APPLICATION NOTE

Samplix

Droplet-based screening for membrane protein antibody discovery

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Summary

- HER2-targeting antibodies identified in a one-day screening workflow of plasma cells in droplets.
- Xdrop enables high-throughput antibody discovery with functional assays.
- Native membrane antigen presentation via co-encapsulation of antigen-expressing and antibody-secreting cells in droplets.

Introduction

Raising antibodies against membrane proteins can be challenging, particularly when only a limited portion of the target is exposed on the cell surface[1]. While some membrane proteins contain large extracellular domains, others present short extracellular loops, restricting the number of accessible epitopes available for immunization and antibody screening. Furthermore, antibodies generated against isolated peptides may not recognize the native target, as many biologically relevant epitopes depend on the three-dimensional conformation of the membrane protein[2].

Here, we demonstrate high-throughput discovery of antibodies targeting membrane proteins with either limited or large extracellular domains via two approaches:

In a bead-based assay, the extracellular domain of human HER2 is conjugated to beads and co-encapsulated with antibody-secreting cells (ASCs) in droplets. Following functional screening and cell recovery, single-cell V(D)J sequencing is used to generate clonotype distribution maps from which HER2-binding antibodies are identified.

In a complementary assay, antigen-expressing cells, rather than antigen-coated beads, are co-encapsulated with ASCs. Unlike bead-based antigen presentation, this approach preserves the native presentation of extracellular epitopes and provides a more broadly applicable solution for antibody discovery against membrane protein targets.

Overall, Xdrop facilitates antibody discovery through adaptable functional screening strategies.

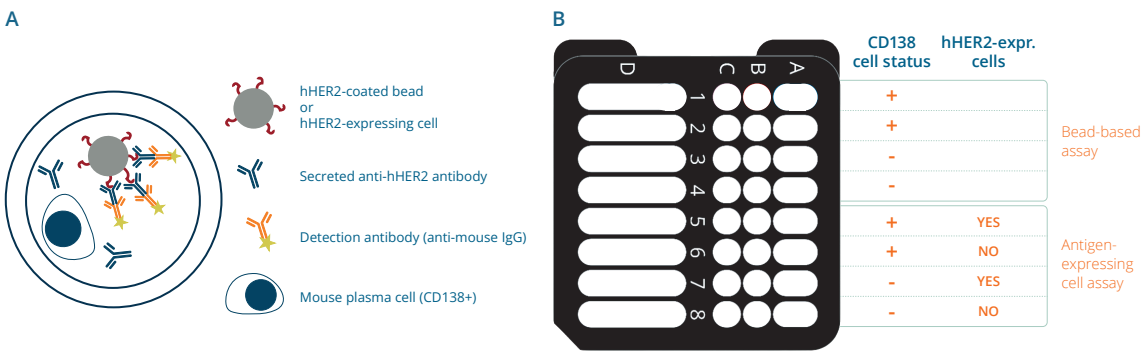

Figure 1. Experimental overview. **A** Overview of the assays performed in droplets for discovery of antibodies against membrane proteins. Secreted anti-hHER2-specific antibodies bind to the hHER2-coated microspheres (bead-based assay) or the hHER2 expressing cell (assay with antigen expressing cells) allowing fluorescent detection via a labeled goat anti-mouse detection antibody. The interaction generates a concentrated and identifiable signal. **B** Summary of the content and loading of samples on the DE50 Cartridge for the two assays.

Immunization and cell preparation

The mouse (NMRI, female) was immunized four times with the extracellular domain of human HER2 (Thr 23 - Thr 652) at two-week intervals using 25 µg per injection and aluminum hydroxide adjuvant. A booster immunization was administered three days before euthanasia. ELISA performed pre- and post- immunization indicated that the mouse had developed a relatively low titer of antibodies against hHER2. Plasma cells were isolated from the spleen using a CD138+ Plasma Cell Isolation Kit (Miltenyi Biotec). Splenocytes that did not bind when using the CD138+ Plasma Cell Isolation Kit were retained as CD138- cells. Cells were counted before and after separation.

Encapsulation in droplets and droplet sorting

Using Xdrop droplet generator and the Xdrop DE50 Cartridge (Samplix), CD138+ plasma cells and CD138- splenocytes were encapsulated separately in double-emulsion droplets (water-oil-water) (see Figure 1B).

