Profiling of the antigen-specific immune response using Dextramer® and dCODE® reagents in combination with flow cytometry and 10x Chromium Single-Cell Analysis System
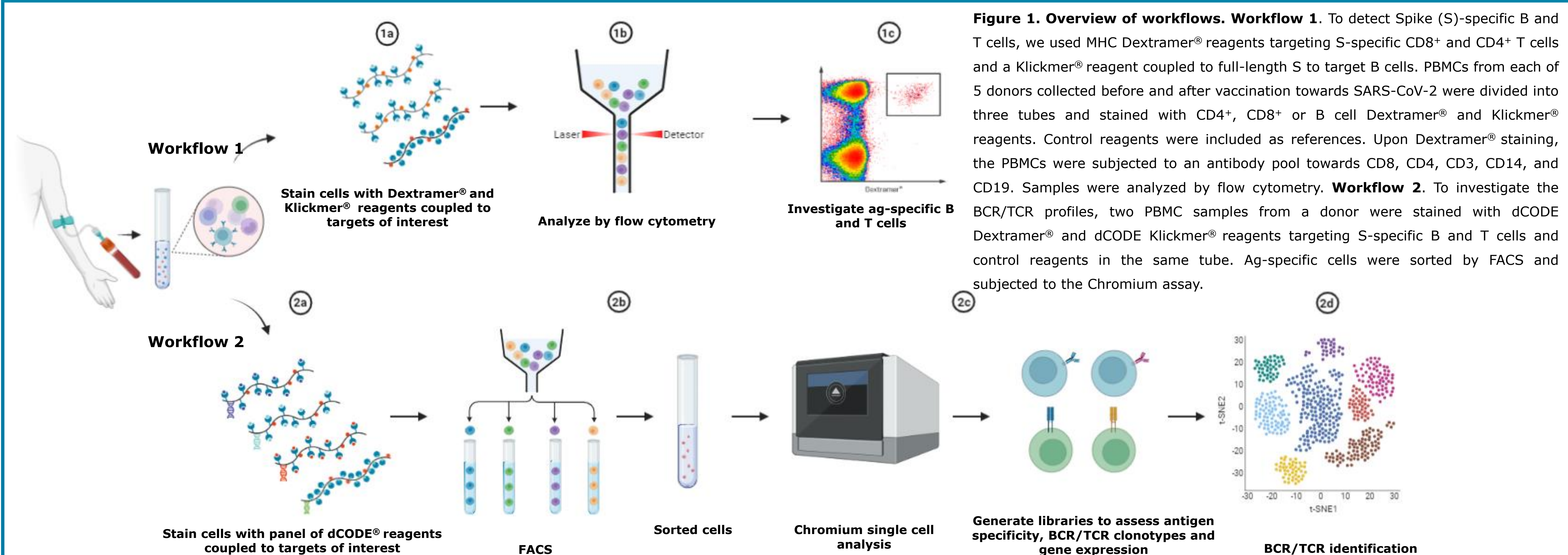
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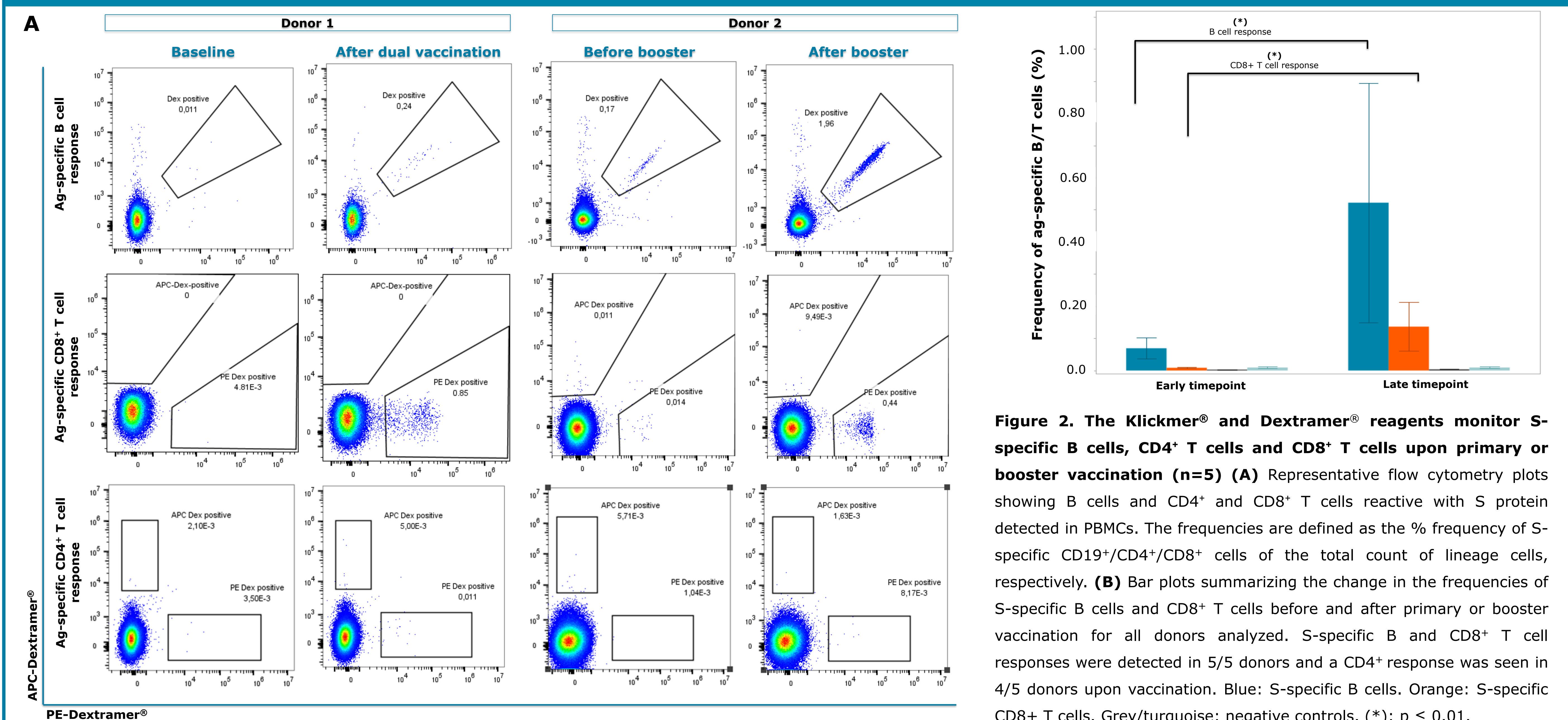[1]Immudex, Copenhagen, Denmark

