00279 Development of Rapid qPCR Profiling of Gut Microbiota From Crude Stool Samples

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Aims: The human gut is host to both commensals and pathogens, and the accurate detection of gut microbes effectively and accurately is increasingly important for research and clinical evaluation. Currently, microbiome analysis is performed using 16S amplicon sequencing or whole genome sequencing. In this study, we aim to develop a real-time PCR assay for qualitative and quantitative comparison of bacterial phyla in the gut microbiome from both extracted DNA and crude stool samples.

Methodology: The qPCR assay employed 16S rRNA-based primer sets to amplify 6 bacterial phyla, namely Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Firmicutes, Actinobacteria and Bacteroidetes. An inhibitor-resistant PCR protocol was developed to allow the detection and amplification of gut microbes from direct stool samples with minimal sample processing. The developed assay was validated against extracted individual and pooled DNA, simulating normal and diseased gut microbiota population, as well as crude faecal samples.

Result: We optimized and tested the primer sets against representative bacteria from the 6 phyla, and the qPCR efficiency of the primers ranged from 1.39 to 1.70. The quantitation of simulated microbiome pools had a log10 accuracy of between -0.3 to 0.8. Using the qPCR method on crude faecal sample, we obtained a similar profile to the extracted DNA sample.

Conclusion: This approach provides the healthcare industry with an affordable and rapid method to study the gut microbiota to understand human health. We detected and quantified specific bacterial phyla directly in faecal samples using PCR, eliminating the need for sequencing-based methods, which are tedious, expensive and time-consuming as it involves DNA extraction, library preparation, sequencing and bioinformatics analysis.