Ligation-mediated PCR Amplification as a Tool to Finish Microbial Genomes

Hope N. Tice[1], Eileen M. Dalin[2], Tanja Woyke[2], Jan-Fang Cheng[2], and Susan Lucas[1]

[1]Lawrence Livermore National Laboratory, [2]Lawrence Berkeley National Laboratory

US Department of Energy Joint Genome Institute, 2800 Mitchell Drive, Walnut Creek, California 94598 USA

As Sanger sequencing is being replaced by higher throughput and lower cost of next generation sequencing, finishing microbial genomes will face two major challenges. First, the technology will need to be fast enough to handle many more drafted genomes. Second, it will have to incorporate a clone-free approach to fill gaps. We have been testing a method that utilizes a universal "bubble-tag" to perform primer walking and gap closure in a clone-free condition. The "bubble-tag" method was first described by Doug Smith (PCR Methods Appl. 2: 21-27, 1992) to amplify and sequence lambda DNA. There is no evidence however, that this approach will work for the more complex microbial genome.

Here we describe the experimentation of this approach in primer walking of the E. coli genome. Genomic DNA was sheared, blunt-end repaired, or digested with frequent cutters, and ligated to bubble adaptors. Site specific primers were used together with the universal bubble primer to amplify and sequence the regions of interest. Different primer concentration was tested in order to generate long amplified templates. This approach enables primer walking and gap filling in a clone-free draft sequencing process. More importantly, the uniformity of this approach is amenable for an automated finishing process.

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