



## Abveris Antibody Discovery Case Study

Human mAb Discovery based on Alloy Humanized Mice and a Function-Forward Hybridoma Workflow

# CASE STUDY: ANTIBODY DISCOVERY WITH ALLOY MOUSE

Function-forward human antibody discovery campaign



## Project Goal:

- Human, single-digit nanomolar mAbs that bind in the presence of a ligand (non-blockers)
- The target is a cell surface receptor with a ~150aa extracellular domain

## Strategy:

- Utilize Alloy's GK humanized mice
- E-fusion to generate a diverse panel of hybridomas
- Function-forward hybridoma screening cascade using HTP flow cytometry in both primary and secondary screens with cell lines of varying expression levels
- Advanced screening identifies functional clones by flow and octet



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# CASE STUDY: ANTIBODY DISCOVERY WITH ALLOY MOUSE

Generation of robust immune response by immunization with Alloy Mice

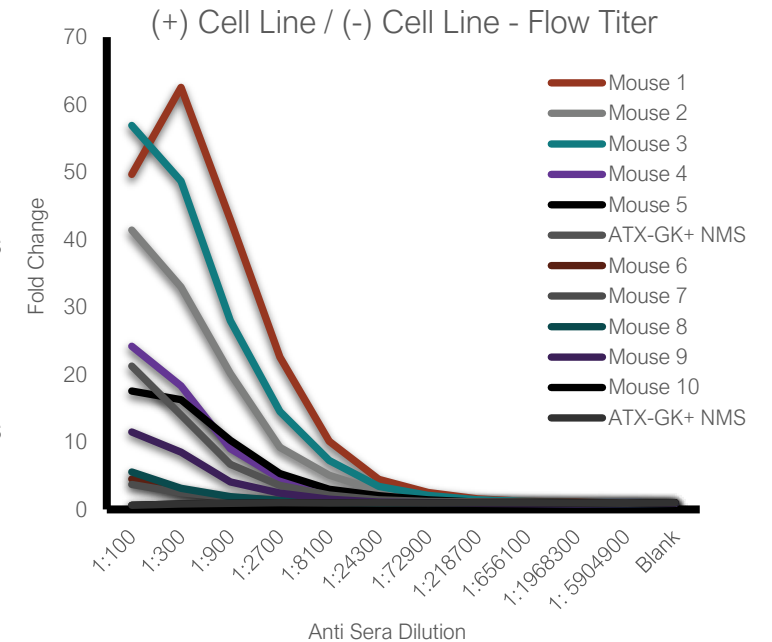
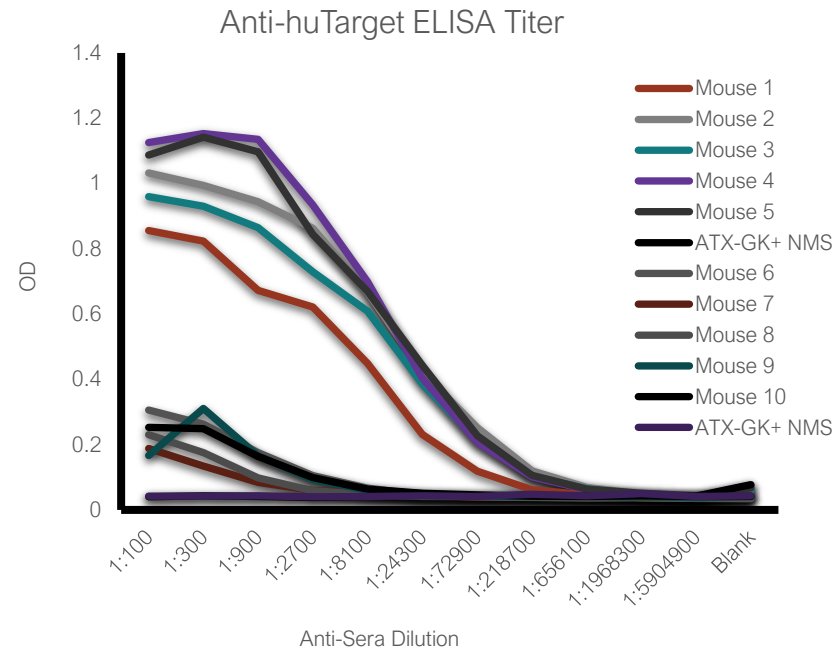
Immunization &  
Titer Test

