mosquito® HTS
application note


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automated low-volume DNA normalisation and NGS library prep for single-cell analysis

introduction

As the per base read cost of next-generation sequencing (NGS) has decreased, the library preparation cost, especially in high-throughput applications, such as single-cell analysis, has become a larger portion of the total cost.

single-cell analysis provides whole genome and transcriptome sequencing from a single cell. Because single cells have small quantities of DNA and mRNA, any degradation, sample loss or contamination can have profound effects on the quality of sequencing results. Recent technical improvements have made single-cell analysis and sequencing a powerful tool. These technologies include the use of automated low-volume liquid handlers.

Nextera XT sample prep kits (Illumina, Inc., USA) are commonly used to prepare DNA libraries for NGS. Insert sizes of generated libraries are dependent on the ratio of DNA and tagmentation enzyme in the reaction. Therefore having a precise and accurate liquid handler is essential in library prep workflows. Normalisation of the libraries prior to sequencing is necessary to ensure there is a similar number of reads and coverage of base pairs per each sample.

To assure high accuracy and precision, most library preparation protocols recommend volumes that are within the range of manual pipettes or that of large volume liquid handlers.

This application note presents data from Prof. Stephen Quake's lab, Stanford University, USA on the use of TTP Labtech's automated low-volume liquid handlers for miniaturising DNA normalisation and library prep volumes for single-cell analysis.

low-volume liquid handlers for miniaturised genomic applications

Sample preparations for high-throughput, low-volume genomics applications require pipetting of very small volumes of various reagents and samples with a varying range of liquid viscosities. Large volume liquid handlers often fail to deliver sub-microlitre volumes of these reagents accurately and precisely. TTP Labtech's mosquito range of liquid handlers (25 nL - 5 µL) uses true-positive-displacement pipetting technology to accurately prepare miniaturised reaction volumes. The low-cost, disposable pipette tips avoid cross contamination (Fig 1).

"mosquito HTS has reduced hands-on time for normalisation and library prep from two weeks to a single day while increasing the accuracy and lowering the cost at the same time."
Dr. Rahul Sinha, Prof. Irving Weissman's group, Stanford University

Fig 1. TTP Labtech's (a) mosquito HTS (8- or 16-channel); and (b) true-positive-displacement tips

key benefits

automating and miniaturising single-cell analysis sample prep

- **reduces volume and cost**
- **enables fast and high-throughput studies**
- **reduces input DNA/RNA to pg values**

case study 1: single-cell DNASeq

Dr. Feiqiao (Brian) Yu, a postdoctorate in Prof. Stephen Quake's lab at Stanford University, CA, USA, has applied the technique of single-cell analysis to identify novel bacterial species from environmental metagenomic samples.

To evaluate the integrity of low-volume sample prep the reactions were prepared at large (12.5 µL) and miniaturised (4 µL) volumes. The large volume sample preps were performed manually (data not shown) while TTP Labtech's mosquito X1 and HTS were used for the lower volume normalisation and library preparation, respectively.

methods

A mixture of 5 known bacterial species (6-20kb) was separated into single cell samples, using limiting dilution method. Genomic DNA (gDNA) was amplified and quantified using Fragment Analyzer (Advanced Analytical Technologies, Inc.). The DNA concentration of each sample was normalised prior to library prep to a desired range of 0.1 – 0.3 ng/µL (optimal for Nextera XT library prep). Single-tip mosquito X1 was used to pool and, at the same time, normalise libraries from 4 different 96-well PCR plates into a single 384-well PCR plate for downstream library prep steps.

Nextera XT libraries were generated using 16-channel mosquito HTS, at a final volume of 4 uL, using only 60 pg of DNA.

results

High quality data were generated with miniaturised volumes for each single-cell library (Fig 2). Lowering the volume for normalisation and library prep not only reduced the cost and enabled higher throughput but also reduced the amount of required input DNA, significantly.

