TECHNICAL NOTE

Quality Control of Cell Surface Protein Labeling using Flow Cytometry

Introduction

Cell surface proteins can be labeled with a specific protein binding molecule, such as an antibody conjugated to a Feature Barcode oligonucleotide, to generate Chromium Single Cell 3' or 5' Cell Surface Protein libraries. This Technical Note outlines methods to perform quality control of cell surface protein labeling with antibody-oligonucleotide conjugates using flow cytometry, along with guidelines for determining optimal labeling concentration.

Method

Quality control for cell surface protein labeling was performed by following two methods:

- Verification of the specificity of the antibody conjugated to the Feature Barcode oligonucleotide.
- Verification of successful conjugation of the antibody and the Feature Barcode oligonucleotide.

Cell surface proteins were labeled as described in Demonstrated Protocol Cell Surface Protein Labeling for Single Cell RNA Sequencing Protocols with Feature Barcoding technology (Document CG000149).

QC: Verification of Antibody Specificity

To verify specificity of TotalSeq™ antibody-oligonucleotide conjugates, a single cell suspension was labeled with TotalSeq antibody followed by a secondary fluorophore antibody specific to the Fc region of the TotalSeq antibody. Simultaneously, another aliquot of the unlabeled single cell suspension was labeled with a fluorophore antibody only (same clone as TotalSeq). Labeled cells were analyzed by flow cytometry and antibody specificity was verified by comparing the presence of positive populations in both groups of labeled cells. Representative flow cytometry results are shown in the Illustrative Overview 1.

Also shown are the sequencing results of the libraries prepared using the cells labeled with TotalSeq and secondary fluorophore antibody.

QC: Verification of Conjugation

Conjugation methods may impact the number of cells labeled with the antibody-oligonucleotide conjugates. To test this, a biotin-oligonucleotide molecule with sequence complementary to the Feature Barcode oligonucleotide sequence was incubated with either TotalSeq or custom conjugated antibodies to generate a biotinylated antibody. A single cell suspension was labeled with the biotinylated antibody, followed by an anti-biotin fluorophore antibody. The labeled cells were analyzed by flow cytometry. Conjugation was verified by comparing the frequency of positive populations in TotalSeq and custom conjugated antibody labeled cells as shown in the Illustrative Overview 2.

Determination of Optimal Concentration of Antibodies

During cell labeling, an optimal amount of antibody-oligonucleotide conjugate is needed to minimize non-specific binding. An antibody titration experiment was performed by labeling a single cell suspension with different dilutions of TotalSeq antibody followed by a fixed amount of a secondary fluorophore antibody (specific to the Fc region of the TotalSeq antibody.) Simultaneously, another aliquot of the unlabeled single cell suspension was labeled with similar dilutions of a fluorophore antibody only (same clone as the TotalSeq). The labeled cells were analyzed by flow cytometry and the separation between the positive and the negative populations at different antibody dilutions was assessed to determine optimum antibody amounts. Representative flow cytometry results at different antibody dilutions are shown in Illustrative Overview 3. Also shown are the UMI counts of libraries generated at different dilutions of TotalSeq antibody.

