JUMPCODE GENOMICS

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Tech Note

DepleteX™ Single Cell RNA Boost Kit

Remove abundant and uninformative fragments prior to scRNA-Seq

Introduction

There are many opportunities to apply single-cell genomics to unanswered questions in biology. With its unique ability to study the individuality of cells, single-cell genomics has become an increasingly common, widely adopted approach.

To profile large amounts of individual cells, transcriptional profiling with single-cell RNA Seq empowers researchers to know what genes are expressed, in what quantity, and how they differ across the cells within a sample. Because of this, single-cell studies require significant sequencing to understand transcript levels within individual cells. It can take anywhere from 50,000 to 150,000 reads per cell to sequence informative, lower abundance genes —with reads for a single sample often exceeding 150 million reads.

Traditionally, single-cell data processing incorporates specific filtering and normalization steps prior to cell clustering and downstream interpretation. Instead of removing those reads in-silico, DepleteX Single Cell RNA Boost Kit removes those reads in-vitro before of sequencing, redistributing sequencing reads to unique biologically relevant transcripts. In this study, using PBMC cell types, we show an additional 2 cell types uncovered in the depleted condition compared to the control sample at the same sequencing depth.

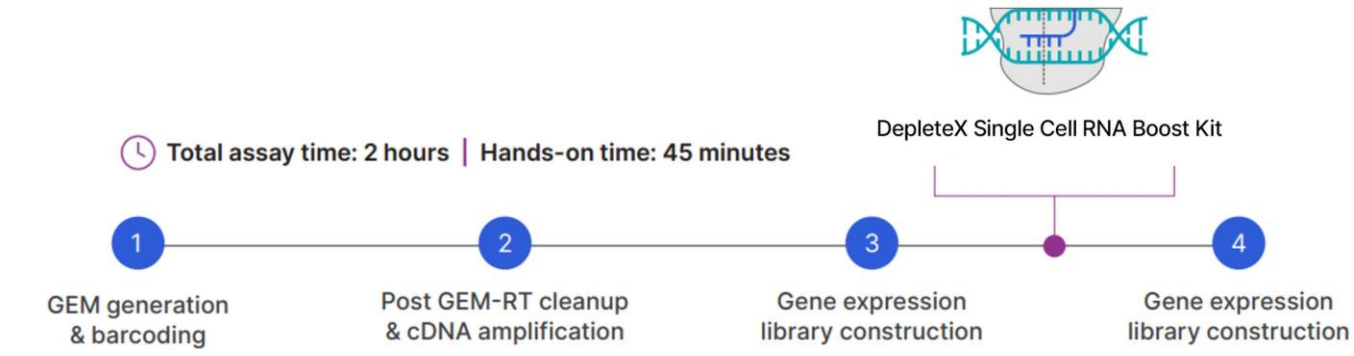
Highlights

- Gain a deeper view of expression profiles of individual cells
- Depletes sequences not used for secondary analysis including: Unaligned reads, ribosomal, mitochondrial, and optionally non-variable genes
- Simple 3-step protocol integrated into any single cell sequencing workflow including 10x Genomics® Chromium™ Next GEM Single Cell 3'

Methodology

DepleteX leverages Cas9 and a specifically designed guide set to remove reads filtered by secondary analysis. DepleteX Single Cell RNA Boost Kit allows you to cut through the noise with minimal impact on your workflow and maximum confidence in your results downstream for either short or long read technologies.

Content for depletion was designed by analyzing a cohort of publicly available single cell 10x Genomics® data. By tailoring guides to deplete genomic intervals in addition to the highest expressed protein-coding ribosomal and mitochondrial genes, which are typically removed informatically downstream, we exhibited the ability to redistribute reads through in-silico depletion across samples representing 14 sample types.



Methods

PBMC samples were isolated from a donor and prepared using 10x Genomics® Chromium™ Next GEM Single Cell 3' Reagent Kit (v3.1) protocol. In summary, barcoded PBMC cDNA from 3 individual channels were processed in parallel. For depleted samples, the barcoded cDNA from the same channels were processed simultaneously. Depletion was performed after adapter ligation.

The 10X v3.1 protocol was resumed at step 3.5. All libraries were sequenced on a NovaseqTM 6000 to achieve a targeted read depth >30,000 reads per cell. We recovered ~9,000 cells per sample with each sample reaching the targeted read depth. Raw sequencing reads were processed through Cell Ranger v7 using default settings and the human transcriptome reference provided by 10x Genomics®. The filtered feature barcode matrix was then used for downstream analysis. Data QC, filtering, normalization, and clustering were performed using the Seurat v4 R toolkit. Cell type annotation and UMAP plots were generated using the python-based single-cell toolkit, Scanpy. Cell type annotations were performed using the semi-automatic annotation tool, CellTypist.

Results and Discussion

Sequencing saturation was compared for the control and depleted samples at different sequencing depths (Figure 1). The depleted condition has more library complexity than the control sample at every sequencing depth.

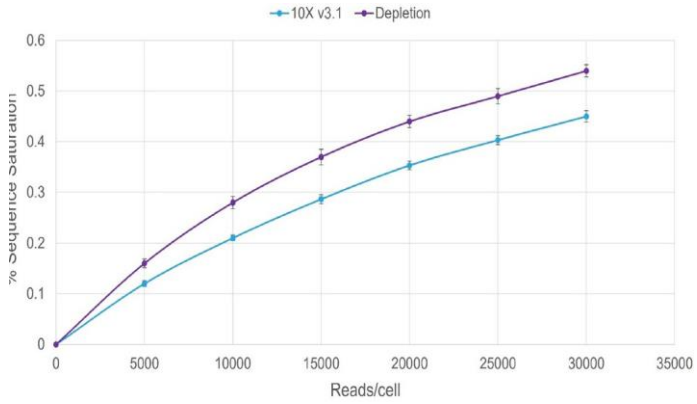


Figure 1: Sequencing saturation curves generated with Cell Ranger v7. Depletion increases the fraction of library complexity extracted at every sequencing depth.