Introduction

Understanding the antigen-specific B and T cell responses is key for development of vaccines and targeted therapies for cancers, encompassing various stages from target discovery to monitoring the treatment efficacy to patient stratification. The Dextramer® and Klickmer® reagents allow simultaneous detection of low-frequency ag-specific B and T cells in the same workflow. For a deeper investigation, the dCODE Dextramer® and dCODE Klickmer® reagents can be used in combination with single-cell RNA sequencing, which provides a deep dive into the ag-specific B and T cells at the individual cell level giving access to BCR/TCR sequences for specific targets.

Here we demonstrate two workflows in a SARS-CoV-2 model system for simultaneous detection of ag-specific B and T cells within the same sample using **(1)** Dextramer® and Klickmer® reagents in combination with flow cytometry or **(2)** dCODE Dextramer® and dCODE Klickmer® reagents in combination with the 10x Single-Cell Analysis System.
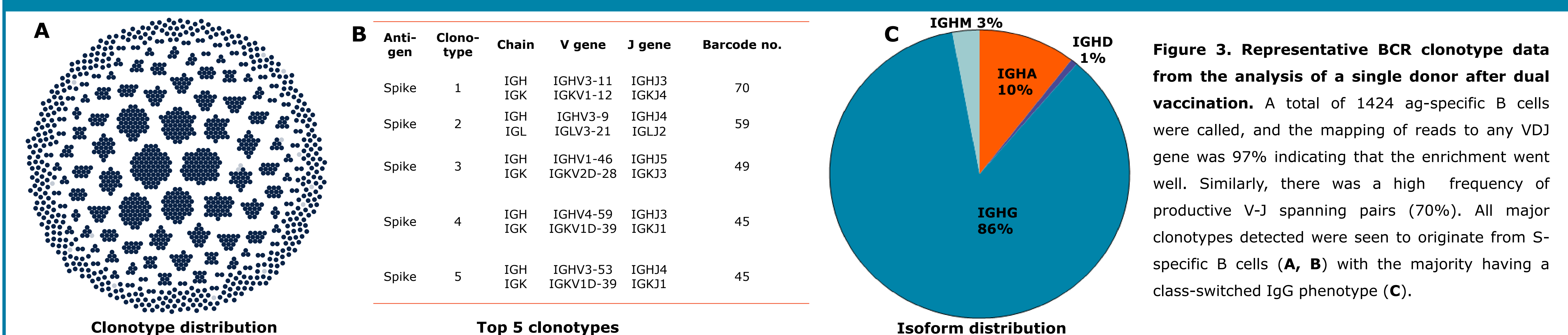
Workflows for simultaneous investigation of antigen-specific B and T cells in blood samples

