Utilizing Next Generation Sequencing to Generate Complete and Accurate Bacterial Genomic Sequences for Evolutionary Analysis

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ABSTRACT:
Many important questions in the field of prokaryotic biology cannot be answered due to the low availability of sequenced and finished genomes. Recent improvements in technology and decreases in price have made the ambition of de novo bacterial genomic sequencing a reality for a wide range of researchers. However, with the advancement of sequencing technology comes the need for an evaluation to determine the most reliable bioinformatics methods in generating a complete and accurate assembly. Biases inherent in the sequencing technology and GC-rich genomes complicate genome assemblies. Here, we sequenced bacterial strains from the GC-rich Caulobacter genus and the closely related Brevundimonas genus. We found that the Pacific Biosciences RS II sequencing systems was the best sequencer to use in conjunction with the HGAP2 assembler. Using our newly acquired sequences, we found that the genus Caulobacter exhibits extensive genome rearrangements giving the appearance of “Genome Scrambling”. We found that these extensive rearrangements had no correlation to genome relatedness within the genus and that they did not disrupt the conservation of NA1000 essential genes between the species. We also found that using the 16S rRNA region to group these bacteria were as accurate as using entire conserved operons spanning thousands of base pairs.