As a direct result of sequencing more library complexity than the control sample, the resulting UMAP plots show additional cell types when compared to the control (Figure 2). The depleted condition was downsampled to 16K reads/cell and compared to the control condition at 32K reads/cell. Even at half the amount of sequencing, the depleted condition has 2 additional cell types. PBMCs were annotated using a semi-automated tool called CellTypist.

Similarly, in Table 1, cell type frequencies are plotted for the control sample at 32K reads/cell and compared to the depleted condition at half of the read depth (16K reads/cell). Even at 16K reads/cell, the depleted condition shows similar cell frequencies compared to the control with additional reads on the NK cells and CRTAM+ gamma-delta T cells.

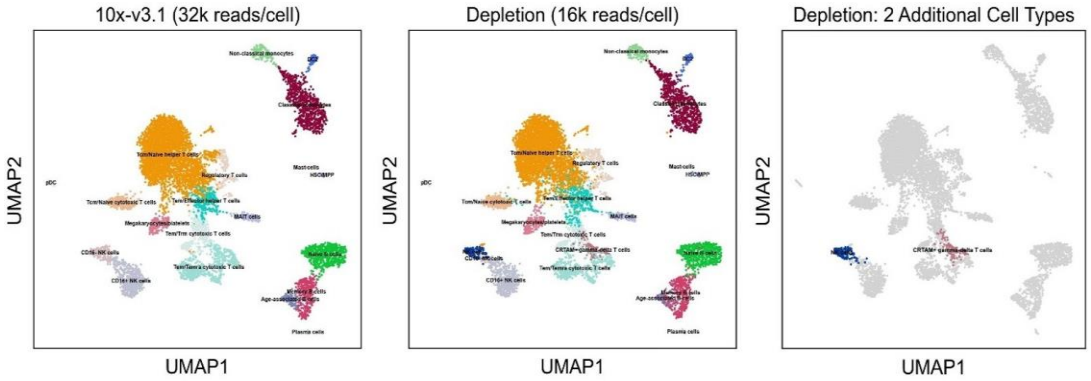


Figure 2: UMAP plots for PBMC samples. 10X v3.1 (32k reads/cell) annotated cell types (left), Depletion (16k reads/cell) annotated cell types (middle), and highlighted additional cell types with depletion (right). PBMCs were annotated using the CellTypist python package here: https://doi.org/10.1126/science.ab15197

Finally, looking at Figure 3, the % of reads aligned to the genome, we have a 1.5X increase in the number of usable reads, or transcriptomic reads without ribosomal, mitochondrial and non-variable genes.

Summary

DepleteX Single Cell RNA Boost Kit for 10x Genomics focuses on the signal, not the noise--to specifically remove uniformative sequences and improve biologically informative signal. Gain a deeper view of the expression profile of cells while still detecting the same number of genes with the DepleteX Single Cell RNA Boost Kit.

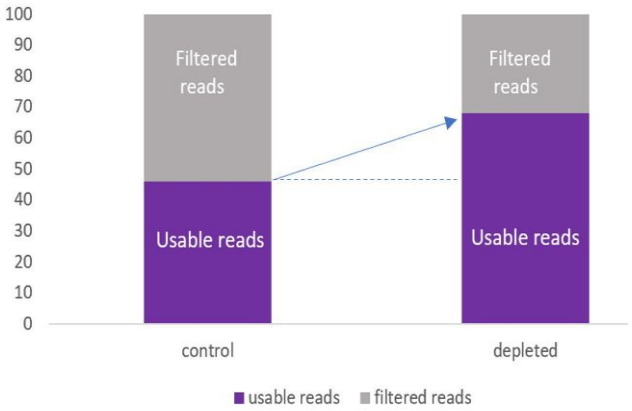


Figure 3. 1.5X boost in usable reads, as defined as transcriptomic reads without ribosomal, mitochondrial, and non-variable genes. Filtered reads are not used in secondary analysis.

Table 1: Cell type frequencies for a matched 10X v3.1 and Depleted library

	10X v3.1 : 32k reads/cell	Depletion : 16k reads/cell
Cell Type	Proportion (% of cells)	Proportion (% of cells)
Tcm/Naive helper T cells	42.98	40.38
Classical monocytes	9.19	9.26
Tem/Temra cytotoxic T cells	7.59	6.98
Naive B cells	7.03	6.55
CD16+ NK cells	6.20	6.13
Tem/Trm cytotoxic T cells	4.01	4.72
Memory B cells	3.93	4.40
Tcm/Naive cytotoxic T cells	3.36	3.43
Tem/Effector helper T cells	3.08	3.25
Regulatory T cells	2.09	3.25
CD16- NK cells	2.02	2.19
Megakaryocytes/platelets	2.01	1.89
MAIT cells	1.92	1.63
Non-classical monocytes	1.81	0.81
Age-associated B cells	1.23	0.80
DC2	0.71	0.39
pDC	0.39	0.34
HSC/MPP	0.22	0.22
Plasma cells	0.14	0.14
Mast cells	0.10	0.09
NK cells	**0.00**	**1.75**
CRTAM+ gamma-delta T cells	**0.00**	**1.39**
Total cells	7865	7865

Ordering information

Catalog	Product name	Samples
NOVA-512850	DepleteX Single Cell RNA Boost Kit	24