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The gut microbiotassay – a high-throughput real-time PCR chip combined with next generation sequencing.

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During the last decade it has become evident that there is a relation between certain medical conditions and the composition of the gut microbiota. To get a better understanding of this complex interaction it is important with high-throughput methods which are sensitive and specific but also informative. Many methods can be used to try to define and characterize the gut microbiota. Here we designed an assay consisting of twenty-four different primer systems targeting the most common bacterial groups of the intestine on different hierarchical levels. The aim of this study was to implement and test this assay with the high-throughput real-time PCR chip "Access Array 48.48" from Fluidigm. The chip executes 2304 individual reactions in parallel and afterwards it is possible to harvest the amplicons for next-generation sequencing. This approach gives a taxonomical overview of the gut microbiota, hence the name: 'the gut microbiotassay'.

The assay was tested on fifteen different bacterial type strains each functioning as target for one or more of the primer systems. In this way the sensitivity and the specificity of the primers were assessed. Next the assay was tested on complex ecosystems by extracting DNA from luminal content from small and large intestine, respectively. A 454-barcode library was added to the samples, and incorporated in the amplicons. Subsequently the amplicons were harvested, and any PCR bi-products were removed in a purification step. Finally detailed information on the bacterial composition for each sample was obtained with the Roche 454 GS FLX sequencing.

The gut microbiotassay had a high specificity and sensitivity, detecting from 50 down to at least 0.05ng/µl when tested on dilution series of pure cultures of bacterial type strains. When applied to complex ecosystems it demonstrated distinct quantities of bacteria in the different gut sections, with the highest number found in colon as expected. From the sequence data it was evident that primer systems targeting lower taxonomical levels, contributed with a higher resolution, revealing species that primer system targeting higher taxonomical levels could not detect. At the same time the results for the different primer systems confirmed one another, as some bacteria were detected on various phylogenetic levels, but all in line with their respective taxonomical classification.

The gut microbiotassay in combination with next generation sequencing both provides a quantitative measure in terms of Cq-values achieved from the real-time PCR, as well as the deeper information obtained from next-generation sequencing of the amplicons. It is quick to perform and offers a high-throughput at a relatively low cost. These features make the gut microbiotassay worth considering, when choosing between current methods used to characterize the gut microbiota.