Hybridoma Fusion &  
Flow Cytometry Screening

Affinity & Ligand Blocking  
(Octet BLI and Flow)

Tertiary Screen  
(Binning by Octet BLI)

- 5-week immunization with cell and protein
- Proprietary Abveris adjuvant
- Alloy mice immunized by protein were selected for fusion (Mouse 1 & 3)

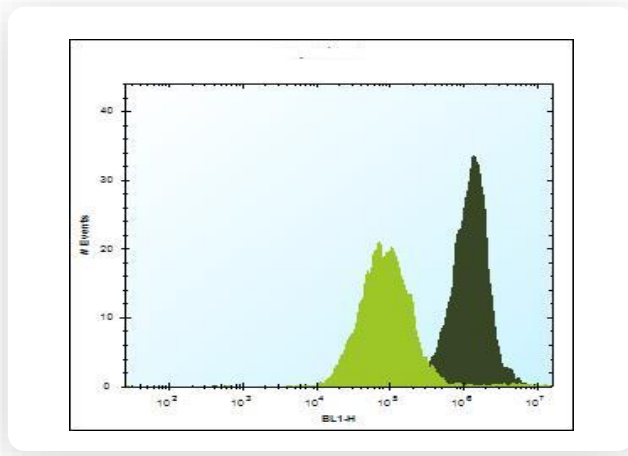


# CASE STUDY: ANTIBODY DISCOVERY WITH ALLOY MOUSE

Primary and secondary screening by HTP flow-cytometry to focus on identifying specific cell binders

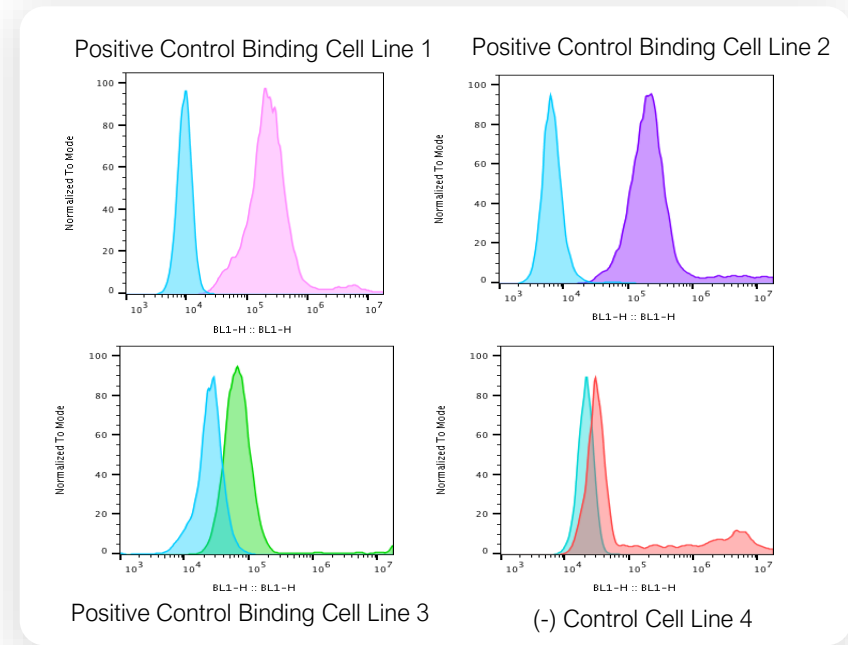
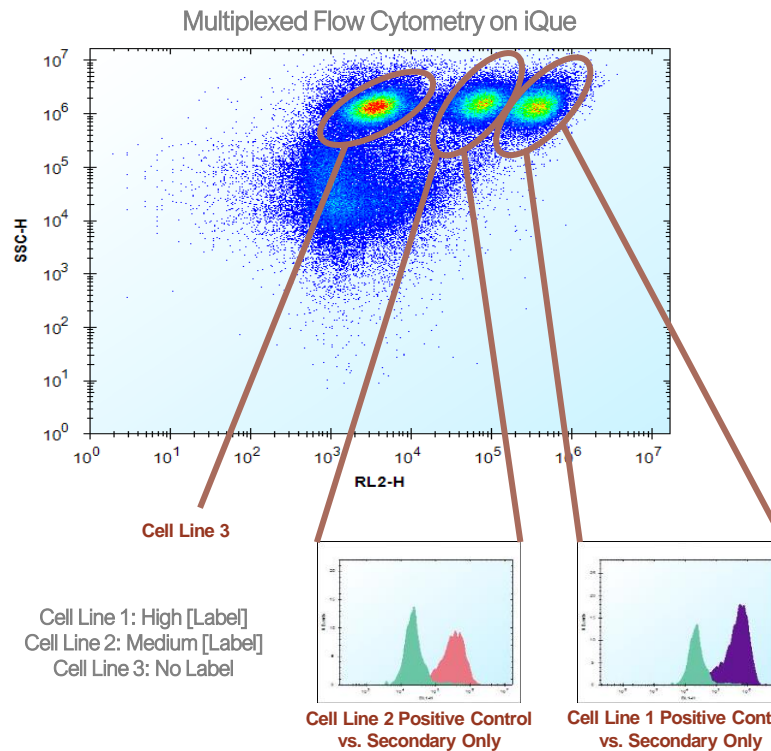