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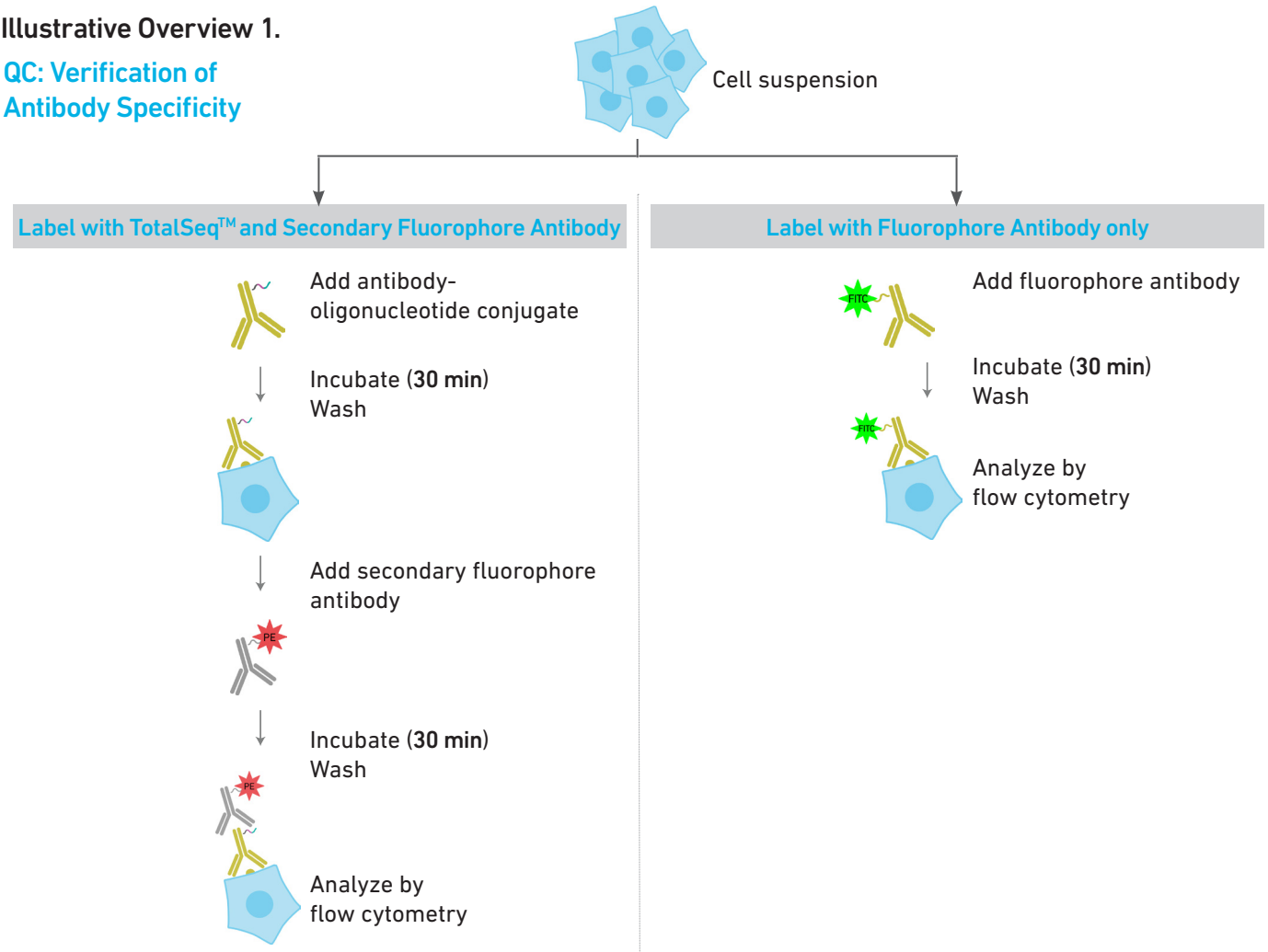
Illustrative Overview 1.

