Characterizing and Developing the Clinical Grade Next Generation Sequencing based Gut Microbiome Assay with the Bioinformatics Solution

Abstract ID. 4704

Danyi Wang[1], Brajendra Kumar[2], Aaron Tenney[3], Ravi Kiron[4], Yang Liu[3], Fei Zhong[3], Juergen Scheuenpflug[5], Zheng Feng [1#],

1. Clinical Measurement Sciences, Global Research & Development, EMD Serono, Billerica, MA, USA, A business of Merck KGaA, Darmstadt, Germany
2. Sigma-Aldrich Chemicals Pvt. Ltd., Bangalore, India (An affiliate of Merck KGaA, Darmstadt, Germany) 3. Bioinformatics R&D, Information Technology, Millipore Sigma, St. Louis, MO, USA
4. Strategic Innovation, Silicon Valley Innovation Hub, EMD Serono, San Jose, CA, USA 5. Merck KGaA, Darmstadt, Germany # Corresponding author

BACKGROUND

It has been recognized that gut microbiome has impact on the cancer immunotherapy efficacy and Cancer Microbiome-Immune Axis is reported. Also, it is important to discover and identify clinically translatable predictive biomarker in gut microbiome to inform the treatment selections. Multiple pre-analytical and analytical steps & factors including the sample collection, DNA extraction, library preparation, sequencing and bioinformatics analysis are associated with the microbiome data interpretation as well as its potential clinical application. 16S amplicon-sequencing coupled with bioinformatics approach for advance analysis provides end-to-end solution.

It is therefore essential to develop a clinical-grade assay for targeting & characterization of taxa at genus and species level microbes in stool samples, which is designed as two-phase approach: firstly, identification the optimal sample preparation reagents using pre-mixed bacteria and healthy donor stool samples coupled with proprietary bioinformatics solution; secondly, exploratory analysis of patient samples.

METHODS

Phase Ia
ATCC® microbiome standards MSA-2002™ and MSA-2003™ → Extraction Kit A, B, C, D &E

Phase Ib
Healthy donor stool samples (n= 30)
- Median age 46 with range 35-75
- Gender ratio 1:1)

→ Extraction Kit A, B & C

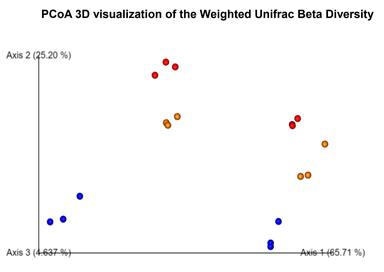
QC → Target-specific PCR amplification of the bacterial 16S V3/V4 regions → QC → MiSeq, 2x300bp paired-end sequencing → QC → Sigma-Aldrich® M-CAMP™

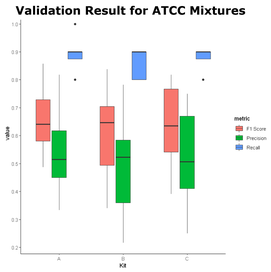
RESULTS – Phase 1a

DNA extraction efficiency varies dependent on extraction methods

- The DNA yield using the extraction kit D and E was below the limit of detection (100 pg/µl) of Qubit assay as both extraction kits are intended for samples with low bacterial counts.
- The pre-mixed bacterial pellets at high concentrations with an input of 2 x10^6 cells for MSA-2002 and 1 x10^6 cells from MSA-2003 were not compatible with the kits D & E.
- Kit D & E were excluded from further analysis.
- Kit A produced the greatest yield, whereas kit B provided the least yield.

Taxonomic Profiles Varies among Extraction Methods

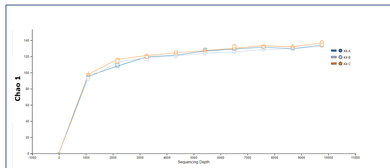




- The PCoA (Principal Coordinate Analysis) 3D visualization of the Weighted Unifrac beta diversity shows that kits A and C cluster closely together while kit B appears as an outlier.
- The kit A sequencing samples cluster more closely together than both the other kits.
- The taxonomic profiles of kit B have lower recall when compared to the known mixture profiles indicating that kit B was inefficient at detecting some of the bacteria.

