Evaluation of the Singleron single-cell RNA, TCR and BCR Libraries Performance on DNBSEQ and another Sequencing Platform

Singleron Biotechnologies developed the GEXSCOPE® Single Cell V(D)J Kit that enables the simultaneous immune profile and whole transcriptome analysis. In this study, Singleron prepared the RNA transcriptomic and V(D)J libraries and sequenced with both MGI DNBSEQ-G400 and Vendor X sequencer. A comprehensive performance comparison indicated that this kit is perfectly compatible with the MGI DNBSEQ™ platform.

Recommended application: V(D)J transcripts and whole mRNA profiling

Recommended sequencers: DNBSEQ-G400RS

- ## A multi-omics approach enabling comprehensive profiling

  The kit enables simultaneous detection of T cell or B cell receptor variable region (CDR3) together with the whole transcriptome expression at single-cell level.

- ## Excellent performance exhibited multidimensionally

  The kit displays high capture efficiency, high specificity and high pairing rate.

- ## Perfect compatibility with DNBSEQ sequencing platform

  MGIEasy Universal Library Conversion Kit enables Singleron kits perfectly work on DNBSEQ sequencing platform.

- ## High-quality sequencing data

  DNBSEQ sequencing technology exhibits many excellent features such as high accuracy, low duplication rate and low index hopping rate.

Background

T-cell receptor (TCR) and B-cell receptor（BCR）sequencing is being used most in disease states where the researcher is looking at whether specific T or B cell clone(s) are expanding. This could suggest specificity against a target related to the disease and is mostly performed in oncology studies, but this in-depth immune profiling information can be utilized for any disease state. Singleron Biotechnologies is a well-recognized company providing single-cell sequencing technologies. It' s RNA profiling and V(D)J kits have been widely used in the study of clinical diagnosis, drug development, and health management.

The Singleron GEXSCOPE® Kits enable a streamlined workflow for single-cell or single nuclei RNA sequencing library preparation. The Singleron GEXSCOPE® workflow starts with preparation of single-cell or single-nuclei suspensions from tissue. Subsequently, single-cell or single-nuclei suspension is loaded onto the Singleron SCOPE-chip®, a portable microfluidic chip with microwells which integrates multiple processing steps such as single cell partitioning, cell lysis and capture of cellular mRNA (Figure 1). The protocol can be performed either manually or can be automated using the Singleron Matrix® instrument. When Matrix® instrument was used, 2 chips could be run in parallel, the process from cell loading to collecting RNA coated beads is 30 minutes.

Singleron GEXSCOPE® kit offerings include the Single Cell V(D)J Kit which enables simultaneous detection of T-cell receptor (TCR) and/or B-cell receptor (BCR) CDR3 sequence, together with whole transcriptome expression at single-cell level. The GEXSCOPE® Single Cell V(D)J Kit utilizes RNA capture beads containing both poly-dT and primers against TCR and BCR constant regions. This allows the capturing of TCR and BCR transcripts to construct the V(D)J library, as well as capturing of all polyadenylated mRNA in the same cell to construct the whole transcriptome library.

There are few reports on the Singleron libraries sequenced in MGI DNBSEQ sequencing platform. In this work, RNA transcriptomic and V(D)J libraries were sequenced by both MGI DNBSEQ-G400 and Vendor X sequencer.To better understand similarities and differences, performance comparison was made for the two platforms.

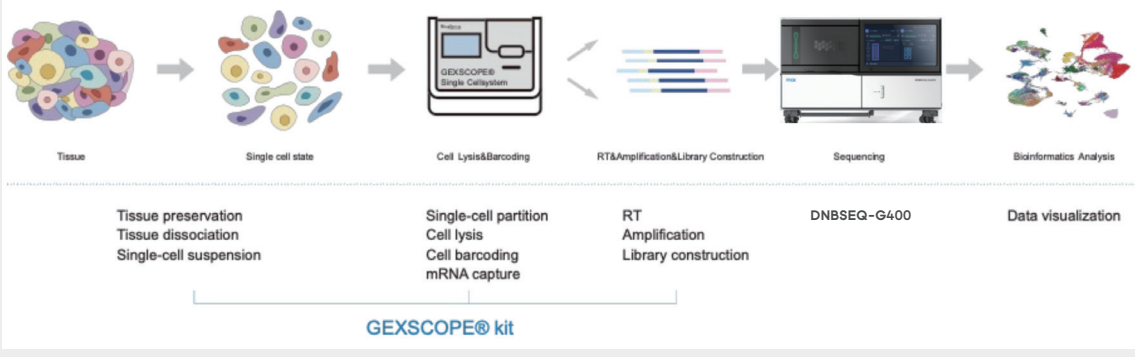

Figure 1. Singleron GEXSCOPE® workflow.