QC: Verification of Antibody Specificity



Results

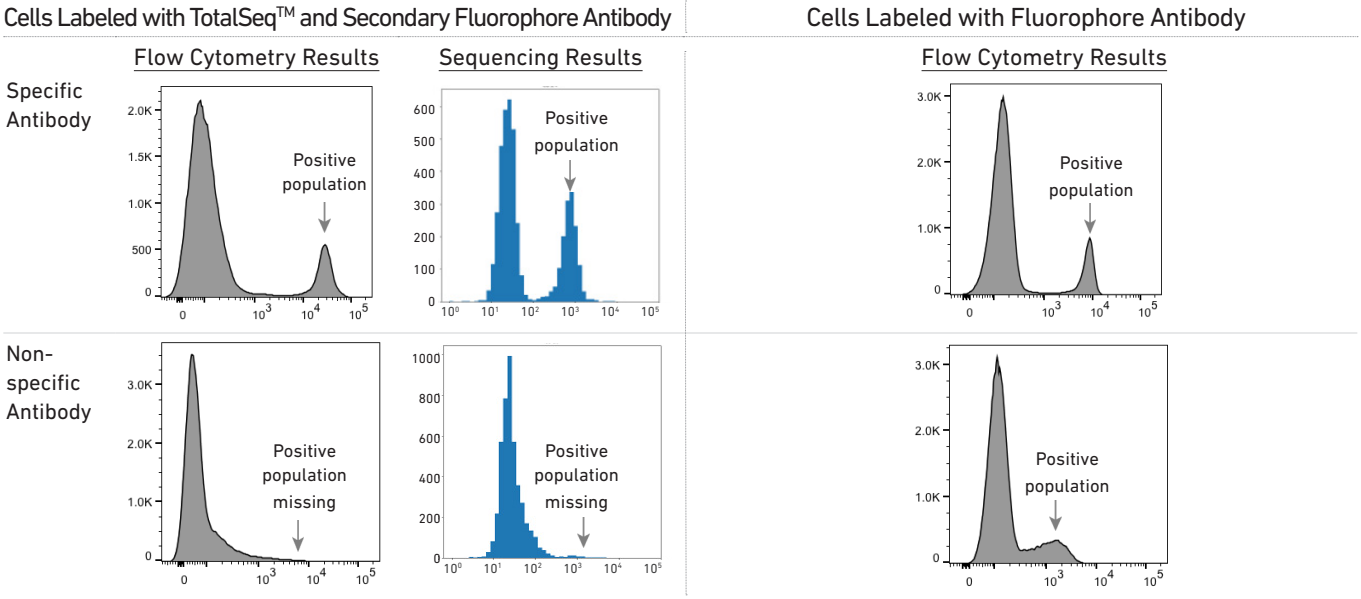


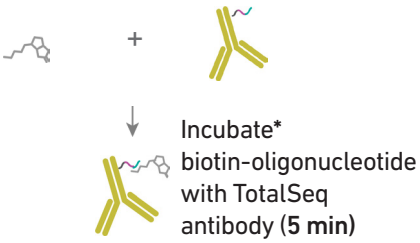
Figure 1. Comparison of TotalSeq and secondary fluorophore antibody labeled cells with fluorophore antibody (same clone) labeled cells. A target-specific antibody-oligonucleotide conjugate shows a distinct positive population that is comparable to the positive population in fluorophore antibody labeled cells. A non-specific antibody-oligonucleotide conjugate shows lack of positive population in TotalSeq and secondary fluorophore antibody labeled cells. In case of TotalSeq and secondary fluorophore antibody labeled cells, the flow cytometry cell counts were also confirmed by UMI counts.

Illustrative Overview 2.

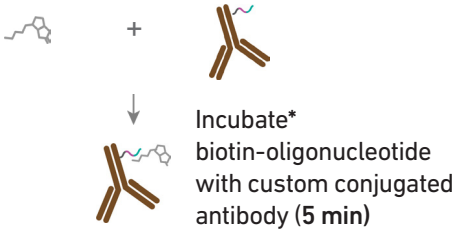
QC: Verification of Conjugation

Prepare Biotinylated Antibody

Prepare TotalSeq™ Biotinylated Antibody

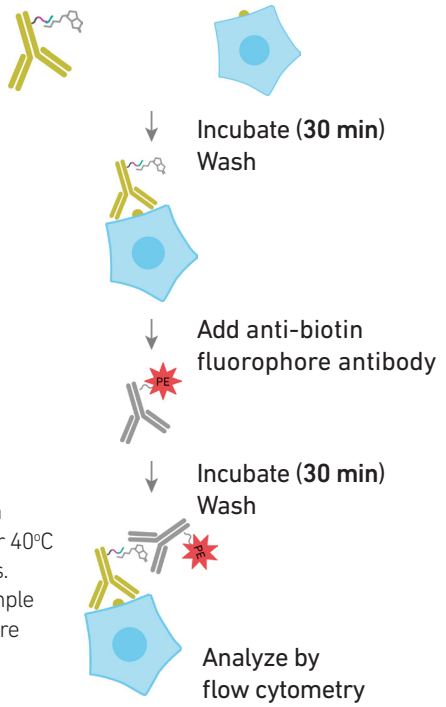


Prepare Custom Conjugated Biotinylated Antibody



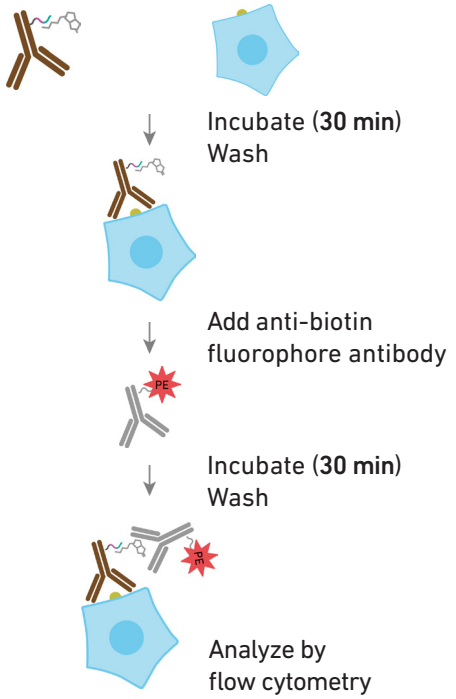
Label Cells

Label with TotalSeq™ Biotinylated Antibody



*Incubation can be performed at room temperature, 4°C or 40°C with similar results. Equilibrate the sample to room temperature before proceeding.

