Multiple B-Cell Pathways to Antibody Discovery by Cellestive™

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Introduction

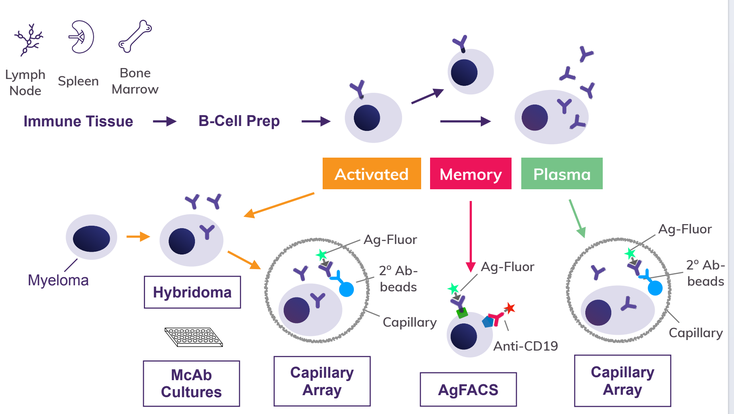
While the superiority of starting with B-cells from immune hosts (vs. synthetic antibody libraries) has become well established, there is growing recognition that more than one type of B-cell population is a potential source for therapeutic antibodies. So, a challenge has remained: How to interrogate – and get antibodies from – different types of B-cells, each with different properties, and each requiring different screening methods.

Starting with a single pool of B-cells, we describe the identification of unique antibodies from activated, memory, and plasma B-cell hits through single-cell RNA (scRNA) sequencing, followed by rapid recombinant antibody expression in a human IgG1 format, and characterization of expression and antigen-binding activity.

Cellestive™ Workflow

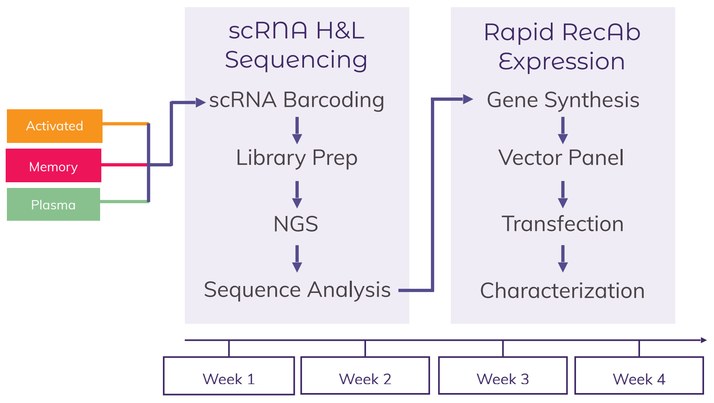
The results presented were generated using Antibody Solutions' platform of integrated services – **Cellestive™** – which is designed to capture all the antigen (Ag)-specific antibodies generated by different B-cell subsets: Activated, Memory & Plasma B cells.

Interrogation and Capture of Ag-specific B-cells by Type



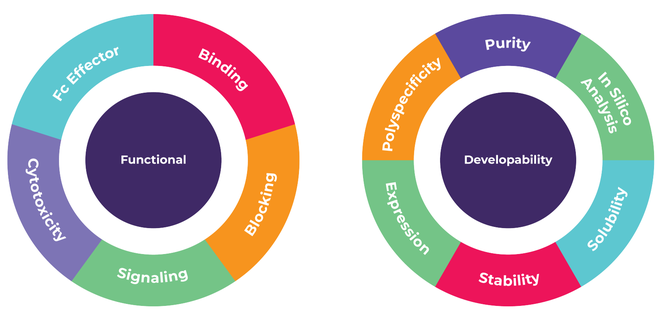
In addition to traditional hybridoma cultures, we utilize the xPloration Capillary Array System and Ag-specific FACS sorting to identify and isolate each B-cell type.

Sequencing & Expression of Ag-specific B-cells



Paired heavy and light chain sequencing identifies unique clonotypes, germline usage, and VDJ diversity. Rapid recombinant antibody expression enables proof of concept testing of reformated, fixed light chain, Fc-modified, and bi-specific antibodies.

Potential Functional and Developability Assays



Sequenced Ag-specific Abs can be filtered via informatics analysis, expressed and put through multiple workflows to analyze the repertoire.