Materials and Methods

DNA Sample Collection

A human peripheral blood mononuclear cell (PBMC) specimen was used for this experiment. Singleron Matrix® instrument was used to automate single cell partitioning, cell lysis and capture of cellular mRNA.

Library Preparation and sequencing

The single-cell RNA, TCR and BCR libraries were constructed using the GEXSCOPE® Single Cell V(D)J Kit following the manufacturer's instructions. As to sequencing on Vendor X platform, the constructed libraries were sequenced with PE150+8 recipe restrictly according to the user manual. As to sequencing on the MGI platform, these libraries were converted to single-stranded circular DNA (ssCircDNA) using MGIEasy Universal Library Conversion Kit (App-A) according to the user manual. SsCircDNAs were later used to generate DNA Nanoballs (DNBs) according to the sequencing protocol and were sequenced on DNBSEQ-G400 with PE150+8 sequencing strategy using High-Throughput Pair End Sequencing Primer Kit (App-A) or High-Throughput Sequencing Primer Kit (App-D). Due to fixed sequences in the libraries, the Singleron libraries were pooled together with 20%-40% MGI standard libraries to improve the diversity.

Bioinformatics Analysis

Paired end FASTQ files generated from the respective sequencing platforms were processed using the CeleScope software which contains pipelines for processing Singleron's single-cell RNA transcriptomes and V(D)J assays to generate gene expression matrix and clonotypes, respectively, for downstream analyses. Cells were identified using the EmptyDrops method while default parameters were used everywhere else in the pipelines (visit Github for more information: https://github.com/singleron-RD/CeleScope). Differential gene expression and dimension reduction were performed using the Seurat package in R.

Sample collection	Library preparation and sequencing	Bioinformatics Analysis	Result analysis
A human peripheral blood mononuclear cell (PBMC) specimen was collected for this study	 GEXSCOPE® Single Cell V(D)J Kit  MGIEasy Universal Library Conversion Kit (App-A)  DNBSEQ-G400 Genetic Sequencer	CeleScope software, Seurat package in R	Performance comparison of the kit on MGI and Vendor X sequencer

Results

Comparison of primary sequencing metrics for RNA libraries

After sequencing, 124 and 837 million paired-end (PE) reads were obtained for Vendor X and MGI, respectively. With an average Q30 of 88.2%, FASTQ files from MGI sequencing were down-sampled to 124 million PE reads to match Vendor X'S sequencing depth. This is then followed by barcode extraction, adaptor trimming, alignment to reference genome and feature counting using CeleScope pipeline. Comparison between these two platforms revealed that most of the metrics have similar performance with the exception on the Trimmed and Reads Too Short parameters that DNBSEQ™ display a slightly better reads quality (Figure 2).

The mapping rate, the percent of sequencing reads that are aligned to the reference genome, is a key metric for the evaluation of sequencing quality. The Uniquely Mapped reads data shows that MGI has more reads than Vendor X that can be uniquely mapped to the genome (Figure 3a), providing more usable information for a given number of sequencing reads. Moreover, the MGI platform has more UMIs detected per cell indicating more data per cell and suggesting overall more genes can be detected by the MGI platform accordingly (Figure 3b).

