

## Introduction

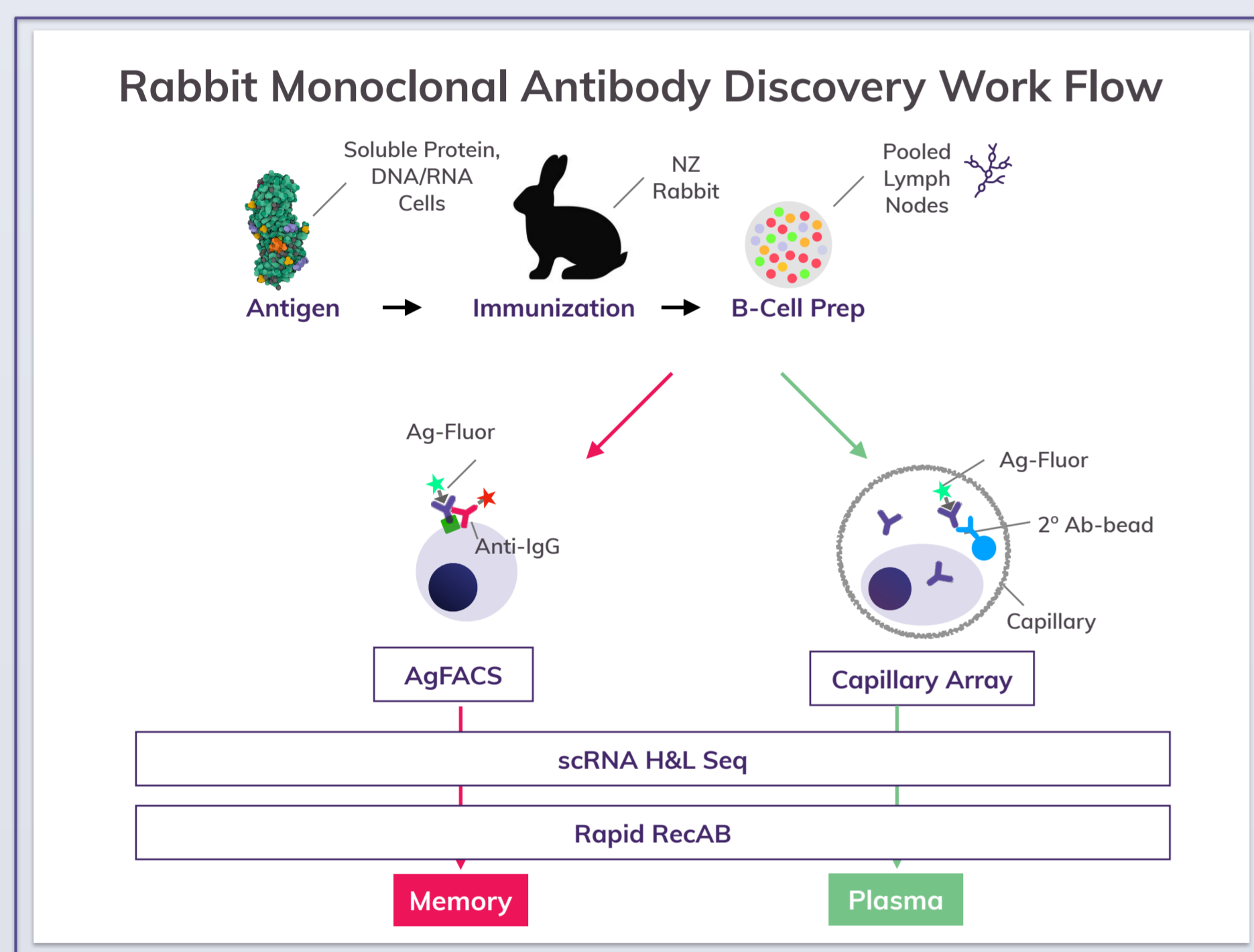
Rabbits generate diverse, high affinity antibodies to a wide variety of antigens using the mechanism of gene conversion, which delivers an antibody repertoire different from mice and humans. However, rabbit monoclonal antibodies have traditionally been difficult to immortalize via hybridoma technology due to challenges such as instability and inefficient antibody secretion. Alternatives such as phage display to generate rabbit antibodies often result in the loss of natural pairing between heavy and light chains, while traditional single B-cell technologies can miss certain B-cell subsets and be cost prohibitive.

