miniaturisation of DNA library preparation for Illumina sequencing using mosquito® liquid handler

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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 of these improvements 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 study describes the application of one of these systems, the mosquito HV system, to successfully implement the preparation of DNA libraries for Illumina sequencing in one tenth the volume of the original manual protocol. The mosquito systems by SPT Labtech pipette liquids using positive displacement via a spool of disposable tips containing a small stainless steel rod inserted into each tip (Fig 1). This pipetting mechanism means that unlike traditional liquid handlers, the mosquito systems can accurately pipette nanoliter volumes without a requirement for specialist liquid classes being assigned by the instrument control software.

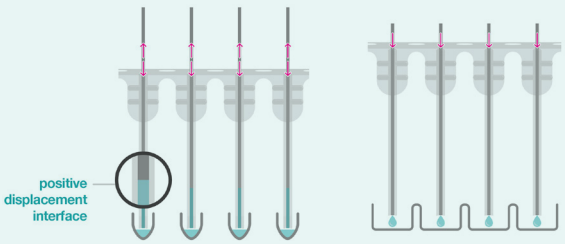
The NEBNext Ultra II FS DNA method was chosen because it uses an enzymatic fragmentation that negates the requirement for a physical fragmentation of the DNA at the start of the library preparation. Using ZymoBIOMICS Microbial Community DNA Standard as genomic DNA the performance of the mosquito HV system was compared to standard volume manually prepared libraries using a range of input amounts of DNA. To determine outcomes when using less standardised 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.

key benefits

mosquito HV genomics, a fully open liquid handler:

- provides low-cost DNA library preparation through miniaturisation of reagent volumes
- increases reproducibility and data quality with accurate pipetting at low volumes irrespective of liquid viscosity
- enables fast and high-throughput microbiome studies

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



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manual vs automated

To test the NEBNext Ultra II FS DNA library kit the ZymoBIOMICS Microbial Community DNA Standard (Cat. No. D6306) was used. This standard contains a known DNA composition of 10 microbial strains which enables users to assess the performance of entire metagenomic workflows, highlighting bias and errors that can occur during processing. It is therefore ideal for testing library preparation kits. It also enabled a direct performance comparison to previously tested library kits utilised at the CGR. Total input amounts of 50ng, 1ng, and 0.1ng were tested in triplicate. A schematic is depicted in Figure 2.

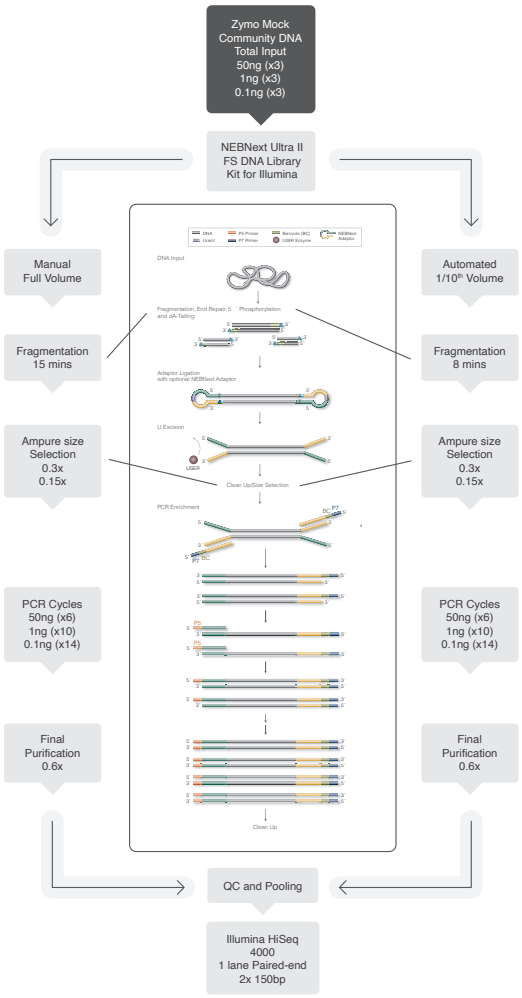


Figure 2. Schematic representation detailing how NEBNext libraries were generated using the ZymoBIOMICS Microbial Community DNA Standard as input DNA. Manual libraries were made using full volumes according to protocol, automated libraries were made using 1 in 10 volume on the SPT mosquito HV platform.