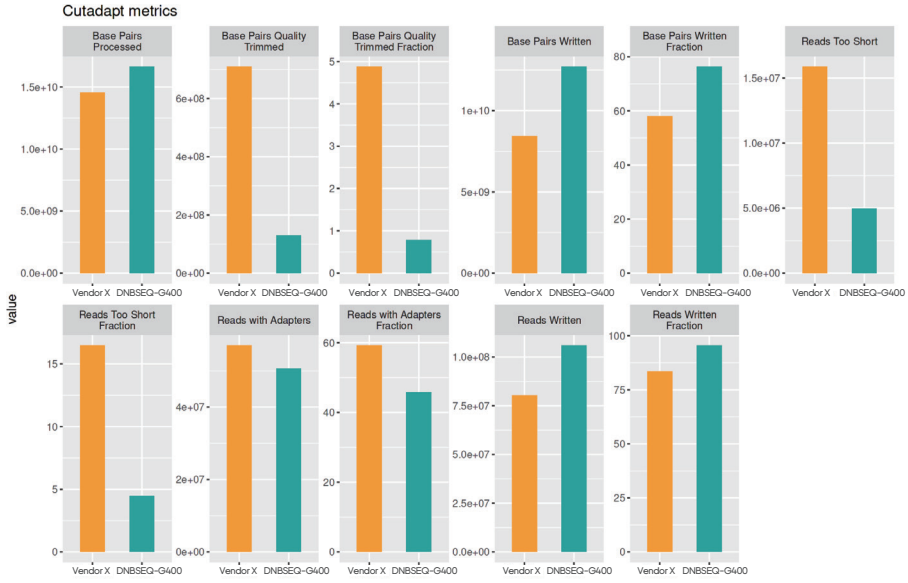

Figure 2. Basic QCs parameters.

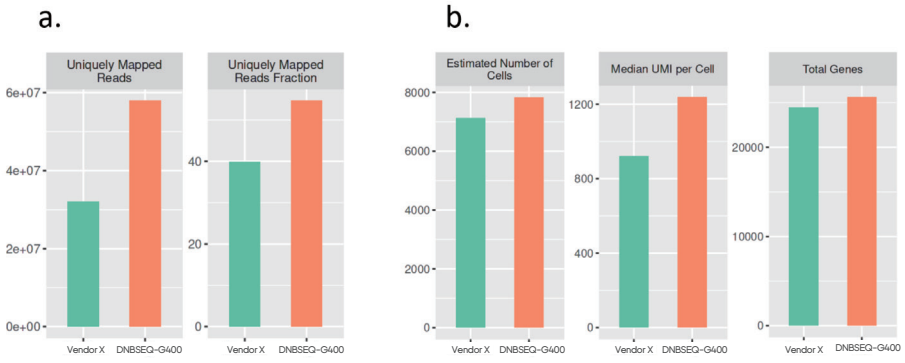

Figure 3. Alignment and UMI analysis. a) unique mapped reads calculation. b) cell and genes expression based on UMI count.

Comparison of secondary metrics for RNA libraries

Feature selection, or identification of informative genes, plays a key role in single-cell studies. In this evaluation, the distribution of features (detected genes) vs RNA counts were calculated, and a similar distribution was found between the two platforms. Notably, DNBSEQ™ has more cells with higher RNA and feature counts, which may be due to the more useable data and more detected cell number for MGI sequencer (Figure 4a). Further UMAP analysis showed that the Vendor X and MGI sequencing data cluster together, demonstrating that the two platforms shared a similar gene expression pattern (Figure 4b). To evaluate the ability of cell type detection, composition of cell types as annotated by SingleR using a public multimodal reference "atlas" of the circulating human immune system was analysed[1]. The result suggests a large similarity between the two systems (Figure 4c). A comparison by the expression of cell type marker genes was also evaluated for a further understanding of the differences and similarities using the two platforms, and the result also shows a similar cell type marker genes expression pattern (Figure 4d).

Comparison of V(D)J libraries

The V(D)J library sequencing results were also analysed for further investigation. Before comparison, all samples were down-sampled to 10 million paired-end reads. The CDR3 region of the BCR and TCR is the most variable region of heavy chain and is used to identify the clonality of B and T cells. In this report, as shown in Figure 5, both the number of cells with detected clonotype and the number of clonotypes detected suggest a large similarity between the two platforms, with the MGI data identifying more unique BCR sequences.