## Primary Screening



- High-efficiency electro-fusion to produce hybridoma
- 17x 96-well plates screened by flow cytometry
- Screened on high expression cell line to move forward a panel of binders

## Secondary Screening

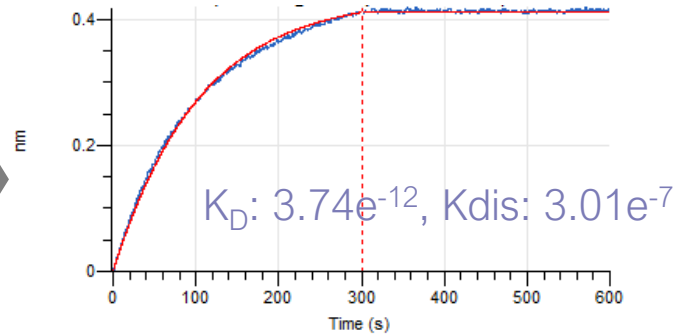
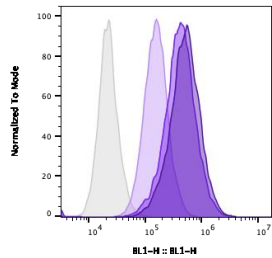


- 3 Positive and 1 Negative Control Cell Lines
- > 50 clones screened in secondary screen on cell lines with decreasing level of target expression

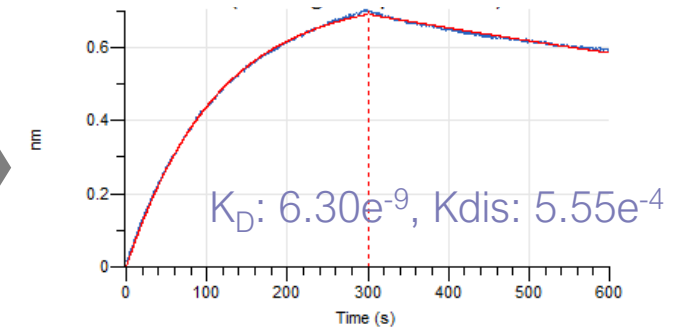
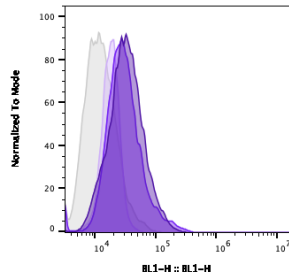
## Affinity ranking by Octet BLI



