Antibody Library Display on a Mammalian Virus : Combining the Advantages of Phage and Yeast Display in One Technology with Full Mammalian Post-translational Modifications

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Abstract

Utilizing a vaccinia virus based library technology we have previously developed an antibody discovery platform that enabled efficient selection of fully functional IgG antibodies from highly diverse immunoglobulin gene libraries expressed in mammalian cells. This technology allowed for the expression of full length IgG on the surface of human cells and, following incubation with antigen, the cells encoding antigen specific antibodies could be isolated by MACS and high speed cell sorting.
We have recently modified our technology by creating an immunoglobulin heavy chain-vaccinia membrane protein fusion protein that enables efficient expression of a library of fully human antibodies on the surface of vaccinia virus; an enveloped mammalian virus. Similar in concept to phage display, conditions are utilized wherein each vaccinia virion expresses a single antibody specificity on its surface. Various panning and magnetic bead based methods have been developed to allow screening libraries of vaccinia-MAb virions to select recombinant virus encoding specific antibodies. Upon infection of mammalian cells the antibody is not only incorporated into newly produced virus, it is also displayed on the surface of the host cell. This enables efficient selection strategies that combine the benefits of selection of vaccinia-MAb virions in a cell free panning system, followed by cell based screening for high specificity and antibody optimization.
Both the vaccinia display and the cell display platforms can be employed for *de novo* antibody selection, affinity improvement of existing human antibodies, or for the robust conversion of a non-human antibody into a panel of fully human antibodies. In all applications, there is a built-in selection for antibodies that are efficiently expressed in mammalian cells.

Vaccinia Virus as an expression host

- Vaccinia virus infects most mammalian cells. Recombinant proteins expressed by vaccinia virus infected cells undergo normal post-translational modifications and trafficking.
- The conventional recombination technology allows for the introduction of *one defined gene* at a time into vaccinia virus. This is sufficient for expression of a single gene product but does not enable functional cloning of unknown genes from a large library.
- Vaccinex has developed proprietary technology that allows for the creation of large and diverse cDNA libraries in a vaccinia virus-based vector.
- Millions of different vaccinia recombinants in each library.
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- Technology for functional cloning in mammalian cells.
- Selection of fully human monoclonal antibodies
- Efficient identification of immune target antigens

Introduction

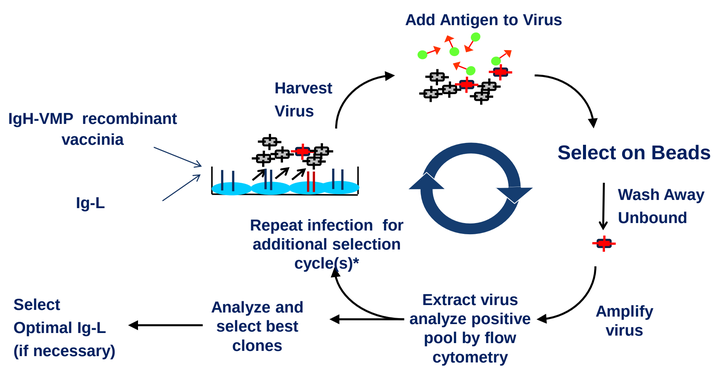
- Variable gene sequences are either isolated from naïve sources or derived synthetically.
- Separate heavy and light chain libraries are created in vaccinia virus
  - 10^7 Ig-H x 10^7 Ig-L = 10^{14} combinations
  - Rapid initial screening of >5X10^9 combinations
  - Alternatively, 10^7 – 10^8 Ig-H can be screened using a limited panel of defined germline Light chains
- Antibody Library Display <u>on</u> Vaccinia Virus (Vaccinia Display): Combines the advantages of phage and yeast display in one technology with full mammalian post-translational modifications.

- We have constructed a fusion protein combining immunoglobulin VH-CH1 with a vaccinia virus membrane protein.
  - This fusion protein is expressed on the surface of the virus.
  - In Vaccinia Display, the antibodies are produced in mammalian cells, but virus is enriched in a cell free system.
  - Vaccinia can be concentrated by standard centrifugation, so that a very high number of combinations can be screened at one time.
  - Selection by Vaccinia Display employs methods, such as binding to antigen coated magnetic beads that have proven effective in phage display.
  - A depletion step can be introduced to remove potentially cross-reactive antibodies.
  - The fusion construct is also expressed on the surface of infected cells which allows specific heavy and light chain pairs to be captured after enrichment of specific heavy chains. This allows phage display like selection of specific binders to be followed by the precision of FACS sorting as in yeast display.

