miniaturization and automation of CEL-Seq2 and SMART-Seq2 using the mosquito liquid handler

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introduction

Plate-based workflows with flow-sorted single cells allow the analysis of rare cell types and subpopulations characterised by cell surface markers. Automation and miniaturisation are key to make such single cell sequencing studies practical and cost-effective at high throughput. Liquid handling steps on robotic platforms must be accurate and robust to avoid any loss of material, prevent cross-contamination, and limit the introduction of technical error.

Here we present the use of SPT Labtech's mosquito® liquid handlers to automate and miniaturise two different single cell RNA-seq protocols with FACS-sorted cells in 384-well plates.

accurate low-volume liquid handling with true-positive displacement

SPT Labtech's positive displacement pipetting technology enables accurate and precise liquid handling in the nanoliter-to-microliter volume range.

mosquito HV has a pipetting range of 500 nL – 5 µL, bridging low- and high-volume liquid handling. mosquito HV is compatible with semi-automated magnetic bead purification.

mosquito LV has a pipetting range of 25 nL – 1.2 µL offering accurate assay miniaturization.

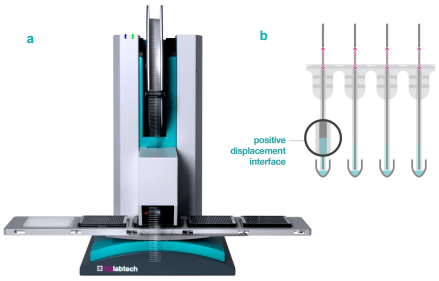

Fig. 1 (a) SPT Labtech mosquito liquid handler, (b) mosquito tips, with positive displacement pistons

Properties of the mosquito LV and HV liquid handlers:

- accuracy and precision with nanoliter to microliter volumes
- accurately handles liquids with high viscosity, such as enzymes in glycerol or genomic DNA
- no cross-contamination or carryover due to sterile, disposable micropipette tips
- future-proof open platform using standard microwell plates

CEL-Seq2 to study macrophage differentiation in granulomas

scRNA-seq with a modified CEL-Seq2 protocol helped uncover the transcription events that produce polyploid macrophages in response to persistent inflammatory stimuli (1,2).

methods

- Macrophages were generated *in vitro* by target stimulation of diploid bone marrow progenitor cells using M-CSF. Inflammatory stimulation was provided by FSL-1 to induce polyploid macrophage cell fate.
- FACS was used to separate the resultant mixed population into 384-well plates. Plates containing 240 nL cell lysis buffer and 1.2 µL Vapor-Lock (Qiagen), as evaporation barrier, per well were prepared with a mosquito HTS.
- 5-fold miniaturised CEL-Seq2 (3) cDNA synthesis reactions were set up with a mosquito LV: 160 nL of reverse transcription mix was used for first strand synthesis; 2.2 µL of second strand reaction mix was then added.
- Twelve libraries (1152 single cells) were sequenced on a single lane (paired-end multiplexing run, 100 bp read length) of an Illumina HiSeq 2500, generating 200 million sequence fragments.

modified CEL-Seq2 protocol for scRNA-seq

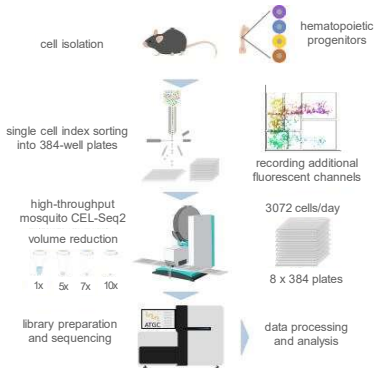

Fig. 2 Miniaturized cDNA synthesis protocol for 384-well scRNA-seq using the mosquito LV, modified from Hashimshony *et al.* (3).

single-cell transcript comparison of macrophage populations

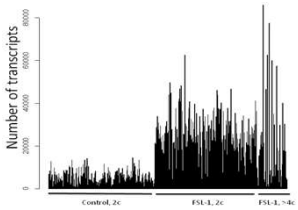

Fig. 3 Total number of transcripts per cell for control macrophages treated with M-CSF alone (control, 2c), and diploid (2c) and polyploid macrophages treated with M-CSF and FSL1 (FSL-1,2c and >4c respectively).

