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Modification of the 454 LT Paired-end Library Protocol for Constructing Longer Insert Size Libraries

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Paired-end library sequencing has been proven useful in scaffold construction during *de novo* assembly of genomic sequences. The ability of generating mate pairs with 8 Kb or greater insert sizes is especially important for genomes containing long repeats. While the current 454 GS LT Paired-end library preparation protocol can successfully construct libraries with 3 Kb insert size, it fails to generate longer insert sizes because the protocol is optimized to purify shorter fragments. We have made several changes in the protocol in order to increase the fragment length. These changes include the use of Promega column to increase the yield of large size DNA fragments, two gel purification steps to remove contaminated short fragments, and a large reaction volume in the circularization step to decrease the formation of chimeras. We have also made additional changes in the protocol to increase the overall quality of the libraries. The quality of the libraries are measured by a set of metrics, which include levels of redundant reads, linker positive, linker negative, half linker reads, and driver DNA contamination, and read length distribution, were used to measure the primary quality of these libraries. We have also assessed the quality of the resulted mate pairs including levels of chimera, distribution of insert sizes, and genome coverage after the assemblies are completed. Our data indicated that all these changes have improved the quality of the longer insert size libraries.

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