High-throughput antibody screening of plasma cells with Xdrop®

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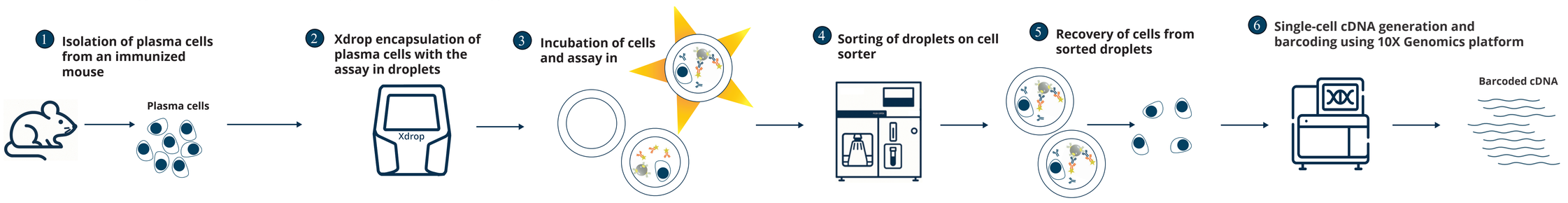
Introduction

High-throughput functional screening of antibody-secreting cells (ASC) accelerates antibody discovery beyond the slow, labor-intensive limits of hybridoma and memory B cell screening while capturing rare, high-affinity clones. We present a new Xdrop protocol that enables rapid functional screening of plasma cells from immunized animals. With a throughput of **>8 million droplets in minutes**, Xdrop compartmentalizes single cells together with the antibody discovery assay inside FACS-compatible droplets. This approach preserves cell viability, **detects secreted antibodies within droplets**, and supports direct V(D)J repertoire analysis as well as recovery of antigen-specific cells. With a streamlined, same-day workflow, this method dramatically reduces discovery timelines and enhances the identification of functional antibody candidates.

Xdrop single-cell antibody screening workflow



Antibody discovery assay

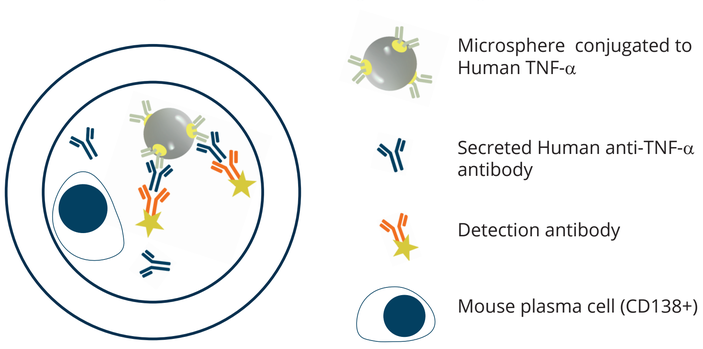


Figure 2: Antibody discovery assay in droplets. Secreted human anti-TNFα specific antibodies bind to the hTNFα-coated microspheres, allowing fluorescent detection via a labeled goat anti-mouse detection antibody, generating a concentrated and identifiable signal on the spheres.

Droplets with encapsulated plasma cells and antibody discovery assay

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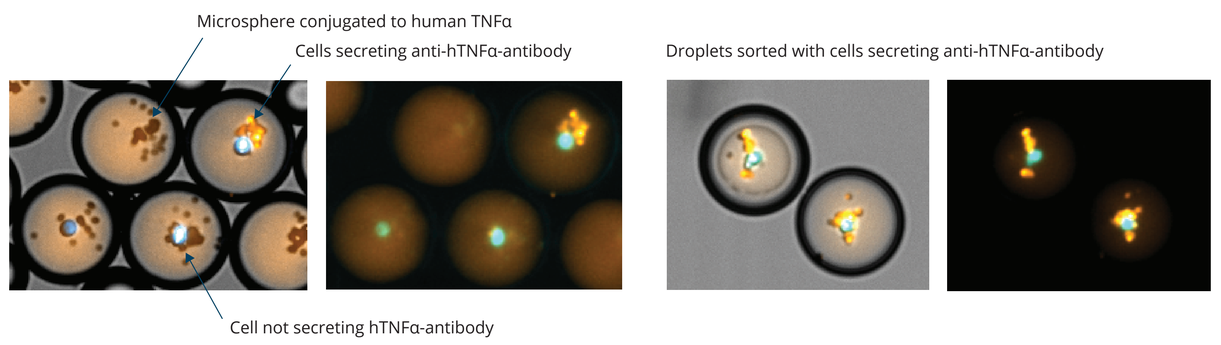


Figure 3: Image of droplets containing the Xdrop ASC screening assay taken with Xcyto®5 (ChemoMetec). For imaging, cells were stained post droplet production with Calcein Blue, AM. Cells secreting anti-hTNFα-antibodies clearly show an accumulated fluorescent signal on the TNFα-coated microspheres.