Bead-based assay: 400,000 CD138+ plasma cells or CD138- cells were loaded in two cartridge lanes each (Figure 1B). The cells were encapsulated in RPMI-1640 with 10% FBS containing microspheres (2 µm, 20-30 spheres per droplet, PolySciences) coated with hHER2 (Thr 23 - Thr 652, AcroBiosystems), SYTOX Green as cell viability indicator (3 nM), Vybrant DyeCycle Violet (5 µM) as cell stain (both dyes Thermo Fisher Scientific), and AF647-labeled goat anti-mouse IgG antibody (Fcγ-fragment specific, Jackson ImmunoResearch) for detection (Figure 1A).

Antigen expressing cell assay: 200,000 CD138+ plasma cells or CD138- cells were co-encapsulated with either 1,700,000 SK-BR-3 cells expressing hHER2 or MDA-MB-468 cells, which do not express hHER2. These two cell lines had been stained with CellTrace Violet (Thermo Fisher Scientific). One cartridge lane of droplets was produced for each of these four co-encapsulation combinations (Figure 1B). The cells were encapsulated in RPMI-1640 with 10% FBS containing SYTOX Green, and AF647-labeled goat anti-mouse IgG antibody for detection (Figure 1A).

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. The DE50 double-emulsion droplets were sorted on an MA900 Multi-Application Cell Sorter from Sony Biotechnology using the Large Particle Sorting Options (Figure 2). The DE50 droplets were identified on a scatter plot. Droplets containing cells were identified using the either Vybrant DyeCycle Violet-stained cells (bead-based assay, Figure 2, top row) or CellTrace Violet-stained cells (antigen-expressing cell assay, Figure 2, bottom row). Droplets containing live cells were gated using the SYTOX Green-stained dead cells. Droplets containing CD138- splenocytes were used as negative controls to define correct gating.

