Bioelectronica

Hypercell® Single-Cell Sorting Platform

For antibody discovery application

Beta-2-Microglobulin (B2M)

ABOUT HYPERCELL® & ABFINDER™

Hypercell® is a high-throughput cell analysis and sorting platform that enables researchers to identify and isolate cells that are secreting proteins of interest quickly and at an affordable price.

AbFinder™ is an advanced downstream solution for selecting promising antibody candidates generated by the Hypercell® single cell sorting platform.

By seamlessly integrating Hypercell® and AbFinder™, Bioelectronica provides an end-to-end solution for antibody discovery by leveraging the power of single cell secretion sorting with next-generation sequencing (NGS).

INTRODUCTION

Monoclonal antibodies have become a function technology for groundbreaking therapeutic modalities, diagnostic methods, and scientific discovery. While many antibody screening technologies exist, there remains an unmet need to produce antibodies with high diversity, at high throughput, and low cost while retaining natural pairing of the heavy and light chains. In this study, we use the Hypercell® platform and the AbFinder™ bioinformatic tool developed by Bioelectronica, to identify high affinity binders to β2 microglobulin (B2M), a crucial component of MHC class I molecules belonging to the beta-2-microglobulin family. High affinity binders leads to a precise quantification of B2M levels in the serum and urine for diagnosis of diabetic nephropathy.

RESULTS

We conducted two separate sorting experiments against B2M, using a cohort of 2 mice , in order to assess the performance and repeatability of the runs. In the first experiment with mouse #1, we utilized the Hypercell® sorter to screen 70,000 CD138+ cells within a single day. The sorted cells were then processed through single cell sequencing and analyzed using the AbFinder™ bioinformatic tool. Out of more than 500 paired sequences identified, we selected 22 antibodies for downstream validation. Subsequently, we confirmed that 17 of these antibodies were potent binders in less than 4 weeks. For the second experiment with mouse #2, we screened 150,000 CD138+ cells. This led to the identification of 1,976 paired sequences, from which 89 antibodies were chosen for validation, and 68 were potent binders.

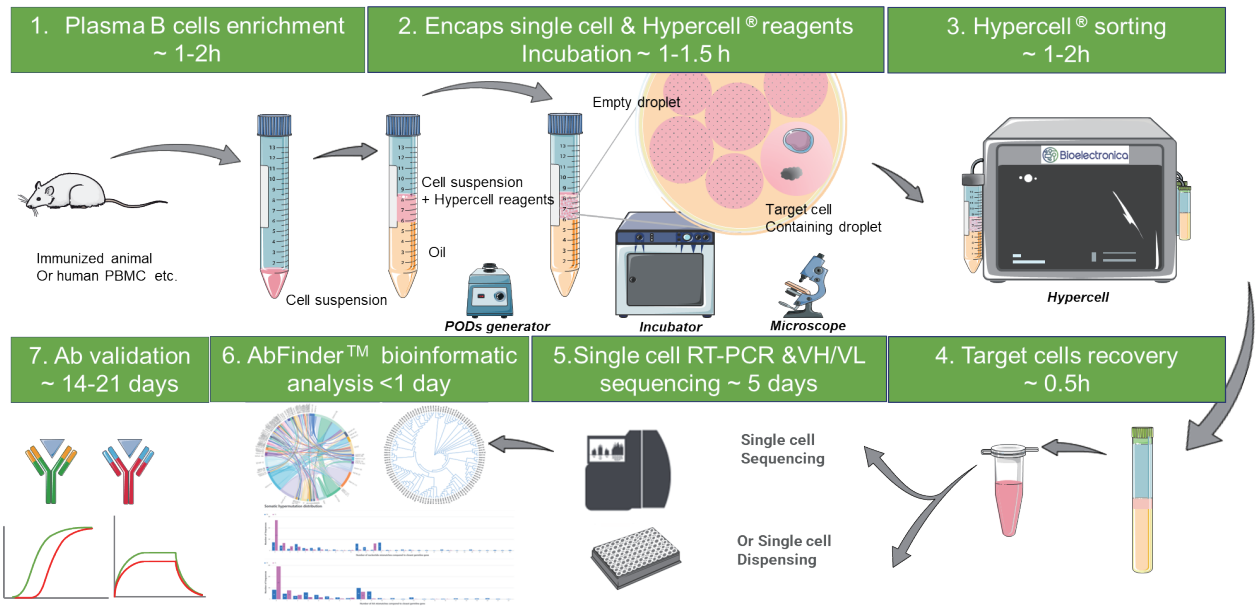


Figure 1. Plasma B Cell Antibody Discovery Workflow. Schematic representation of the Hypercell® sorting process (steps 1-4), which is completed within one day. The subsequent steps include single-cell RT-PCR and VH/VL sequencing (step 5), bioinformatic analysis (step 6), and antibody validation (step 7), all of which can be completed in less than one month. In this study, we employed single-cell V(D)J sequencing for high-throughput recovery. As an alternative downstream workflow for low- to mid-throughput, a single-cell dispensing solution in a well plate format can also be used.