## Cellestive™ Platform

The results presented here were generated using Cellestive™ Antibody Solutions' platform of integrated services designed to capture all the antigen (Ag)-specific antibodies generated by different B-cell subsets including both Memory & Plasma B cells.

Our Cellestive™ approach begins with an optimized immunization protocol robustly targeting the rabbit's popliteal lymph nodes. Following harvest, B-cell subsets (activated, memory, and plasma cells) can be interrogated via multiple screening platforms using bespoke reagents including fluorescent-labeled peptides and protein or cells expressing target antigen. Hundreds to thousands of antigen-specific B-cells can be identified and unique paired antigen-specific sequences obtained. Antibodies can be re-expressed as fully rabbit IgG or humanized IgGs, and evaluated for target specificity, affinity, and developability.

## Rabbit Monoclonals in the Cellestive™ Workflow

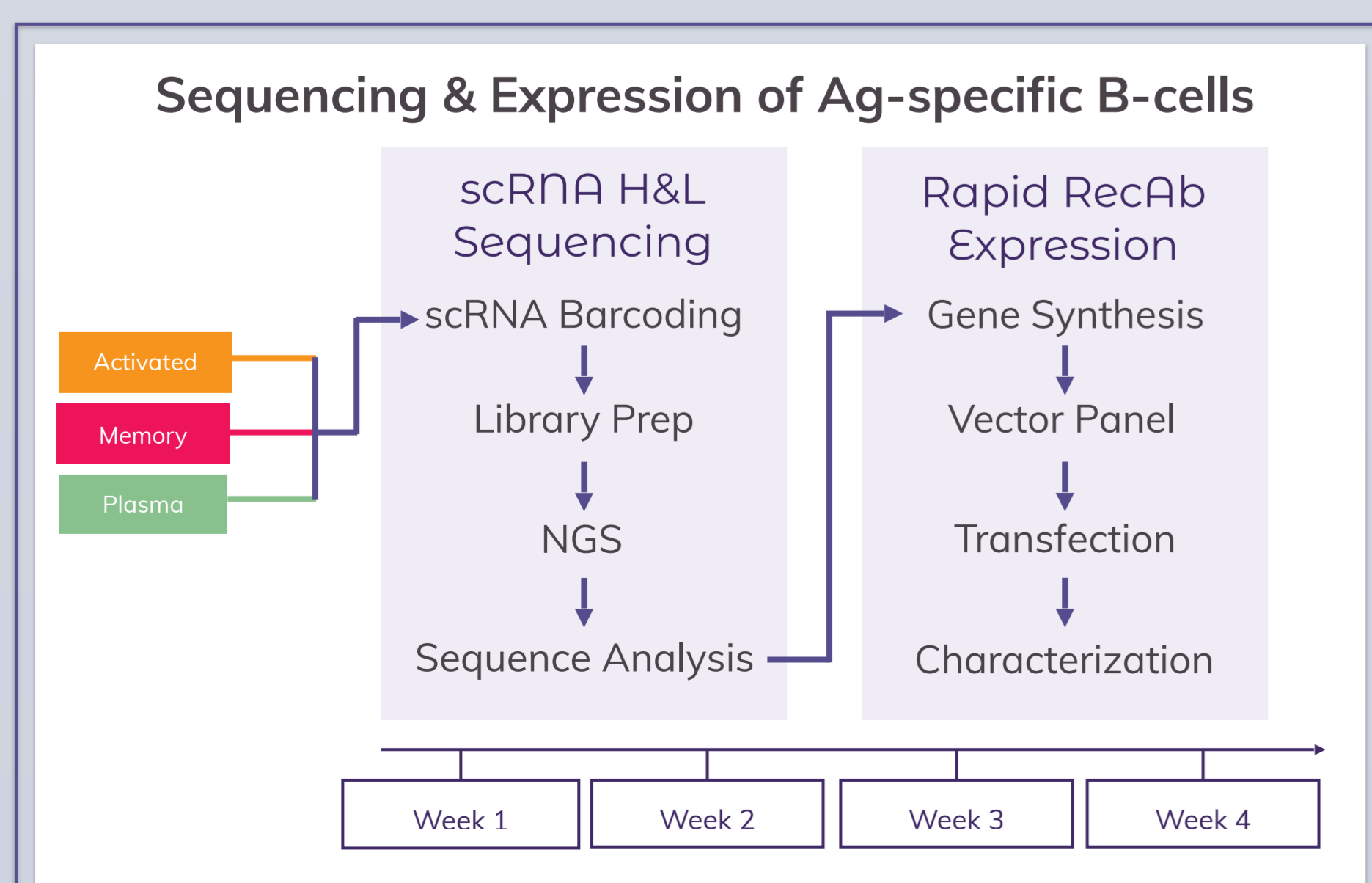


Our rabbit Cellestive™ platform benefits multiple applications, including the generation of anti-idiotype antibodies and antibodies to challenging targets including enzymes and phosphorylation-specific antibodies. The resulting antibodies can be used in PK and anti-drug antibody (ADA) assays, in IHC for target validation and localization, for western blots and for analysis of cell signaling and phosphorylation pathways. Using the Antibody Solutions rabbit Cellestive™ platform can unlock the discovery of unique monoclonal antibodies that may be difficult to generate in other animal models.

## Sequencing and High Throughput Expression

The Cellestive™ platform supports antibody discovery from multiple species and includes optimizations rabbits. Paired heavy and light chain sequencing identifies unique clonotypes, germline usage, and VDJ diversity.

Sequenced antigen specific monoclonal antibodies can be filtered via informatics and repertoire analysis to select lead candidates and rapidly expressed to enable proof of concept testing.

