Native complex membrane antigen expression on poxvirus for antibody discovery

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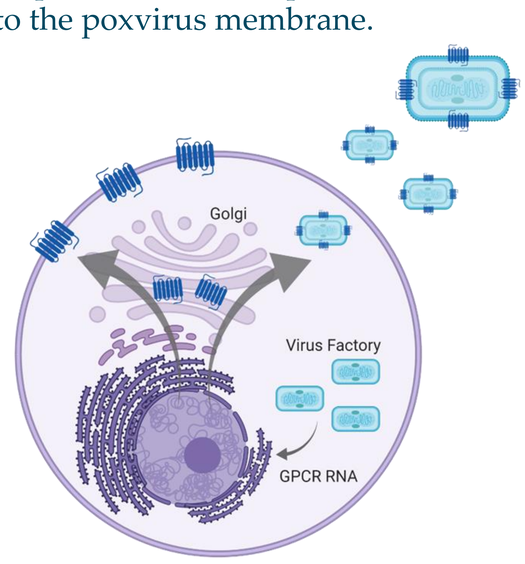
ActivMAb Technology

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 poxviruses. This method naturally embeds the protein of interest in a cell derived viral membrane and does not require detergents or refolding. Antigen expressing virus can be produced in two antigenically distinct strains to facilitate in vitro antibody selection.

Introduction

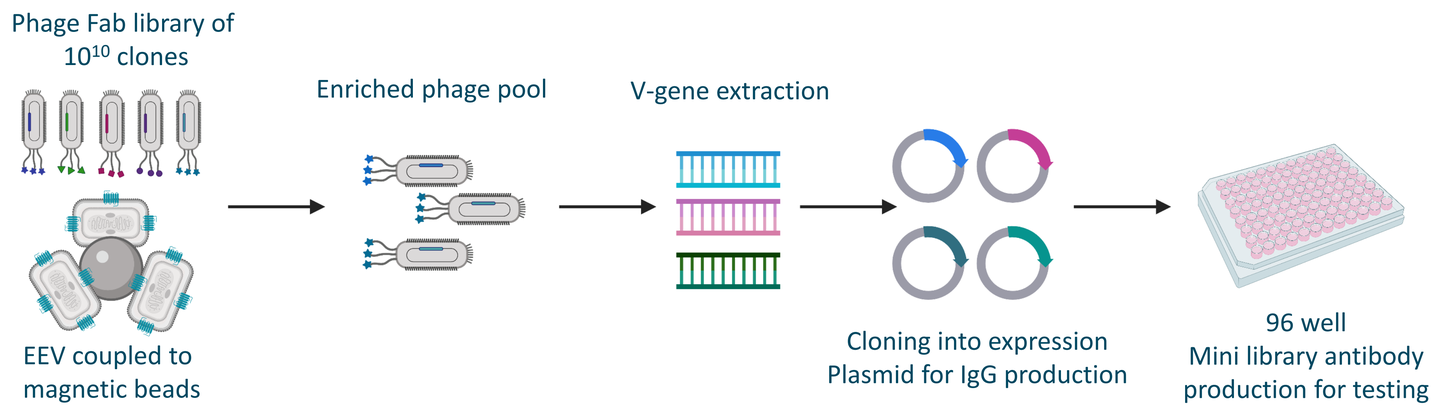
Vaccinex fusion protein technology provides for efficient incorporation of multi-pass membrane proteins into the poxvirus membrane.



- Untagged molecules do not incorporate efficiently
- Easy to generate virus expressing antigen
- 2 months from sequence to antigen virus
- No detergents or refolding
- Ag-virus harvested by simple centrifugation
- Essentially one size fits all
- Transient (inducible) expression system
- Highly attenuated strains
- Suitable for antibody selection technologies
- Specific viral membrane complexity (only 4 viral proteins)
- Recombinants are made in two antigenically distinct poxviruses

