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Automated High-throughput 384-well Format 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. This method uses 384-well format plates from cell growth, DNA isolation to sequencer loading, significantly increases the throughput comparing to the method using 96-well format plates. Using Beckman's Biomek FX without stacker carousel, our throughput is 6 384-well plates in 2 hours per instrument. After the fosmid DNA is eluted, cycling sequencing was performed using reduced reagents and according to our standard production protocol. We are able to achieve a pass rate (Q20 > 50) of over 95% and average read length (Q20) over 650 bp. Next steps will be to utilize stacker carousels to double our throughput to 12 plates in same amount of time and to further reduce sequencing reagents while maintaining high quality.