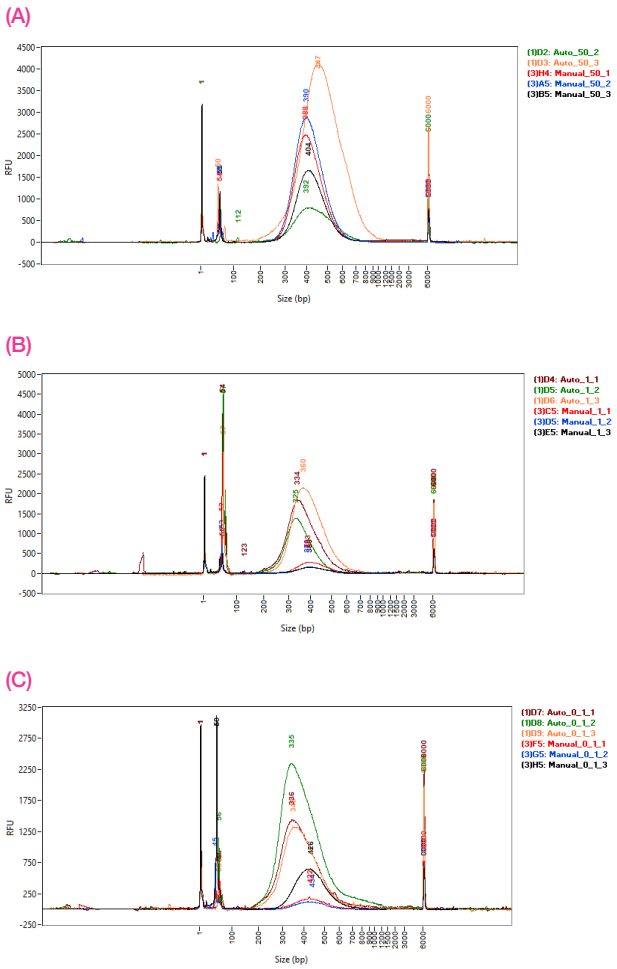


Figure 3. Fragment Analyzer traces comparing the size distribution of manual and 1/10 automated libraries for A) 50ng, B) 1 ng, and C) 0.1 ng of input DNA.

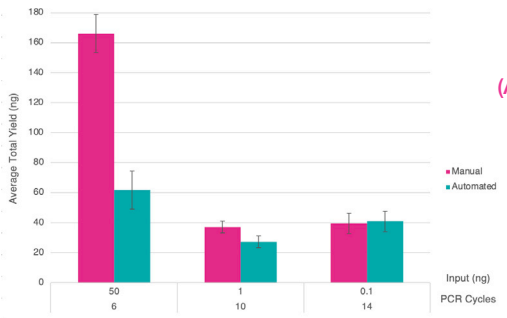


Figure 4. Bar chart showing average yields for the manual and 1/10 automated libraries with differing input amounts and PCR cycle numbers. Error bars indicate standard deviation.

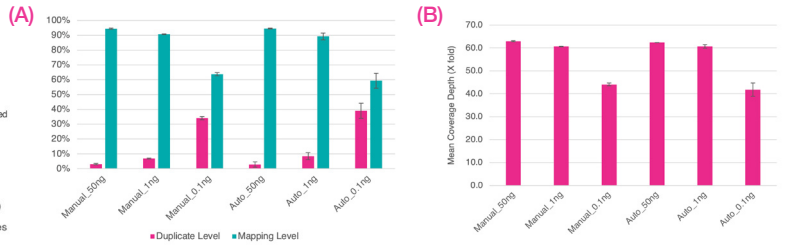


Figure 5. Sequence data were normalised to 13M reads per library, A) indicates average levels of duplicate and mapping percentages, B) shows average fold coverage. Error bars indicate standard deviation

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 in 10 volume NEBNext Ultra II FS DNA method with 10 ng of input DNA, and the results compared.

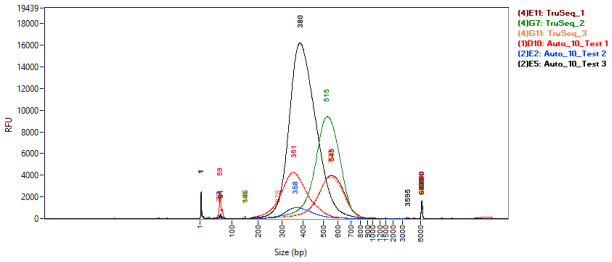


Figure 6. Fragment Analyzer traces comparing the size distribution of manual TruSeq Nano and 1/10 volume automated NEBNext Ultra II FS DNA libraries.