### Clone 13

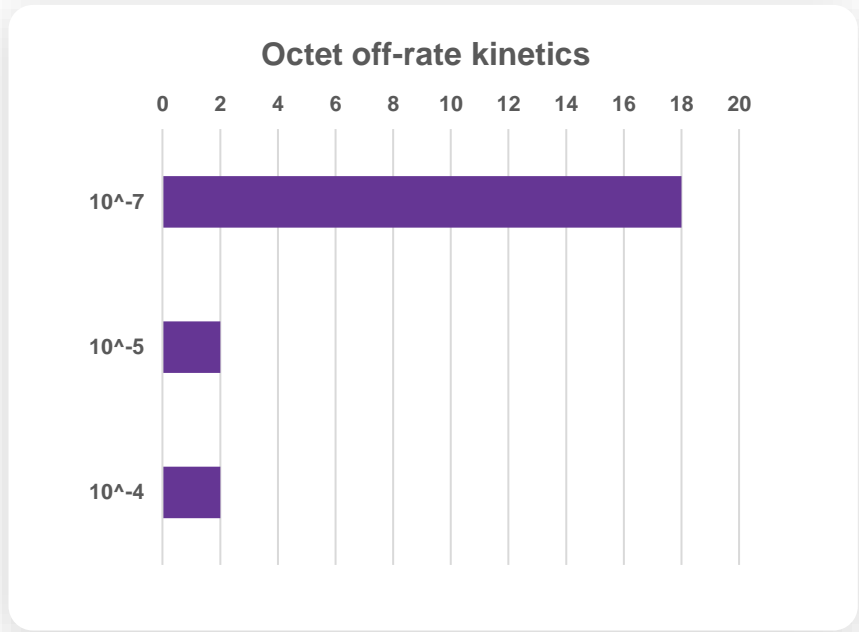


### Clone 2



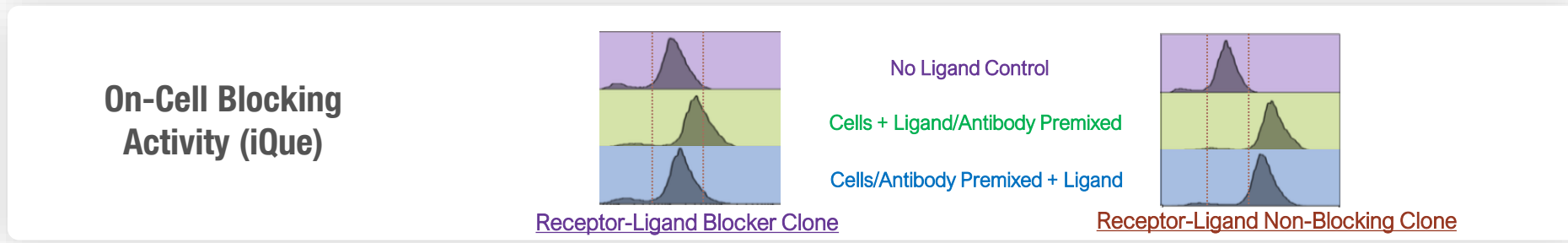
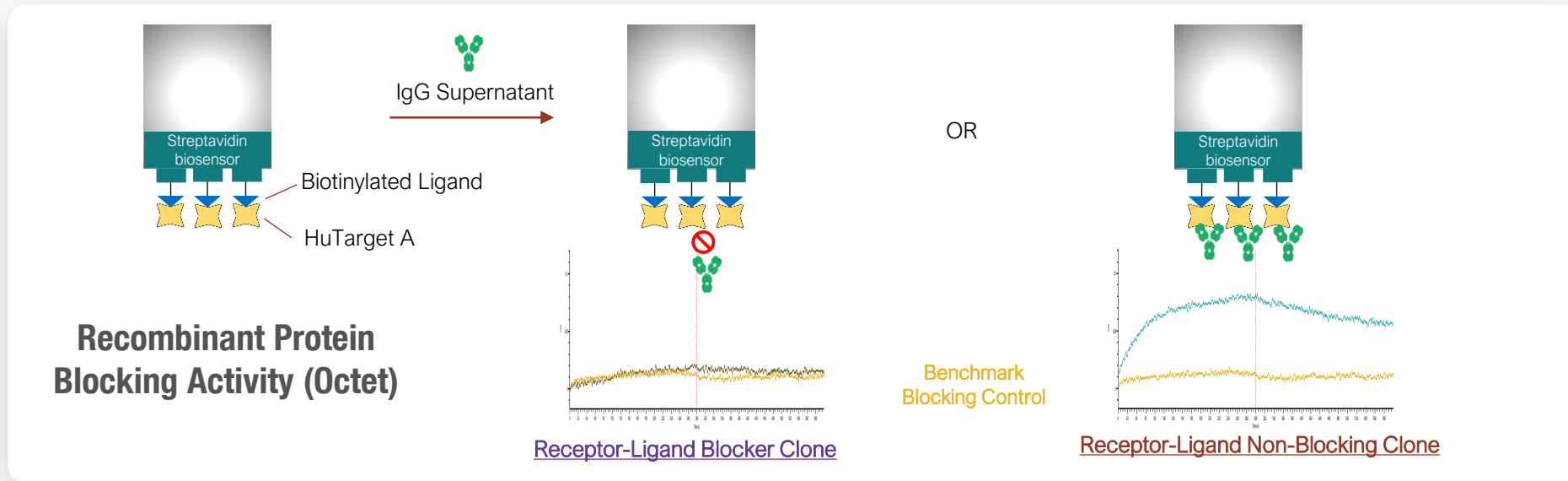
Selected examples for binding kinetics

