APPLICATION NOTE

Samplix

Thousands of V(D)J sequences in a one-day plasma cell screening workflow

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Summary

- Plasma cells from immunized mice are screened for antigen-specific antibody secretion in a one-day workflow.
- V(D)J sequencing yield 2,510 and 1,153 sequences from ~1 million screened plasma cells per mouse.
- This large-scale data generation provides a strong foundation for applying AI in antibody discovery.

Introduction

Monoclonal antibodies are foundamental to today's therapeutic approaches in a wide range of medical conditions. Antibody-secreting cells (ASCs) are a rich source of highly specific, high-affinity antibodies; however, their use has been limited due to technological constraints and their low abundance in peripheral blood[1]. Isolation of plasma cells producing desired antibodies is not compatible with conventional cell sorting, such as FACS, as antibodies are secreted and few or no immunoglobulins are expressed on the surface[2]. Existing methods for studying antigen-specific ASCs are often low throughput, costly, labor-intensive, or technically complex, limiting their broader accessibility and application[3].

Here, we present a new Xdrop® high throughput and FACS compatible protocol for screening and isolating antigen specific ASCs. This one-day protocol enables functional screening of millions of plasma cells individually encapsulated in droplets followed by cell recovery and subsequent single-cell V(D)J sequencing (Figures 1 & 2).

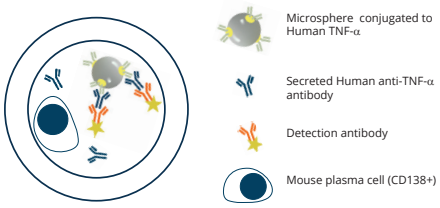

Figure 1. Antibody discovery assay in droplets. Secreted human anti-TNFα specific antibodies bind to the hTNFα-coated microspheres, allowing fluorescent detection via a labeled goat anti-mouse detection antibody, generating a concentrated and identifiable signal on the spheres.

Immunization and cell preparation

Mice (NMRI, female) were immunized four times with human TNFα (25 µg per injection) with three-week intervals and received a booster vaccine three days before euthanasia. The spleen of each mouse was mechanically dissociated on a 100 µm cell strainer (Sarstedt) followed by a 70 µm cell strainer (Sarstedt) using Wash buffer (dPBS with 0.5% BSA and 2 mM EDTA) to wash cells through the strainers. Plasma cells in Wash buffer were isolated using a CD138+ Plasma Cell Isolation Kit (Miltenyi Biotec). Cells were counted before and after cell separation using a CytoSmart (Axion Biosystems).

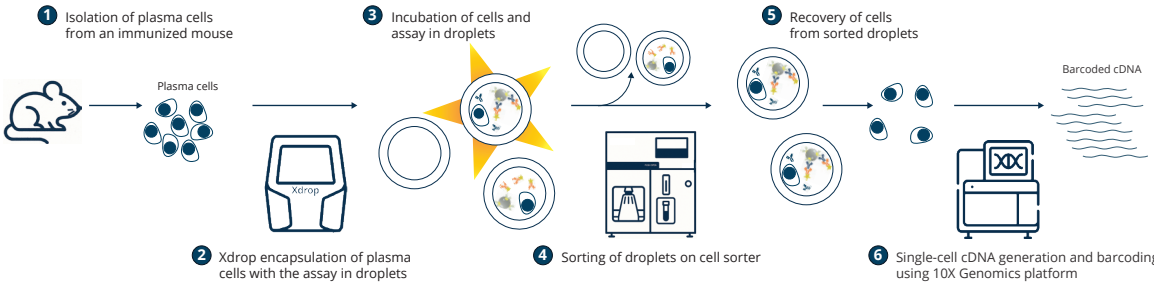

Figure 2. Xdrop-based one-day workflow enabling screening, recovery of antigen-specific plasma cells, and single-cell sequencing.