Label with Custom Conjugated Biotinylated Antibody



Results

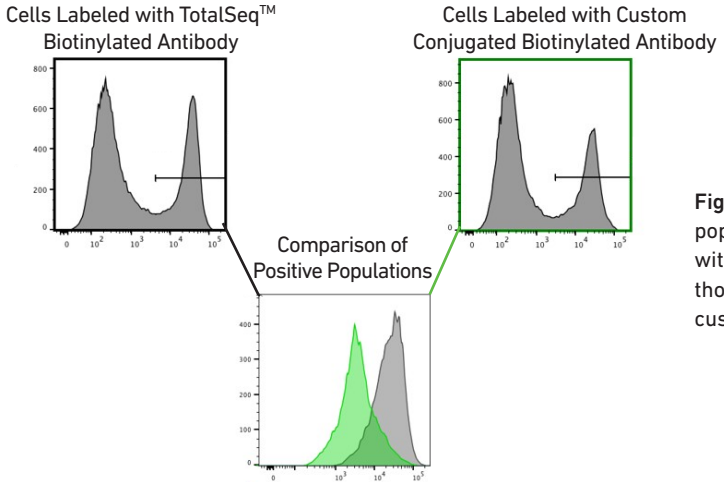
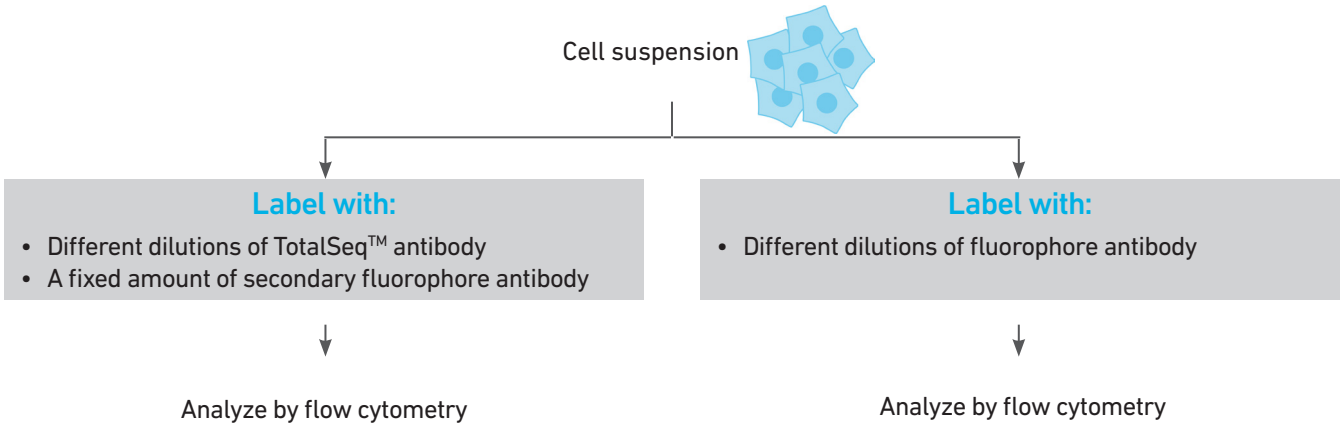


Figure 2. Comparison of positive populations obtained by labeling with TotalSeq antibodies with those obtained by labeling with custom conjugated antibodies.

Illustrative Overview 3.