transcript detection from reduced-volume cDNA synthesis

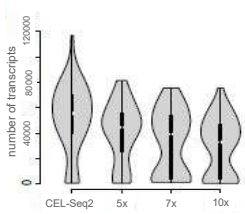

Fig. 4 Violin plots showing a comparison between manual CEL-Seq2 and the robotic high-throughput version at different volume reductions for the distribution of the number of transcripts per mESC. 5x miniaturized CEL-Seq2 maintains high sensitivity (2)

low-volume SMART-Seq2 workflow with challenging single-cell samples

The MIT BioMicro Center is an integrated genomics core facility that provides a broad range of services. Core facilities face many unique challenges due to the broad spectrum of sample types and qualities they must accept. The center has utilized SPT Labtech's mosquito HV liquid hanler to provide an automated scRNA-seq workflow in this environment.

An existing manual SMART-Seq2 workflow (4) was adapted and performed with FACS-sorted cells in 384-well format using the mosquito HV liquid handler.

miniaturized SMART-Seq2 / Nextera XT using mosquito HV

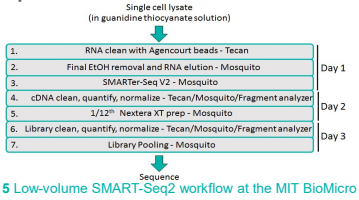

Fig. 5 Low-volume SMART-Seq2 workflow at the MIT BioMicro Center

SMART cDNA quality

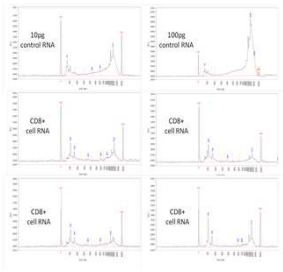

Fig. 6 cDNAs were generated by miniaturized SMART cDNA synthesis in 5 µL final volume from 10 pg or 100 pg control RNA, or four individual quiescent CD8+ T cells, and analyzed on a Fragment Analyzer (Advanced Analytical). The SPT Labtech mosquito SMART- Seq2 protocol is compatible with a range of input amounts of RNA, including single-cell RNA.

Nextera XT library analysis

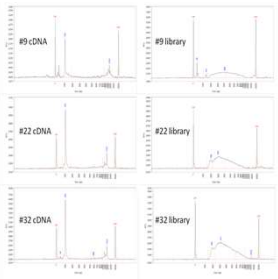

Fig. 7 Fragment Analyzer data from three representative single cells showing the cDNA profile before library preparation (left traces) and the resulting Nextera XT libraries (right traces). Effective library preparation was achieved with an automated, miniaturized protocol in 4 µL final volume using mosquito HV.

single-cell RNA-seq metrics

Mapped	CDS	UTR5	UTR3	Intron	Flanking (+-3k)	Intergenic
67%	18%	9%	20%	36%	6%	9%

Exon/intron	exon/intergenic	rRNA	Top30Count	Genes detected
15.45	43.42	7%	8%	1635

Fig. 8 scRNA-seq metrics from libraries generated on SPT Labtech mosquito liquid handler from 384 single CD8+ cell RNA-seq libraries.

number of transcripts detected per cell

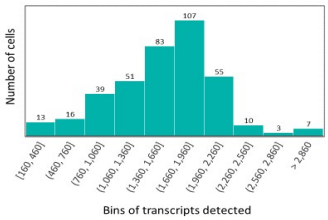

Fig. 9 Number of transcripts detected per cell from the above project (Fig. 8), with the majority of analyzed cells exceeding 1000 detected transcripts.

conclusions

- automation and miniaturisation of CEL-Seq2 cDNA synthesis with mosquito LV increased throughput, improved process consistency and reduced cost ~5-fold, while maintaining high sensitivity and accuracy.
- MIT BioMicro Center's SMART-Seq2 workflow with mosquito HV overcomes challenges with cost, throughput and diversity of samples, and delivers the required data quality for high-throughput single-cell RNA-seq.
- liquid handlers with positive displacement pipetting technology facilitate a wide range of miniaturized workflows in an adaptable microwell plate format.

References

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