

TotalSeq™: Standardized oligonucleotide barcode antibody conjugates for multiplex immunophenotyping

Abstract

The CITE-Seq (Cellular Indexing of Transcriptomes and Epitopes by Sequencing) platform is a recent advance in single cell analysis, which is based on high-throughput single cell sequencing (scSeq); it combines measurements of cellular proteins and transcriptomes. This platform will potentially transform how complex cell populations (e.g., lineage differentiation or tumor infiltrating lymphocytes) are studied. Published data indicates that scSeq analysis on cell surface marker expression is comparable to multi-color flow cytometry, but enables superior multiplexing capabilities. Currently, individual investigators use their choice of oligo barcodes for different protein markers, and there is no standardized control available. Comparison of data from different studies is difficult in the absence of such controls. The availability of standardized oligonucleotide barcode-labeled antibodies enables reliable comparisons of data across longitudinal and multi-site studies. After assigning a unique oligo barcode to each of our monoclonal antibodies, we prepared conjugated reagents: the TotalSeq™ products. Our standardized barcoding system and ready-to-use oligo-antibody conjugates support scSeq based multiplex immunophenotyping. In this study, TotalSeq™ products are validated on the scSeq platform.

Methods

Barcode design and assignment: 15-bp oligonucleotide barcodes were selected based on stringent bioinformatic criteria. Each barcode is assigned to a specific mAb clone. The 5' and 3' ends of the barcode are flanked by universal sequences used for library amplification and cDNA generation, respectively.

Oligo-antibody conjugate preparation: Highly specific, flow cytometry tested monoclonal antibodies (mAbs) were directly conjugated to oligonucleotides containing assigned barcode sequences (synthesized by Integrated DNA Technologies). After purification, conjugated antibodies were supplemented with sodium azide and EDTA, and stored at 4°C.

Fluorophore-conjugated antibody staining and flow cytometry: Single cell suspensions from human PBMCs or mouse tissue were stained with fluorophore-conjugated antibodies for cell surface markers as indicated. Flow cytometry data were acquired on BD™ LSR II, Fortessa, or Canto II instruments, and analyzed using FlowJo®.

TotalSeq™ oligo-conjugated antibody staining and flow cytometry: Human PBMCs were incubated with barcode oligo-conjugated mAbs. After wash, Alexa Fluor® 647 conjugated secondary antibody was used to detect the binding. Alternatively, barcode oligo-conjugated monoclonal antibodies were hybridized with oligo-dT (which anneals to the poly-A at the 3' end of barcode oligos) that is conjugated with Alexa Fluor® 647. The mixture was then diluted and used to stain human PBMCs. Flow cytometry data were acquired as above.

Single cell partitioning and sequencing: Human PBMCs were incubated with barcode oligo-conjugated antibodies. After wash, single cell partitioning was performed with the 10X Genomics Chromium Controller according to the manufacturer's instructions. cDNA library preparation was done using the 10X Genomics Chromium Single cell 3' solution package according to manufacturer's instructions with modification. Sequencing was performed on an Illumina sequencer.

Conclusions

We provide TotalSeq™ to the scientific community, a portfolio of oligonucleotide-conjugated antibodies, each with a unique barcode sequence, to support multiplex immuno-phenotyping on scSeq platforms. Our studies demonstrate that these conjugates meet our stringent manufacturing standards, and perform as expected compared to classical flow cytometry. Experiments performed with both mouse and human cells clearly show this correlation. The wide utility and applications of TotalSeq™ conjugates and scSeq workflows will be further demonstrated in current and future studies by BioLegend and multiple collaborators.

Figure 1. TotalSeq™ oligo-conjugated antibody and typical workflow.