Competitive Advantages

Challenge	Vaccinex Technology Advantage
Efficient selection of fully human, high affinity antibodies.	• Vaccinex selects antibodies from libraries constructed in a vaccinia virus vector and expressed in mammalian cells with full post-translational modifications. • With Vaccinia Display, virions displaying antibodies are rapidly selected in one or more rounds of binding to magnetic beads in a cell free system. This is followed by a final cell-based FACS sort of cells to capture specific pairs of highly enriched immunoglobulin heavy and light chain recombinant virus.
Conversion of non-human antibodies to fully human Affinity improvement of existing human antibodies	• Selection of multiple antibodies derived from distinct VH and VL germ line genes with different biochemical properties. • Converting mouse antibodies to fully human antibodies with conserved epitope specificity and with similar or improved affinity and functional activity.
Manufacturing	• Intrinsic selection for high expression in mammalian cell lines, easily adaptable to manufacturing.

Vaccinia Display with Germline Ig-L



* Cell sorting is used as a final step

Vaccinia Display. Mammalian cells are co-infected with human Ig-VMP Library and with a cocktail of vaccinia recombinant human light chains at m.o.i.=1. Virus is released from infected cells and concentrated by centrifugation. Virus is then incubated with antigen and magnetic beads. Unbound antigen is washed away, and the recombinant viruses that express a specific antibody are isolated using magnetic bead separation. The selected virus is rapidly expanded and further enriched by an additional round of selection. The Ig-VMP fusion protein is also expressed on the cell surface, so in a final step, infected cells can be stained with antigen and binders selected by cell sorting. The specific VH and Ig-L can be isolated and characterized. If necessary, the selected Ig-H can be used to select optimal Ig-L

Magnetic Bead Based Selection

<u>Streptavidin beads</u>
Use Biotinylated antigen and Streptavidin coated beads
<u>Protein G beads</u>
Use Fc fusion protein
<u>Tosylactivated magnetic beads</u>
No Tags required; direct coating of antigen onto the beads

Ig-Vac Bead Selection

CD100-Fc / ProG Bead Selection	
Virus	**% Bound**
MAb 2408 anti-C35	0.36%
MAb 2368 anti-CD100	58%
CD100--His / Tosyl Bead Selection	
Virus	**% Bound**
MAb 2408 anti-C35	0.1%
MAb 2368 anti-CD100	47%

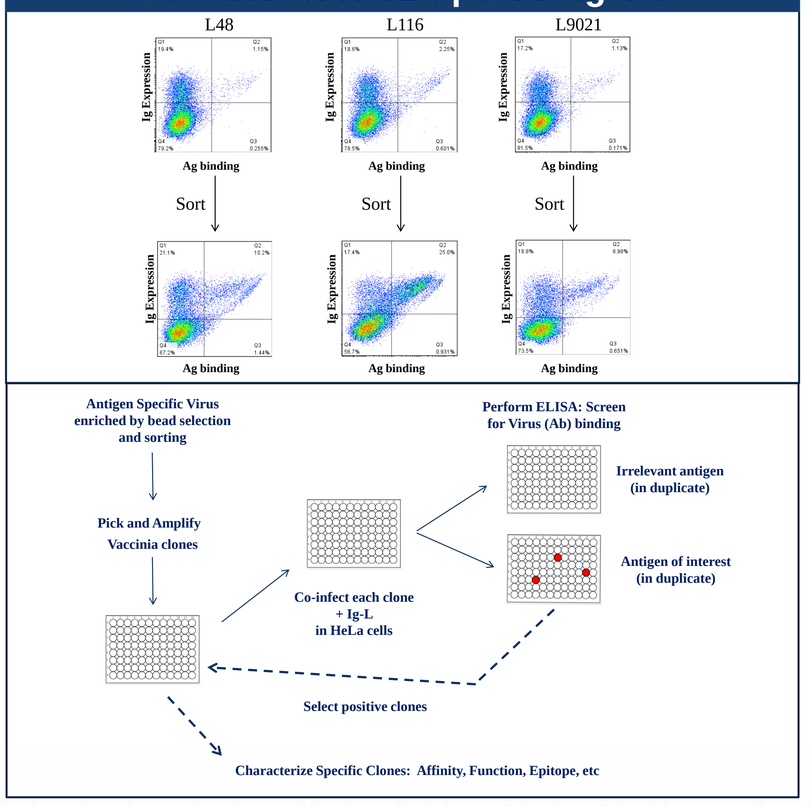
Fusion protein expressing virus was produced and purified. CD100-Fc protein or CD100-His were loaded onto Dynal Protein G /tosyl Beads. The virus was added to the coated beads and incubated at 25ºC for 2 hours. Unbound virus was removed by 5 X1ml washes in PBS, and bound virus was collected. The efficiency of recovery was determined by titering bound and unbound virus.

