# Native complex membrane antigen expression on poxvirus for antibody discovery

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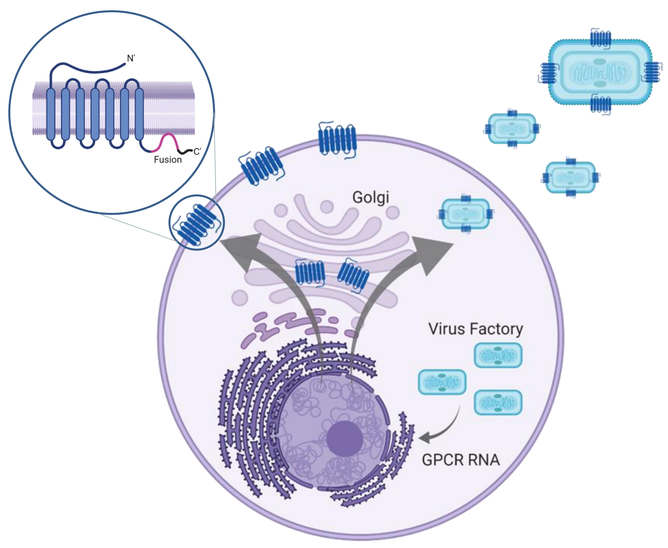
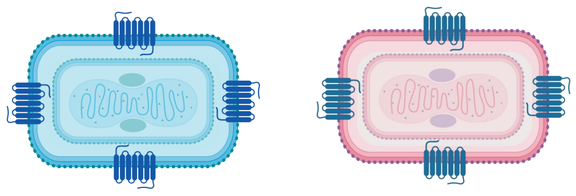
Vaccinex has developed a fusion protein technology to enable the direct incorporation of multi-pass membrane proteins such as GPCRs and ion channels into the membrane of two antigenically distinct poxviruses. The protein of interest is correctly folded and expressed in the cell-derived viral membrane and does not require any detergents or refolding before downstream use. Antigen expressing virus can be readily purified and used for antibody selection using any in vitro display platform where alternating between the two strains eliminates any anti-viral antibodies from being selected.
Antigen virions can also be used for in vivo antibody discovery methods. Immunization with the viral strains produce potent antibody responses. The resulting immune cells can then be used for Single B Cell sorting, Plasma Cell interrogation, or to create an immunized phage library for in vitro panning. Here we describe the discovery of functional antibodies against CXCR5 and P2X2 utilizing our antigen virions post-immunization in mice by all three methods.

## Technology Introduction

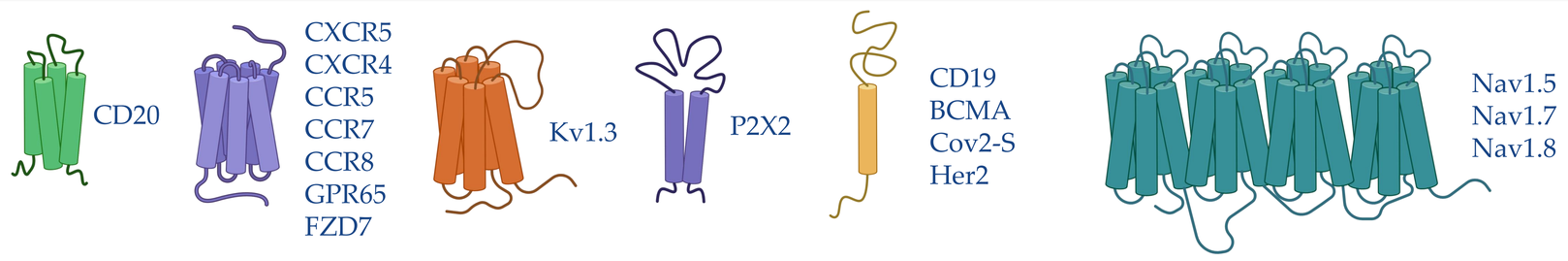
Vaccinex fusion protein technology provides for efficient incorporation of multi-pass membrane proteins into the poxvirus membrane. This system ensures native conformations for antibody discovery both *in vitro* and *in vivo*.




Antigens are produced in two antigenically distinct, highly attenuated BSL1 poxviruses to eliminate anti-viral background. Additionally, the poxvirus membrane has limited protein complexity with 4 known proteins incorporated.