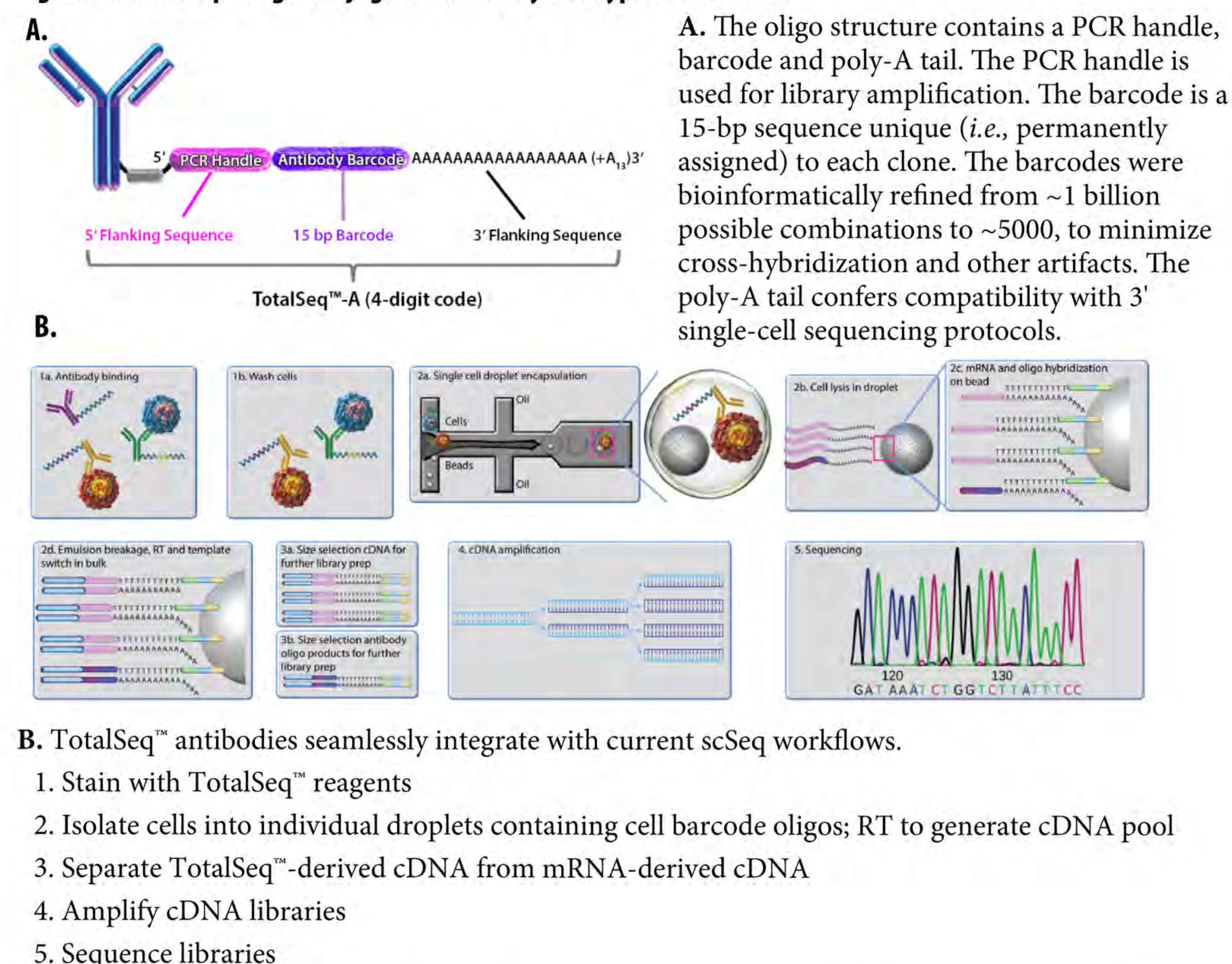
