Miniaturization of DNA library preparation for Illumina sequencing using automated positive displacement liquid handling

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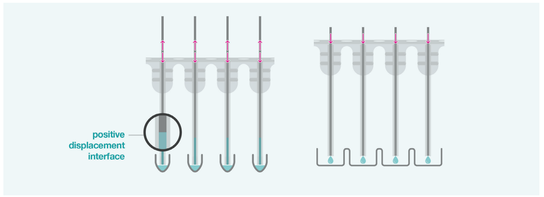
Introduction

Advances in next generation sequencing have resulted in huge increases in throughput with associated decreases in costs. As a result, the process of library preparation has become even more of a financial and time constraint to high throughput sequencing core facilities. Automation, in the form of liquid handling robots, has been able to alleviate some of these bottlenecks. This work describes the application of SPT Labtech's mosquito® HV positive displacement nanoliter liquid handler to successfully implement the preparation of DNA libraries for Illumina sequencing at one tenth the volume of the original manual protocol.

The NEBNext® Ultra™ II FS DNA method was chosen because it uses an enzymatic fragmentation that negates the requirement for a physical fragmentation of the DNA at the start of the library preparation. Using ZymoBIOMICS Microbial Community DNA Standard as genomic DNA the performance of the mosquito HV system was compared to standard volume manually prepared libraries using a range of input amounts of DNA. To determine outcomes when using less standardized input material, reduced volume NEBNext Ultra II FS DNA libraries were generated from individual bacterial isolates and compared to manually prepared Illumina TruSeq Nano libraries generated from the same input material. In addition, the NEBNext Ultra II FS DNA automated libraries were compared to TruSeq Nano and TruSeq PCR Free libraries produced manually, or with traditional automation.



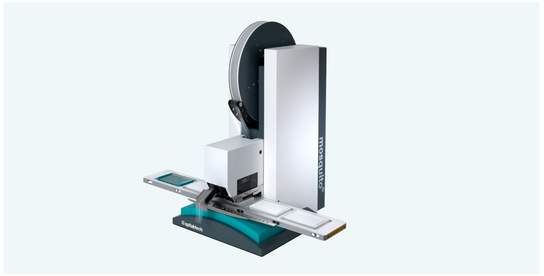


Figure 1. mosquito® positive displacement tip technology (top) and mosquito® HV genomics (bottom).

Acknowledgements

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Manual vs. Automated

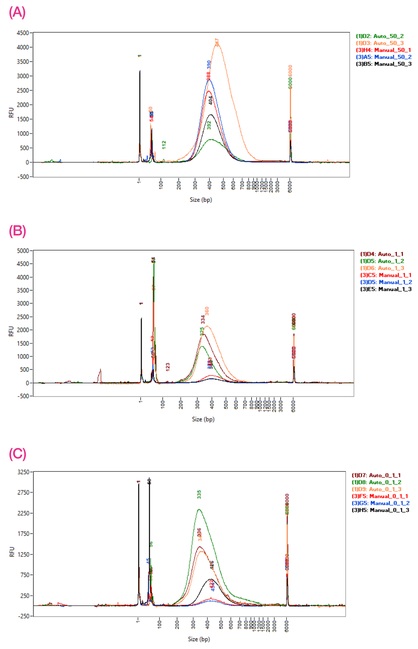

Figure 2. Fragment Analyzer traces comparing the size distribution of manual and 1/10 automated libraries for A) 50 ng, B) 1 ng, and C) 0.1 ng of input DNA.

