Achieving cost-effective NGS through automated miniaturization of Illumina Nextera XT library preparation

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Introduction

While progress in next-generation sequencing (NGS) technology has led to improved speed and throughput, the library preparation process remains a significant challenge in terms of cost and manual labor for high-throughput applications.

SPT Labtech's mosquito® liquid handler uses true positive displacement technology to accurately handle low volumes (500 nL-5 µL) of samples and reagents, irrespective of liquid viscosity or environmental conditions (Figure 1). It has been widely used to miniaturize a variety of library preparation protocols without compromising accuracy and reducibility.

This poster highlights how mosquito HV genomics has been used to automate the Illumina Nextera XT protocol at SEQ-IT, achieving 5X miniaturized reaction volumes while delivering reproducible, high-quality libraries. This miniaturized method was developed to identify and screen mutations with oncogenic potential and drug resistance (HIV, HBV, HCV), SNP detection, as well as in HLA-typing, among other applications.

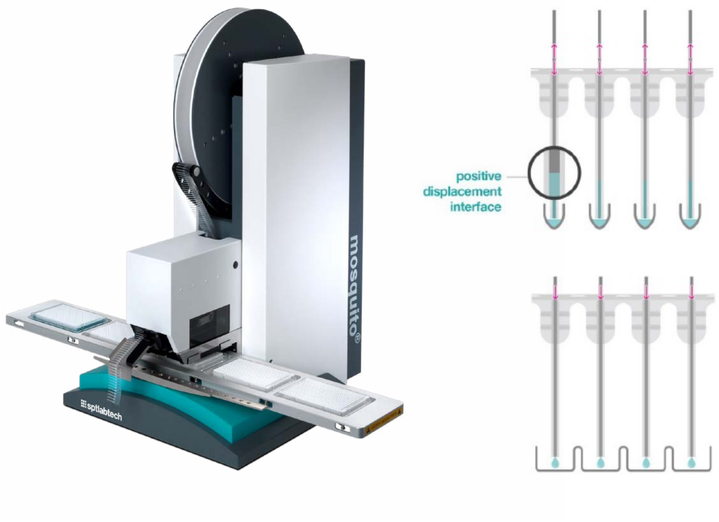

Figure 1. mosquito HV genomics liquid handler equipped with 5 deck positions (left), schematic representation of positive replacement technology (right).

Method

DNA extracted from patient samples is subjected to amplicon sequencing, targeting a range from 300 bp to 10 kb, across different areas of diagnostic interest, including immunogenetics, oncology, coagulation disorders, and infectious diseases.

The 5X miniaturized Illumina Nextera XT protocol on mosquito HV genomics is used to prepare libraries for 660 samples per month on average, amounting to almost 8,000 library preparations per year.

Library preparation for 48 samples takes 5.5 hours, while processing 96 samples requires 6.5 hours, including PCR cycles. Sequencing is performed using the Illumina MiSeq system.

Figure 2. Graphical representation of the Illumina Nextera XT protocol indicating the pre-PCR steps (pink) and post-PCR steps (teal).

A representative sequencing run of 50 samples was prepared and sequenced on MiSeq using 500cycle v2 sequencing reagent in a 2x250nt mode. A breakdown of the 50 samples is shown in Table 1. The DNA concentration as input for tagmentation was 0.2 ng/µL.

Table 1. A breakdown of the 50-sample representative sequencing run prepared by the 5X miniaturized Illumina Nextera XT protocol on mosquito HV genomics.

Sample type	Size (kb)	Number of samples
HIV-1 amplicons	2.5	42
HIV-1 whole genome	9	6
Negative controls	N/A	2

Results

Sample library size was analyzed by Bioanalyzer after amplicon tagmentation and PCR amplification of fragmented DNA (before normalization). The results show a typical size distribution with an average size of approximately 500 bp, indicating good tagmentation quality (Figure 3).

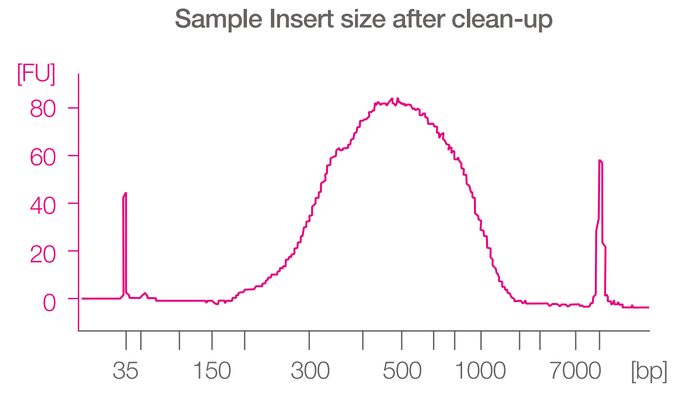

Figure 3. The Fragment Analyzer trace shows a typical size distribution of the sample insert pool after tagmentation and bead clean-up.