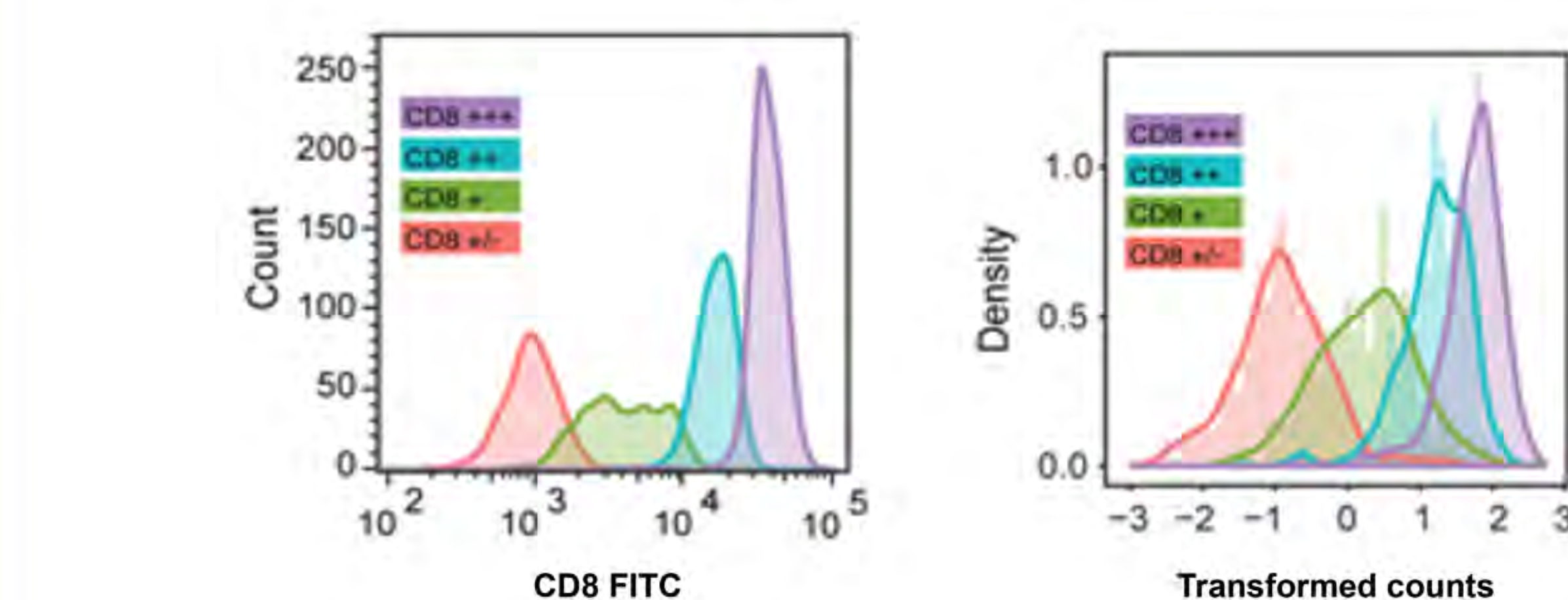