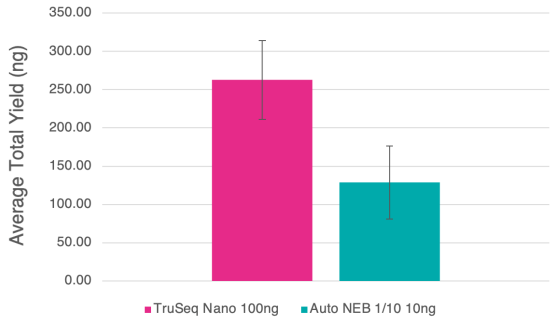


Figure 7. Bar chart detailing average yields for the manual TruSeq Nano and 1/10 automated NEB libraries generated from clinical bacterial isolates. 100 ng and 10 ng of input DNA were used for the TruSeq Nano and NEB 1/10 libraries, and 8 and 10 cycles of PCR, respectively. Error bars are standard deviation.

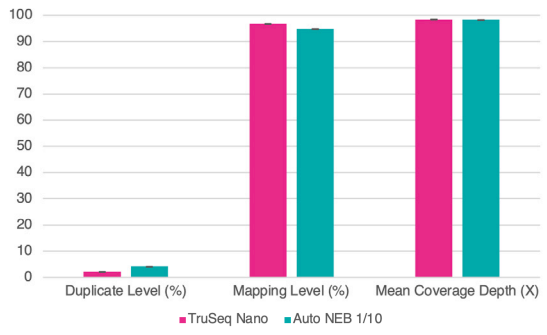
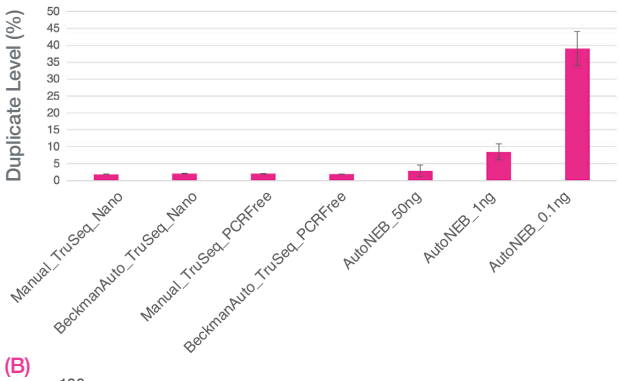


Figure 8. Sequence data were normalised to 1.3M reads per library. Bar chart shows averages of duplicate and mapping percentages, and fold coverage of the genome. Error bars are standard deviation.

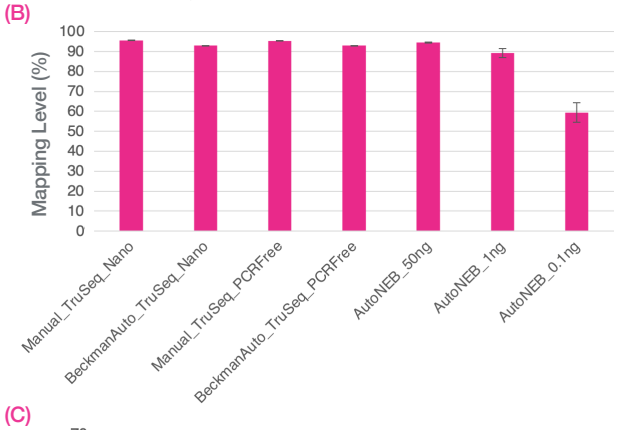
comparison of automated 1/10 volume NEB Ultra II FS libraries to full volume automated and manual TruSeq Nano and TruSeq PCR free libraries

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 1µg 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 in 10 volume NEB NEBNext Ultra II FS DNA libraries.

(A)



(B)



(C)

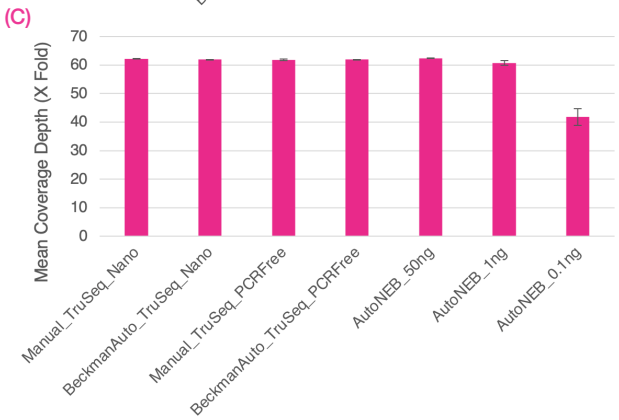


Figure 9. Sequence data were normalised to 13M reads per library. Bar chart shows averages of A) percentage duplicates, B) percentage mapping, and C) fold coverage (X) of the mock community. Error bars are standard deviation.

conclusion

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 miniaturisation 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.

acknowledgements

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