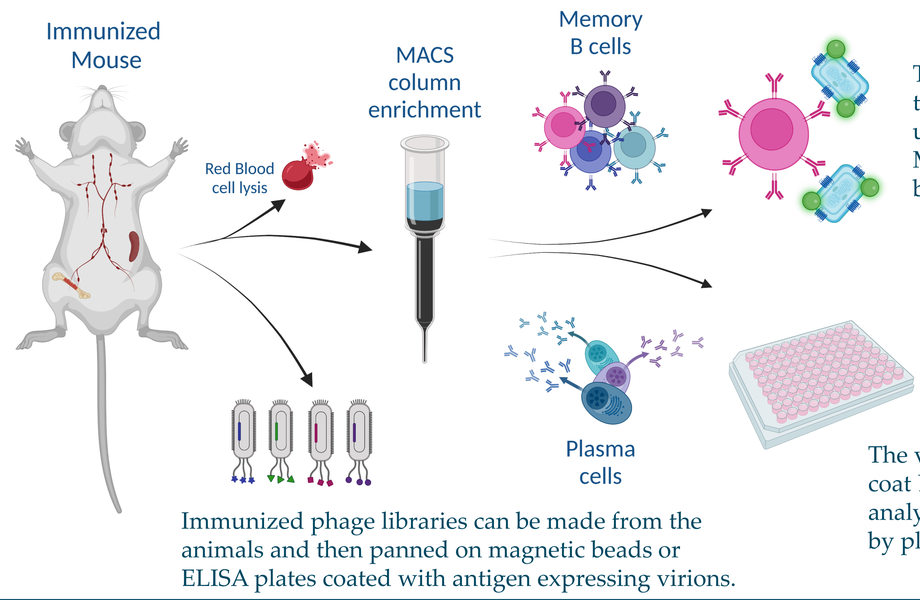

## Examples of Successful Targets




## *In vivo* Antibody Discovery Methods

Antigen virions are excellent immunogens. With two antigenically distinct poxvirus strains available, we can immunize with one strain and perform downstream applications with the other to avoid anti-virus background.

Post immunization, antigen virions can be used to isolate antigen specific B cells by Fluorescent Activated Cell Sorting (FACS), immunized phage library panning or plasma cell analysis.



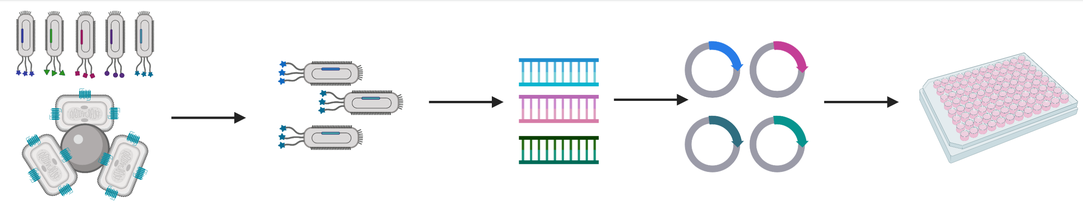

The virions can be coupled to fluorescent beads and used to stain isolated Memory B cells for sorting by antigen binding.

Immunized phage libraries can be made from the animals and then panned on magnetic beads or ELISA plates coated with antigen expressing virions.

The virions can be used to coat ELISA plates or beads for analysis of antibodies secreted by plasma cells.

## *In vitro* Antibody Discovery Methods

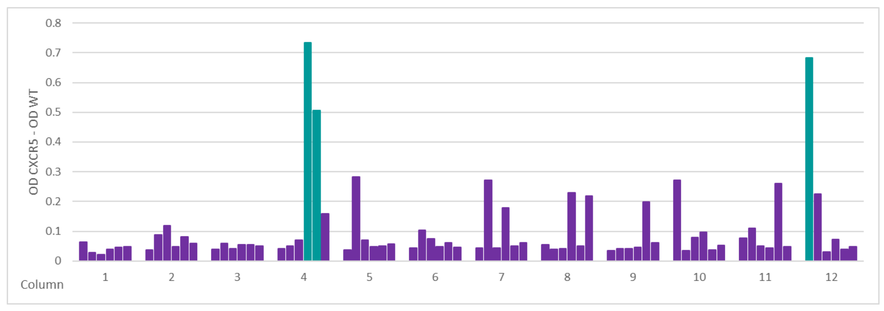
Antigen virions can be coupled to magnetic beads for in vitro phage display panning. After DNA extraction and transfection, secreted antibody clones are tested by flow cytometry.



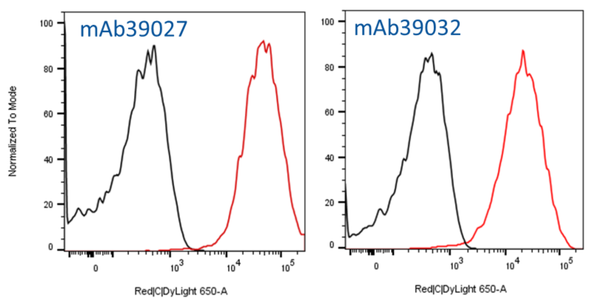
## Example: Antibody Discovery for CXCR5

Balb/c mice were immunized with CXCR5 antigen virions and mice with sufficient anti-CXCR5 titer were sacrificed and their spleens and bone marrow taken for B cell sorting, immunized phage library generation and plasma cell ELISA screening.

Plasma cells were isolated from the spleens and bone marrow and seeded into 96 well plates at 100-1000 cells per well. Supernatants were then tested by ELISA on CXCR5 virion versus Negative virion coated ELISA plates.