Encapsulation in droplets and sorting

Using the Xdrop droplet generator and the Xdrop DE50 Cartridge (Samplix), plasma cells were encapsulated in double-emulsion droplets (water-oil-water). Xdrop can produce droplets in 8 separate lanes simultaneously. Here, 400,000 plasma cells were loaded in each lane. The inner buffer in the droplets was RPMI-1640 with 10% FBS. Monovalaent human TNFα-coated microspheres (2 µm, 20-30 spheres per droplet, PolySciences), SytoxGreen as cell viability indicator (0.5 µM, ThermoFisher), and AF594-labeled goat anti-mouse antibody (FCγ-fragment specific, Jackson ImmunoResearch) for detection were encapsulated in the droplets together with the plasma cell and buffer. RPMI-1640 with 10% FBS and with DE stabilizer C (Samplix) was used as the outer buffer surrounding the droplets.

The cells and assay reagents were incubated inside the droplets in a CO_2 incubator at 37°C for 1–2 hours to allow antibody secretion. Double-emulsion droplets containing cells are compatible with flow cytometry. The droplets with the plasma cells were sorted on a MA900 Cell Sorter using the Large Particle Sorting upgrade (Sony) (Figure 3). Sorted droplets were collected in 100 µl DE Flow buffer (Samplix) in a 2 ml DNA LoBind Eppendorf tube.

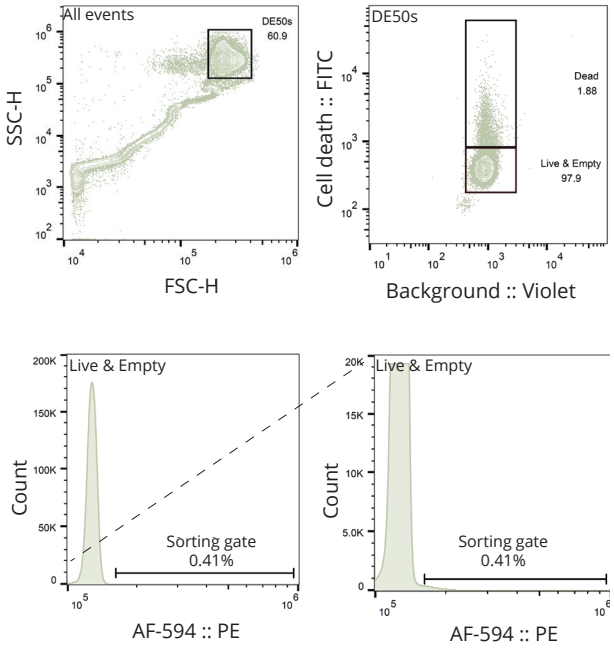

Figure 3. Gating strategy for sorting DE50 droplets containing plasma cells sorted on Sony MA900 Cell Sorter with Large Particle Sorting upgrade. The DE50 droplets are identified on a scatter plot. Since only dead cells are labeled, both droplets containing live cells as well as empty droplets are gated. 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.

Cells and antibody secretion were distinguishable by imaging of DE50 droplets loaded with plasma cells and assay reagents using a Xcyto®5 (ChemoMetec) (Figure 4A-B). Enrichment by sorting was confirmed through imaging (Figure 4C-D).

