

Research

# **Comparative efficacy of full-length and partial 16S rRNA gene sequencing in gut microbiome analysis of children with varied weight status**

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# **Abstract**

## **Background**

Obtaining consistent outcomes in the analysis of bacterial microbiomes through next-generation sequencing (NGS) poses challenges due to the diversity of synthetic platforms for 16S rRNA genes and the associated analytical pipelines. This study aimed to tackle this issue by comparing partial-length segments (V3–V4 regions) with full-length segments (V1–V9 regions) in sequencing synthetic 16S rRNA genes extracted from human gut microbiomes. The primary objective of this research was to investigate the differences between these two methods in terms of taxonomic categorization and weight status prediction for twelve children with obstructive sleep apnea.

## **Results**

The full-length NGS technique by Pacbio® successfully captured 118 genera and 248 species from the V1–V9 regions, both with a 100% coverage rate. In contrast, the partial-length NGS platform by Illumina® identified 142 genera (with a coverage rate of 61%) and 6 species (with a coverage rate of 1%) using the V3–V4 regions of the 16S rRNA gene. These methods revealed significant differences in gut microbiome composition. Remarkably, the full-length method was able to differentiate between children with obesity and those without based on the Firmicutes/Bacteroidetes ratio, a

recognized obesity marker ( $p = 0.046$ ). In comparison, the partial-length method showed less conclusive results ( $p = 0.075$ ).

## **Conclusions**

Our findings highlight disparities in NGS-based assessments, emphasizing the value of the full-length NGS method with amplicon sequence variant analysis for clinical research involving the gut microbiome. This underscores the need of meticulous consideration of methodological differences in forthcoming meta-analyses.

**Keywords** Gut microbiome, Next-generation sequencing, Synthetic 16S rRNA gene, Read lengths, Analysis pipelines, Firmicutes/Bacteroidetes ratio, Obesity, Obstructive sleep apnea, Children