Direct Sequencing of Large Insert Size Clones Using Templates Generated by Rolling Circle Amplification

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Rolling circle amplification (RCA) has been widely used in production sequencing facilities for preparing high quality sequencing templates from small insert size (3 and 8 Kb) clones. This approach, however, has not been successful in preparing sequencing templates from large insert size clones (fosmids and BACs) due to the inconsistency of generating high quality reads. In the attempt to optimize this process, we have tested several conditions including heat lysis of cells, lysis buffer, addition of DMSO, premix to cell lysate volume ratio, and cycle sequencing. We will describe in detail how the various conditions influence the quality of the reads. The results show that a 15 second heat lysis at 95C, with $MgCl_2$ and TE, 10% DMSO, 2 to 1 ratio of premix to cell lysate, and 38 cycles of sequencing reaction give the best sequencing quality. We have applied this condition to sequence 42 plates of fosmid clones derived from two libraries including the soybean and an environmental sample. We have obtained an average read length of 649 bp and a pass rate of 89%. In the initial testing, using clones from 12 different fosmid libraries, we have found that different libraries could generate very different sequence quality under the same condition. We also found that the induction of the fosmid copy number may actually lead to a decrease of sequence quality in particular fosmid libraries. The differences in sequencing quality resulted from different libraries are being investigated. We have begun to test conditions that are suitable for BAC end sequencing using the RCA templates. The preliminary data shows that it is possible to sequence up to 700 bp directly from BACs using the RCA products.

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