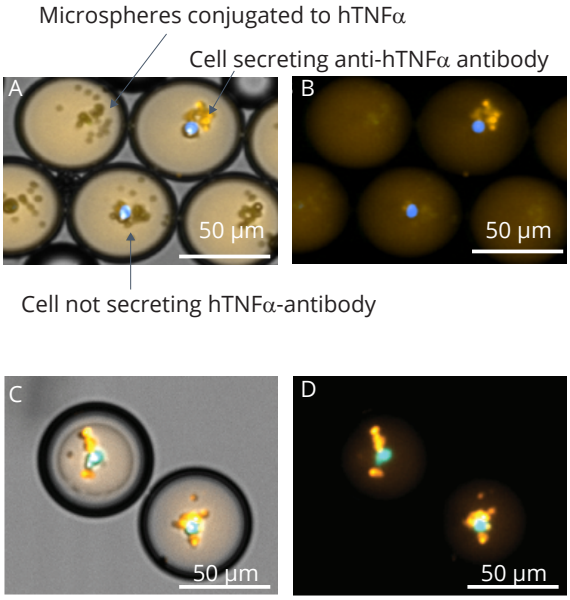

Figure 4. Image of droplets containing the Xdrop ASC screening assay taken with Xcyto5, ChemoMetec. For imaging, cells were stained with Calcein Blue, AM post droplet production. Cells secreting anti-hTNFα-antibodies clearly show an accumulated fluorescent signal on the TNFα-coated microspheres (A and B). Panels C and D show two droplets retaining a highly concentrated signal following sorting. Panels A and C were overlaid with brightfield images.

Cell recovery and sequencing

Cells were recovered from the sorted droplets by breaking the droplets with Droplet Break solution (Samplix), and an aliquot was analyzed on a Novocyte Quanteon flow cytometer (Agilent) using Calcein Blue, AM and SytoxGreen (ThermoFisher) staining to estimate cell numbers and viability, respectively.

The remainder of the cells were used as input for single-cell V(D)J sequencing using Chromium (10X Genomics) and NextSeq (Illumina). In parallel, unscreened ASCs from one mouse were subjected to V(D)J sequencing. Since hTNFα-secreting cells were not isolated from this control sample, it served as a negative control for droplet-based enrichment.

	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 ASC screen.

Screening results

Approximately 1 million CD138+ plasma cells were screened from each mouse. Flow cytometry sorting of DE50 droplets with the highest hTNFα (AF594) signal resulted in ~16,000 (Mouse A) and ~10,000 (Mouse B) sorted droplets (Table 1). V(D)J sequencing of the cells recovered from the sorted droplets after droplet breaking resulted in paired V(D)J sequences from 2,510 and 1,153 cells, giving 739 and 432 clonotypes (unique combinations of V(D)J sequences), respectively. Only cells containing a complete variable heavy (VH) and variable light (VL) chain sequence were included in the analysis. As only approximately one-third of the total CD138+ cells purified from the two mice were screened (~1 million CD138+ cells/3 million per mouse), the total theoretically obtainable number of V(D)J sequences could exceed 10,000.

Sequencing analyses revealed that antibody-secreting cells (ASCs) selected for secretion of human TNFα-specific antibodies predominantly comprised class-switched plasma cells expressing IgG heavy chains, whereas unscreened ASCs exhibited a more heterogeneous heavy chain repertoire, with approximately 50% expressing IgM. 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).

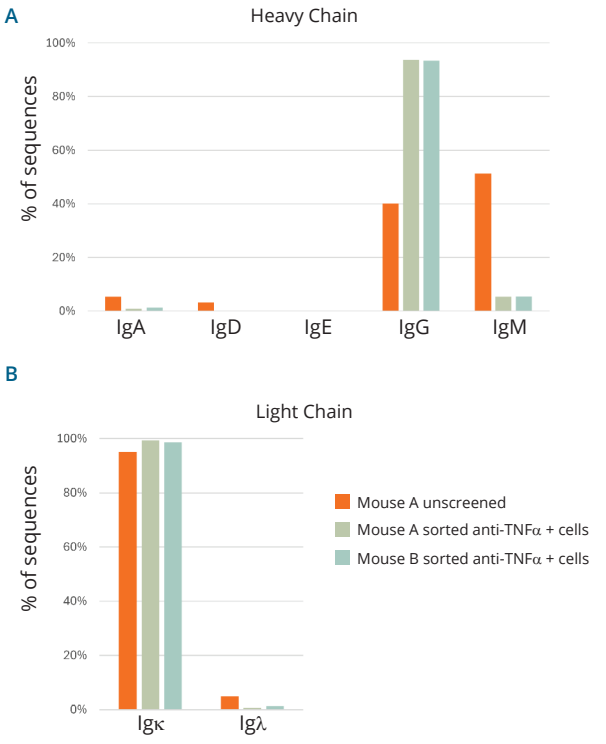

Figure 5. A: Heavy chain isotype distribution showing enrichment of IgG in TNFα-specific ASCs. B: The light chain isotype distribution frequency was as expected