- Recovery of antigen specific virus is very high
- Background binding of control virus is very low
- Similar data observed with other antigen-antibody combinations

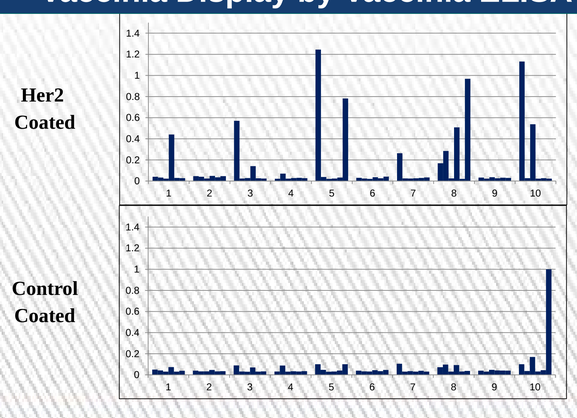
Example: Selection of Her2 specific MAbs

- Her2-Fc coupled to Protein G beads as described
- Naïve Vaccinia Ig-H-VMP fusion protein library co-infected with a cocktail of Ig-L recombinant virus and subjected to 2 rounds of selection using “Vaccinia Display”

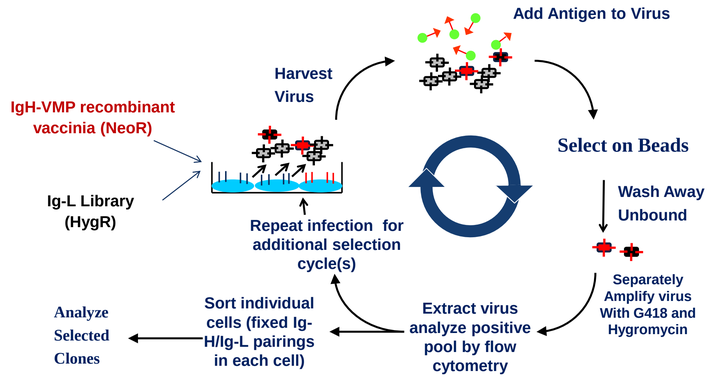
Selection of Her2 Specific Ig-Vac



Identification of Specific L116 Clones Following Vaccinia Display by Vaccinia ELISA



