APPLICATION NOTE

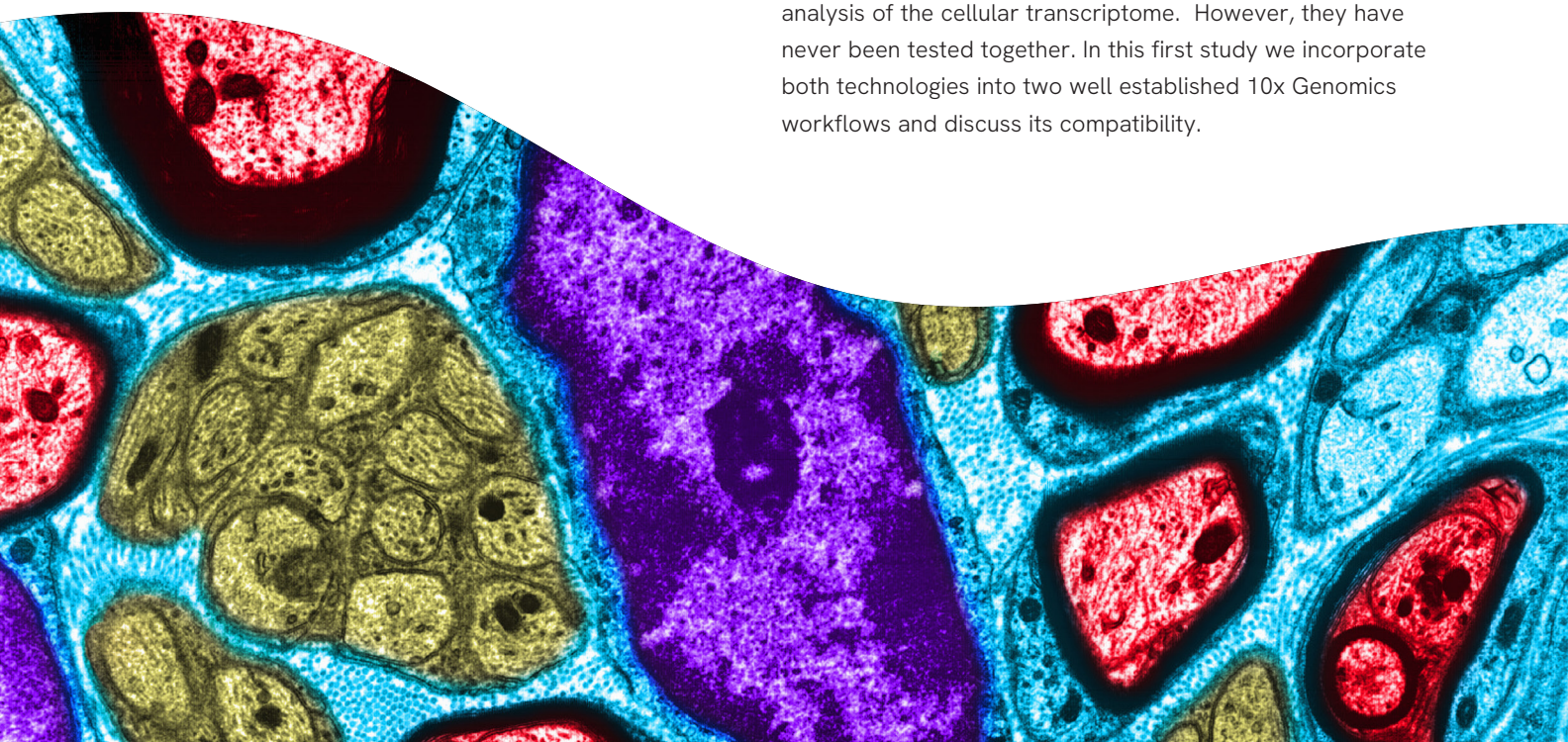
TotalSeq™ and DepleteX® technologies for efficient analysis of the cellular transcriptome

Proteins represent the main functional machinery of cells, so how the expressed proteome differs from cell to cell is a question of high interest. CITE-Seq oligo-conjugated antibodies enable the expression profiling of large number of protein markers in parallel to transcriptomes of thousands of individual cells. The protein and RNA readouts are plotted together to deliver a high-resolution image of heterogeneous cellular populations, which has been shown to outperform RNA clustering, reduce dropouts and enhance cell type identification.

