Examining the Specificity and Robustness of Site-Specific and Non-Site-Specific DNA-Labeled Antibodies

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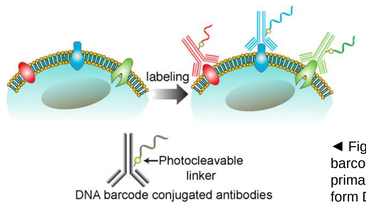
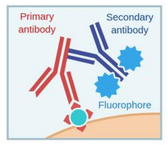
Background

- DNA barcode-antibody conjugates (DNA-Abs) are used when identifying multiple specific biomarkers on single cells. However, it is uncertain whether such conjugates lose sensitivity and specificity during DNA labeling.[1]
- DNA labels reside in amino acid residues (lysine) on antibodies.[2,3] For non-site-specific labeling, if the labels reside in the antigen-binding site, it can interfere with antibody binding.[2]
- Therefore, we investigated non-site-specific labeled antibodies (AbSeq, TotalSeq-A) and compared them to site-specific labeled antibodies (Oyo-Link Alpha Thera) to identify whether non-site-specific labeling interferes with DNA-Ab bindings.
- DNA-Abs: Primary antibodies that directly attaches to biomarkers and contain specific DNA barcodes (Figure 1). They are non-fluorescent to avoid spectral overlap and bleed-through.[4]
- Indirect immunofluorescence: When a fluorophore-conjugated secondary antibody (sAb) binds with a DNA-Ab, it emits a fluorescent wavelength that is detected through a flow cytometer (Figure 2).[4]



◄ Figure 1. DNA barcodes bind with primary antibodies to form DNA-Abs.



▲ Figure 2

Objectives

- Use fluorescent polydT to validate the specific DNA barcodes are successfully conjugated to their primary antibodies (Figure 3).
- Use sAb to validate the DNA-Abs are bound to the specific biomarkers.

▲ Figure 3. The polydT anneals to the poly(A) sequence of the barcodes.

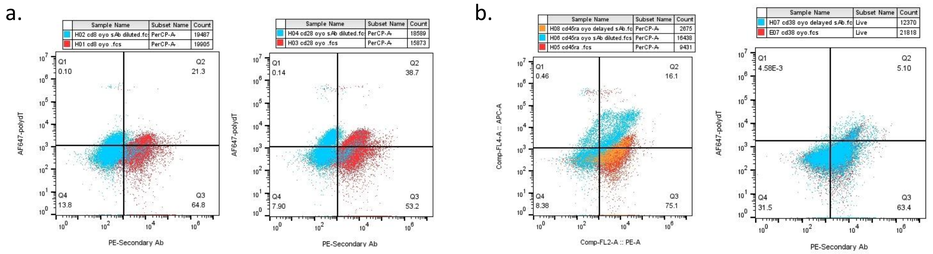
- Verify the specificity and robustness of the DNA-Abs through sAb and polydT signal strength:

  Test various combinations of concentrations for sAb and polydT:
  1) Original concentrations of polydT and sAb
  2) Diluted polydT 1:100 and sAb 1:100 (TotalSeq/Abseq conditions)
  3) Diluted polydT 1:100 and sAb 1:10 (Oyo-Link Conditions)
- Compare signal results of different DNA-Abs.
- Delay 15 minutes in adding sAb for certain conditions to verify whether dT binding interferes with sAb binding.

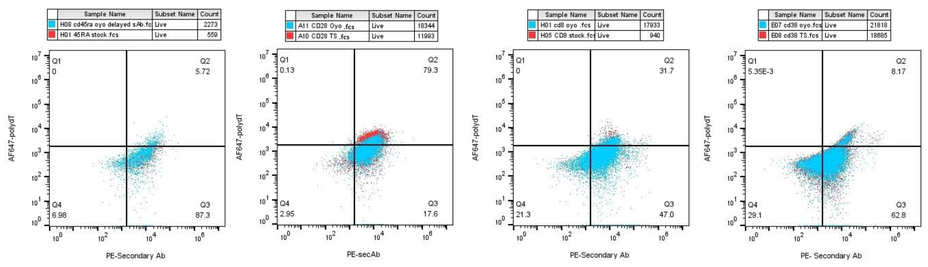
Materials and Methods

- CD8 cells were isolated from human peripheral blood mononuclear cell (PBMC) samples through the stem cell isolation kit.
- The CD8 cells were stained with primary (DNA-labeled) antibodies. The results of CD45RA (Abseq), CD8 (TotalSeq-A), and CD28 (TotalSeq-A) stains were compared to the Oyo-Link (Alpha Thera) antibody stains (CD45RA, CD8, CD28, CD38). All conditions were also stained with secondary antibody (anti-mouse PE sAb) and polydT (AF647).
- Gated for CD8 cells in flow cytometry analysis.
- Also compared the signal of unconjugated and fluorescent antibodies. (QC)

