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Automated High-Throughput Fosmid Isolation and End-Sequencing Using Magnetic Beads and Reduced Terminator Cycling Sequencing Reaction Kit

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Automated High-Throughput Fosmid Isolation and End-Sequencing Using Magnetic Beads and Reduced Terminator Cycling Sequencing Reaction Kit

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High quality fosmid end-sequencing plays an important role in whole genome shotgun assembly. Accurate paired end information at the size of about 40 kb is crucial in building large genome scaffolds. We have developed an automated high-throughput fosmid DNA isolation and sequencing protocol using a magnetic bead prep (Agencourt) and terminator cycling sequencing. Using Beckman's Biomek FX without stacker carousel, our throughput is 8 96-well plates in less than 1.5 hours per instrument. After the fosmid DNA is re-arrayed into 384-well format, cycling sequencing performed using reduced reagents and according to our standard production protocol. We are able to achieve a pass rate ($Q_{20} > 50$) of over 95% and average read length (Q_{20}) over 610 bp. Next steps will be to utilize stacker carousels to increase our throughput to 16 plates in one hour and to further reduce sequencing reagents while maintaining high quality. A protocol for 384-well format fosmid preps is also under development.

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