Genome Sequence Completion Strategy for Recalcitrant Regions Causing Segmented Data

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Background: Completion of bacterial genomic data generated with next generation sequencing technologies is of substantial importance to comparative genome analyses. *Desulfovibrio desulfuricans* ND132 is an important sulfate-reducing bacterium that generates high levels of toxic methylmercury and is currently being elevated to model organism status. *D. desulfuricans* ND132 genome sequencing was performed by the DOE Joint Genome Institute using a combination of Illumina and 454 DNA technologies and produced data consisting of six contiguous segments separated by hard stops. The goal of this work was to develop technologies to overcome these issues and complete the genome.
Methods: Exhaustive PCR optimization with Taq DNA polymerase, several PCR additives and a novel ramped extension cycle PCR amplification protocol were developed as well as the application of thermal-stable strand displacing polymerases.
Results: Successful amplification across the six gaps demonstrated that one gap resulted from a 261 nucleotide assembly error and generated 13 bases of sequence data required for gap closure. PCR products were generated with distinctive electrophoretic hard stop signatures for 3 additional gaps using Taq DNA polymerase. Application of thermal-stable strand displacing polymerases supported amplification across all gaps, without strong stop signatures.
Conclusions: Amplification of all gap regions using strand displacing polymerases confirmed circularity of the *D. desulfuricans* ND132 genome and showed that the genome was within a few hundred bases of completion. With ever more genomes being sequenced and such cases becoming more prevalent, a sequence acquisition strategy tailored to progress toward genome project completion such as that described here is presently relevant and may well become even more important in the near future.