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Modification of the Roche/454 Titanium Paired-end Protocol for a Scale Up Library Production

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Paired-end library sequencing has been proven useful in scaffold formation during the *de novo* assembly of genomic sequences. The current protocol for constructing 454 Titanium paired-end libraries (Roche/454 unpublished) has allowed a routine production of libraries with 3 to 20 Kb insert sizes, which is suitable for assembling large eukaryote genomes with large segments of repeats. This protocol however was designed for manual operation that handles individual tubes, which is laborious and low in throughput. To increase the productivity, we modified the procedure by adopting an 8-strip tube, multiple channel pipette, and 96 magnetic O-ring plate. Other modifications include using the Promega Wizard SV 96-well column instead of gel electroelution after gel separation; replacing nebulization with sonication; and applying AMPure-Beads in certain steps for DNA purification. These changes could increase the productivity of our manual library construction process to 24 libraries a week. These modifications also make it possible for the future development of automatic or semi-automatic procedures. Furthermore, we have also modified some of the steps to improve the library quality and productivity. These steps include applying UV cross linked DNA as carrier DNA and using barcoded adapters in library construction for sequencing pooled libraries.

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