### Affinities are ranked by the off-rate



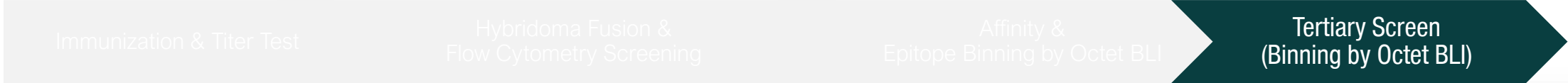
# CASE STUDY: ANTIBODY DISCOVERY WITH ALLOY MOUSE

100% Correlation between recombinant protein and cell-based ligand blocking activity

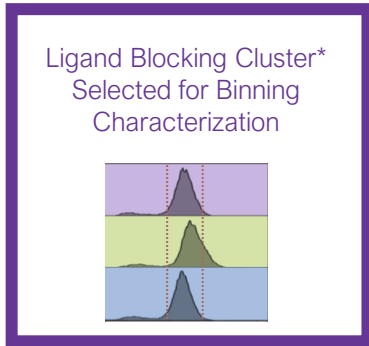
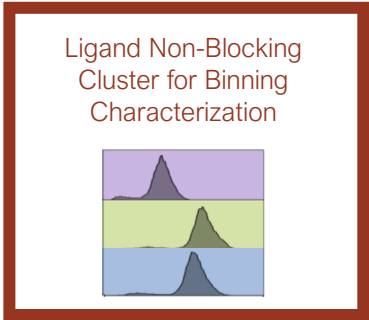


# CASE STUDY: ANTIBODY DISCOVERY WITH ALLOY MOUSE

Octet BLI assay in classical binning format of antibodies based on blocking assay results