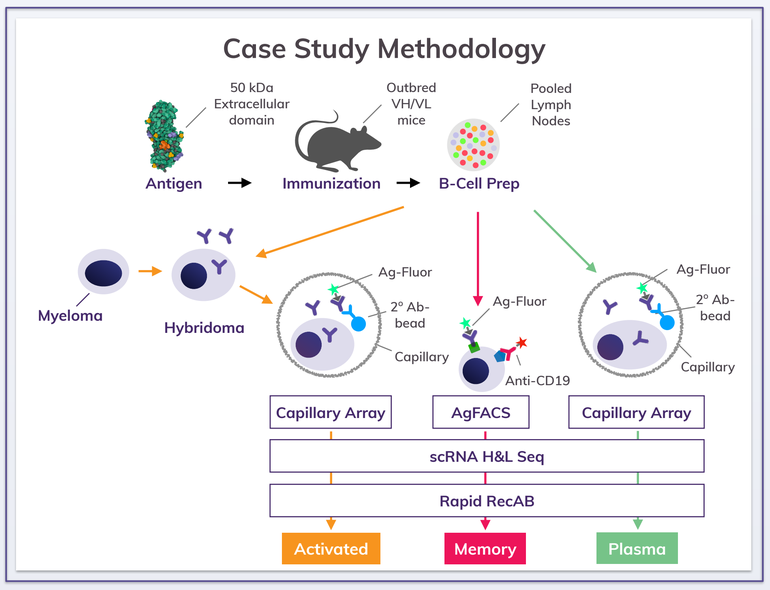
Applicability of Cellestive™ for Host B-cell types

	Activated	Memory	Plasma
Mouse	X	X	X
Rat	X	X	X
Transgenic Rodent	X	X	X
Rabbit		X	X
Other		X	X

Cellestive™ interrogation of Activated, Memory, and Plasma B-cells has broad applicability across multiple host models.

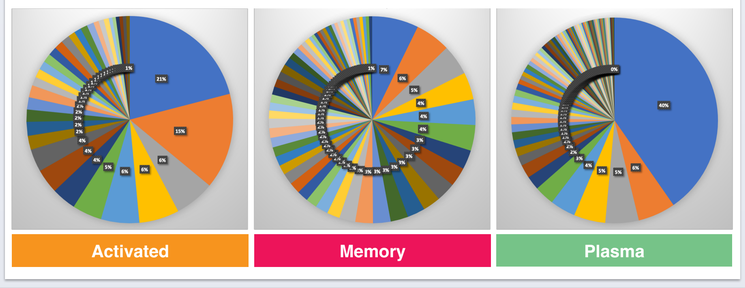
Multiple B-Cell Pathways: Case Study

Case Study Methodology



Sequencing Results

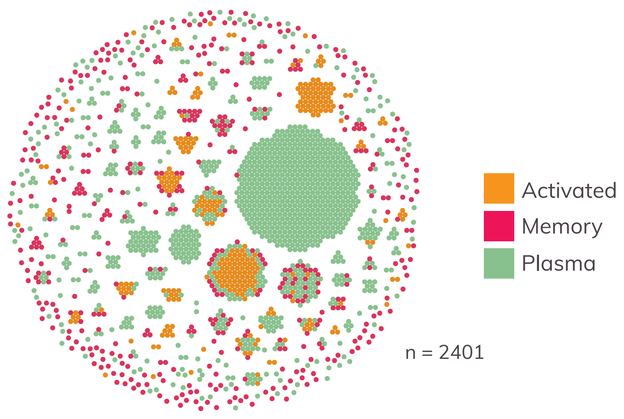
Frequency of Paired VH/VL Sequences in B-cell Types



Diversity of paired VH/VL sequences:
Memory > Activated > Plasma

Pie sections illustrate the frequency of paired VH/VL chains for each B-cell type. Sequencing results yield 400-1400 Ag-positive cells sequenced in each group. Since it is a population you can expect multiple cells with the same clonotype due to proliferation, resulting in a range of 50-200 unique clonotypes per B-cell type

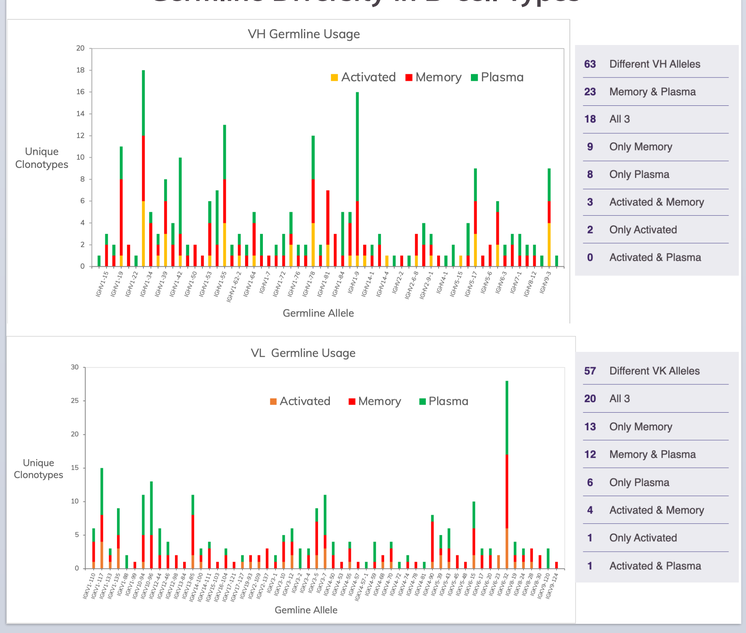
Heavy-Chain CDR3 diversity in B-cell Types



Most CDR3 clonotypes are unique
to each B-cell type

Each dot represents a unique VH CDR3 with identical sequences clustered and color-coded for B-cell type. Diversity of unique VH CDR3s was greatest in the Memory B-cell population. Activated and Plasma B-cells showed greater expansion (i.e., multiple copies) of specific VH CDR3s.