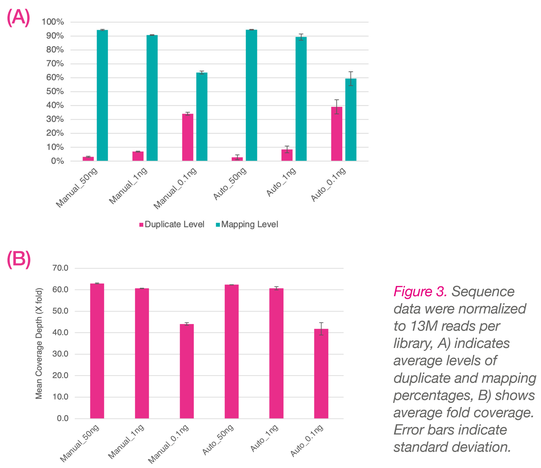

Figure 3. Sequence data were normalized to 13M reads per library, A) indicates average levels of duplicate and mapping percentages, B) shows average fold coverage. Error bars indicate standard deviation.

Comparative performance on clinical and bacterial isolates

Analyses using the ZymoBIOMICS Microbial Community DNA Standard were encouraging, however this is not always indicative of performance when using "real world" samples. Data had previously been generated from clinical isolates of bacterial samples using manually prepared TruSeq Nano libraries, using 100 ng of input DNA. The same samples were used as input into the automated 1/10 volume NEBNext Ultra II FS DNA method with 10 ng of input DNA, and the results compared.

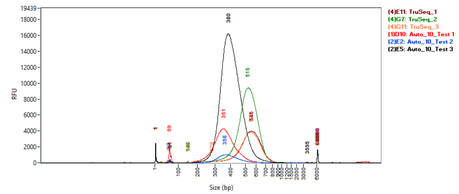

Figure 4. Fragment Analyzer traces comparing the size distribution of manual TruSeq Nano and 1/10 volume automated NEBNext Ultra II FS DNA libraries.

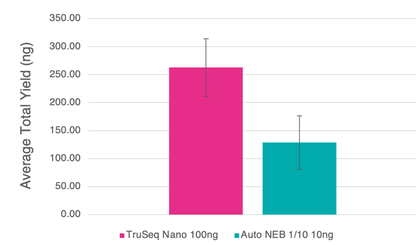

Figure 5. Average yields for the manual TruSeq Nano and 1/10 automated NEB libraries generated from clinical bacterial isolates. 100 ng and 10 ng of input DNA were used for the TruSeq Nano and NEB 1/10 libraries, and 8 and 10 cycles of PCR, respectively. Error bars are standard deviation.

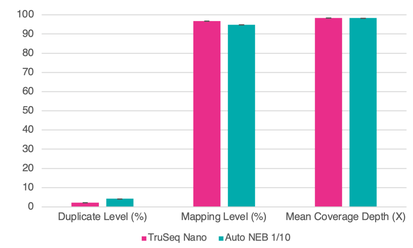

Figure 6. Sequence data were normalized to 1.3M reads per library. Bar chart shows averages of duplicate and mapping percentages, and fold coverage of the genome. Error bars are standard deviation.

Comparison of automated 1/10 volume NEB libraries

to full volume automated and manual TruSeq Nano and TruSeq PCR free libraries

Previously, workflows in the CGR used Illumina TruSeq Nano or TruSeq PCR Free libraries. Data was generated using these methods (manually and with Beckman full volume automation on the FXP system). An input of 100 ng and 1 µg of ZymoBIOMICS Microbial Community DNA Standard was used for the TruSeq Nano or TruSeq PCR Free libraries, respectively. Data was comparable between all three methods when 50 ng of DNA was used as input into the 1/10 volume NEB NEBNext Ultra II FS DNA libraries.

Figure 9. Sequence data were normalized to 13M reads per library. Bar charts show averages of A) percentage duplicates, B) percentage mapping, and C) fold coverage (X) of the mock community. Error bars are standard deviation.

Conclusions

This work demonstrates that NEBNext Ultra II FS DNA libraries prepared at 1 in 10 performed as well as the full volume manually prepared libraries, whilst providing significant cost savings through miniaturization of reaction volumes. The percentage duplicates, mapping levels, fold coverage and GC skew (not shown here) were comparable down to 1 ng of input DNA. The full workflow can be completed within a day enabling larger projects with a greater number of samples, with less technical bias.

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