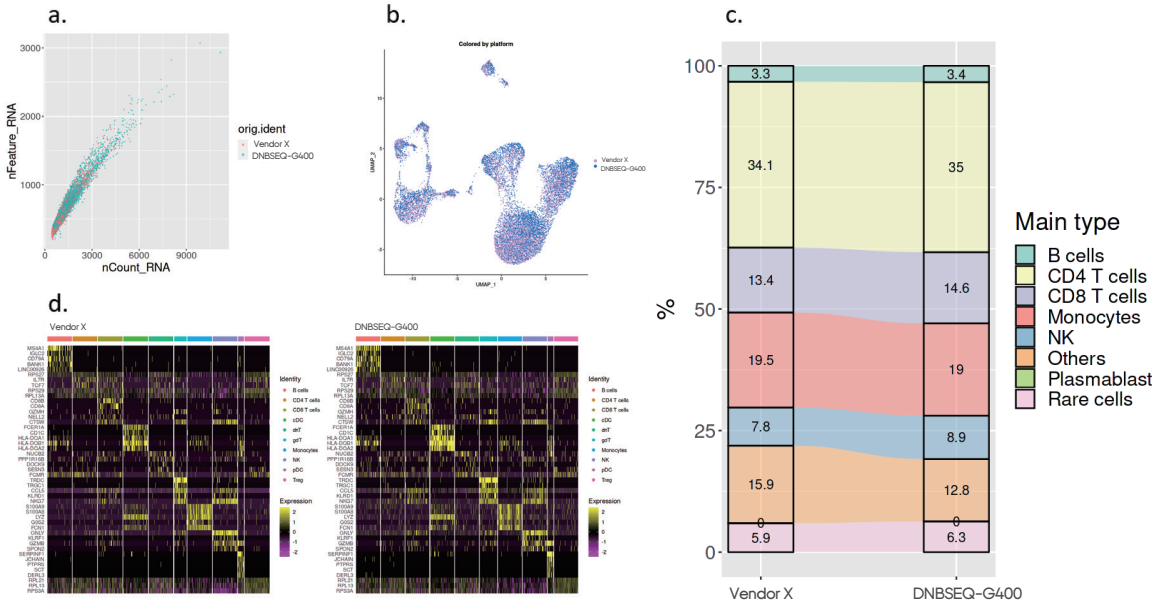

Figure 4. Comparison of secondary metrics. a) the Distribution feature vs RNA counts. b) UMAP analysis for two platforms. c) Composition of cell types as annotated by SingleR using a public reference dataset. "Rare cells" are MAIT, ILC, HSPC, ASDC, Doublet, platelet and erythrocytes d) expression pattern for Cell type marker genes.