Determination of Optimal Concentration of Antibodies



Flow Cytometry Results

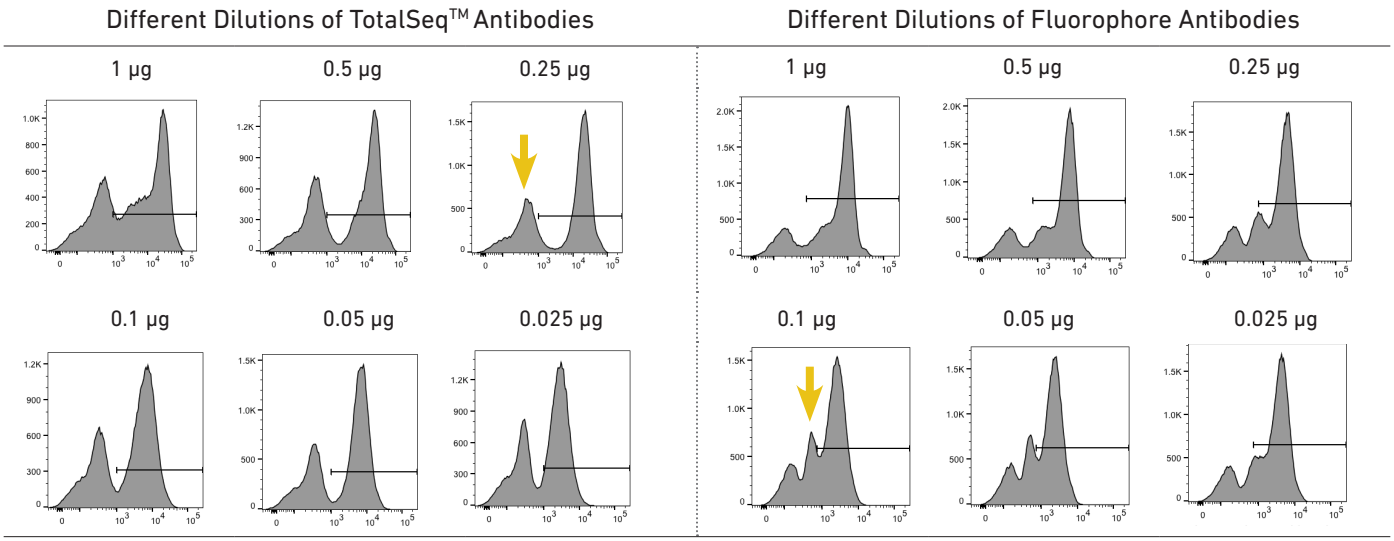


Figure 3. Flow cytometry cell counts using different antibody dilutions. A stable negative population (yellow arrow) denotes optimal working concentration. Decreasing the amount of antibody increases staining of the negative population without improving staining of the positive population.

Sequencing Results

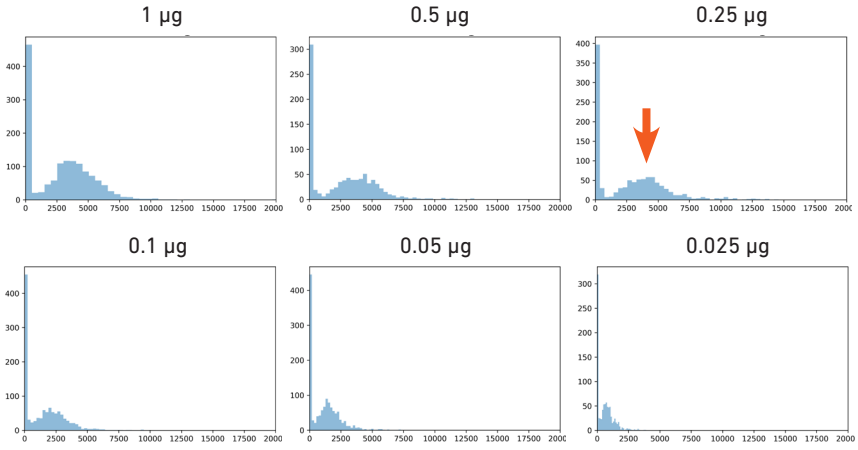


Figure 4. UMI counts using different dilutions of TotalSeq antibody. Orange arrow indicates optimal working concentration as decreasing the amount of antibody causes a shift in positive population to the left.

Results & Conclusions

In summary, the experiments and results described in this Technical Note serve as a guide to perform quality control of cell labeling with antibody-oligonucleotide conjugates using flow cytometry. The results show that flow cytometry can be used to:

- Verify the specificity of antibody-oligonucleotide conjugates using a secondary fluorophore antibody (Illustrative Overview 1).
- Confirm the presence of the Feature Barcode oligonucleotides in antibody-oligonucleotide conjugates using a specific biotin-oligonucleotide and an anti-biotin fluorophore antibody (Illustrative Overview 2).
- Determine the optimal concentration of antibody-oligonucleotide conjugates for cell labeling protocol (Illustrative Overview 3).

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