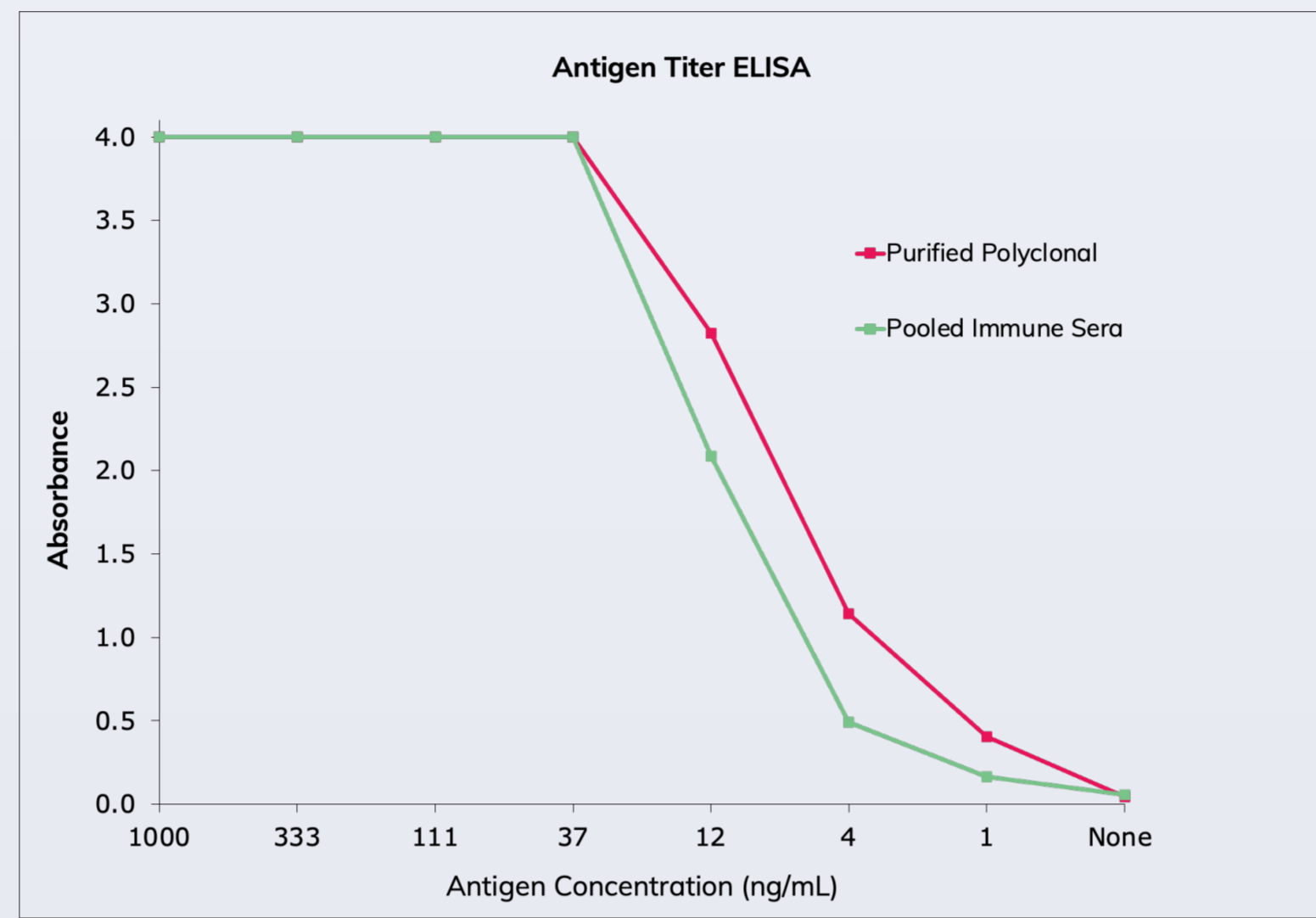


## Rabbit Monoclonals using AgFACS

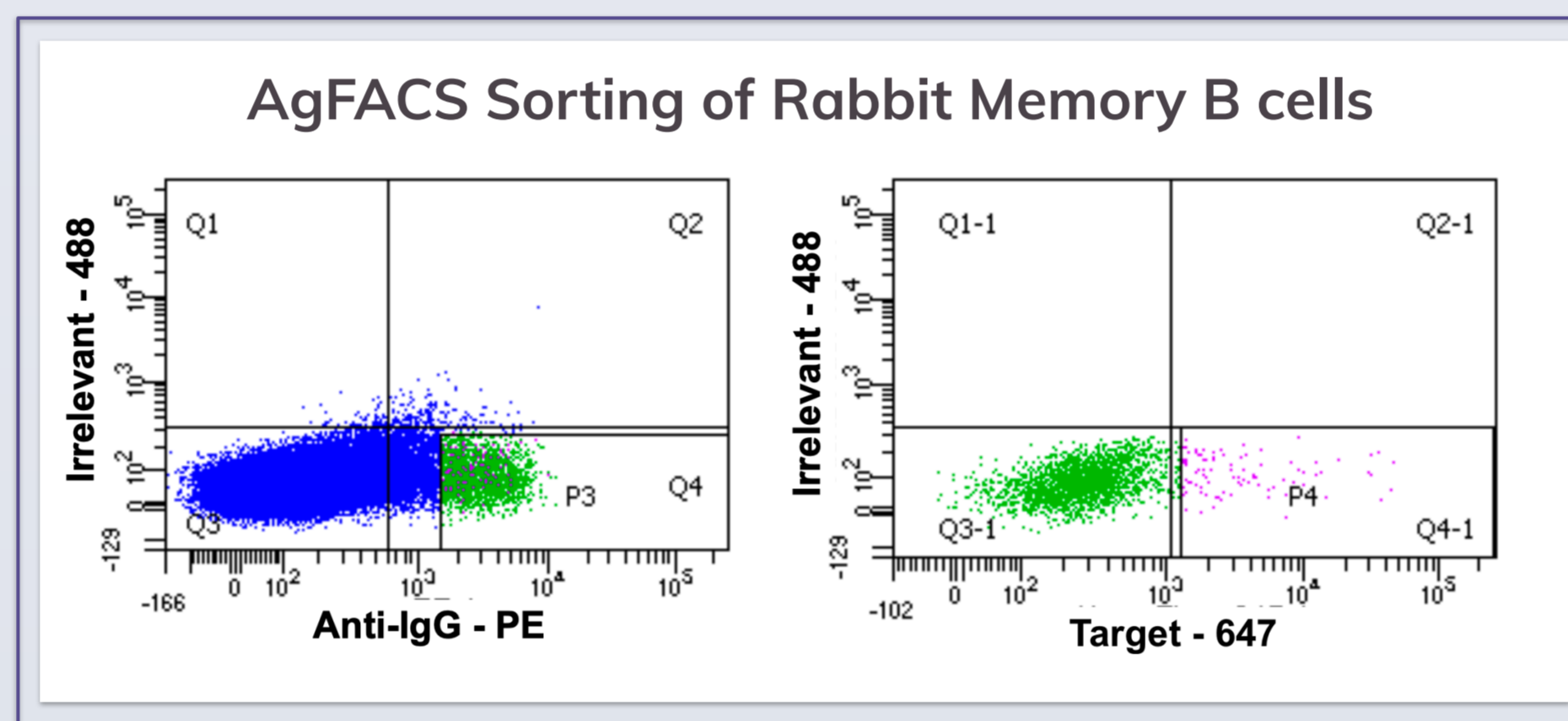
### Therapeutic Enzyme Target

Historically rabbit polyclonals and monoclonals are well established for the detection of therapeutic targets to support biotherapeutic candidate development as well as critical reagents in pre-clinical and clinical stage studies including biodistribution, pharmacokinetic analysis, and as controls for ADA (anti-drug antibody).

