Human Microbiome Controls

Development of AcroMetrix™ Human Microbiome Quality Control Materials

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Abstract

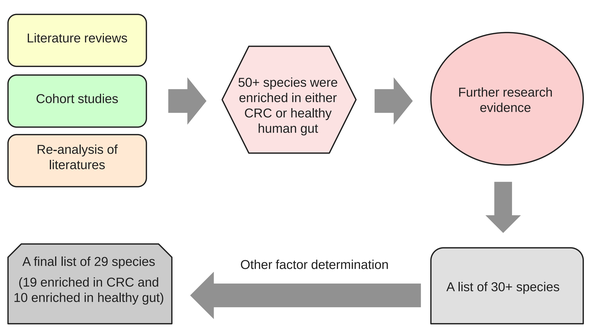
Introduction: The human microbiome is receiving significant attention given its critical roles in the maintenance of human health. The compositional and functional alterations of the human microbiota have been associated with various pathological conditions including colorectal cancer (CRC). As the number of human metagenomic data increases, the interest to utilize these metagenomic biomarkers for diagnosis of colorectal cancer and other diseases has also elevated. Studies that evaluated the defined fecal microbial markers for CRC detection showed the test accuracy could vary from 73% to 98% depending on the detection panels of microbiome biomarkers. The measurement inconsistencies between laboratories due to the use of different detection platforms, the coverage of various assay panels, the biases in different steps of microbiome workflow all indicated the critical need for reliable quality control materials. Currently, the commercially available microbial standards usually contain limited species and also mixed with fixed ratios which may not reflect the microbial composition in the clinical samples. There is no commutable reference material that recapitulates the dysbiotic microbial composition under healthy or pathological conditions (e.g., CRC). Therefore, in this study, we developed DNA quality control materials (QCMs) that recapitulate the dysbiotic microbial composition for colorectal cancer to assess the technical and non-technical variation for part of the microbiome workflow.
Method: We first identified the specific bacterial species that either are enriched in CRC patients or healthy human gut based on comprehensive literature search and the other available dataset. We then created microbiome QCMs to recapitulate their dysbiotic microbial composition in comparison to the compositions in healthy condition. The QCM consists of a mixture of DNA from the selected bacterial species, the copy number of DNA was determined by Bio-Rad™ droplet digital PCR (ddPCR) with the assays that target to species-specific regions to reflect the microbial composition. In addition, the microbiome controls are further evaluated and validated by Microbiome Health Research Assay on Ion Ampliseq and GeneStudio S5 platforms.

Results: The AcroMetrix™ human microbiome QCMs have been carefully formulated to mimic dysbiotic microbial composition for colorectal cancer or healthy human gut. This CRC QCM covers 29 different species with a wide range of guanine-cytosine content sequences, belonging to 10 phyla and 20 genera. 25 out of these 29 species (86.2%) were covered by Microbiome Health Research Assay (MHRA). To mimic the trends of dysbiotic microbial composition, the DNA frequency among these 29 species was targeted from 0% to 15% according to the microbial composition. Droplet digital PCR results confirmed the target frequencies within the accepted tolerance range and the results were compared with the data from the Next Generation Sequencing platform.

Conclusions: The human microbiome QCMs that cover 29 bacterial species and mimic dysbiotic microbial composition for colorectal cancer or healthy human gut have been designed and developed. These QCMs could provide essential elements to verify, validate and monitor test performance, technical and non-technical variation of microbiome workflows, as well as product QC releases and method development for ddPCR & NGS assays.

Introduction

Figure 1. Workflow to identify CRC-associated species



Comprehensive literatures (reviews and cohort studies) were first used to identify a list of potential species that were either enriched in colorectal cancer (CRC) or healthy human gut. Then further research evidence and other factor determination were conducted to finalize the list.

Materials and methods

Figure 2. Manufacturing workflow of the human microbiome controls

Control development and manufacture

Bio-Rad QX200 AutoDG ddPCR system

Ion Chef and GeneStudio S5
Ion 550 chip
Ion AmpliSeq Microbiome Health Research Kit