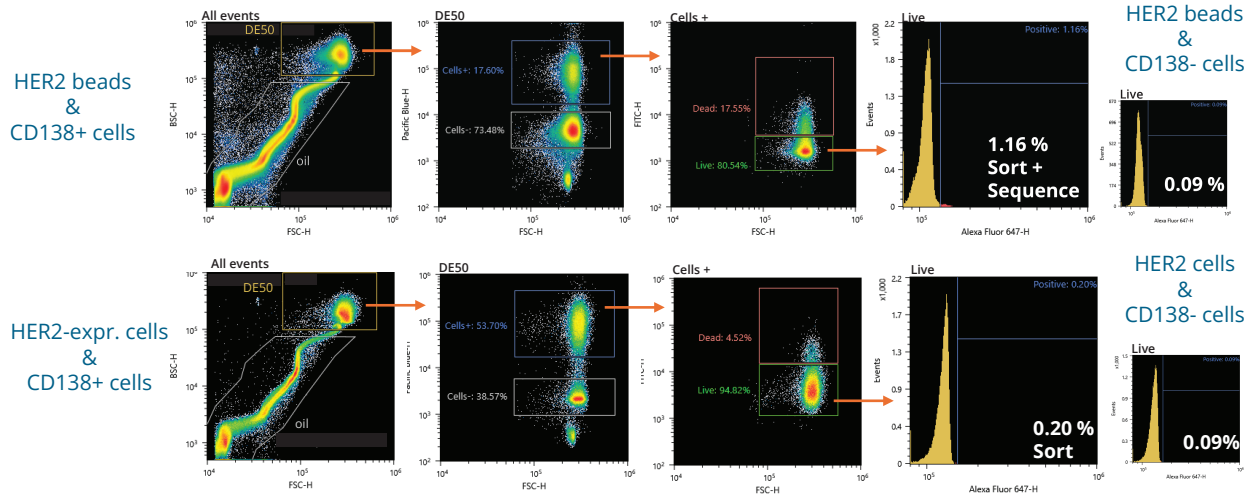

Figure 2. Sorting of DE50 droplets containing plasma cells. With both the bead-based assay (top row) and the assay with hHER2-expressing cells (bottom row), a similar gating strategy is applied. The DE50 droplets are identified on a scatter plot of all events. Droplets with cells are identified on the plot of DE50 gated droplets using Vybrant DyeCycle Violet stain (plams cells, for bead assay) or CellTrace Violet (HER2-expressing SK-BR-3 cells), both in the Pacific Blue channel. Droplets with live cells are gated on the Cells+ plot using the SYTOX Green Dead Cell Stain (FITC channel). Droplets containing plasma cells secreting anti-hHER2-antibodies show an accumulated fluorescent signal from the AF647-labeled detection antibody in the histogram from the Live gate. Background signal from the AF647-labeled detection antibody is measured in droplets with CD138- cells to assist in defining the sorting gate for CD138+ cells.

HER2 specific antibody secretion by plasma cells was visualized by microscopy of DE50 droplets for both types of assays. For droplets with the bead-based assay, fluorescent signal was evident on beads that were aggregated near the cell (Figure 3A). In addition, a clear AF647 signal was observed in droplets containing HER2-expressing cells (SK-BR-3), validating the co-encapsulation approach, where antigen-expressing cells present the antigen in its native configuration (Figure 3B,C).

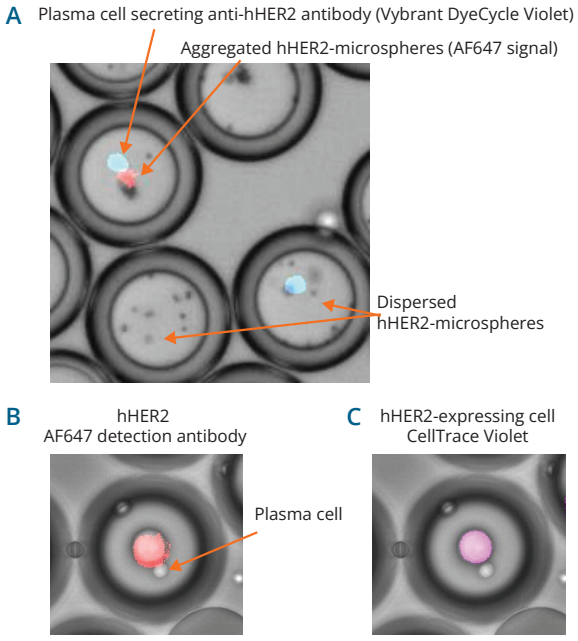

Figure 3. Microscopy of droplets with plasma cells producing HER2-specific antibodies. **A** Bead-based assay image with blue (Vybrant DyeCycle Violet) plasma cell and aggregated beads with AF647 fluorescence. **B** Assay with co-encapsulation of HER2-expressing SK-BR-3 cell and unstained plasma cell showing HER2 specific antibody signal (AF647) on the SK-BR-3 cell stained with CellTrace Violet (**C**) .

Cell recovery and sequencing

Plasma cells were recovered from the sorted droplets containing the bead-based assay using Droplet Break solution (Samplix). An aliquot was analyzed by flow cytometry to determine cell number and viability.

The released cells were used as input for single-cell V(D)J sequencing using Chromium (10X Genomics) and NextSeq (Illumina). In parallel, unscreened plasma cells from the same mouse were subjected to V(D)J sequencing.

Screening results

Approximately 800,000 CD138+ plasma cells were screened from the hHER2 immunized mouse. Flow cytometry sorting of DE50 droplets with the highest anti-hHER2 (AF647) signal resulted in ~700 sorted droplets (Table 1). The relatively low number of AF647-positive droplets reflected the low titer following immunization of the mouse. V(D)J sequencing of the cells recovered from the sorted droplets generated paired V(D)J sequences from 271 cells, giving 54 clonotypes, based on CDR3 sequences as defined by Loupe V(D)J Browser (10X Genomics).

Workflow step	Result
Screened CD138+ plasma cells	800,000
Sorted droplets with antigen-specific antibodies	700
Single-cell V(D)J sequences	271
Number of clonotypes	51

Table 1. Summary of antibody-secreting cell (ASC) screening.