Clonotype distribution

Clonotype analysis indicate that the Xdrop selection process enrich for the antigen specific subset of clonotypes. This is evident from the clonotype distribution plots, where certain clonotypes appear almost exclusively in the unscreened (non-encapsulated) population of ASCs and are absent from the sorted fraction (Figure 6A). Clonotypes in the sorted (enriched) population are, as expected, also present in the unscreened sample. This is consistent with the fact that the screened ASCs represent a subpopulation of the broader ASC pool. The analysis also enabled a comparison of the hTNFα-specific antibody clonotypes between the two different mice, revealing distinct and non-overlapping clonotype repertoires, illustrating the biological variation (Figure 6B).

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, it is estimated that over 10,000 sequences can be acquired from the full plasma cell population of the two animals. This level of throughput and specificity support AI-driven antibody development by providing the diverse data needed for training and optimization.

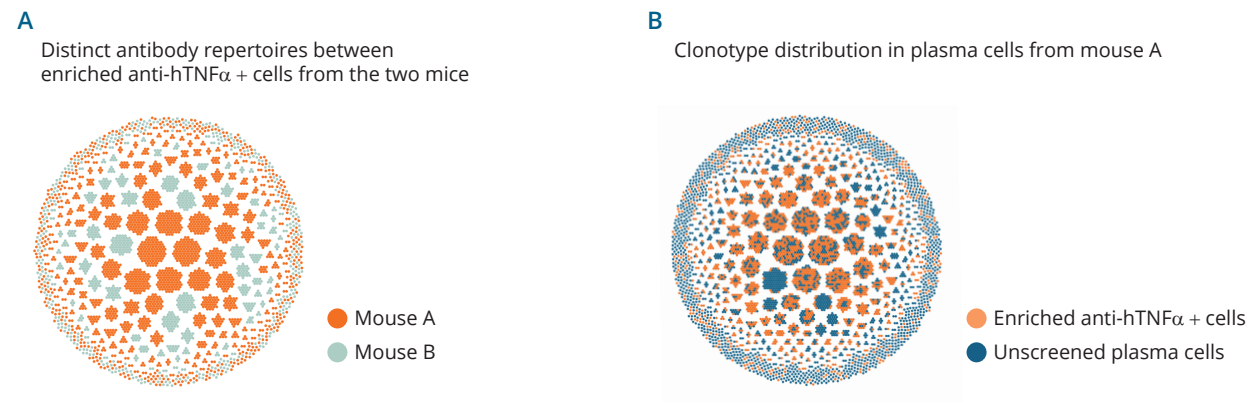

Figure 6. Clonotype distribution plots. Each cluster represents a unique clonotype, with cluster size proportional to its relative frequency within the repertoire. Expanded clonotypes are represented by larger clusters concentrated toward the centre, whereas rare clonotypes appear as smaller symbols 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.

Notes and references

1. Pedrioli, A. & Oxenius, A. Single B cell technologies for monoclonal antibody discovery. Trends Immunol. 42, 1143–1158 (2021).
2. Pinto, D. et al. A functional BCR in human IgA and IgM plasma cells. Blood 121, 4110–4114 (2013).
3. Fischer, K. et al. Rapid discovery of monoclonal antibodies by microfluidics-enabled FACS of single pathogen-specific antibody-secreting cells. Nat Biotechnol. 43(6), 960-970 (2025).