Genomic GC-content affects the accuracy of 16S rRNA gene sequencing based microbial profiling due to PCR bias

Profiling of microbial community composition is frequently performed by partial 16S rRNA gene sequencing on benchtop platforms following PCR amplification of specific hypervariable regions within this gene. Accuracy and reproducibility of this strategy are two key parameters to consider, which may be influenced during all processes from sample collection and storage, through DNA extraction and PCR based library preparation to the final sequencing. In order to evaluate both the reproducibility and accuracy of 16S rRNA gene based microbial profiling using the Ion Torrent PGM platform, we prepared libraries and performed sequencing of a well-defined and validated 20-member bacterial DNA mock community on five separate occasions and compared results with the expected even distribution. In general the applied method had a median coefficient of variance of 11.8% (range 5.5-73.7%) for all 20 included strains in the mock community across five separate sequencing runs, with underrepresented strains generally showing the largest degree of variation. In terms of accuracy, mock community species belonging to Proteobacteria were underestimated, whereas those belonging to Firmicutes were mostly overestimated. This could be explained partly by premature read truncation, but to a larger degree their genomic GC-content, which correlated negatively with the observed relative abundances, suggesting a PCR bias against GC-rich species during library preparation. Increasing the initial denaturation time during the PCR amplification from 30 to 120 s resulted in an increased average relative abundance of the three mock community members with the highest genomic GC%, but did not significantly change the overall evenness of the community distribution. Therefore, efforts should be made to optimize the PCR conditions prior to sequencing in order to maximize accuracy.

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