4 Sorting of plasma cells in droplets

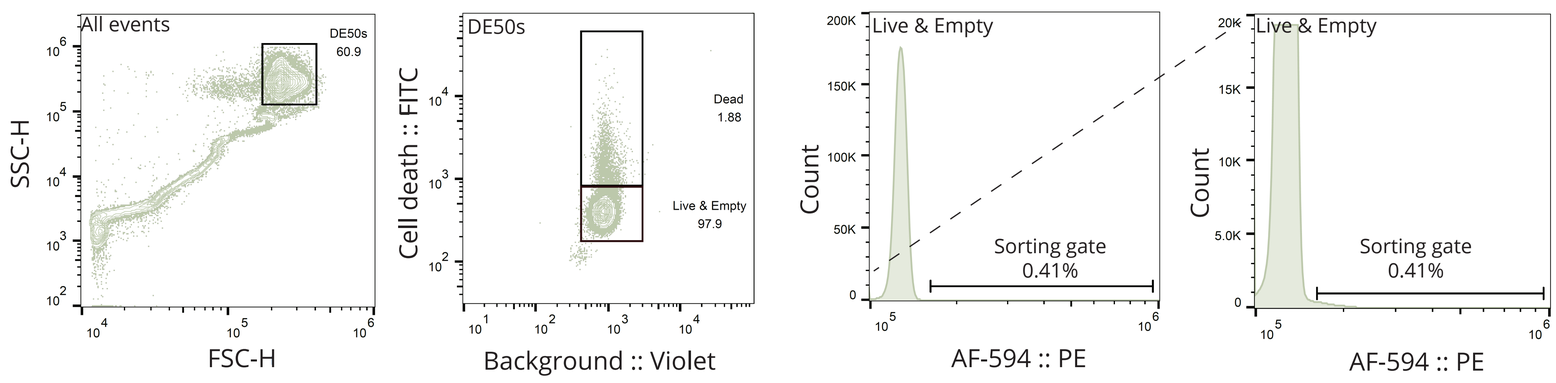


Figure 4: Gating strategy for sorting Xdrop DE50 droplets containing plasma cells sorted on Sony MA900 Cell Sorter with Large Particle Sorting upgrade. The DE50 droplets are identified on a scatter plot. Since only dead cells are labeled, both droplets containing live cells as well as empty droplets are gated.
From these, droplets containing plasma cells secreting anti-hTNFα-antibodies clearly show an accumulated fluorescent signal from the AF594-labeled detection antibody. The plot on the far right is a zoom-in.

6 V(D)J sequencing of sorted plasma cells

	Mouse A	Mouse B
Macs-purified plasma cells (CD138+)	**3,530,000**	**3,640,000**
Screened plasma cells	**1,149,418**	**993,906**
Sorted droplets with antigen specific antibodies	**16,449**	**10,048**
Obtained single-cell V(D)J sequences	**2,510**	**1,153**
Number of clonotypes	**739**	**432**
Theoretical obtainable sequences	**7,709**	**4,223**

Table 1: Summary of results of ASC screening in two mice.

Result of ASC screening

From ~1 million CD138+ B cells screened per mouse, droplets with the strongest TNFα-specific signal yielded ~16,000 (Mouse A) and ~10,000 (Mouse B) sorted droplets (Table 1). V(D)J sequencing produced 2,510 and 1,153 paired sequences, corresponding to 739 and 432 clonotypes, respectively. This represents roughly one-third of each mouse's plasma cell pool, suggesting >10,000 sequences could be obtained in total.

Sequencing revealed strong enrichment for class-switched IgG plasma cells in the antigen-specific fraction, while non-screened ASCs showed a more mixed repertoire dominated by IgM (Figure 5).These findings suggest that the screening approach selectively enriches for mature, class-switched plasma cells capable of secreting high-affinity anti-TNFα antibodies.

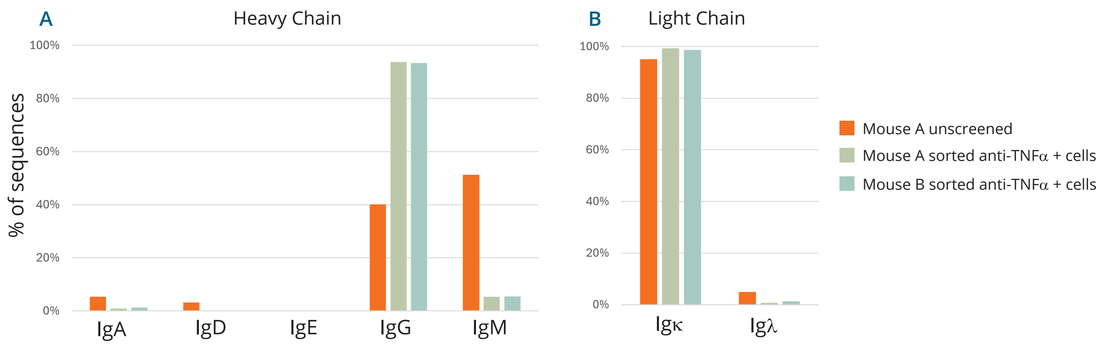


Figure 5: A. Heavy chain isotype distribution showing enrichment of IgG in TNFα-specific ASCs.
B. Light chain isotype distribution frequency was uniform as expected.