The ability to generate simultaneously proteomic and transcriptome data has driven single cell studies (scRNA-seq) to a new level, but still many technical challenges remain ahead. Just as an example, it is known that in scRNA-seq experiments up to 50% of the reads can be filtered out prior to secondary analysis, obscuring the detection of low abundancy transcripts in the cell that might be crucial to understand many cellular states. This is not a problem that can be resolved simply by increasing the number of reads per cell.

A new depletion technology based on the CRISPR-Cas9 system (DepleteX®) has been recently launched. This approach degrades abundant, uninformative sequences in libraries prior to sequencing, redistributing sequencing clusters to unique biologically relevant transcripts. It has been shown previously to be applicable to scRNA-seq, reducing the amount of reads per cell by half while maintaining cell type resolution.

The combination of TotalSeq™ oligo-conjugated antibodies and DepleteX® technologies can potentially lead to a more efficient analysis of the cellular transcriptome. However, they have never been tested together. In this first study we incorporate both technologies into two well established 10x Genomics workflows and discuss its compatibility.



Methods

PMBC samples were obtained from healthy donors and stained using either TotalSeq™-B Human Universal Cocktail, v1.0 (cat# 399904, BioLegend) or TotalSeq™-C Human Universal Cocktail, v1.0 (cat# 399905 from BioLegend). TotalSeq™-B is designed to be compatible with the 10x Genomic Single Cell 3′ workflow, whereas TotalSeq™-C is compatible with the 10x Genomics Single Cell 5′ workflow. For additional information about TotalSeq™ please visit Multiomics and TotalSeq™ Reagents.

Libraries were prepared according to the standard CITE-Seq workflow, where the cDNA derived from the antibodies (also known as antibody-derived tags or ADTs) are separated from the mRNA-derived libraries and prepared independently. Adapter-ligated mRNA-derived libraries were split in two aliquots. One of them (control) proceeded directly to PCR and the other one was incubated with DepleteX before PCR (Figure 1).

Pooled control cDNA libraries, depleted cDNA libraries and ADT libraries were quantified using Qubit® (Thermofisher) size was assessed using 4200 TapeStation (Agilent Technologies) and loaded onto Illumina® NovaSeq6000™ SP flow cell (30-8-0-92 cycles), targeting 25,000 reads/cell for control and depleted cDNA libraries (300 million reads) and 10,000 reads/cell for ADT libraries (120 million reads). Data

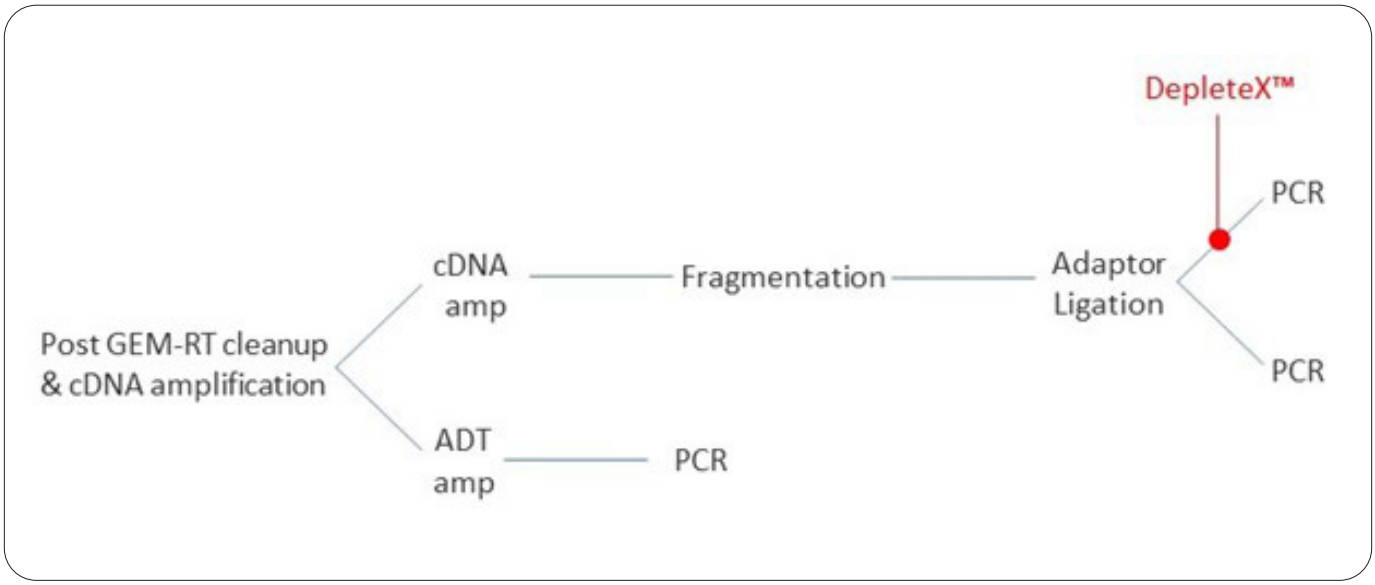


Figure 1: Incorporation of DepleteX® into the CITE-Seq workflow.

