ESTABLISHMENT OF PROBE-BASED QPCR ASSAYS FOR EVALUATION OF BACTERIAL MARKERS IN HUMAN FECAL SAMPLES
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BACKGROUND: With the widespread sequencing-based investigation of microbiota, disease-associated bacterial markers are emerging. There is an urgent need to develop a reliable qPCR platform for quantification of fecal bacterial markers to facilitate their application in non-invasive diagnosis. METHODS: We designed a VIC-labeled probe-based internal control qPCR assay targeting 16S rDNA with high coverage of the eubacterial population. We further established a duplex qPCR assay by incorporating a FAM-labeled primer-probe set targeting the colorectal cancer (CRC)-associated F. nucleatum. The abundance of F. nucleatum was examined in feces from 175 healthy subjects and 117 CRC patients. RESULTS: The control assay could evaluate total bacteria with DNA 10 ng/µL in final reactions; higher concentration showed inhibitory effect from the general impurities within fecal DNA. The duplex qPCR assay could quantify F. nucleatum using template 0.1 ng/µL to avoid false-negative assessment for samples low in F. nucleatum. The new qPCR assays were not affected by human DNA contamination thus could also be applied to mucosa samples. Using this platform, enrichment of F. nucleatum was confirmed in both tumorous mucosa and feces from CRC patients. The value of fecal F. nucleatum as a non-invasive marker for CRC diagnosis was also corroborated for the first time in Chinese population. CONCLUSIONS: This study established a reliable probe-based 16S qPCR assay for bacterial marker quantification, which was further refined in the detection of the CRC marker F. nucleatum in fecal samples. This study allows easy and reliable evaluation of bacterial markers using duplex/multiplex qPCR in the future.