miniaturization and automation of CEL-Seq2 and SMARTer-Seq using the mosquito HTS/HV liquid handler

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introduction

Automating single-cell RNA-sequencing (scRNA-seq) protocols on robotic platforms has the potential to enable unbiased, transcriptomic analyzes of individual cells at an unprecedented scale. Liquid handling steps for scRNA-seq on robotic platforms must be accurate and robust to avoid any loss of material, prevent cross-contamination and limit the introduction of technical error into sequencing data. Furthermore, miniaturization of sample preparation results in a significantly reduced cost-per-cell that is essential for large-scale single-cell studies.

Here we present the use of TTP Labtech mosquito® liquid handlers to automate and miniaturize two very different scRNA-seq protocols, CEL-Seq2 and SMARTer-Seq. The mosquito liquid handler is a versatile and easy-to-use platform that is capable of a broad-range of liquid-handling functions, including both dispensing and aspiration. Semi-automated magnetic bead purification at low volume is also facilitated on the system. This setup allows for high-throughput, parallel processing of single cells on 384-well plates, providing a significant reduction in reaction volume and hands-on time, while maintaining or improving data quality compared with manual liquid-handling.

accurate low-volume liquid handling with true-positive displacement

TTP Labtech's true-positive displacement 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 HTS has a pipetting range of 25 nL – 1.2 µL offering accurate assay miniaturization.

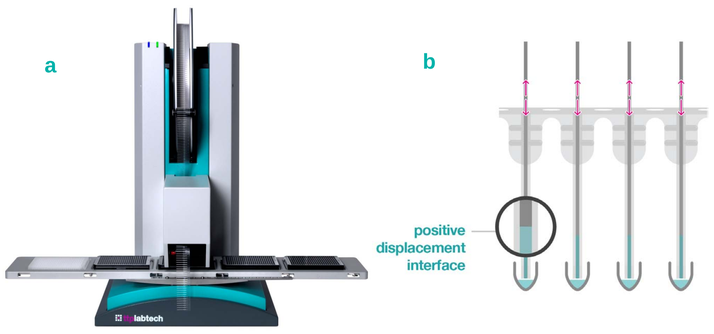

Fig 1. (a) TTP Labtech mosquito liquid handler, (b) mosquito tips, with true-positive displacement pistons

Properties of the mosquito HTS 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

conclusions

- reduced-volume CEL-Seq2 cDNA synthesis with TTP Labtech mosquito HTS increased throughput, improved process consistency and increased transcript detection levels compared to full volume set-ups.
- MIT BioMicro Center's SMARTer-Seq2 workflow with TTP Labtech 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 true-positive displacement technology facilitate a wide range of miniaturized genomics workflows in an adaptable microwell plate format.

References

1. Herrtwich *et al*., 2016. DNA Damage Signaling Instructs Polyploid Macrophage Fate in Granulomas. Cell 167(5), 1264-1280.
2. Hashimshony *et al*., 2016. CEL-Seq2: sensitive highly-multiplexed single-cell RNA-Seq. Genome Biology 17:77.
3. Picelli *et al*., 2014. Full-length RNA-seq from single cells using Smart-Seq2. Nature protocols. 9(1):171-81.

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).

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 (2) cDNA synthesis reactions were set up with a mosquito HTS: 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 (pair-end multiplexing run, 100 bp read length) of an Illumina HiSeq 2500, generating 200 million sequence fragments.

modified CEL-Seq2 protocol for scRNA-seq

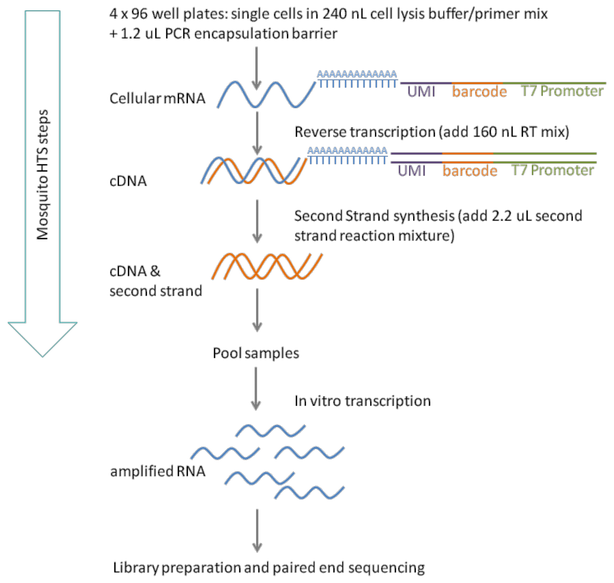

Fig 2. Miniaturized CEL-Seq2 protocol for 384-well scRNA-seq using the mosquito HTS, modified from Hashimshony *et al*. (2).

single-cell transcript comparison of macrophage populations

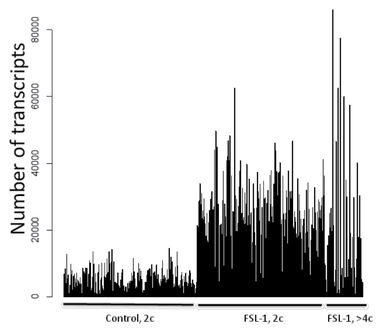

Fig 3. Total number of transcripts per cell for control macrophages treated with M-CSF alone (control, 2c), and diploid (2c) and polyploid macrophages treated with M-CSF and FSL1 (FSL-1,2c and >4c respectively).