Phage Panning for Antibody Discovery

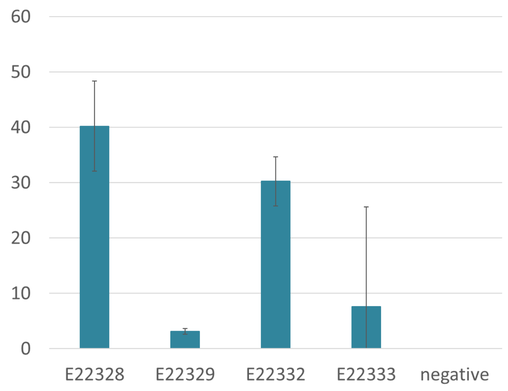
Virions can be coupled to magnetic beads or to ELISA wells to facilitate in vitro panning by phage display.



in vitro Antibody Discovery

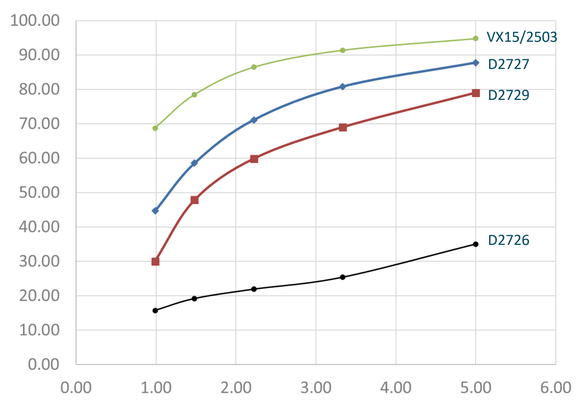
Phage panning was performed for various antigens using antigen EEV, including CXCR4, SEMA4D and SARS-CoV2 S. Selected antibodies show functionality and variable affinity for target antigens.

% Blocking of Jurkat cells (CXCR4+) migration toward SDF-1



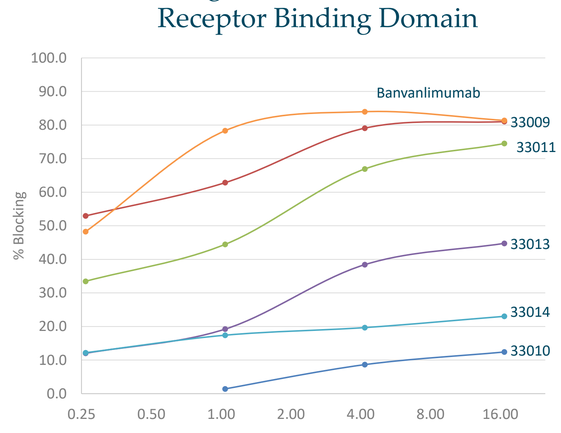
Affinity range: 0.84 – 19.6 nM

% Blocking of huSEMA4D binding to PlexinB1



Affinity range: 6-22 nM

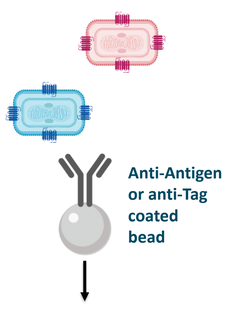
% Blocking of ACE-2 to SARS-CoV2-S Receptor Binding Domain

