Gap Closure with 454 Reads

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As the Sanger sequencing in the *de novo* assemblies is being replaced by the next generation sequencing reads, finishing of remaining gaps in the 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 be a clone-free approach. We have been testing a method that utilizes a universal "bubble-tag" to perform extended amplification from contig ends and gap closure in a clone-free condition. The "bubble-tag" method was first described by Doug Smith (PCR Methods Appl. 2, 1992) to sequence lambda DNA. The advantage of this approach is that gap closure can be performed from all contig ends without the prior knowledge of contigs' order and orientation. It has not been demonstrated, however, that this approach would work for the complex genomes.

Here we describe the experimentation of this approach in closing gaps of the draft Ktedonobacter racemifer genome. Genomic DNA was sheared and ligated to the bubble adaptors. Primers derived from 96 contig ends were used with the universal bubble primer to amplify the gaps. We applied the AMPure beads to reduce the amount of small fragments. The remaining large amplified DNA appears to be suitable for sequencing. Different bead-to-DNA ratios were tested in order to generate long amplified templates. This approach enables the finishing of complex genomes in a clone-free process with the new sequencing platforms. More importantly, the uniformity of this approach is amenable for a massive, parallel finishing operation.

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