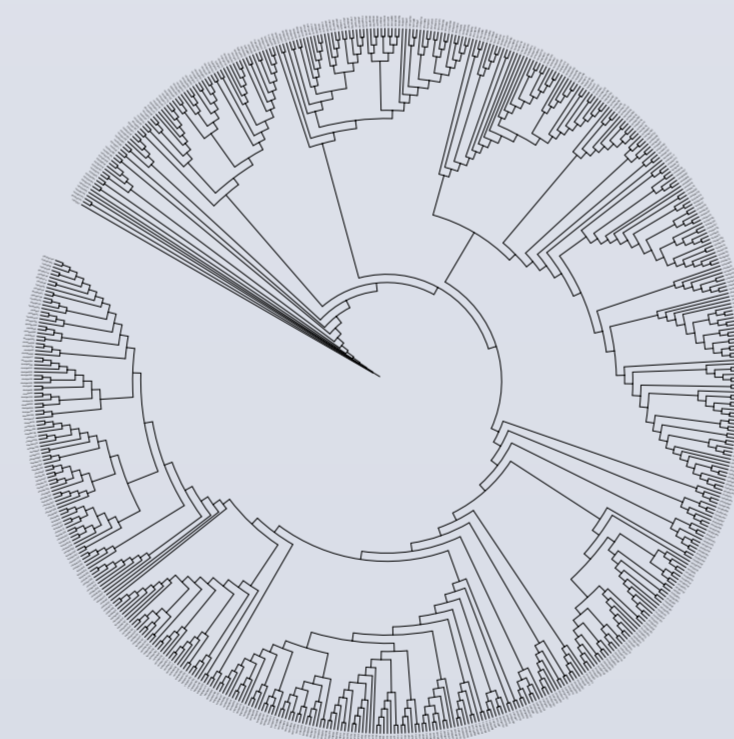
In one such application, rabbits were immunized with an engineered enzyme therapeutic candidate. Robust titers were quickly generated via Rabbit Rapid immunization and polyclonal antibody from serum was purified via traditional affinity chromatography.



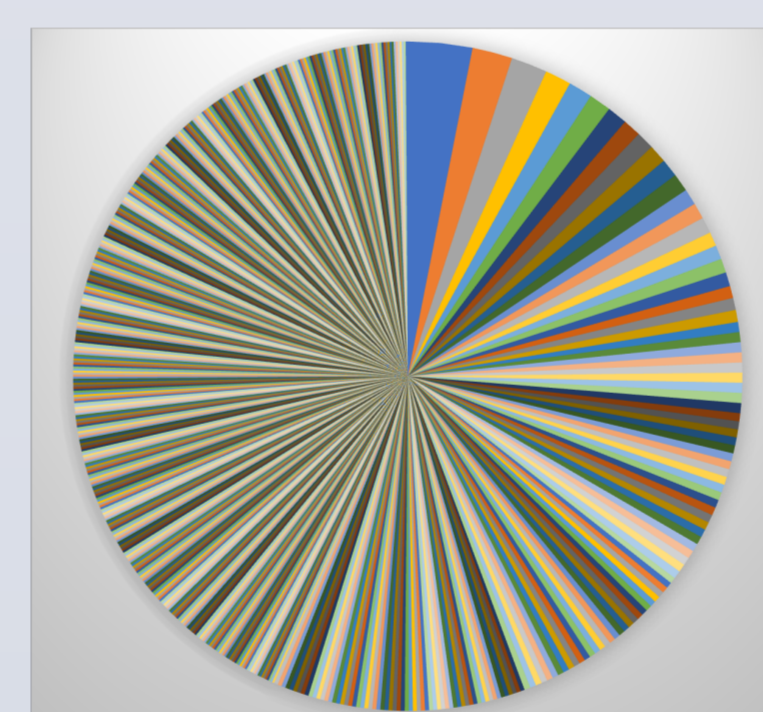
To isolate monoclonal antibodies, rabbit lymph nodes were harvested and binders were identified via AgFACS B-cell sorting technique using fluorescently labeled enzyme and followed by paired heavy/light chain sequencing.