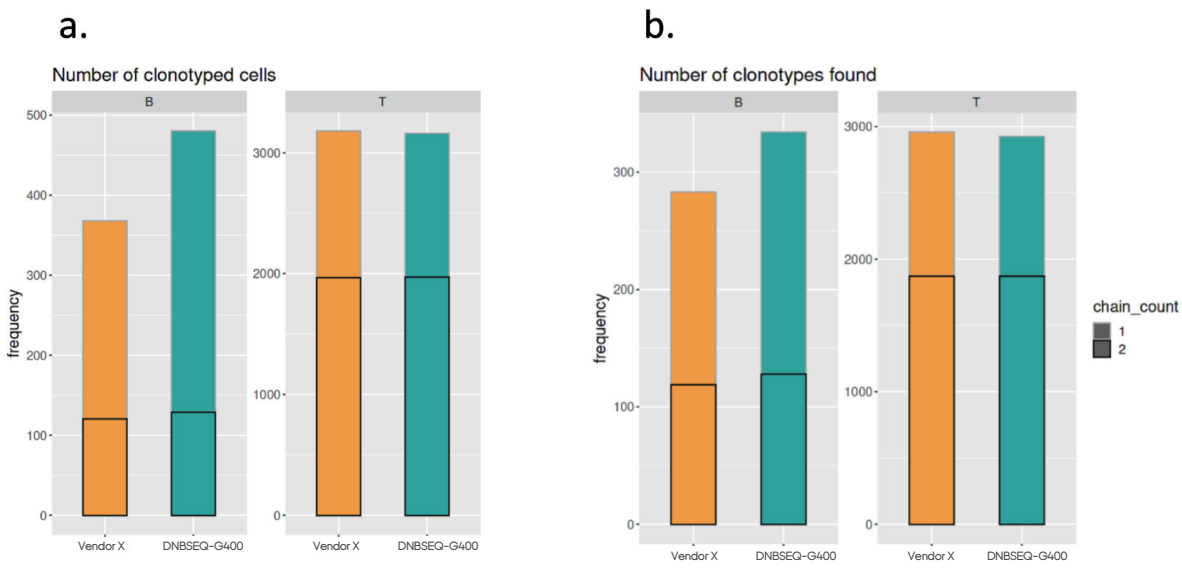

Figure 5. Comparison of V(D)J libraries. All samples were down-sampled to exactly 10 million reads before processing. a) number of cells recognized as clonotypes. Grey framed: cells with only 1 chain being detected. Black framed: cells with 2 chains being detected. b) number of clonotypes found. Grey framed: clonotypes with 1 chain being detected. Black framed: clonotypes with 2 chains being detected.

Summary

In summary, combined analyses of Singleron GEXSCOPE RNA and V(D)J libraries on human PBMCs shows comparable data performance between the MGI DNBSEQ™ and Vendor X sequencing platforms although DNBSEQ™ displayed higher performance in certain parameters such as the range of the useable data, higher mapping rate, UMI number, and RNA count number.

This report shows that Singleron Biotechnologies'GEXScope® Single Cell RNA and Single Cell V(D)J kits are highly compatible with MGI DNBSEQ™ platforms and provides a valuable resource for researchers aiming to produce high quality data with convenient to use workflows and cost savings.

DNBSEQ-G400 Genetic sequencer

Acknowledgement

1.We thank Jonathan Scolnick, Singleron Biotechnologies, and MGI-Tech APAC for providing support and materials, as well as comments and suggestions to this comparative study.

2.The MGI sequencing run was performed at MGI-Tech Training and Demo laboratory at NSG BioLabs, Singapore.

Reference

1. Hao, Yuhan et al. "Integrated analysis of multimodal single-cell data." Cell vol. 184,13 (2021): 3573-3587.e29. doi:10.1016/j.cell.2021.04.048

Recommended Ordering Information

Category	Product	Cat. NO.
Instruments	DNBSEQ-G400 Genetic Sequencer (Configuring A)	900-000168-00
	Matrix Single Cell Preparation Instrument	MT1001001
Software	MegaBOLT Bioinformatics analysis accelerator	900-000555-00
	CeleScope® Software	https://github.com/singleron-RD/CeleScope
Library Prep	GEXSCOPE® Single Cell Human V(D)J Kit	5183121
	MGIEasy Universal Library Conversion Kit (App-A)(16 RXN)	1000004155
	Standard library reagent V3 (26 ng/EA)	1000005033
Sequencing Reagents	DNBSEQ-G400RS High-throughput Sequencing Set (FCL PE150)	1000016952
	High-Throughput Pair End Sequencing Primer Kit (App-A) (1 RXN)	1000020832
	High-Throughput Sequencing Primer Kit (App-D) (1 RXN)	1000028550

MGI Tech Co.,Ltd

Building 11, Beishan Industrial Zone, Yantian District, Shenzhen, CHINA, 518083

+86-4000-688-114

en.mgi-tech.com

MGI-service@mgi-tech.com

*1. For StandardMPS and CoolMPS: Unless otherwise informed, StandardMPS and CoolMPS sequencing reagents, and sequencers for use with such reagents are not available in Germany, Spain, UK, Sweden, Belgium, Italy, Finland, Czech Republic, Switzerland, Portugal, Austria and Romania. Unless otherwise informed, StandardMPS sequencing reagents, and sequencers for use with such reagents are not available in Hong Kong. No purchase orders for StandardMPS products will be accepted in the USA until after January 1, 2023.

2. For HotMPS sequencers: This sequencer is only available in selected countries, and its software has been specially configured to be used in conjunction with MGI's HotMPS sequencing reagents exclusively.

3. For HotMPS reagents: This sequencing reagent is only available in selected countries.