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

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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.

RESULTS

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 50% 40% 236 base pairs (bp) in length, making it compatible with a number of Ion Torrent sequencing technologies.

We demonstrated highly reproducible genus level identification using the 16S Pool with the commercially available ATCC microbial standard, Gut Microbiome Genomic Mix, ATCCYA-1006. Here we show 2 representative replicates (R1-R2) from 2 sites including 2 external Early Access (EA) labs.

DISCUSSION

Disruption of microbial diversity in the human gut can lead to disease (a) The healthy human gut is filled with various microbial species. When the diversity is disrupted (b) the gut microbiota 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.

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 prep, operators, and sequencing chips, with robust quantitative detection of high and low abundance species. Overall species detection is at 100% specificity and sensitivity.

CONCLUSIONS

We describe the molecular biology and design underlying our new best-in-class sample to result microbiome 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 relative measure for researching human disease.

REFERENCES