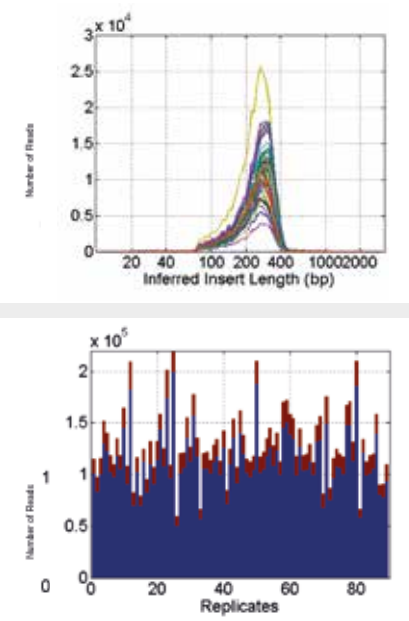

Fig 2. (a) Inferred insert length for 4 µL Nextera XT libraries. Mean insert sizes are ~300 bp, as expected in standard volume libraries. (b) The percentages of trimmed reads show less than 10% of reads being rejected for each library, confirming high quality data. The red and blue denote the number of rejected and accepted reads, respectfully.

case study 2: single-cell RNASeq

In a second study Dr. Feiqiao (Brian) Yu characterised the differences in gene expression levels between healthy and abnormal mouse macrophages using low-volume single-cell RNAseq methods.

methods

96 cells were sorted and cDNA were synthesised and amplified using the C1 Single-Cell Auto Prep System (Fluidigm Inc.). Resulting cDNAs were extracted from the C1 chip and quantified using Fragment Analyzer (Advanced Analytical Technologies, Inc.). mosquito X1 was used to normalise cDNA to a final concentration of 0.2 ng/µL, using an easy-to-use and automated software interface. The software determines the amount of cDNA and buffer required based on original and final concentrations, making the normalisation calculation seamless. 4 µL Nextera XT libraries were prepared using mosquito HTS, with only 80 pg of cDNA.

results

All the reads mapped at over 78%, indicating reliability of the data at 4 µL total Nextera XT library prep volume (Fig 3).

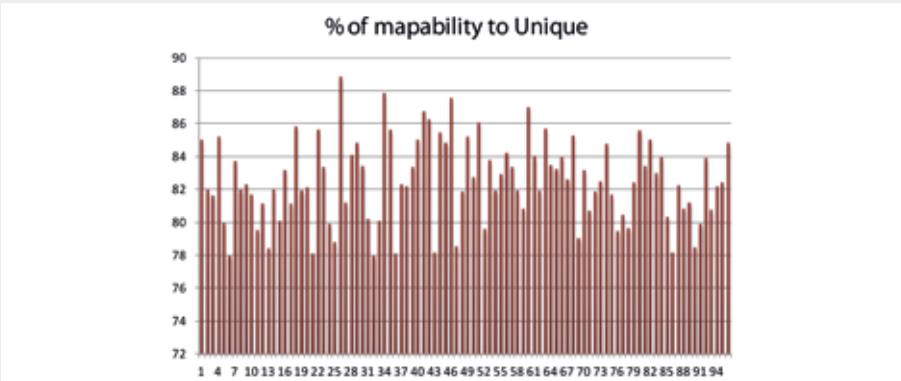

Fig 3. Percentage of reads from 96 different macrophage cells mapped uniquely to the mouse reference genome (exons, introns and translation start sites). All the reads mapped at over 78%, indicating reliability of the data at 4 µL total Nextera XT library prep volume.

conclusions

This work demonstrates that gDNA and cDNA Nextera XT libraries prepared at low volumes such as, 4 µL retain the complexity and integrity of the samples. This highlights the advantages of using an accurate, nanolitre-scale liquid handling system based on true-positive displacement for both the normalisation and library preparation steps of NGS applications.

TTP Labtech's true-positive-displacment liquid handlers (25 nL - 5 µL) provide:

- reduced cost
- low dead volume
- decreased DNA/RNA input
- automated cherry picking and plate reformatting
- fast, accurate and reliable pipetting
- simplicity of use and a small footprint
- a low cost instrument, fully integrable
- gentle pipetting

acknowledgements

We would like to thank Dr. Feiqiao (Brian) Yu, Dr. Rahul Sinha, Prof. Stephen Quake and Prof. Irving Weissman at Stanford University, USA, for their collaboration and providing the data presented in this application note.

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