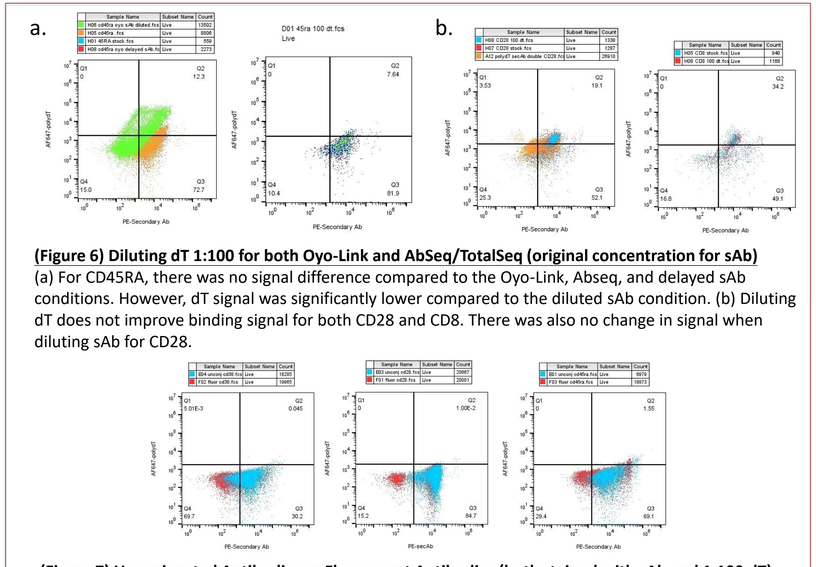
Results



(Figure 4) Oyo-Link conditions: Original sAb concentration vs 1:10 sAb dilution (both 1:100 dT)
(a) The population of the diluted sAb for CD8 and CD28 shifted left from the original population. It shows no improvement in dT signal, and sAb signal decreased. (b) Double positive signal increased significantly with diluted CD45RA, so the sAb dilution caused dT signal to increase. There was no significant signal difference when delaying in adding sAb for CD45RA and CD38.



(Figure 5) Comparing TotalSeq and Oyo-Link Conditions (both 1:100 dT and original sAb concentration)
CD28 TotalSeq had a slightly greater dT signal than Oyo-Link but in general, there were no significant signal difference between TotalSeq and Oyo-Link conditions, which suggests non-site-specific labeling were not interfering with DNA-Ab bindings.



(Figure 6) Diluting dT 1:100 for both Oyo-Link and AbSeq/TotalSeq (original concentration for sAb)
(a) For CD45RA, there was no signal difference compared to the Oyo-Link, Abseq, and delayed sAb conditions. However, dT signal was significantly lower compared to the diluted sAb condition. (b) Diluting dT does not improve binding signal for both CD28 and CD8. There was also no change in signal when diluting sAb for CD28.

(Figure 7) Unconjugated Antibodies vs Fluorescent Antibodies (both stained with sAb and 1:100 dT)
Since there was no DNA labels in these antibodies, there was no dT signal. The sAb signal for unconjugated antibodies matches the signal of the fluorescent antibodies.

Conclusions

- As seen in the Oyo-Link conditions, diluting sAb for CD45RA is effective in expressing dT binding signal, which can improve the identification of DNA-Ab bindings.
- Excluding CD45RA, diluting dT and sAb showed no signal change for the DNA-Abs tested, which indicates signal expression are antibody dependent.
- When diluting sAb, the concentration of sAb decreases, so when the dT signal increased with lower sAb concentration (for CD45RA Oyo-Link), it indicates potential interference between sAb and polydT binding could have occurred.
- **Future work:** More DNA-Abs can be tested (such as CITE-Seq/Abseq) and for different biomarkers. Different concentrations of sAb for CD45RA can also be tested.

References

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