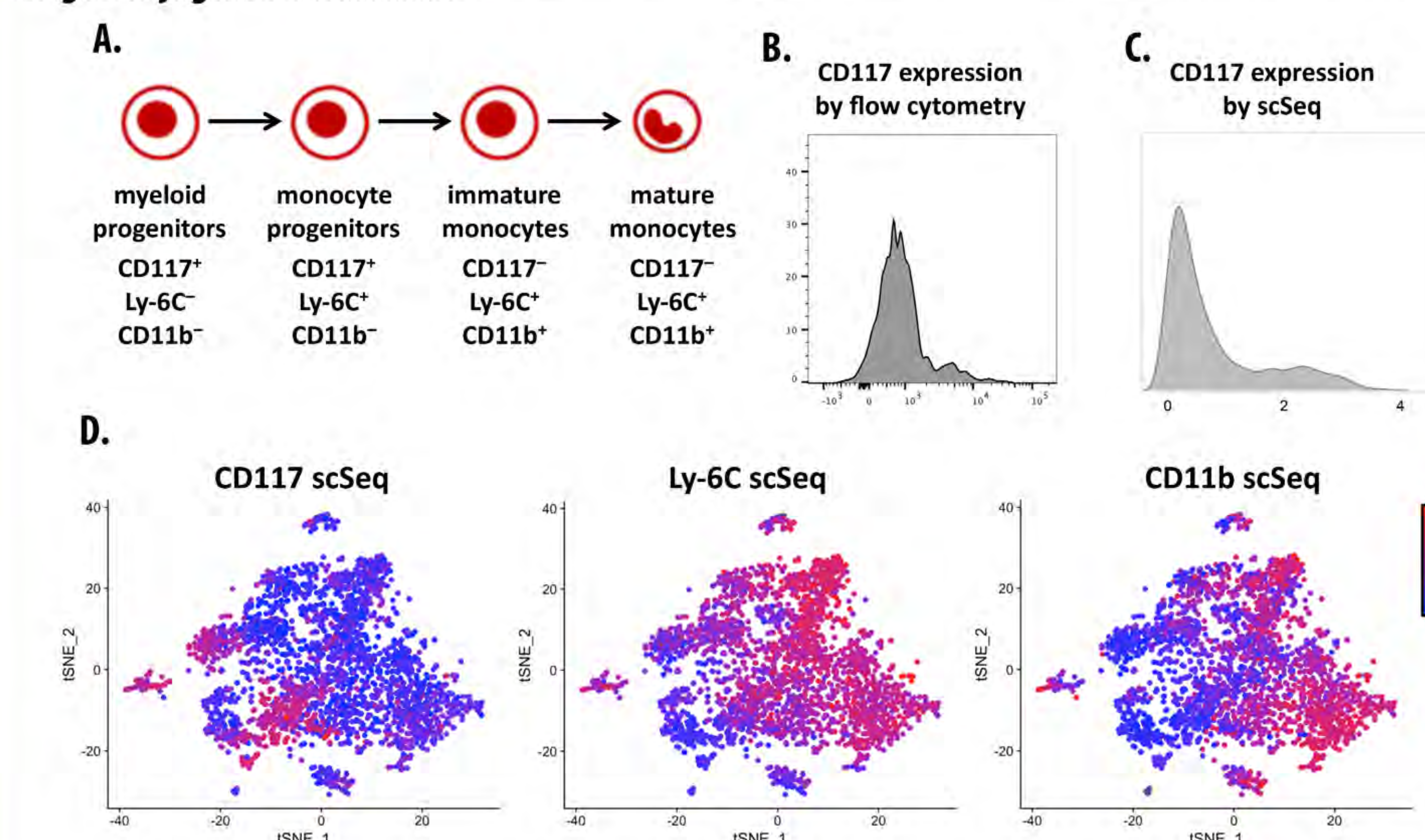