Conclusion

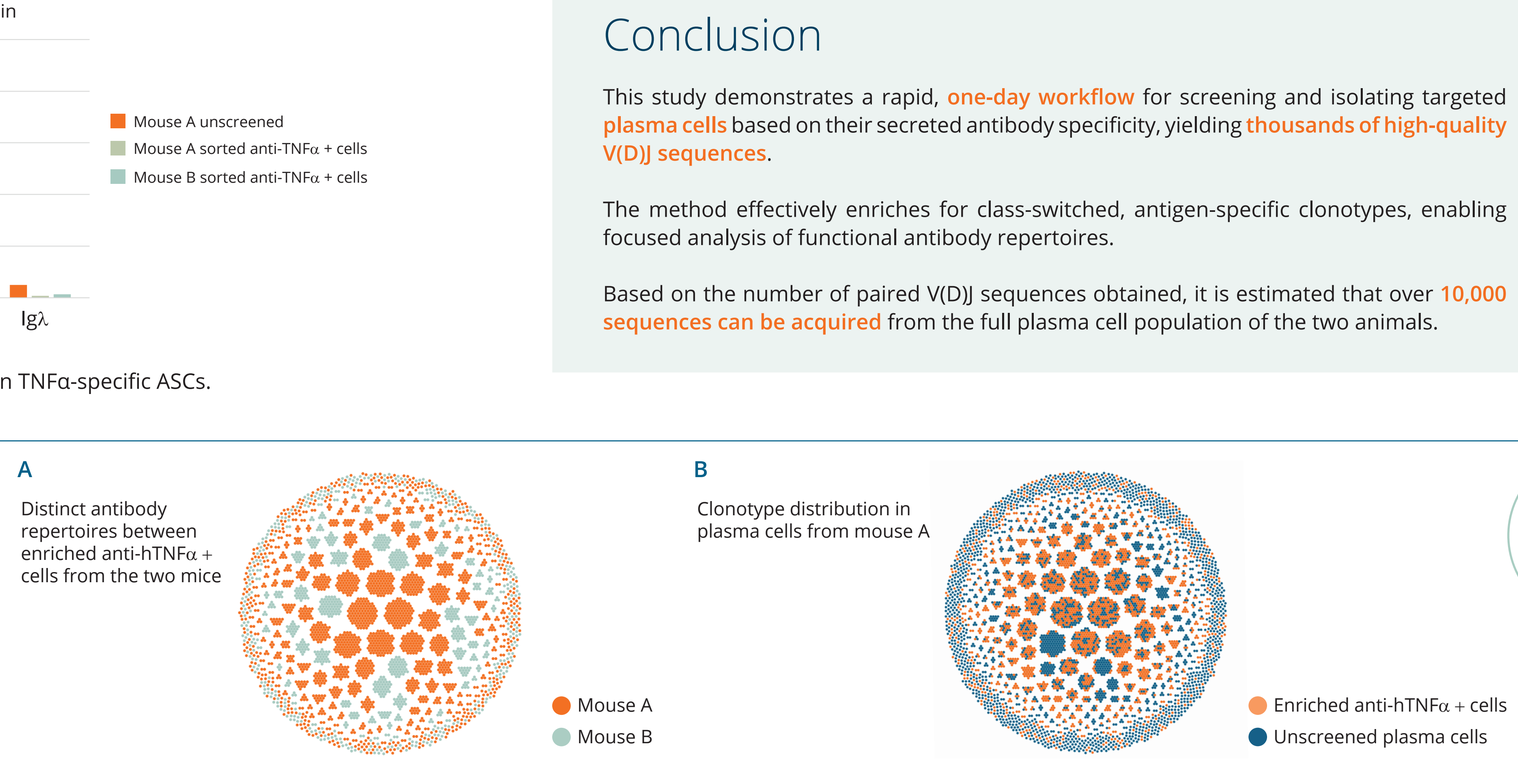
This study demonstrates a rapid, **one-day workflow** for screening and isolating targeted **plasma cells** based on their secreted antibody specificity, yielding **thousands of high-quality V(D)J sequences**.

The method effectively enriches for class-switched, antigen-specific clonotypes, enabling focused analysis of functional antibody repertoires.

Based on the number of paired V(D)J sequences obtained, it is estimated that over **10,000 sequences can be acquired** from the full plasma cell population of the two animals.

Clonotype distribution

Figure 6: Clonotype distribution plots. Each cluster represents a unique clonotype, with cluster size proportional to its relative frequency within the repertoire. Expanded clonotypes are represented by larger clusters concentrated toward the centre, whereas rare clonotypes appear as smaller symbols at the periphery. A. Enriched anti-hTNFα plasma cells from Mouse A and Mouse B display distinct clonotype repertoires. B. The Xdrop ASC screen enriches specific clonotypes shared with the unscreened population, while others remain dominant only in unscreened ASCs and are not enriched by the screen.



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