Next Generation Sequencing (NGS) is expanding its applications. Laboratories are increasingly implementing NGS and incrementing the number of samples to process. The ability to construct high quality sequencing libraries in a fast turnaround time has become critical. Automation streamlining library preparation reduces bottlenecks, enables high throughput and minimizes human errors. This work describes the flexible, automated NEBNext Ultra™ II DNA library preparation protocols on the TECAN® Freedom EVO® NGS workstation. This simple workflow allows for highly reproducible library preparation from a wide range of DNA input (picograms to a microgram), and with variable quality (from intact to heavily degraded FFPE samples). Automation of this protocol on the Freedom EVO NGS workstation enables flexible sample numbers from 1-96, and minimizes hands-on time with minimal user intervention. High input (200ng) and low input (500pg) human and yeast genomic DNA libraries generated on the Freedom EVO NGS workstation have comparable library performance (high yield, absence of adaptor dimer) to those obtained from manual libraries. NEXTSeq® sequencing data shows high quality libraries. The high percentage of aligned reads (>97.7% mapped reads and >99.03% mapped in pairs) and the low percentage of chimeras (<1%) and adaptor-mapping reads (<0.001%) observed indicate that the Tecan automation of the NEBNext Ultra II DNA Library Prep workflow enables high quality sequence data, even with very low input amounts. GC content information obtained indicates that automated Ultra II DNA libraries have very uniform coverage across the range of GC content. This automated method provides a much-needed resource for the reliable preparation of DNAseq libraries from a broad range of sample types and input amounts.

ABSTRACT

The indicated observed high workstation preparation quality other distribution amplified Figure 2 results 75 404 Kit sources processing 500pg Input Sequencing with paired amplification which also from libraries the Total Reads Total Reads a 223 High Input Libraries 96 Freedom 99.11% 99.06% 99.11% 99.03% 99.11% Pairs Data, libraries were assessed from a 200ng workflow and This results show that the automated Ultra II DNA libraries have very uniform coverage across the range of GC content regardless of the input amount.

Library Performance and Sequencing Metrics

(A) Library Yield

(B) Library profile and size distribution

(C) Sequencing Metrics

(D) Uniformity of Coverage

CONCLUSIONS

The results presented in this poster demonstrate that automation of the NEBNext Ultra II DNA library preparation workflow on the Freedom EVO NGS workstation provides a fast and efficient solution for library preparation. This setup enables generation of high quality libraries from a broad range of input samples – from 500 pg to 1 pg – while reducing the number of PCR cycles required. The Tecan’s TouchTools interface ensures a user-friendly experience, reducing training needs, minimizing the risk of manual errors and increasing process reproducibility. Combined with flexible processing of up to 96 samples (with or without size selection) and a number of safe stopping points within the protocol, this setup provides highly reproducible, sequencing-ready libraries to suit a variety of laboratory workloads.