Rabbit Memory B-cells were stained and sorted for surface IgG expression and binding to fluorescently labeled target enzyme. Single B-cells were isolated from the indicated gate and barcoded for Paired Antibody heavy/light chain next generation sequencing, resulting in the recovery of 1490 paired heavy/light chain clonotypes, of which there were 605 unique IgG clones.



CDR3 Phylogenetic Analysis



Frequency of VH/VL Clones

Further analyses of the resulting sequences including frequency, germline usage, CDR3 phylogenetic analysis, and sequence liabilities were performed to select 24 sequences for recombinant expression and testing. The vast majority (20 of 24) successfully expressed and exhibited strong target specific binding by ELISA.

### ELISA Testing of Expressed Rabbit mAbs

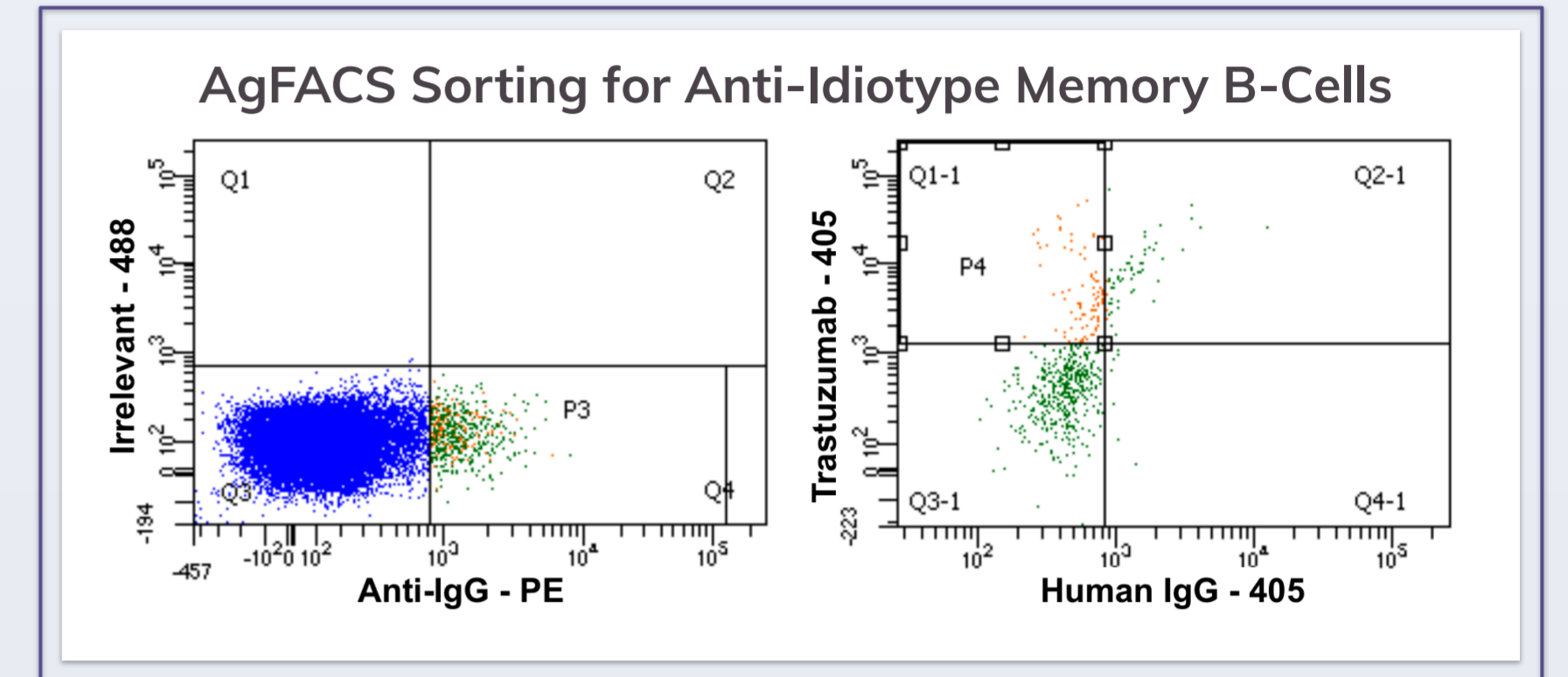


