Methods for reduced cost and lower sample prep volumes for genetic analysis applications

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introduction

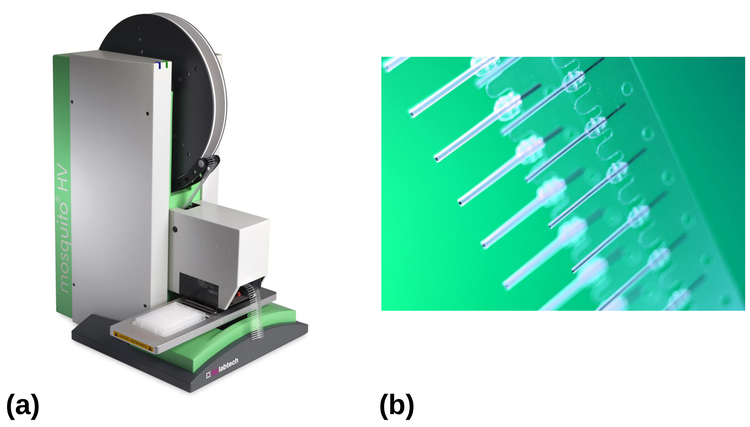
As the cost of NGS has decreased, the library preparation cost has become a larger portion of the total expenditure. This is especially true for high-throughput applications, such as single-cell analysis. Therefore, there is a need to develop methods that can not only study the transcriptomes of single cells, but can also feasibly analyze large numbers of single cells.

Miniaturizing the sample preparation volume provides the opportunity for significant cost savings. Using TTP Labtech's mosquito liquid handlers, reagent and sample quantities can be scaled down to picogram values.

automated, low volume liquid handling

mosquito® HTS (25 nL – 1.2 µL) and **mosquito HV** (0.5 – 5 µL) are automated 8- or 16-channel liquid handlers. Being based on true-positive displacement technology they enable fast, accurate, gentle and contamination-free liquid transfer, essential for genetic analysis applications (Fig1a).

mosquito® X1 (25 nL – 1.2 µL or 0.5 – 5 µL) is an automated single channel liquid handler. It's low dead volume (< 0.5 µL) is ideal for DNA normalization. mosquito's easy-to-use software calculates the required volumes of buffer and DNA in order to perform the normalization process seamlessly. This is especially beneficial for precious samples, such as genomic materials derived patient specimens.



(a) (b)

Fig 1. (a) mosquito HV liquid handler, (b) mosquito tips, with true-positive displacement pistons

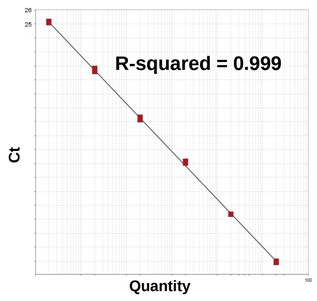
1. low-cost KAPA library quantification sample prep

qPCR-based quantification kits from Kapa Biosystems Inc. (Wilmington, USA) are commonly used to accurately quantify NGS libraries. In this work, utilizing a precise and accurate automated liquid handler, such as mosquito HV, the qPCR reaction volumes are accurately miniaturized, resulting in significant cost savings during the quantification process. The results were compared to those from manual pipetting.

methods

6 different DNA libraries at an average size of 490 bp were prepared using TruSeq Nano DNA library prep kit (Illumina Inc., USA). The libraries were quantified using KAPA library quantification kit *(*code: KK4824) at a total volume of 10 µL in triplicate, both manually and using mosquito HV. This is a two-fold reduction in volume compared to standard reactions.

results



(a)

Manual Concentration (nM)	mosquito HV Concentration (nM)	%Error
28.10	30.70	9.25
24.70	26.00	5.26
32.50	30.90	-4.92
31.60	28.60	-9.49
32.30	32.60	0.93
41.30	44.00	6.54

(b)

Fig 2. (a) Standard curve, (b) concentrations of DNA libraries determined manually and using mosquito HV. Percent of errors for mosquito HV vs. manual are shown.

2. low-cost SYBR-Green qPCR

A novel qPCR gene expression (GEX) system was used to analyze the effects of peptide stimulation on the transcriptome of human-derived immunological cells. In these types of studies, the ability to interrogate a large range of target genes is important to ensure robust data.

For high-throughput analysis, robotic liquid handlers greatly simplify the process, assist with sample tracking, and reduce pipetting errors, as well as operator fatigue.

This study uses TTP Labtech's mosquito HV to minimise reaction volumes, demonstrating its role in high-throughput qPCR assays. Most importantly it allows valuable and limited samples to be analyzed multiple times from the same up-stream reaction (for example, a patient-derived or archival sample).

methods

11 human immune cell lines were simulated with up to 6 different peptides. 6 different genes (including 2 housekeeping genes) were studied via GEX analysis using FastStart Universal SYBR Green master (Roche Diagnostics, USA).

The qPCR reactions were prepared using 1 µL cDNA in a total volume of 5 µL and in 384-well plates, using mosquito HV.

results

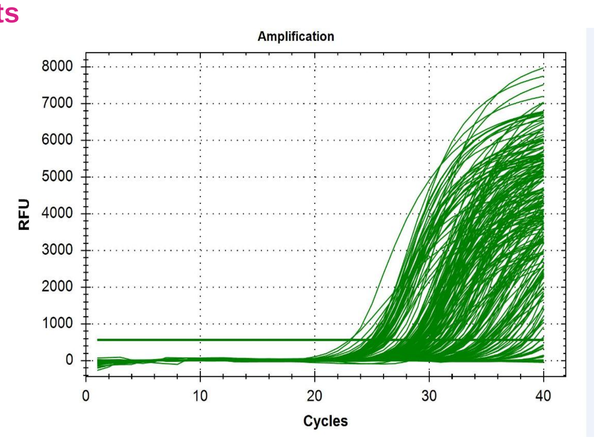


Fig 3. The amplification results of 56 samples for 6 genes of interest. Using the mosquito HV did not affect the amplification profile of these genes. A 4-fold reduction was achieved in reaction volume, sample input and cost.