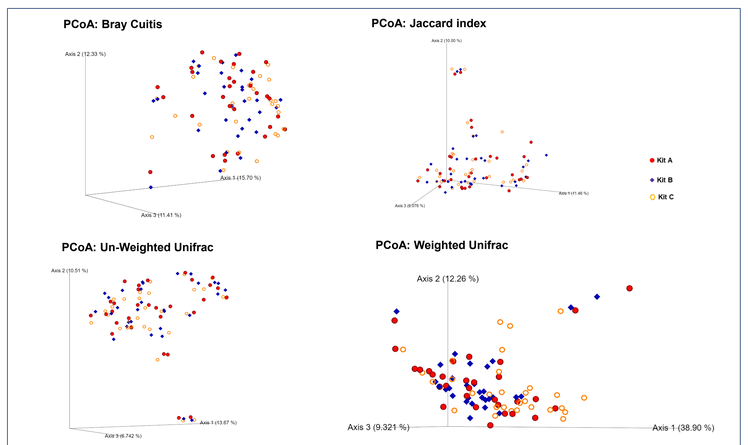
RESULTS – Phase 1b

Alpha diversity measures indicates richness and evenness within the kits



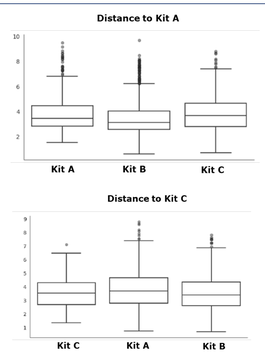
p-value and H-test does not show the statistically significant differences between groups

Dis(similarity) metrics-based ordination analysis for PCoA



- Beta diversity distant metrices (Bray Curtis, Jaccard & Un-weighted Unifrac) based group significance test using pairwise PERMANOVA indicates no significant group separation between the kits.
- P-value and the F-ratio value of pairwise permanova indicates the significant group separation based upon the based on weighted unifrac (P = 0.046)

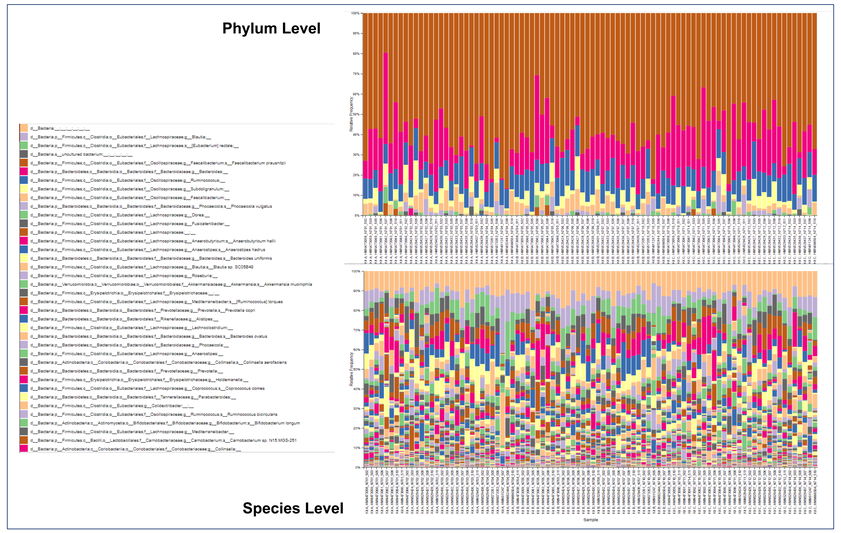
Weighted Unifrac metric-based Beta group significance



Group 1	Group 2	Sample size	Permutations	pseudo-F	p-value
Kit A	Kit B	60	999	0.205	0.991
Kit A	Kit C	60	999	2.621	0.027
Kit B	Kit C	60	999	3.121	0.017

pseudo- F ratio value in Pairwise Permanova results indicates that Kit C species abundance is significantly diverged from both Kit A and Kit B respectively.

Relative frequency-based group-sample level composition at phylum and species level show high level similarity



CONCLUSIONS

The comprehensive qualification approaches including the analytically optimized extraction condition and post-analytically implement the bioinformatics solution assures the characterization of microbiota for enabling biomarker driven precision oncology. Analytical performance assessment using colorectal cancer patients' samples is ongoing for further exploring its potential clinical utilities.

Phase 1
Identification of the optimal sample preparation reagents using pre-mixed bacteria and healthy donor stool samples coupled with proprietary Sigma-Aldrich® bioinformatics solution.

Phase 2
Exploratory analysis of patient samples for enabling precision medicine

Identify and develop microbiome derived **clinical biomarker** for patient selection & enrichment

- Detection of stable organisms in microbiome
- Identification of changes in stables microbiome with cancer risk or development
- Develop suitable techniques to detect these signatures
- Retrospective, Prospective, Randomize clinical Trials

"Fit for purpose" assay development & validation

clinical validation & application

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2. Wang D, Andrew SE, O'Rourke D, Brajendra K, Liu Y, Zhong F, Scheuenpflug J, Feng Z. Characterizing and Developing the Clinical Grade Microbiome Assay with Robust Bioinformatics Pipeline for Supporting Precision Medicine Driven Clinical Development. Presented at the 2020 International Conference on Human Microbiome. September 24-25, 2020

ACKNOWLEDGEMENTS AND DISCLOSURES

This study was sponsored by EMD Serono, a business of Merck KGaA, Darmstadt, Germany.