Low-volume, automated single cell RNA-seq library preparation with mosquito liquid handlers

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Introduction

Single-cell sequencing requires an individual cell per sample and therefore a large sample size is necessary to provide valid results.

Reproducibility and sensitivity can be challenges for these single-cell assays in addition to the scalability and cost when large numbers of cells are analysed. Automation and miniaturisation can address these limitations.

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. However, only a small proportion of each of the libraries prepared will be required for the sequencing, therefore reducing the library prep volume will reduce costs.

This poster presents a low-cost and miniaturised method to create RNA sequencing libraries of single human pancreatic embryonic stem cells using mosquito automated low-volume liquid handlers (TTP Labtech, Melbourn, UK). Technical and biological variability was compared between three different reaction volumes (2, 4 and 8 µL) and between four replicates.

automating low-volume single-cell library preparation

Using low-volume liquid handling in sample preparation and bead purification for genomics applications is especially beneficial when working with precious and low abundance samples such as DNA/RNA derived from patient specimens or single-cell analysis.

mosquito® HTS (25 nL – 1.2 µL) and **mosquito® HV** (0.5 – 5 µL) are automated 8- or 16-channel liquid handlers. Based on true positive-displacement technology they enable fast, accurate, gentle and contamination-free liquid transfer, essential for genetic analysis applications where viscose gDNA and reagents are being handled (Figure 1).

The benefits of mosquito for single-cell analysis are:

1. **speed** rapid pipetting (1.5 min per reagent in 96-well plate, 3 min per reagent in 384-well plate)
2. **accuracy** having an inaccuracy below 5% for any liquid type
3. **high-throughput** compatible with 96 and 384-well plates and therefore can rapidly process large numbers of samples

Figure 1. TTP Labtech's mosquito (a) HTS and (b) HV low-volume, automated liquid handlers

Case study: Single-cell analysis of differentiated human pancreatic stem cells

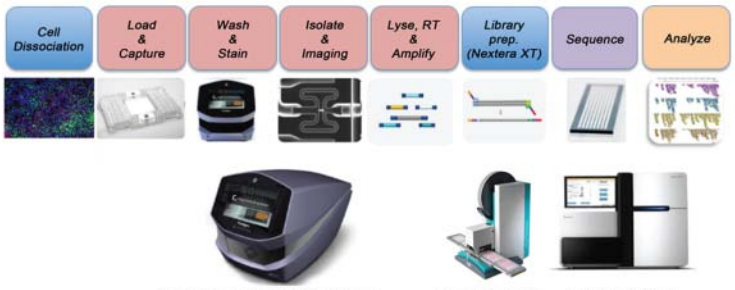

Figure 2. experimental design

method

WA09 human embryonic stem cells were differentiated *in vitro* to the pancreatic lineage. Cells from stages 1 and 2 were collected and analyzed as follow: An average cell concentration of 2.5 x 105 cells/ mL was loaded into the C1 Single-Cell Auto Prep System (Fluidigm, San Francisco, USA), which generated amplified cDNA in a sequence of single cell sorting, cell lysis, reverse transcription and cDNA amplification. Two independent cells from stage 1 (cell A and cell B) and two cells from stage 2 (cell C and cell D) were selected and yielded with similar cDNA concentrations of 0.1 ng/µL and then converted to Illumina sequencing libraries using the Nextera XT kit (Illumina, San Diego, USA) on mosquito HTS liquid handler and at reduced reaction volumes and lower sample input. Libraries were generated in three different final reaction volumes (2, 4 and 8 µL) in quadruplicate in 384-well plates using 20, 40 and 80 pg of cDNA per reaction (Fluidigm recommend volumes is 12.5 µL with 125–375 pg minimum input cDNA) (Figure 3).

Mosquito HTS and HV enabled accurate and reproducible pipetting for each of the Nextera XT (and bead clean up steps) in the sequencing process as illustrated in Table 1.

The resulting sequencing data was analysed and correlations between the three different reaction volumes were assessed for quality and reproducibility.

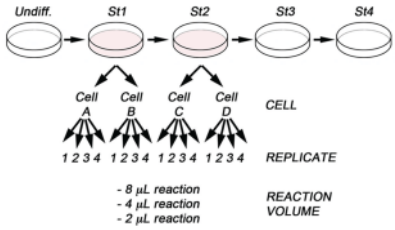

Figure 3. WA09 human embryonic stem cells were differentiated *in vitro* to the pancreatic lineage.

Table 1. Volumes of reagents and cDNA pipetted by mosquito HTS for total reaction volumes of 2, 4 or 8 µL.

	Reaction volumes		
	2 µL	4 µL	8 µL
Atm buffer (nL)	200	400	800
TD buffer (nL)	400	800	1,600*
cDNA 0.1 ng/µL (nL)	200	400	800
NT buffer (nL)	200	400	800
NPM buffer (nL)	600	1,200*	2,400*
Index N#/S# (nL)	200 N# 200 S#	400 N# 400 S#	800 N# 800 S#

*total volume was made up of multiple pipetting of smaller volumes

results

miniaturisation does not affect technical reproducibility

The mean correlation coefficient was > 0.936 between each replicate for each cell at each reaction volume. The correlation coefficients between different reaction volumes for a given cell were all greater than 0.918 (data not shown). There were no significant differences between the overall CVs and the CVs from each reaction volume separately.

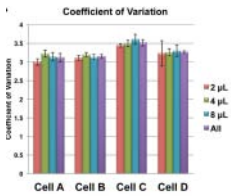

Figure 4. Mean coefficients of Variation(CVs) for each reaction volume for each cell calculated from DESeq normalised data. The purple bar is the mean CVs for each cell, irrespective of reaction volume.

miniaturisation does not affect biological variation

Using two different clustering methods (principal component analysis and Hierarchical clustering), a clear separation was observed between the libraries from each of the four cells.

Importantly, the libraries did not cluster according to reaction volume, even within a single cell.

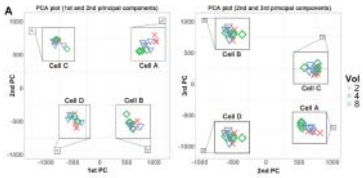

Figure 5. Principal component analysis (PCA).

miniaturisation does not affect the RNAseq data complexity

Greater than 78% of all transcripts were present for all three reaction volumes .

conclusions

- mosquito HTS and HV enable automated library preparation and bead clean up for single-cell transcriptome sequencing at markedly lower reaction volumes and cost, without compromising reproducibility, quality, or complexity of the resulting libraries
- automating and miniaturising single-cell RNAseq library prep volumes with mosquito liquid handlers
  - enables a substantial reduction in input cDNA
  - provides potential cost savings of over 4-fold and library prep costs of less than $2 USD per single cell
  - high reproducibility and accuracy for high viscosity solutions
- this technical advance will make analysis of hundreds to thousands of single cells more feasible

acknowledgments

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