Figure 3. TotalSeq™ oligo-conjugated antibody and sequencing can recapitulate dynamic range of classical flow cytometry.



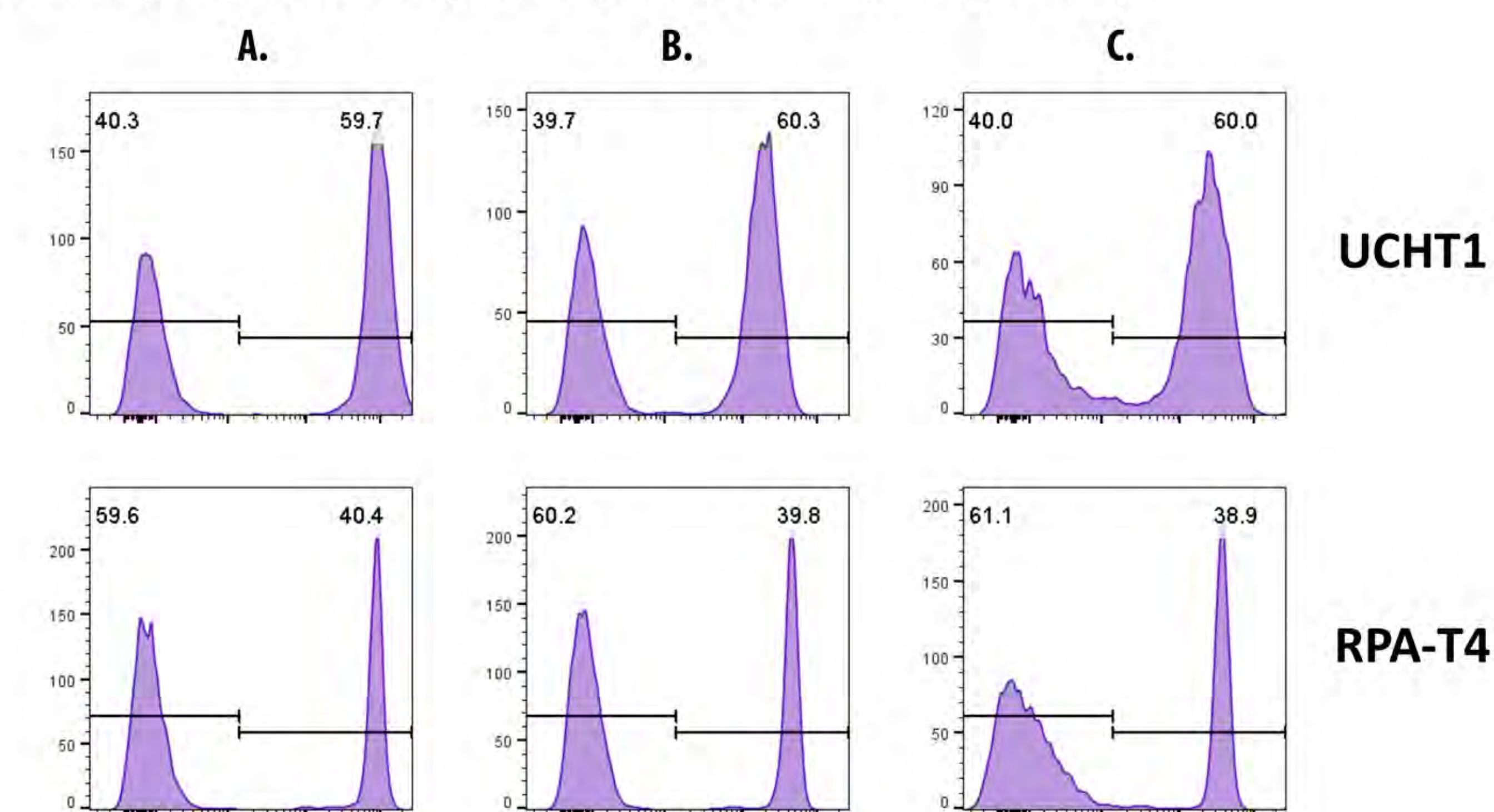
Human PBMCs were stained with a 1:1 mixture of FITC-conjugated or oligo-conjugated anti-human CD8. After washing, one half of the sample was analyzed by classical flow cytometry (left) and the other half was analyzed by sequencing (right).