## Memory and Plasma Cell Screening

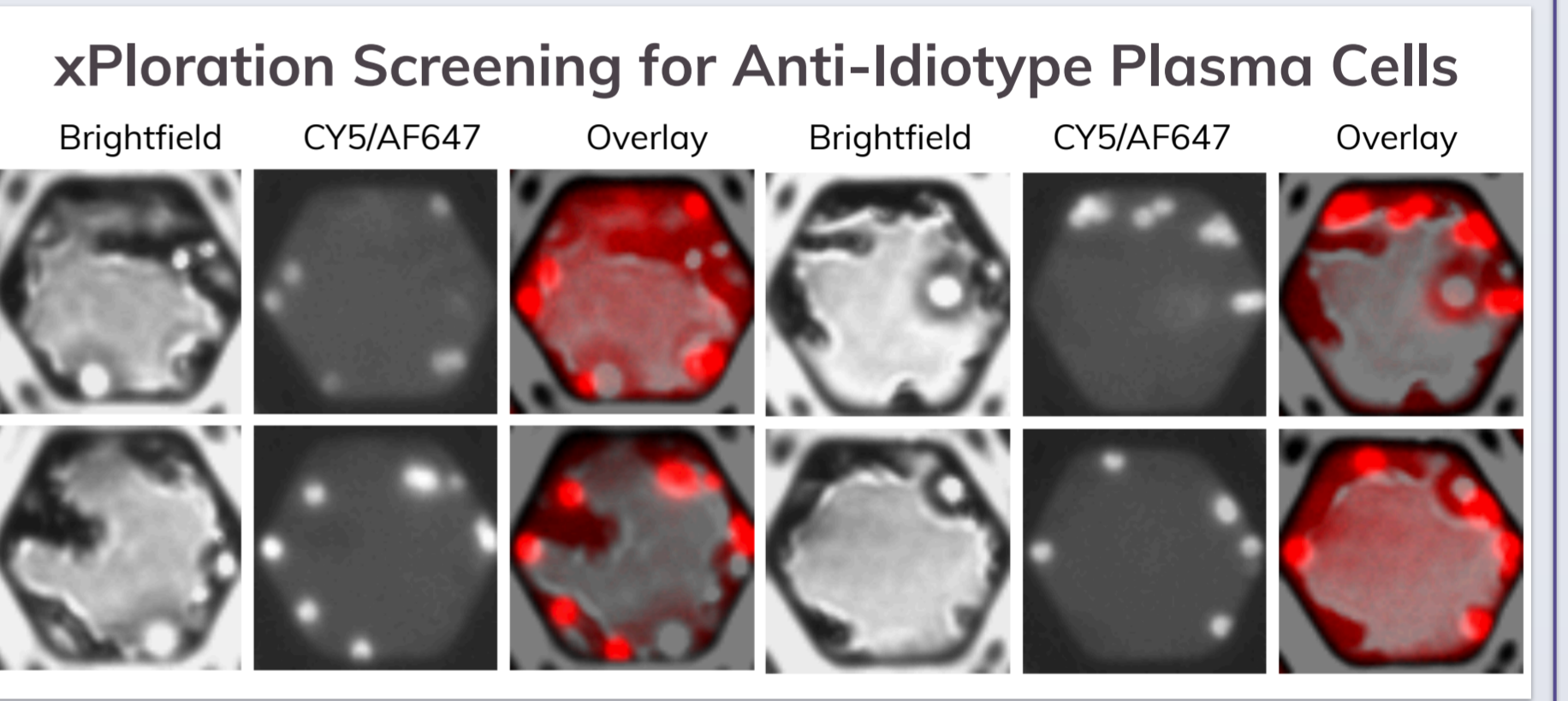
### Anti-Idiotype Monoclonals

Rabbits are often considered the "gold-standard" model to generate positive controls for anti-drug antibody (ADA) assay development. Often these are affinity purified polyclonal antibodies, yet the Cellestive™ platform enables efficient discovery of monoclonal antibodies from the same rabbits which can be used as critical reagents for multiple assays include pharmacokinetics (PK) and neutralizing antibody assays. In the study below, rabbits were immunized with Anti-HER2 humanized antibody trastuzumab with the goal of generating anti-idiotype specific monoclonals.

Rabbit lymph nodes were harvested and binders were identified via AgFACS B-cell sorting technique using fluorescently labeled enzyme and followed by paired heavy/light chain sequencing.