Results

Depletion has no significant impact on mRNA sequencing metrics

Post ligation cDNA libraries showed comparable size distribution and yield in both control and depleted aliquots, for both 10x Genomics 3′ and 5′ workflows (Figure 2).

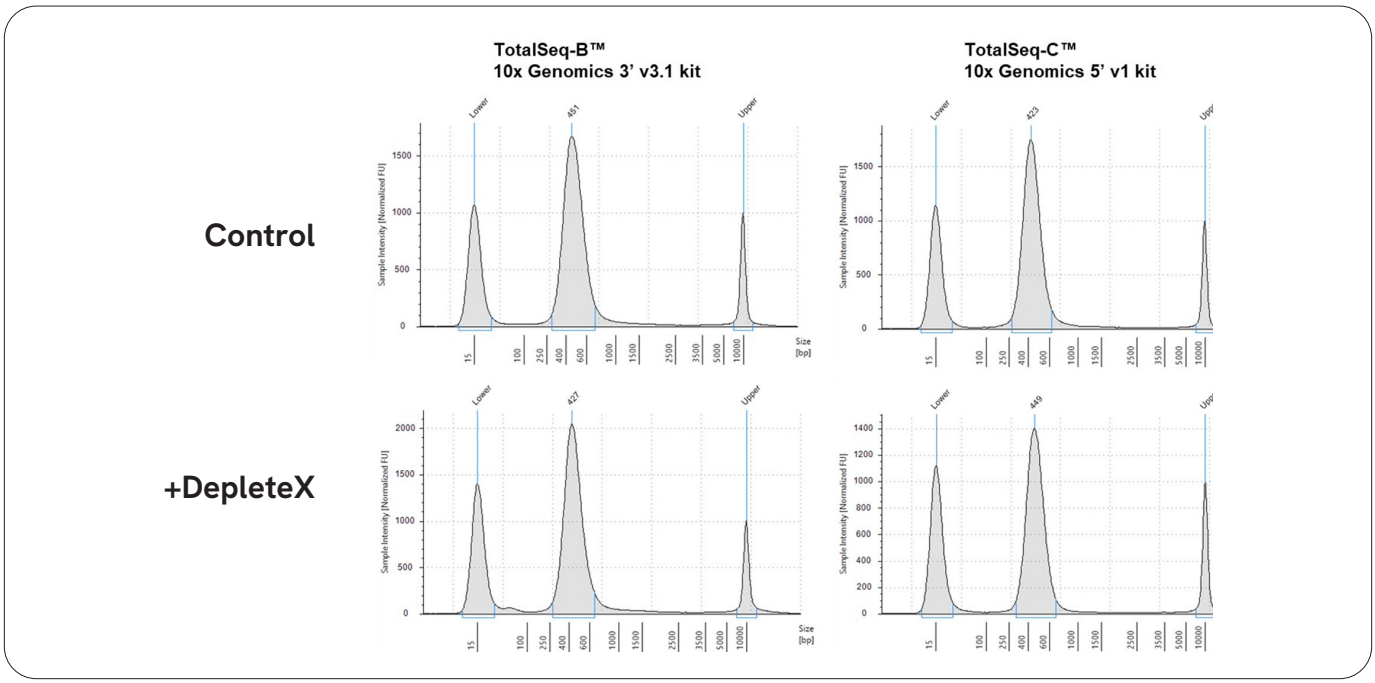


Figure 2: Size distribution of TotalSeq™-B and -C libraries, control or treated with DepleteX®

Control and depleted libraries were pooled and sequenced simultaneously. No significant difference in sequencing metrics quality was found among both groups.

Depletion improves detection of many cell surface protein genes

We did not perform an in-depth differential gene expression analysis comparing control and depleted samples as this was not the goal of the study. However, we compared the expression levels of several cell surface protein genes.

We found that in depleted samples the expression levels of cell surface protein genes where higher (IL7R, CD52, CD3E) or similar (CD40, CD84, ANPEP) to the control samples.

Only in the case of known DepleteX targets such as HLA-E, CD48 or B2M there was a significant decrease of expression level in depleted versus control samples.