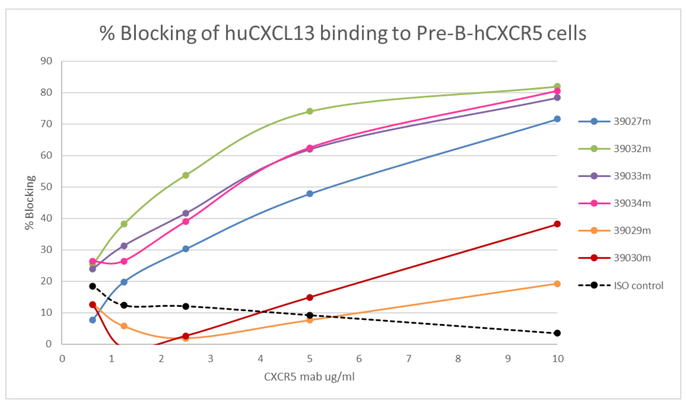

Representative ELISA data. Plasma cells from the wells highlighted were taken for downstream processing.




Individual phage libraries were created from whole spleens, sorted B cells and CXCR5+ plasma cell pools and panned on CXCR5 antigen virions. DNA from the CXCR5 enriched phage was cloned into a CHO expression vector for transfection. Individual antibody clones were tested for binding to CXCR5+ cells (red histogram) vs Negative cells (black histogram) by flow cytometry.

A total of 30 antibodies were discovered. Antibodies exhibited functionality in the ability to prevent migration of CXCR5 expressing cells toward hCXCL13 and blockade of CXCL13 binding. Antibody affinity was determined by flow cytometry using the Scatchard method. Additional antibodies are pending purification and testing.

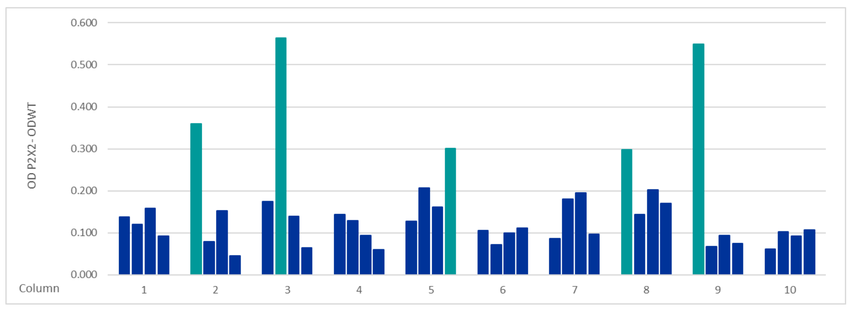
| Antibody | Affinity (nM) | % Migration blocking |
|---|---|---|
| 39027 mouse IgG2a | 0.04 | 36% |
| 39032 mouse IgG1 | 0.53 | 44% |
| 39033 mouse IgG2a | 0.34 | 45% |
| 39034 mouse IgG2a | 0.35 | 53% |
| 39029 mouse IgG1 | 1.66 | 29% |
| 39030 mouse IgG1 | 0.89 | 49% |




## Example: Antibody Discovery for P2X2

Balb/c and C57Bl/6 mice were immunized with P2X2 antigen virions. Mice with sufficient anti-P2X2 titer were sacrificed and their spleens and bone marrow taken for immunized phage library generation and plasma cell ELISA screening.

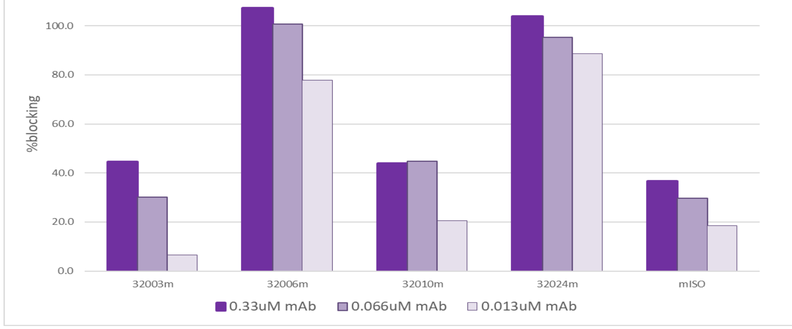
Plasma cells were isolated from the spleens and bone marrow and seeded into 96 well plates at 100-1000 cells per well. Supernatants were then tested by ELISA on P2X2 virion versus Negative virion coated ELISA plates.




Representative ELISA data. Plasma cells from the wells highlighted were taken for downstream processing.

A total of 128 unique antibodies were discovered. Select antibodies were purified and tested for affinity and their ability to block ATP induced membrane potential changes.

| mAb | Affinity (nM) |
|---|---|
| 32003 mouse IgG1 | 0.3 |
| 32006 mouse IgG2a | 0.4 |
| 32009 mouse IgG2a | 0.9 |
| 32010 mouse IgG2a | 1.2 |
| 32024 mouse IgG2a | 0.7 |




## Conclusions

Poxvirus display of antigens is a versatile tool to express a variety of complex membrane proteins for antibody discovery, including GPCRs, Ion Channels and ECDs in their native conformation. The use of two antigenically distinct poxviruses with limited membrane diversity facilitates antibody selection by *in vitro* display technologies such as phage and yeast. Antigen poxvirus can additionally be used for *in vivo* discovery methods such as single B cell analysis, plasma cell analysis and hybridoma screening.

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