Figure 1. Overview of workflows. Workflow 1. To detect Spike (S)-specific B and T cells, we used MHC Dextramer® reagents targeting S-specific CD8+ and CD4+ T cells and a Klickmer® reagent coupled to full-length S to target B cells. PBMCs from each of 5 donors collected before and after vaccination towards SARS-CoV-2 were divided into three tubes and stained with CD4+, CD8+ or B cell Dextramer® and Klickmer® reagents. Control reagents were included as references. Upon Dextramer® staining, the PBMCs were subjected to an antibody pool towards CD8, CD4, CD3, CD14, and CD19. Samples were analyzed by flow cytometry. **Workflow 2**. To investigate the BCR/TCR profiles, two PBMC samples from a donor were stained with dCODE Dextramer® and dCODE Klickmer® reagents targeting S-specific B and T cells and control reagents in the same tube. Ag-specific cells were sorted by FACS and subjected to the Chromium assay.

Dextramer® and Klickmer® reagents reveal changes in magnitude and kinetics of antigen-specific B and T cells upon vaccination

Figure 2. The Klickmer® and Dextramer® reagents monitor S-specific B cells, CD4+ T cells and CD8+ T cells upon primary or booster vaccination (n=5) (A) Representative flow cytometry plots showing B cells and CD4+ and CD8+ T cells reactive with S protein detected in PBMCs. The frequencies are defined as the % frequency of S-specific CD19+/CD4+/CD8+ cells of the total count of lineage cells, respectively. **(B)** Bar plots summarizing the change in the frequencies of S-specific B cells and CD8+ T cells before and after primary or booster vaccination for all donors analyzed. S-specific B and CD8+ T cell responses were detected in 5/5 donors and a CD4+ response was seen in 4/5 donors upon vaccination. Blue: S-specific B cells. Orange: S-specific CD8+ T cells. Grey/turquoise: negative controls. (*): $p \leq 0.01$.

dCODE Klickmer® reagents identify BCR clonotypes

Anti-gen	Clono-type	Chain	V gene	J gene	Barcode no.
Spike	1	IGH	IGHV3-11	IGHJ3	70
		IGK	IGKV1-12	IGKJ4	
Spike	2	IGH	IGHV3-9	IGHJ4	59
		IGL	IGLV3-21	IGLJ2	
Spike	3	IGH	IGHV1-46	IGHJ5	49
		IGK	IGKV2D-28	IGKJ3	
Spike	4	IGH	IGHV4-59	IGHJ3	45
		IGK	IGKV1D-39	IGKJ1	
Spike	5	IGH	IGHV3-53	IGHJ4	45
		IGK	IGKV1D-39	IGKJ1	

Clonotype distribution — **Top 5 clonotypes** — **Isoform distribution**

Figure 3. Representative BCR clonotype data from the analysis of a single donor after dual vaccination. A total of 1424 ag-specific B cells were called, and the mapping of reads to any VDJ gene was 97% indicating that the enrichment went well. Similarly, there was a high frequency of productive V-J spanning pairs (70%). All major clonotypes detected were seen to originate from S-specific B cells (**A, B**) with the majority having a class-switched IgG phenotype (**C**).

Conclusion

We have demonstrated two workflows for the simultaneous detection and characterization of ag-specific B and T cells in blood samples. **(1) Workflow 1** combines Dextramer® and Klickmer® reagents with flow cytometry to detect and characterize ag-specific B cells, CD4+ and CD8+ T cells. The workflow was demonstrated using SARS-CoV-2 as a model system to detect changes ag-specific B and T cells upon vaccination.

(2) Workflow 2 combines dCODE Dextramer® and dCODE Klickmer® with single-cell RNA seq to enable the examination of T and B cells in the same sample at the individual cell level and facilitates the evaluation of specific target BCR/TCR clonotypes. Although we are in the process of finalizing the sequencing of all samples, as an example we have presented data on BCRs for a single donor upon vaccination.

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