PREPARING POWERSEQ® AUTO/MITO/YSTR LIBRARIES WITH THE NEOPREP™ LIBRARY PREP SYSTEM

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The Illumina® NeoPrep™ Library Prep System prepares DNA libraries through digital microfluidics technology for sequencing on the MiSeq®. The TruSeq® Nano DNA Library Prep Kit for the NeoPrep™ was used to successfully add adapter sequences to the ends of the amplicons created by the Promega PowerSeq® Auto/Mito/YSTR kit, ranging in size from 129-332 bp, for sequencing. The Illumina® NeoPrep™ instrument (beta test version) was tested and demonstrated to provide results comparable to other automated digital microfluidics platforms while offering enhanced features such as automated quantification and normalization, increased samples per run, and reduced DNA input requirements. Multiple test parameters, including varying concentration of input PCR material, bead concentration, PCR cycles, and sample concentration, were evaluated for sequencing on the MiSeq®. While all conditions gave accurate and reproducible data, runs that included post- PCR input of 25 ng, standard bead concentrations for 350 bp fragments, 6-cycle on-deck PCR, and 10 nM input for sequencing produced optimum results. Inter-locus balance was reduced when using the NeoPrep™ System to generate libraries; however, coverage for all loci was sufficient for accurate genotyping. Comparison of data from the automated library prep workflow to manual library prep workflow, a modified version of the TruSeq DNA PCR-Free adapted by Promega, suggests the imbalance may be the result of size selection that occurs during automated library preparation.