the 30 genomic DNA mixes and controls as mentioned earlier. Here we show the expected versus observed abundance for the same microbial mixture sample demonstrated in Figure 2. Overall species detection across all samples and replicates is at 100% specificity and sensitivity.

Figure 5. Reproducibility Of Assay Across Replicates Of Stool Samples



16S Pool - Spearman's rho values

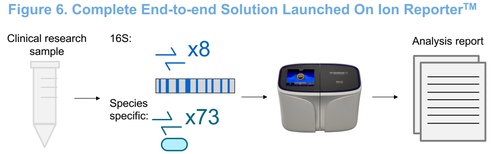
	Rep1	Rep2	Rep3	Rep4
Rep1	1	0.91	0.99	0.99
Rep2	0.91	1	0.94	0.91
Rep3	0.99	0.94	1	0.98
Rep4	0.99	0.99	0.98	1

Species Pool - Spearman's rho values

	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 pool against healthy stool samples. Here we display the intra-sample abundance correlations for one sample. In the 16S pool, Spearman values for Genus level identification (inset) are all above 0.90. In the targeted species pool, we see an improvement to Spearman values (inset) all at or above 0.99 for species level identification.

Figure 6. Complete End-to-end Solution Launched On Ion Reporter™



Simple and easy to use full analysis solution

- Sample --> Sequencing --> Analyses --> Results!
- Two separate result tabs for 16S rRNA Gene and Target Species pools
- Improved 16S amplicon mapping and summarization algorithm (8 HV regions) for easier interpretation
- Taxonomic classification collated summary table for multiple samples

Use of up-to-date and maintained reference databases

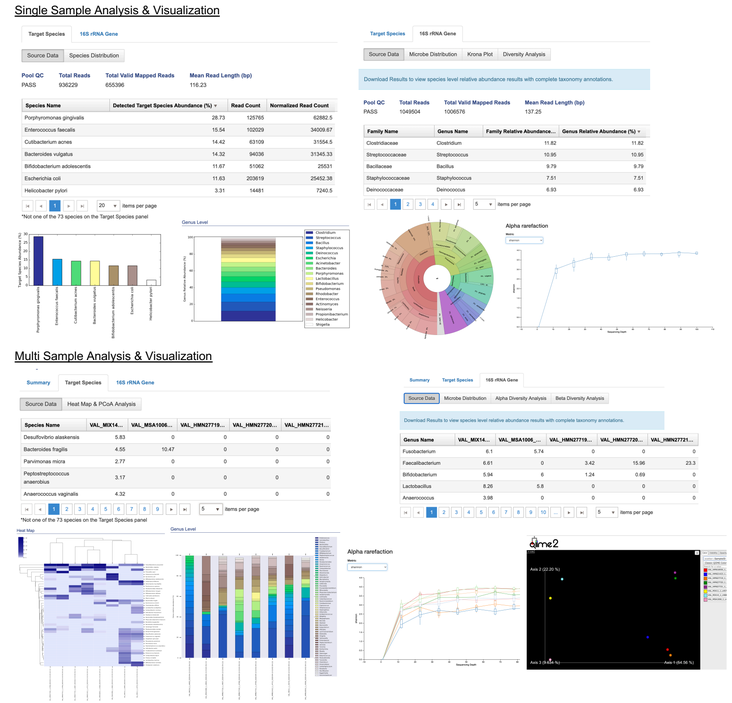
- Curated SILVA, MicroSeq, GreenGenes database

Single and Multi-sample analysis and visualization

- Full taxonomic profiling - Interactive Krona plot for taxonomic classification
- Microbiome composition analysis - Relative abundance stacked bar plots
- Microbiome diversity analysis - Alpha and Beta diversity visualization via QIIME2
- Comparative analysis - Heat map and PCoA plots for unsupervised clustering of multiple samples

Figure 7. Features Of The End-to-end Ion Reporter™ Workflow Solution

Single Sample Analysis & Visualization



Multi Sample Analysis & Visualization

CONCLUSIONS

- **We have launched a full end-to-end solution for sample to result detection and quantification of bacteria in the gut microbiome, with diversity and abundance visualizations for multiple samples.**
- **This highly multiplexed solution is accurate, reproducible, and affordable with 100% sensitivity and 100% specificity for the added species content relevant to research in Cancer, Immuno-Oncology (I/O) response, Gastrointestinal Disorders (like celiac disease, irritable bowel disease etc.), Immune Health and Auto-Immune disorders; making it more suited for complex disease research than current 16S solutions.**

REFERENCES

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2. Gopalakrishnan, V., et al. Science, 5 Jan. 2018; Vol. 359, Issue 6371, pp. 97-103
3. Matson, Vyara, et al. Science, 5 Jan. 2018; Vol. 359, Issue 6371, pp. 104-108

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