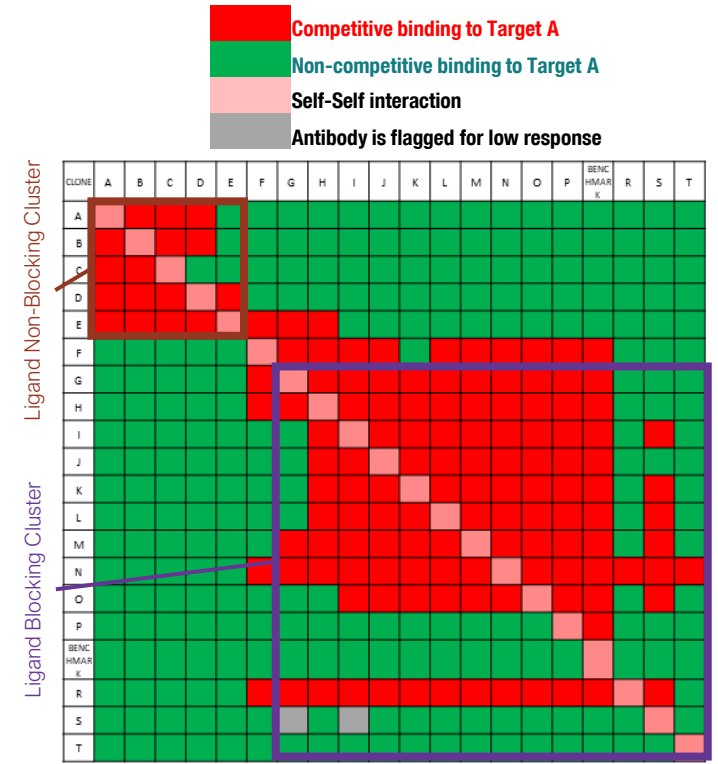
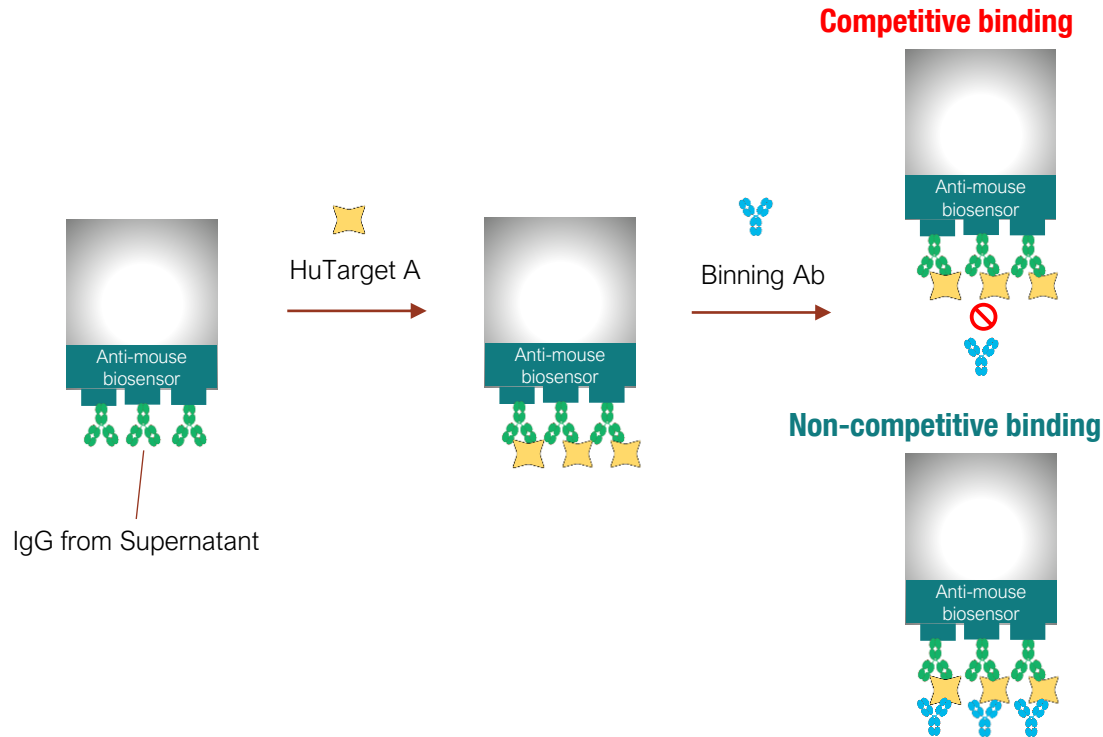


19 clones selected



\*Clone F designated as a partial blocker

## Binning Analysis (Octet, Classical Format)



Binning of parental clones

Immunization &  
Titer Test

Hybridoma Fusion &  
Flow Cytometry Screening

Affinity &  
Epitope Binning by Octet BLI

Tertiary Screen  
(Binning by Octet BLI)

## Project Goal:

- Human, single-digit nanomolar mAbs that bind in the presence of a ligand (non-blockers)
- The target is a cell surface receptor with a ~150aa extracellular domain

## Results:

- Immunized Alloy GK mice, achieved titer for fusion
- All screening performed by flow cytometry (both primary and secondary screens) and Octet BLI
- Top affinity clones were analyzed in a ligand blocking assay by Octet and flow cytometry
- 14 high value clones identified – sub nM affinity and non-ligand blocking activity
  - 8 unique paired HC/LC sequences delivered
- Immunization start to functional screening completed in less than 3 months



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