Figure 5. Identifying myeloid progenitor populations in mouse bone marrow by scSeq using TotalSeq™ oligo-conjugated antibodies.



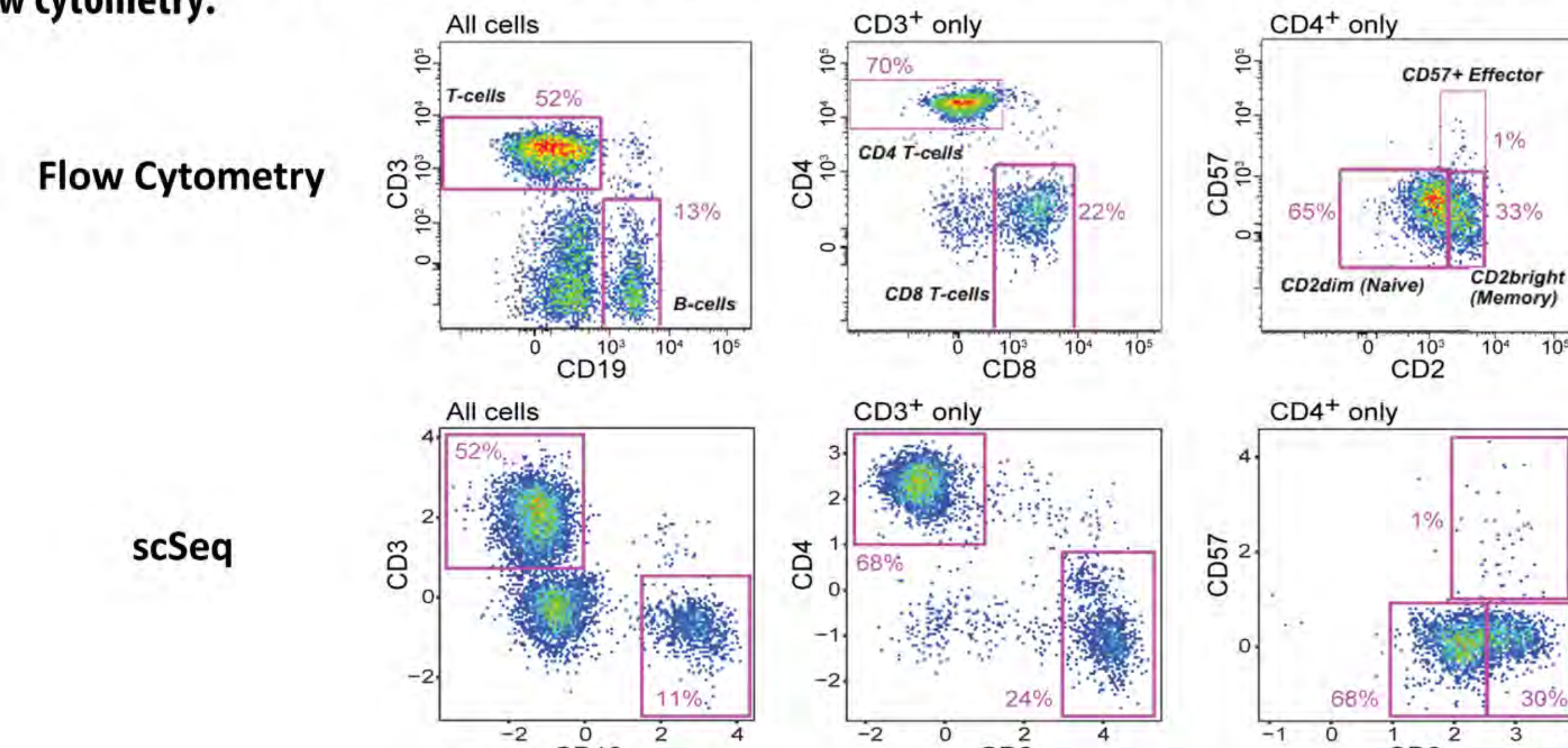
- The CD3 B220 Ly-6G CD115⁺ fraction of mouse bone marrow contains multi-lineage myeloid progenitors and monocyte-committed progenitors, as well as immature and mature inflammatory monocytes.
- Mouse bone marrow cells were analyzed by classical flow cytometry. CD117 expression is shown on CD3 B220 Ly-6G CD115⁺ gated cells.
- CD3 B220 Ly-6G CD115⁺ cells were FACS-sorted from mouse bone marrow. Cells were incubated with oligo barcode-conjugated anti-CD117, anti-Ly-6C, and anti-CD11b mAbs. scSeq results showed similar CD117 expression profile.
- Data from C were clustered on the most variable genes in the transcriptome. Protein expression detected by TotalSeq™ antibodies are superimposed on the clusters as indicated. The results demonstrate the identification of different populations as shown in A (*i.e.*, the presence of CD117⁺ Ly-6C⁻ myeloid progenitors, CD117⁺ Ly-6C⁺ monocyte progenitors, and Ly-6C⁺ CD11b⁺ monocytes).

Figure 2. Testing antibody reactivity after oligo-conjugation by flow cytometry.