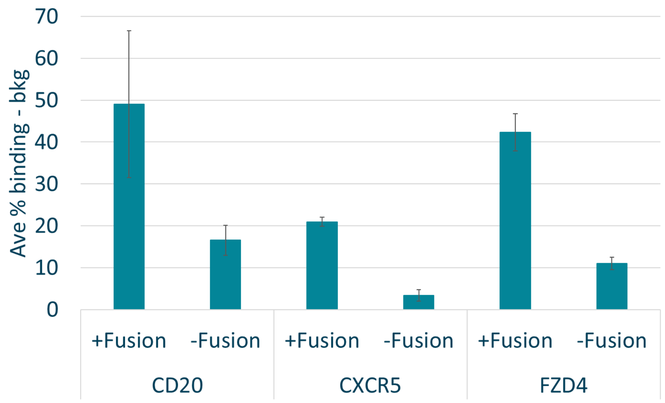


Affinity range: 0.5 – 6.7 nM

Antigen Virus Expression

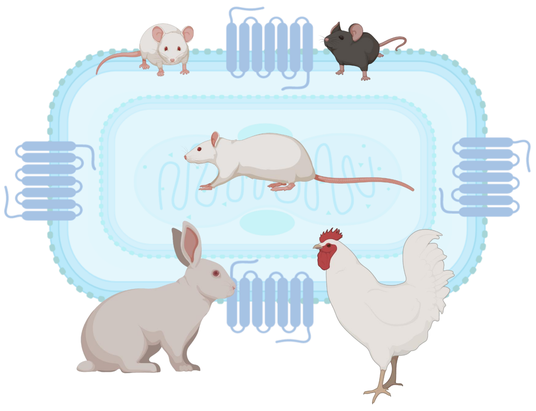
Virions expressing various antigens can be assayed by a pull-down assay, similar to an immunoprecipitation, using magnetic beads. The virions are captured by either an anti-antigen or anti-tag antibody and the number of bound virions is determined by plaque assay.



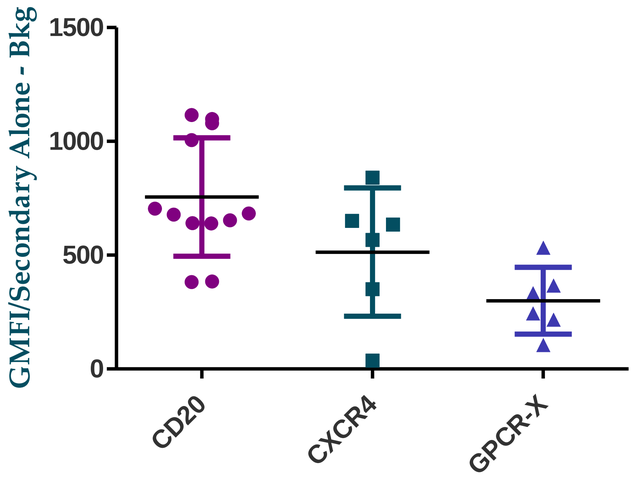


In vivo Antibody Discovery

Animals immunized with antigen expressing virions can be used to generate *in vitro* display libraries, hybridomas, NGS, plasma cell screening or B cell sorting.



Balb/c sera for select antigens (single dilution) by flow cytometry

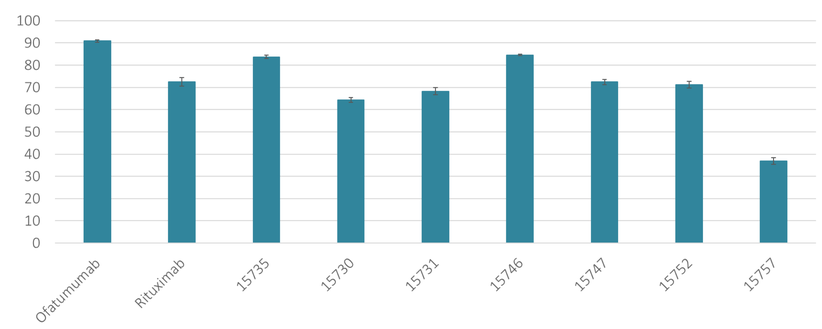


Immunization and *in vitro* Immune Library pan for CD20 antibodies

Two Balb/c and two C57Bl/6 mice were immunized with PV1 antigen virions and their sera were analyzed for titer by flow cytometry on cells. Mice with sufficient titer were sacrificed and their spleens used to make phage library. The library was used to pan on PV2 virions (alternate poxvirus) and then screened as a Mini library by flow cytometry.
Unique antibodies were purified and tested for affinity and functionality in a CDC assay against the clinical antibodies Ofatumumab and Rituximab.

Mab Number	Affinity (nM)	No. of variants in HCDR3 family	No. of different VL pairings
15735	27.3	1	1
15730	>50	6	8
15731	26.1	6	6
15746	38.4	6	8
15747	19.2	2	4
15752	31.5	2	2
15757	13.5	1	1
Rituximab	33.1	-	-

CDC Assay: % Cytotoxicity at 5 ug/ml antibody



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. The production in mammalian cells ensures the proteins are correctly folded and contain all modifications that would be on the native antigen. There is no need for detergents or refolding, and the limited membrane diversity facilitates antibody selection by *in vitro* display technologies such as phage and yeast. Antigen poxvirus can additionally be used to immunize animals for *in vivo* discovery methods such as single B cell analysis and hybridomas.

VACCINEX

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