transcript detection from reduced-volume cDNA

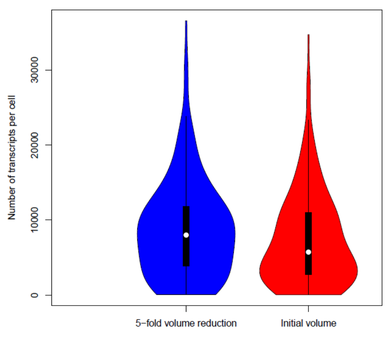

Fig 4. Distribution of transcript numbers per cell using the initial CEL-Seq2 protocol volume (red) compared to 5-fold reduced volume with the mosquito HTS (blue). The data illustrates improved transcript detection with reduced-volume cDNA synthesis.

results

- Whole-transcriptome comparison demonstrates that polyploid and diploid macrophages cluster together based on transcript similarity.
- Polyploid macrophages exhibited additional signature expression, indicating an advanced stage of differentiation.

low-volume SMARTer-Seq 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 TTP Labtech mosquito HV liquid hanler to provide an automated scRNA-seq workflow in this environment.

An existing manual SMARTer-Seq2 workflow (3) was adapted and performed with FACS-sorted cells in 384-well format using the mosquito HV liquid handler.

miniaturized SMARTer-Seq / Nextera using mosquito HV

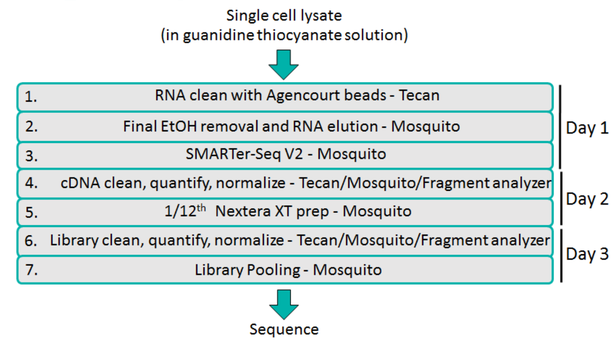

Fig 5. Low-volume SMARTer-Seq workflow at the MIT BioMicro Center

SMARTer cDNA quality

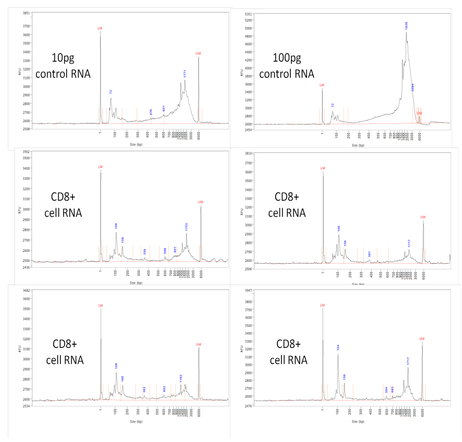

Fig 6. cDNAs were generated by miniaturized SMARTer cDNA synthesis in 5 µL final volume from 10 pg or 100 pg control RNA, or four individual quiescent CD8+ T cells, and analyzed on a Fragment Analyzer (Advanced Analytical). The TTP Labtech mosquito SMARTer-Seq2 protocol is compatible with a range of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

Nextera XT library analysis

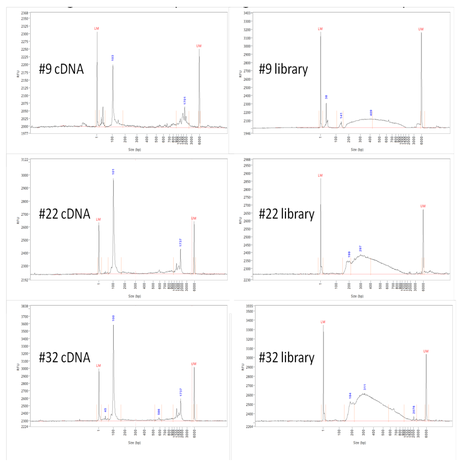

Fig 7. Fragment Analyzer data from three representative single cells showing the cDNA profile before library preparation (left traces) and the resulting Nextera XT libraries (right traces). Effective library preparation was achieved with an automated, miniaturized protocol in 4 µL final volume using 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 TTP Labtech mosquito liquid handler from 384 single CD8+ cell RNA-seq libraries.

number of transcripts detected per cell

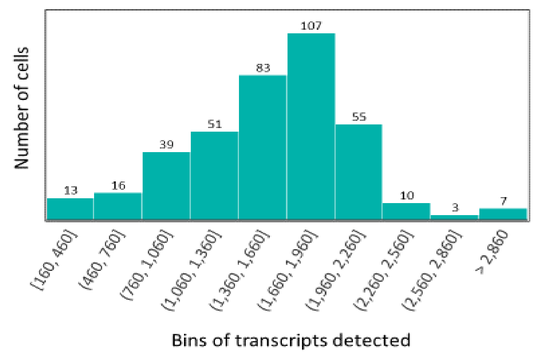

Fig 9. Number of transcripts detected per cell from the above project (Fig. 8), with the majority of analyzed cells exceeding 1000 detected transcripts.

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