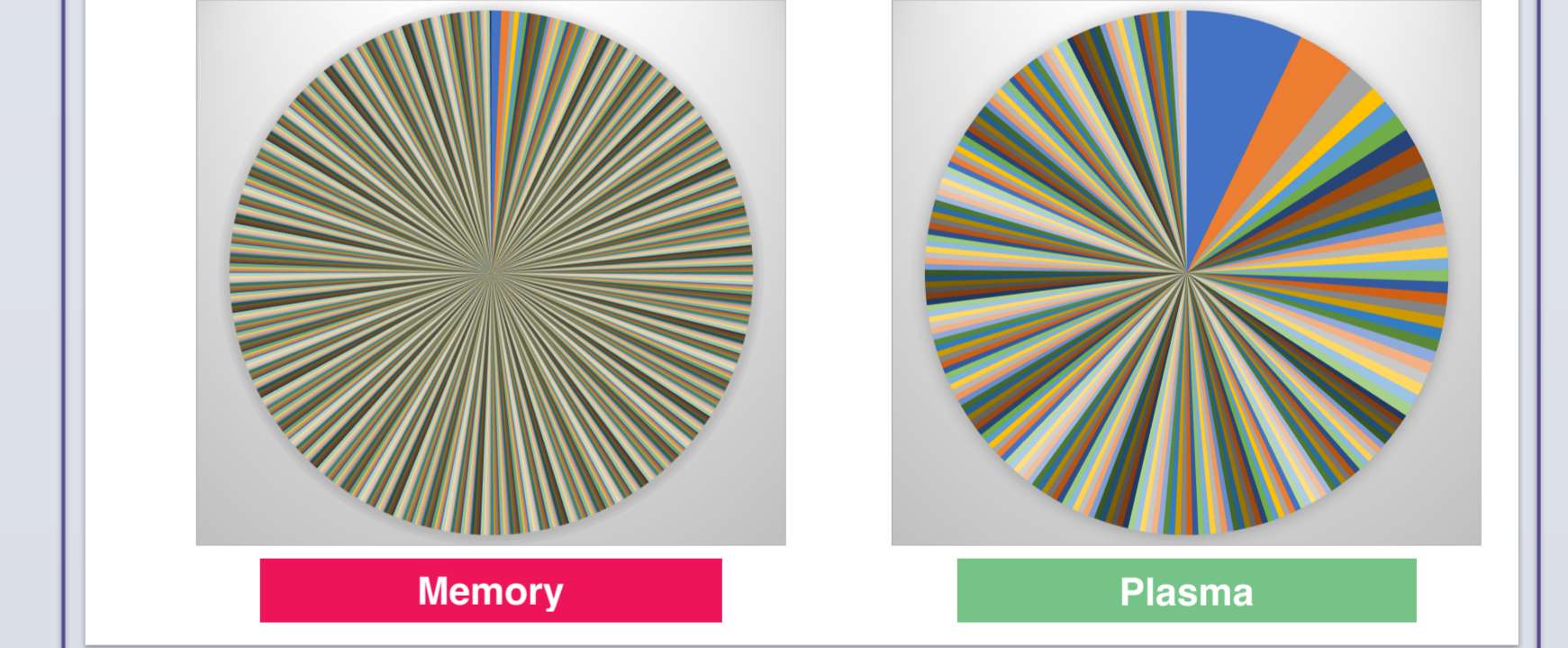


From the same rabbit lymph nodes, antibody secreting plasma cells were screened via xPloration microcapillary array, utilizing beads to capture secreted rabbit antibody and fluorescent labeled antigen to identify binders.



Memory B-cells sorted via AgFACS and Plasma cells isolated via xPloration were barcoded for paired heavy/light chain sequencing. Memory B-cells yielded 987 paired antibody sequences, of which there were 338 unique IgG clones. Plasma cells yielded 278 paired antibody sequences, of which 95 were unique IgG clones.

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



After sequence analysis, 10 unique Memory sequences and 10 unique Plasma sequences were selected for recombinant expression and ELISA binding analysis.

### ELISA Testing of Expressed Rabbit mAbs



From this initial panel, a total of 11 unique antibody sequences (5 from memory, 6 from plasma) exhibited strong anti-trastuzumab specific binding and robust IgG expression.

## Benefits of Cellestive™

Thousands of antigen-specific rabbit B-cells can be isolated and unique paired antigen-specific sequences obtained with the Cellestive™ platform. Antibodies can be rapidly re-expressed and evaluated for target specificity, affinity, and developability.