99.54% of the reads were properly mapped (Figure 4) and 84.3% of the sequencing data presented a QC score ≥30, which is in the expected range of this amplicon approach (Figure 5).

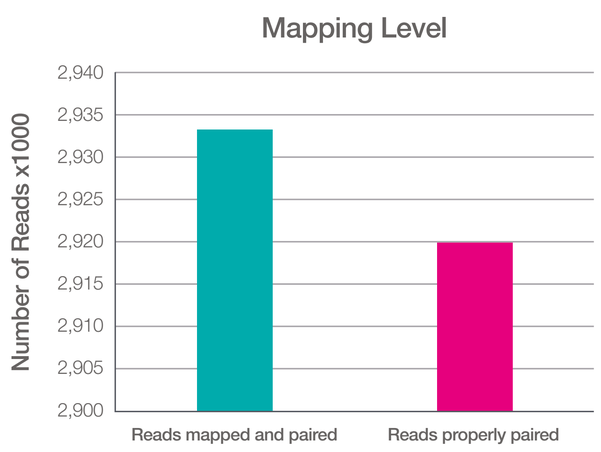

Figure 4. Quality control data showing that 29,200,000 out of 29,333,582 total reads were properly mapped, corresponding to a mapping level of 99.54%.

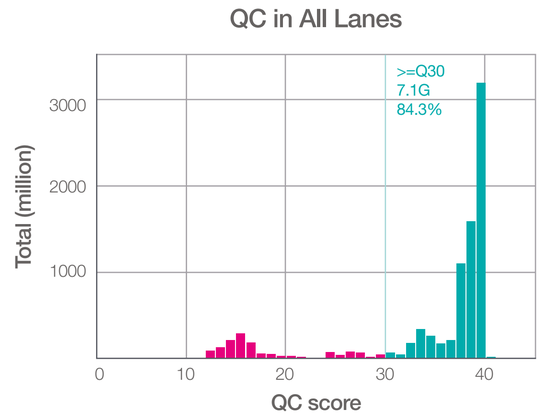

Figure 5. 84.3% of the sequencing data presented a QC score ≥30.

The desired output of each sample had been calculated in advance to evaluate the accuracy of mosquito's performance. The proportion of the 42 HIV-amplicons (2.5 kb) were calculated to match approximately 2% each, whereas the 6 HIV-1 genomes should have a proportion of 1% each (Figure 6). The proportion of each individual sample to the total output agrees with the expected percentage, highlighting the high accuracy.

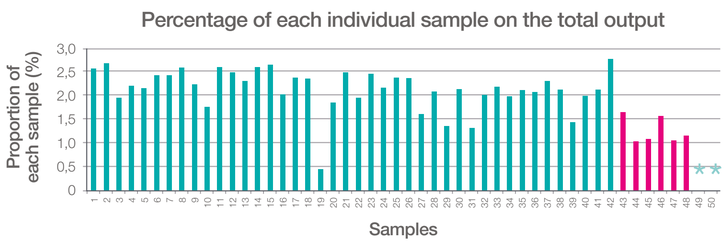

Figure 6. Proportion of each sample on the total output. The 42 HIV amplicons are presented by blue and the 6 HIV whole genome samples are highlighted by purple. Sample 19 had a low DNA input concentration. The negative controls are indicated by teal asterisks.

Conclusions

Miniaturizing Illumina Nextera XT library preparation on mosquito has delivered significant cost savings of up to 80% without compromising sequencing data quality. Metrics such as sample fragmentation levels, mapping rates, QC scores, and the distribution of each sample in the total output demonstrated excellent results, even at 5X miniaturized volume. Moreover, the entire workflow can be completed within a few hours, supporting higher throughput for larger projects while requiring minimal sample input.

Additionally, this miniaturized workflow reduces the burden of manual pipetting and minimizes plastic waste, making it both a cost-effective and a more environmentally sustainable solution. By addressing common bottlenecks, this approach facilitates the broader adoption of NGS technologies across diverse applications.