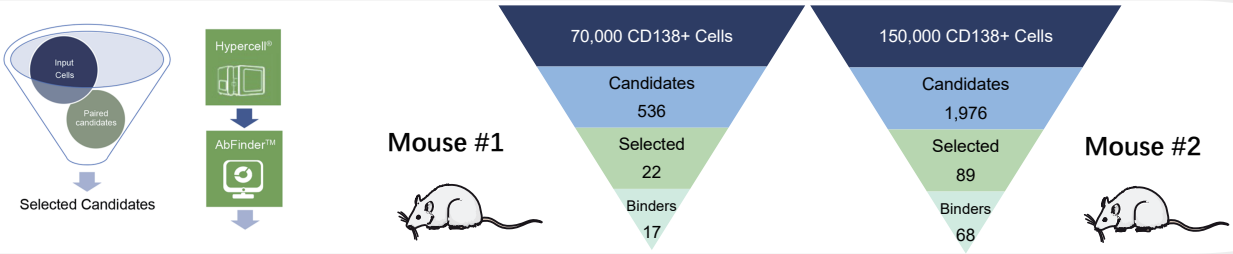


Figure 2. Key numbers in the Hypercell® and AbFinder™ workflow. A magnetic bead-based CD138+ positive selection kit (Stemcell) was used to enrich plasma B cells from the spleen of the immunized mouse. For mouse #1, 70,000 CD138+ cells were screened on the Hypercell®. All sorted cells were encapsulated with DNA-barcoded gel beads using a 10x Chromium controller and processed using the B cell single cell V(D)J solution. After library construction and sequencing (NovaSeq 6000), 536 non-redundant antibodies were obtained. Based on the sequence analysis and antigen docking analysis via AbFinder™, 22 candidates were selected for downstream synthesis, expression and binding validation, and 17 binders were confirmed (77.3%). For mouse #2, the ratio was similar at 76.4%

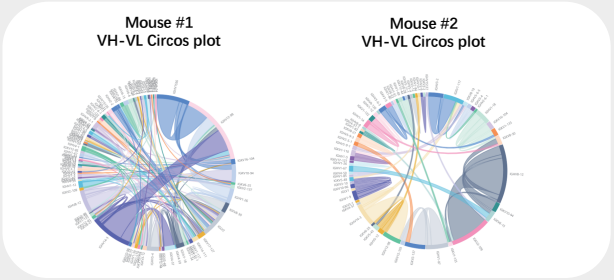
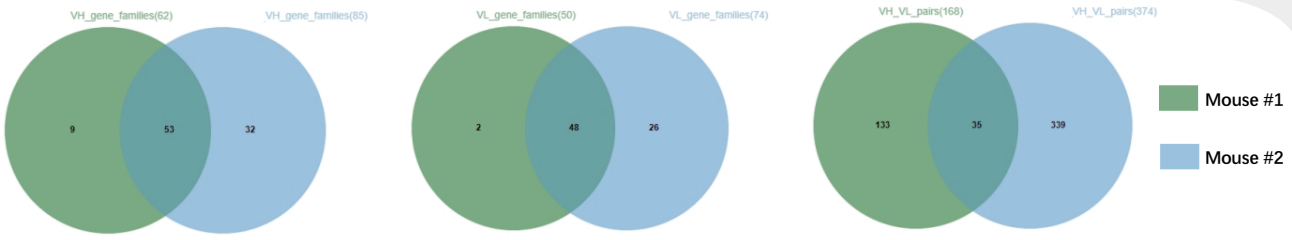


Figure 3. VH-VL Circos plot presenting the associations of V-genes between pairs from the paired antibodies. A high diversity of IgGs covering multiple V-gene families were observed with the sorted population for both mouse #1 and mouse #2. Heavy chain V-genes are spanned to the left of the circle and light chain V-genes to the right. The color of the ribbon indicates different heavy chain V-gene families. The width of the ribbon connecting heavy and light chain V-genes is proportional to the number of pairs using those genes.



	Cells #	VH families #	VL families #	Combined VH-VL families #
Mouse #1	536	62	50	168
Mouse #2	1,976	85	74	374

Figure 4. By comparing the V-gene usage in the heavy and light chains, a significant difference was identified between mice from the same cohort which indicates high stochasticity in the immune response of individual mice. This suggests that a pooling strategy of mice can be adopted to further enhance the diversity of antibody candidates per sort when using the Hypercell® sorter.

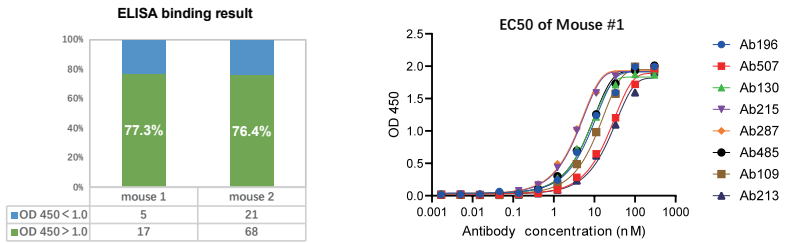


Figure 5. (a) ELISA binding result of culture supernatant, after 5 days culture of anti-B2M IgGs expressed from cloned genes. The result showed similar binding (OD 450 > 1.0) ratio for mouse #1 (77.3%) and mouse #2 (76.4%) . (b) For mouse #1, 8 antibodies were randomly selected for EC50 affinity measurement which showed low nM concentration.

SUMMARY

Using the Bioelectronica Hypercell® platform and plasma B workflow, hundreds to thousands of antibodies candidates can be identified in less than four weeks time. Two separate sorts using mice from the same cohort demonstrated reliable and reproducible results.

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