Depletion has no impact on ADT metrics

Analysis of ADT-only sequencing data confirmed that depletion has no obvious effects on the fraction of antibody reads usable, antibody read in cells or median UMI per cells detected (Table 1).

Table 1. No impact of DepleteX® on ADT quality metrics.

Workflow	Sample	Mean reads per cell	Fraction antibody reads usable	Fraction unrecognized antibody	Antibody reads in cells	Median UMIs per cell
TotalSeq™-B	Control A	12,984	34.80%	4.70%	36.70%	2,842
TotalSeq™-B	Depleted A	13,530	33.40%	4.70%	35.20%	2,857
TotalSeq™-B	Control B	13,736	32.80%	4.70%	34.60%	2,865
TotalSeq™-B	Depleted B	13,841	32.70%	4.70%	34.40%	2,873
TotalSeq™-C	Control A	10,204	52.90%	3.30%	57.40%	2,833
TotalSeq™-C	Depleted A	10,652	51.40%	3.30%	55.80%	2,895
TotalSeq™-C	Control B	12,280	50.30%	3.30%	54.60%	3,222
TotalSeq™-C	Depleted B	11,982	49.80%	3.30%	54.10%	3,146

We confirmed that depletion does not affect detection of cell surface proteins, an example of which is shown in Figure 3.