Clonotype distribution and sequence validation

The clonotype distribution map (Figure 4) showed that several specific clonotypes were enriched by the Xdrop assay (blue clusters) indicating that these clonotypes represent hHER2-specific antibody producers. These clonotypes also contained a smaller fraction of unscreened plasma cells cells (orange dots) since cells enriched by the Xdrop assay represent a subpopulation of the unscreened plasma cell pool. A larger number of clonotypes consisted almost exclusively of sequences from the unscreened plasma cell pool (orange clusters), suggesting that they did not contain producers of HER2-specific antibodies.

Antibody sequences were selected for validation from clonotypes enriched for Xdrop-isolated V(D)J sequences (Figure 4). The selection criteria required that the sequence was present in more than three cells within the clonotype. In most cases, the most abundant sequence was selected for validation from each clonotype. Validation was performed by cloning and transiently expressing the selected antibodies, followed by biolayer interferometry (BLI) measurements of their binding affinities to HER2 (Thr 23 - Thr 652). 67 % of the selected sequences were confirmed as HER2 binders, with affinities in the micro- to nano- molar range (Table 2).

Clonotype ID	K_D (M)
#1	**1.07E-08**
#2	**8.97E-09**
#21	**1.18E-07**
#59	**2.51E-06**

Table 2. Binding affinities of antibodies identified in four selected clonotypes.

The results demonstrate that, even using an immunization strategy that was not specifically optimized for a membrane protein target such as HER2 and which yielded relatively low titers and a limited number of positive sorted droplets, it was nevertheless possible to successfully identify HER2-binding antibodies. This highlights the robustness of the platform and its potential to discover relevant binders. Binding affinities were variable with the highest affinity in clonotype #2 showing more than half the affinity of the drug Trastuzumab[3].

Conclusion

Using the membrane protein HER2, this study demonstrates how Xdrop enables discovery of antibodies against membrane proteins using either antigen-coated beads or antigen-expressing cells. Functional screening combined with single-cell V(D)J sequencing successfully identified HER2-specific clonotypes and recovered high-affinity antibodies. These two Xdrop workflows provide an efficient platform for antibody discovery against membrane proteins, including targets with large extracellular domains or smaller and more challenging cell-surface epitopes.

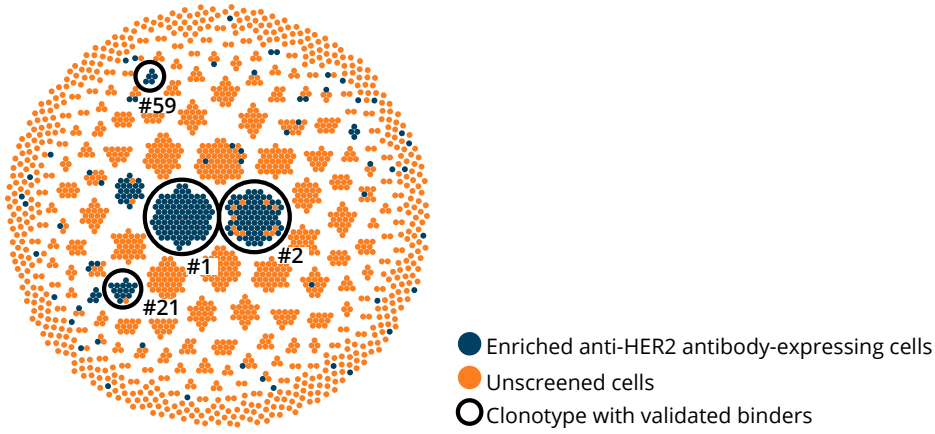

Figure 4. Clonotype distribution plot. Each cluster represents a unique clonotype comprising V(D)J sequences with identical or highly similar CDR-H3 regions. Cluster size is proportional to the clonotype's relative abundance within the antibody repertoire. Expanded clonotypes are represented by larger clusters concentrated toward the center, whereas rare clonotypes appear as smaller clusters at the periphery. The Xdrop ASC screen (blue dots) enriches for specific clonotypes shared with the unscreened population (orange dots), while other clonotypes remain dominated by the unscreened ASCs and are not enriched by the screen. Selected single-cell V(D)J sequences from the encircled clonotypes (black circles) were validated as binders of hHER2. The #number refers to the clonotype ID in Table 2. The data was visualized with Loupe V(D)J Browser (10X Genomics).

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References

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