Germline Diversity in B-cell Types



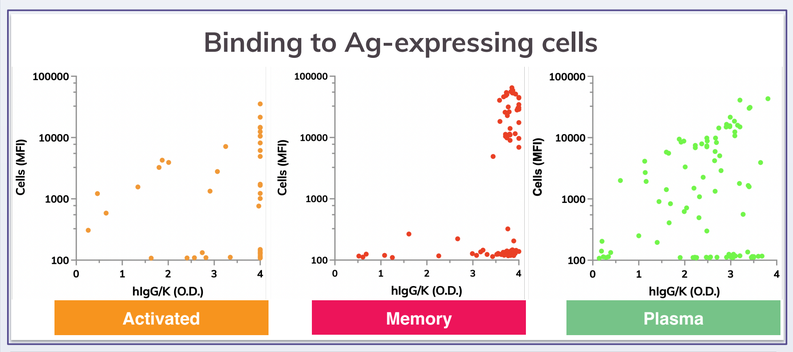
30% of VH and 35% of VL alleles are unique to one B-cell type.

The bar height indicates the frequency of each unique allele (VL or VH), color-coded for B-cell type.

Expression Results

IgG expression and soluble Ag binding

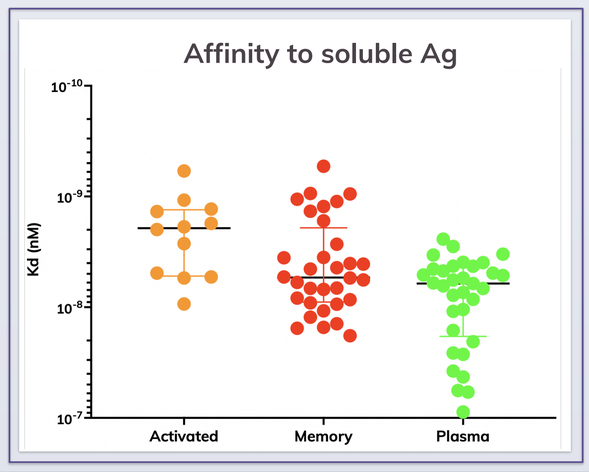
Binding to Ag-expressing cells



Most sequences are expressed after reformatting as human IgG. Approximately 50% bound to soluble Ag or Ag-expressiing cells

Expressed sequences were analyzed for human IgG/K expression by ELISA, soluble Ag binding by ELISA, and binding to Ag-expressing cells by Flow Cytometry.

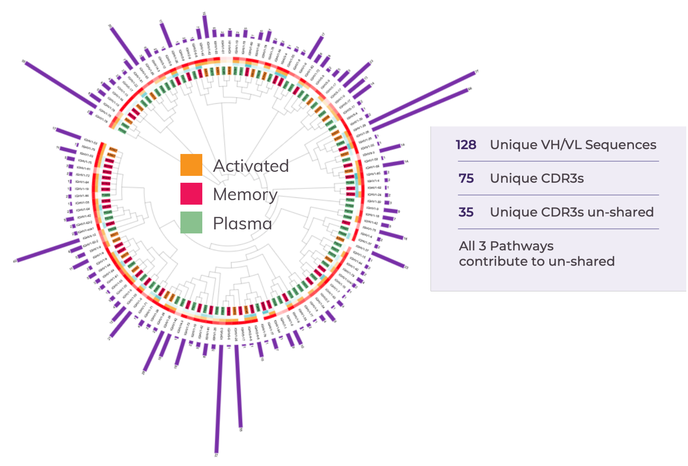
Affinity to soluble Ag



Median Affinity of B-cell types: Activated >
Memory = Plasma

The Kinetics of Ag binding was determined using the soluble Ag used for immunization.

VH CDR3 Diversity of Ag-Positive B-cells



All 3 B-cell pathways contribute to unique Ag-binding clones, some of which are only found in one B-cell type

Results for VH CDR3 are represented in the phylogenetic tree above, with branches indicating common lineage and evolutionary distance. Results for binding and secretion assays are indicated in the middle circle followed by VH germlines. Purple bars indicate frequency of each clonotype in the original population of cells.

Benefits of the Cellestive™ Workflow

We have three pathways to exploit the three major subtypes of antigen-specific B-cells for antibody discovery. Combined with sequencing and high throughput recombinant expression, we are able to deliver a broad diversity of unique antibody candidates in as little as 8 weeks. We found that unique antigen-specific antibodies (i.e., clonotypes) were obtained from each B-cell population, with many germline alleles and CDRs being unique to each population. This demonstrates the power of interrogating activated, memory, and plasma B-cells to optimize antibody discovery in as little as 8 weeks.