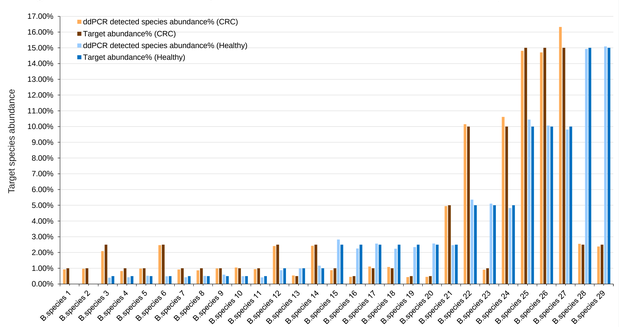
Results

Table 1. The 29 species that enrich in colorectal cancer or human healthy gut

Enrichment	Species	Genus	Phylum
Colorectal cancer	B.species 1	Helicobacter	Campylobacterota
	B.species 2	Thermanaerovibrio	Synergistota
	B.species 3	Collinsella	Actinomycetota
	B.species 4	Campylobacter	Proteobacteria
	B.species 5	Clostridium	Bacillota
	B.species 6	Enterococcus	Firmicutes
	B.species 7	Fusobacterium	Fusobacteriota
	B.species 8	Peptostreptococcus	Bacillota
	B.species 9	Porphyromonas	Bacteroidetes
	B.species 10	Porphyromonas	Bacteroidetes
	B.species 11	Parvimonas	Firmicutes
	B.species 12	Clostridium	Bacillota
	B.species 14	Prevotella	Bacteroidetes
	B.species 21	Streptococcus	Firmicutes
	B.species 22	Akkermansia	Verrucomicrobia
	B.species 24	Escherichia	Proteobacteria
	B.species 25	Bacteroides	Bacteroidetes
	B.species 26	Bacteroides	Bacteroidetes
	B.species 27	Bacteroides	Bacteroidetes
Healthy gut	B.species 13	Lactobacillus	Firmicutes
	B.species 15	Bifidobacterium	Actinobacteria
	B.species 16	Bifidobacterium	Actinobacteria
	B.species 17	Lactobacillus	Firmicutes
	B.species 18	Lactobacillus	Firmicutes
	B.species 19	Lactococcus	Firmicutes
	B.species 20	Lactobacillus	Firmicutes
	B.species 23	Bifidobacterium	Actinobacteria
	B.species 28	Faecalibacterium	Firmicutes
	B.species 29	Parabacteroides	Bacteroidetes

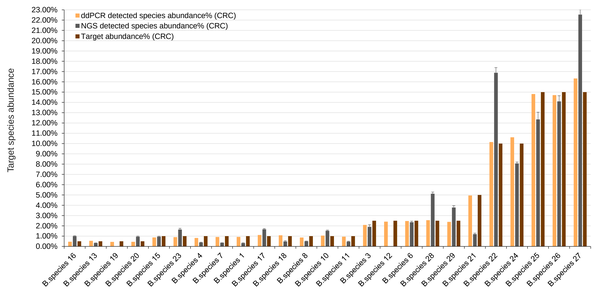
Among these 29 species, 19 were enriched in colorectal cancer and 10 were enriched in human healthy gut. 86.2% of these 29 species (25) was covered by Ion AmpliSeq Microbiome Health Research Assay (MHRA), this makes it reasonable to use NGS MHRA to evaluate the microbiome controls.

Figure 3. ddPCR detected species abundance in both CRC and healthy controls



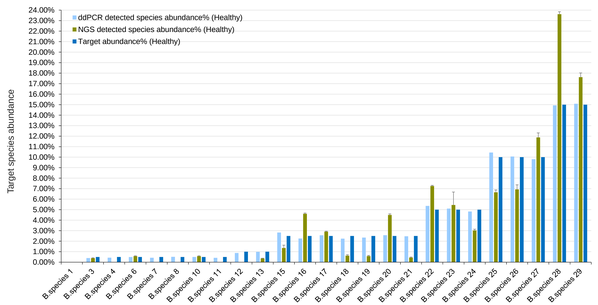
The controls were tested with ddPCR Supermix for Probes (No dUTP) on the Bio-Rad QX200 AutoDG Droplet Digital PCR System. The copy number of species gDNA was quantitated using QuantaSoft (version of 1.7.4.0917) ddPCR data software. N=3 for ddPCR replicates.

Figure 4. NGS detected species abundance in CRC control



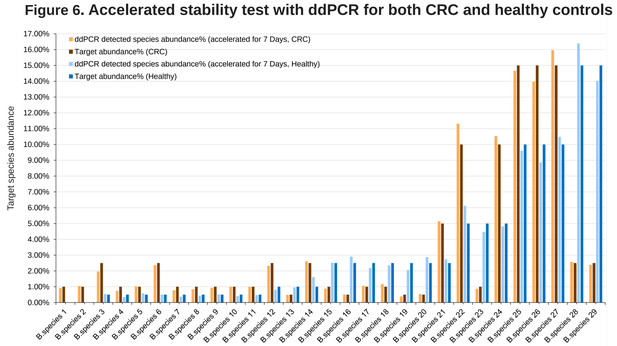
The controls were tested with Ion AmpliSeq Microbiome Health Research Kit and Ion Torrent Ion 550 chip on the Ion Chef and GeneStudio S5. The NGS results will be evaluated using Ion Reporter software. N=3 for ddPCR and N=4 for NGS replicates.

Figure 5. NGS detected species abundance in healthy control



The controls were tested with Ion AmpliSeq Microbiome Health Research Kit and Ion Torrent Ion 550 chip on the Ion Chef and GeneStudio S5. The NGS results will be evaluated using Ion Reporter software. N=3 for ddPCR and N=4 for NGS replicates.

Figure 6. Accelerated stability test with ddPCR for both CRC and healthy controls



The controls were stressed at 37°C for 7 days (equivalent to 12 months stored at -20°C). And then the samples were tested on the Bio-Rad QX200 AutoDG Droplet Digital PCR System and quantitated using QuantaSoft (version of 1.7.4.0917) ddPCR data software. N=3 for ddPCR replicates.

Conclusions

- The human microbiome quality control materials that cover 29 bacterial species to mimic dysbiotic microbial composition for colorectal cancer or healthy human gut have been designed and developed.
- Accelerated stability studies of the AcroMetrix™ human microbiome controls supported a 12-months shelf-life stored at -20° C.
- These quality control materials could provide essential elements to verify, validate and monitor test performance, technical and non-technical variation of microbiome workflows, as well as product QC releases and method development for ddPCR & NGS assays.

References

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