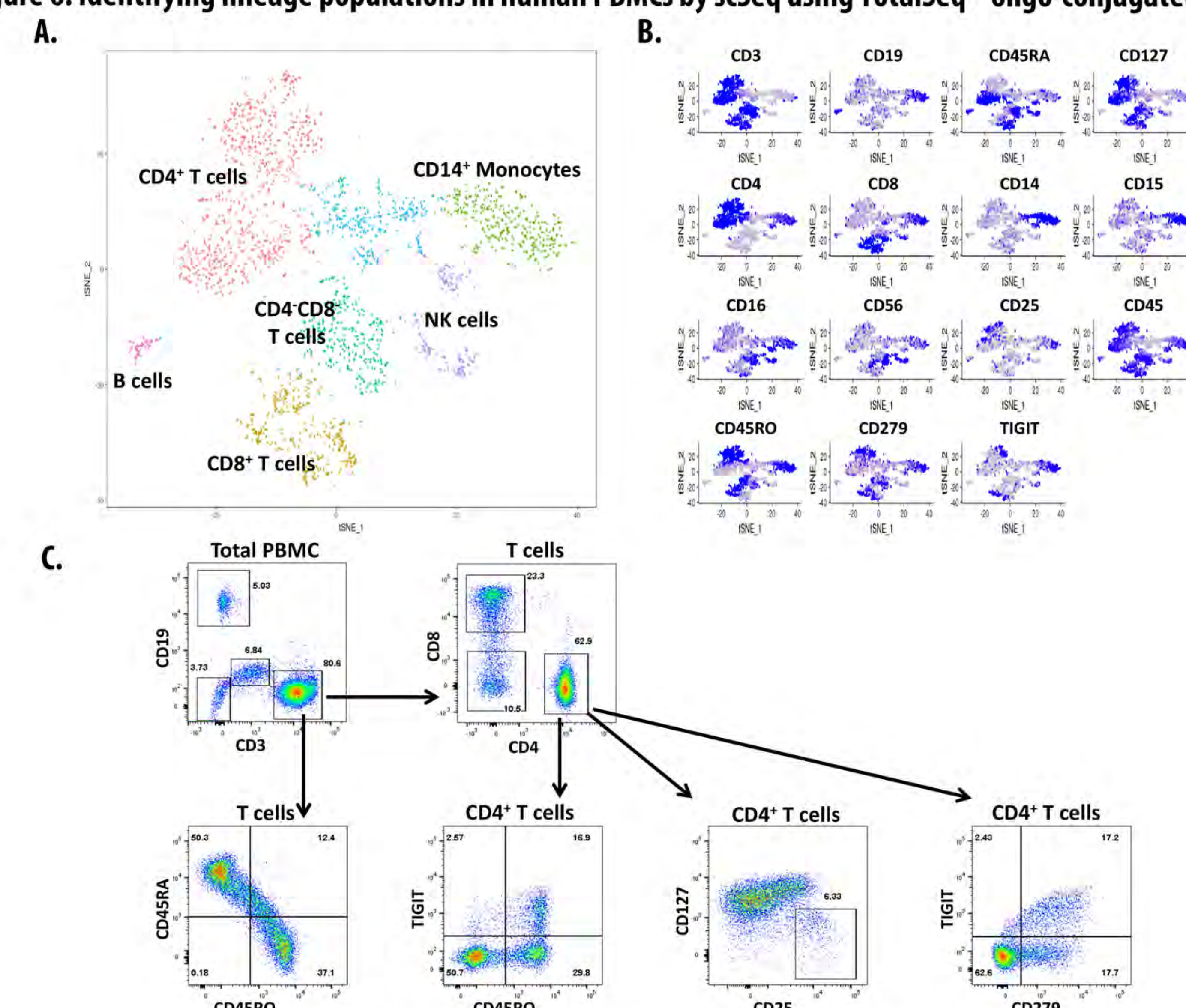
- Human PBMCs were stained with purified anti-human CD3 (clone UCHT1, upper row) or purified anti-human CD4 (clone RPA-T4, lower row), and detected by Alexa Fluor® 647-conjugated goat-anti-mouse IgG.
- Human PBMCs were stained with TotalSeq™ oligo-conjugated UCHT1 or RPA-T4 and detected by Alexa Fluor® 647 conjugated goat-anti-mouse IgG.
- Human PBMCs were stained with oligo-conjugated antibody (UCHT1 or RPA-T4) hybridized with Alexa Fluor® 647 conjugated oligo-dT. Cells were then analyzed by flow cytometry.

Figure 4. Oligo-conjugated antibody and sequencing can recapitulate cell population profile of classical flow cytometry.



Human PBMCs were stained with fluorophore-conjugated or TotalSeq™ oligo-conjugated antibodies as indicated. Cells were analyzed by flow cytometry or scSeq.

Figure 6. Identifying lineage populations in human PBMCs by scSeq using TotalSeq™ oligo-conjugated mAbs.



Human PBMCs were stained with fluorophore-conjugated or TotalSeq™ oligo-conjugated antibodies as indicated. The panel included antibodies to the following surface molecules: CD3, CD4, CD14, CD15, CD16, CD19, CD25, CD45RO, CD45RA, CD56, CD127, CD279, TIGIT. Cells were analyzed by flow cytometry or scSeq, respectively.

- Clustering of cell populations based on proteomic scSeq profiling.
- Cells expressing indicated markers in the clusters.
- Cell populations identified by classical flow cytometry.