3. low-cost SMARTer® ultra low RNA input sample prep

SMARTer ultra low input RNA kit (Clonetech Laboratories, Inc., USA) is a reverse transcription cDNA synthesis kit designed for ultra low input RNAseq down to single-cell input levels.
For cost-saving purposes, in recent years, it has been of interest to sort cells using a cell sorter and then perform low-volume cDNA synthesis, instead of costly microfulidics solutions.

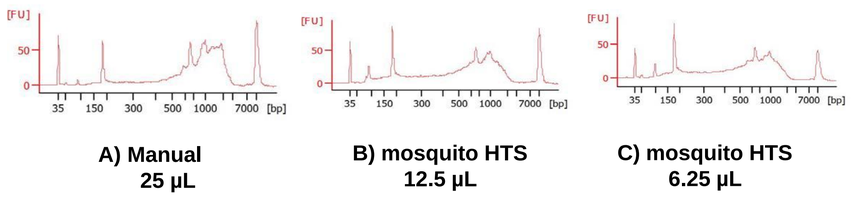
This study set out to reduce the reaction volumes for the cell lysis, reverse transcription and cDNA synthesis using mosquito HTS liquid handler and compare the results of manual reaction set ups.

methods

In order to reduce the cost and required sample input, mosquito HTS liquid handler was used to miniaturize the cDNA synthesis reaction volumes down to 12.5 and 6.25 µL, using limiting dilution of K562 cells.

The samples consisted of minimally diluted K562 single cells. As a comparison, a 25 µL reaction volume was set up manually with 10 of K562 cells.

results



A) Manual 25 µL B) mosquito HTS 12.5 µL C) mosquito HTS 6.25 µL

Fig 4. Validation of the quality of reverse transcription process of total RNA for limiting dilution of K562 cells, using Bioanalyzer data (Agilent Technologies, Inc., USA).
The graphs clearly show that lowering the reaction volumes by 2X, 4X and 8X did not affect the quality of cDNA synthesis, while providing 8 times cost saving per sample prep, compared to the original kit.

4. low-volume DNA normalization & low-cost Nextera XT library prep

Nextera XT sample prep kit (Illumina, Inc., USA) is commonly used to prepare DNA libraries. During this process, it is essential to have a precise and accurate ratio of tagmentation enzyme to DNA sample to obtain fragments of the correct size. Almost all library prep protocols recommend reagent volumes that are within the range of manual pipettes, or that of larger volume liquid handlers.

Here, we present automated miniaturized DNA normalization and then Nextera XT library prep at sub microliter volumes.

methods

The differences between macrophages associated with healthy and abnormal mouse tissues were studied using single-cell RNAseq.

96 cells were sorted and cDNAs were synthesized using C1 system (Fluidigm Corp., USA). mosquito X1 (Fig. 6) was used to normalize cDNAs to a final concentration of 0.2 ng/µL, using an easy-to-use software interface.
The software determines the amount of cDNA and buffer required based on original and final concentrations making the normalization calculation seamless. 4 µL Nextera XT libraries were prepared using mosquito HTS, using only 80 pg of cDNA.

results

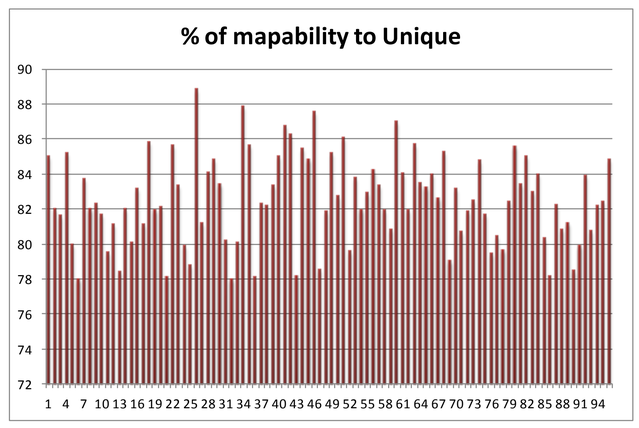


Fig 5. The percentage of mappability of reads from 96 different macrophages to unique mouse reference genome (exons, introns and translation start sites). All the reads mapped over 78% at 4 µL, indicating reliability of the data.

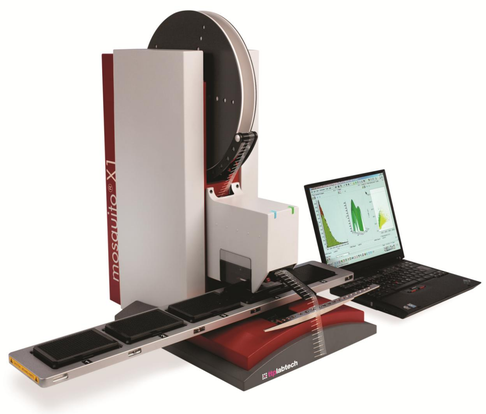


Fig 6. TTP Labtech's mosquito X1 single tip automated liquid handler

conclusions

mosquito liquid handlers (TTP Labtech, UK) have been validated in setting up reduced cost and reduced reaction volumes in genetic analysis applications.
The system provides:

- low cost through minimizing reagent volumes
- reduced sample input down to pg values
- gentle pipetting
- fast, accurate and reliable low volume liquid handling
- simplicity of use, small footprint, low cost of the instrument and being fully integrable
- accurate normalization of DNA at very low volumes, essential for variety of library prep protocols

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