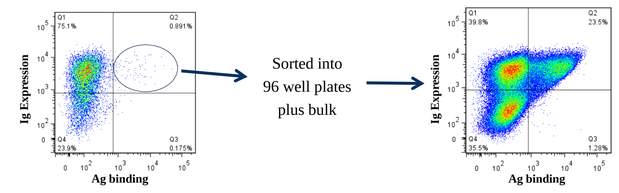
Vaccinia Display with Ig-L Library



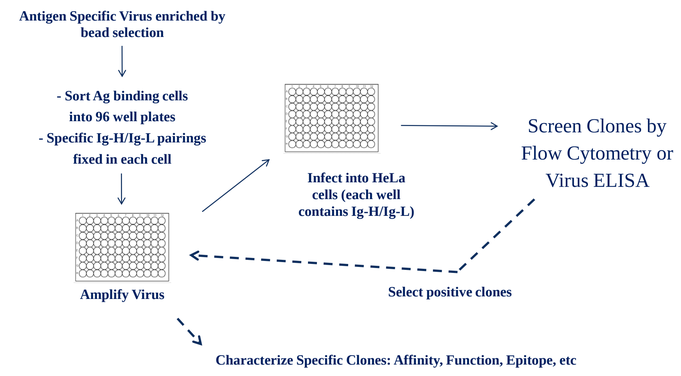
Vaccinia Display. Mammalian cells are co-infected with human Ig-VMP Library and with a diverse library of Ig-L. Virus is released from infected cells and concentrated by centrifugation. Virus is then incubated with antigen and magnetic beads. Unbound antigen is washed away, and the recombinant viruses that express a specific antibody are isolated using magnetic bead separation. The Ig-H-VMP virus contains a NeoR gene, and the Ig-L contains a Hygromycin resistance gene. The selected virus is rapidly expanded using G418 and hygromycin and further enriched by an additional round of selection. The Ig-VMP fusion protein is also expressed on the cell surface, so in a final step, infected cells are stained with antigen and binders selected by cell sorting. The specific VH and Ig-L can be isolated and characterized. If necessary, the selected Ig-H can be used to select optimal Ig-L

Example: Selection of C35 specific MAbs

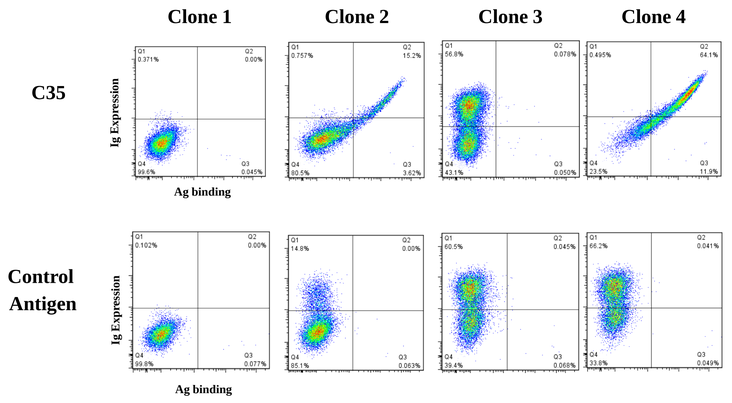
- C35 (C17orf37) used as target antigen for *de novo* selection with vaccinia display
- Selection used C35 coupled to tosyl beads as described
- Vaccinia fusion protein library co-infected with Library of Ig-L at a total of **3x10^9** combinations was subjected to 2 rounds of selection
- Enrichment for C35 specific binders tested by flow cytometry



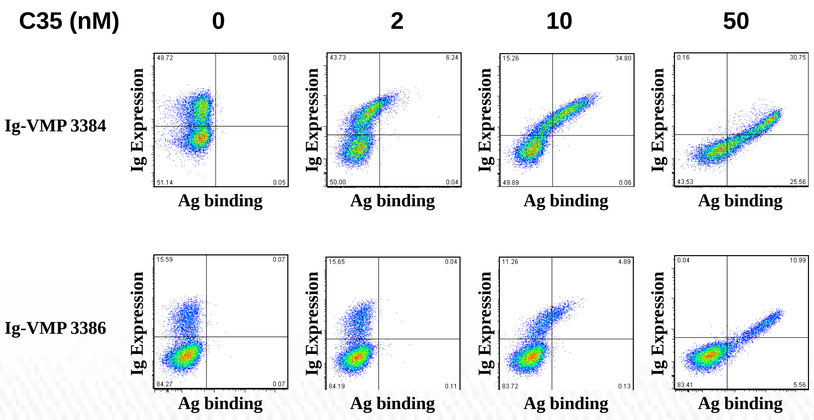
Identification of Specific Ig-H/Ig-L



Example of Screening Single Cell Clones



Preliminary Analysis of Antibody Affinity



Summary of Selected Mouse to Human Projects

Antigen	Comments
TNFα	Antibodies superior to Remicade and Humira Selected
IL6	50pM affinity antibody selected (Equivalent to current clinical anti-IL6 MAbs)
VEGF	Antibodies superior to Avastin selected
CXCL13	High Affinity antibodies Selected
EGFR, Her2 and others	High Affinity antibodies Selected