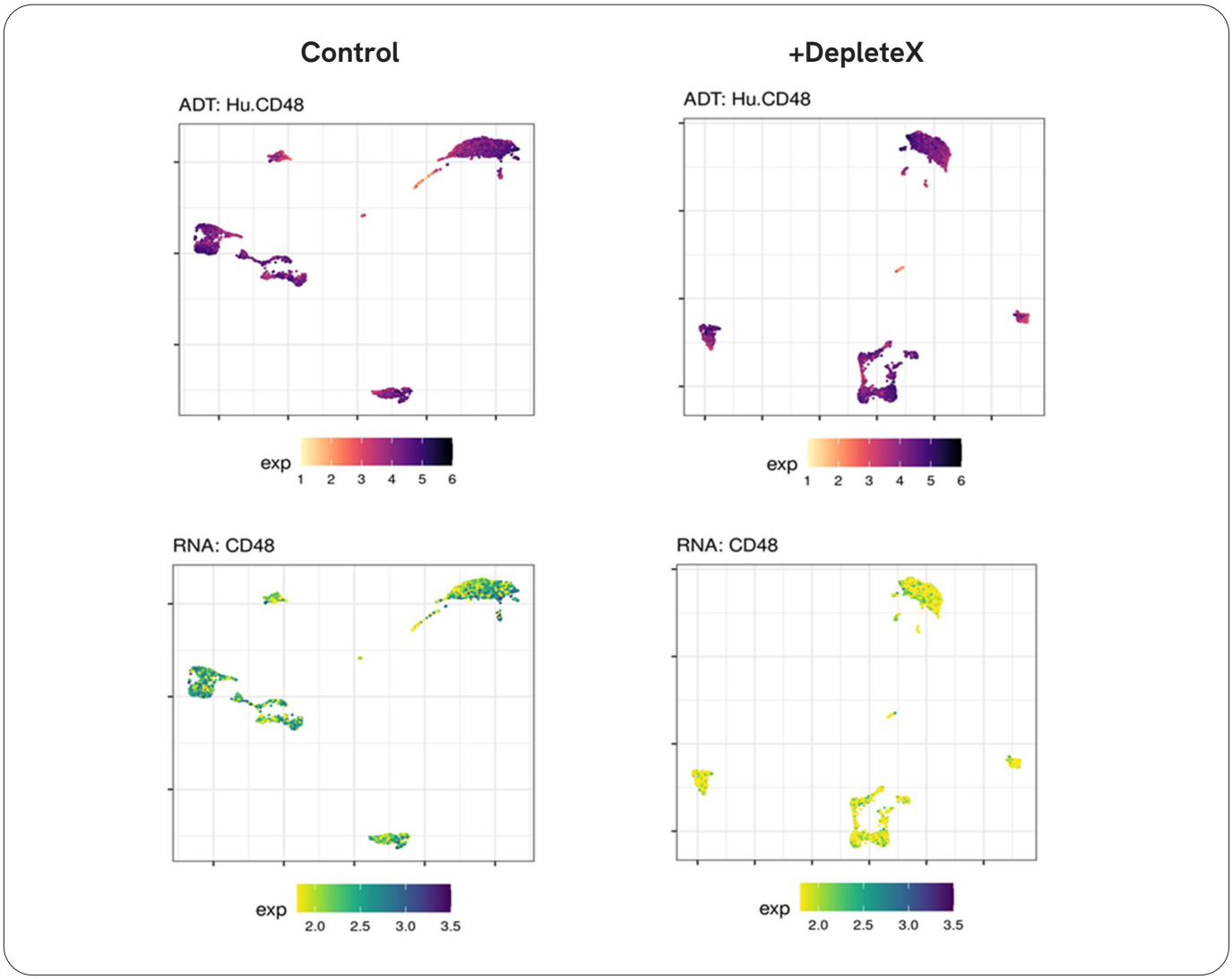


Figure 3: CD48 protein (ADT) and RNA expression analysis of sample processed with TotalSeq-B™. Surface protein levels are not affected by DepleteX® treatment. CD48 mRNA is a known target of DepleteX and shows a significant decrease in expression after depletion, although still is detectable.

Depletion does not modify the cell types previously observed

We confirmed that depletion did not affect the determination of cell types that have been previously observed on PBMC samples using CITE-Seq (Figure 4).

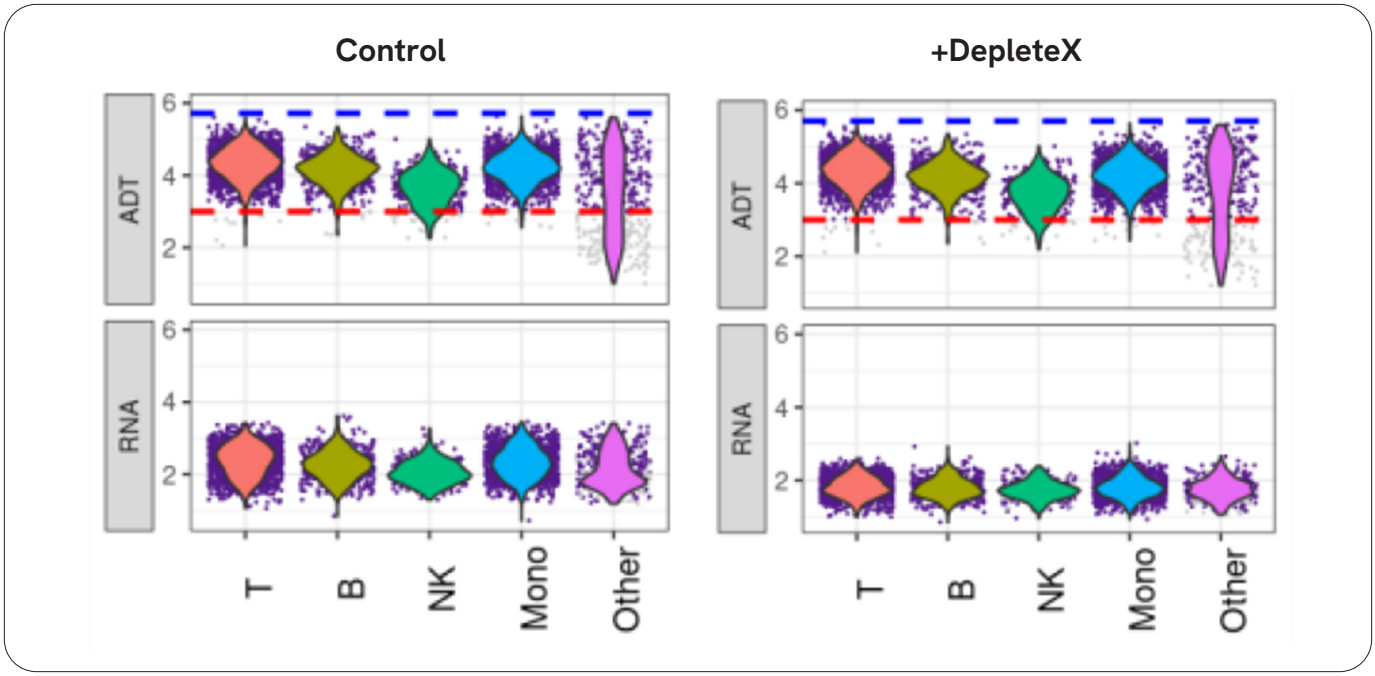


Figure 4: CD48 protein (ADT) and RNA expression analysis of sample processed with TotalSeq-B™. Cell types identified are are not affected by® DepleteX treatment.

Conclusions

DepleteX® is compatible with TotalSeq™ and can be incorporated into CITE-Seq workflows by adding an extra step after adapter ligation of mRNA-derived libraries. We find that this depletion does not interfere with ADT metrics and improves mRNA detection of many cell surface protein genes, without affecting the cell types previously observed