Antibody Library Display: Combining the Advantages of Panning and Cell Sorting in One Technology


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Abstract
Using a vaccinia virus-based library technology we have developed an antibody discovery platform that enables efficient expression of a library of human antibodies in full length format on the surface of vaccinia virus; an enveloped mammalian virus. Similar in concept to phage display, but allowing display of full length IgG synthesized and processed in mammalian cells, conditions are utilized where each vaccinia virion will express a single antibody specifically on its surface. Various panning and magnetic bead based methods have been developed to allow screening of a library of vaccinia-Mb vectors and selection of recombinant vaccinia 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. In a final purification step, the cells displaying vaccinia encoded antibody can be selected by cell sorting, and the virus encoding the specific antibody readily reassembled and analyzed. This technology allows for rapid enrichment of vaccinia-Mb vectors in a cell free protein system, and then incorporates a cell based screening assay for isolation of optimal, highly specific antibodies. This technology has been employed for de novo antibody selection, affinity improvement of existing human antibodies, and 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.

Competitive Advantages

<table>
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<tr>
<th>Challenge</th>
<th>Vaccinex Technology Advantage</th>
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<tbody>
<tr>
<td>Efficient selection of Fully Human, high-affinity full length IgG antibodies</td>
<td>- Vaccinex selects high affinity antibodies from libraries constructed in a vaccinia virus vector and expressed in mammalian cells with full-length translational modifications. - With Vaccinex Display, virions displaying antibodies are rapidly selected in one or more rounds of binding to magnetic beads in a cell-free system, followed by a final cell-based FACS sort of cells infected by highly enriched virus to select specific antibodies.</td>
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<td>Affinity improvement of existing human antibodies</td>
<td>- Selection of multiple antibodies derived from distinct VH and VL germ line genes with different biological properties. - Converting mouse antibodies to fully human antibodies with conserved epitope specificity and with similar or improved affinity and functional activity.</td>
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<td>Conversion of non-human antibodies to fully human</td>
<td>- Intrinsically selected for high expression in mammalian cell lines easily adaptable to manufacturing.</td>
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Introduction

- We have constructed a fusion protein of full length IgG heavy chain with a vaccinia virus protein (VMP) that coats the surface of vaccinia virus. This fusion protein is expressed both on the surface of the virus and on the culture media.
- Antibody Library Display on Vaccinia Virus: Combining the advantages of virus panning and cell sorting in one technology with intrinsic selection for antibodies with good mammalian cell expression.
- Virus panning: Allows for rapid screening of billions of antibody combinations in a cell-free selection system.
- Vaccinia is a large enough virus that infects a mammalian virus and contains cells that can be rapidly processed by centrifugation in 1ml for antibody selection.
- Cell Sorting: Allows for tunable affinity selection by adjusting the amount of antigen used to stain the cells and allows for easy confirmation of antibody expression on the cell surface during selection.
- Heavy and light chain libraries are derived from naïve or synthetic sources and represent all major germline families (including kappa and lambda constant).
- Heavy chain diversities are on the order of 10^9 and are paired with either pools of germline light or low diversity light libraries to ensure that each heavy pair has multiple functional light chains.
- The use of germline light chains creates opportunities for the independent selection of heavy chains with specificity for diverse antigens being assembled in bispecific form.

Vaccinia Virus as an expression host

- Vaccinia virus infects most mammalian cells. Recombinant proteins expressed by vaccinia virus undergo normal post-translational modifications and trafficking.
- The conventional recombination technology allows for the introduction of a single gene into a vaccinia virus. This is sufficient for expression of a single gene product, but does not allow cloning of unknown genes from a large library.
- Vaccinex has developed proprietary technology that allows for the creation of constructs that can be transfected into mammalian cells.
- Many vectors for loading into mammalian cells.
- Selection of highly human recombinant antibodies.
- Efficient identification of immune target antigens.

Vaccinia Display Process Flow

A. Light chain recombinant vaccinia (vaccinia IgM)

1. Seed virus infected with MB vector into mammalian cells
2. Generates cell line infected with MB vector
3. Selects cell line by cell type
4. Generates virus infected with MB vector
5. Generates virus infected with MB vector

B. Affinity selection

1. Add antibody to virus
2. Select on beads
3. Membrane bound antibodies
4. Examine antibodies
5. Purify antibodies

Characterization of Clones

- The VH genes contained in the sorted virus were PCR amplified and cloned into a mammalian expression plasmid containing the complete domain of IgG1.
- 239k-neutralizing clones were sequenced for each paired light chain.
- >120 unique clones were identified that bind FZD4 by ELISA and have affinities better than 100nM.
- About 40 of these affinity clones have affinities better than 10nM.
- 30 of these higher affinity clones were identified only 1X, so there are no questions many additional specific antibodies that were selected.

Magnetic Bead-Based Selection

Streptavidin beads
Use biotinylated antibody and Streptavidin-coated beads

Protein G beads
Use Fc fusion protein

Protein G beads
Use Streptavidin beads

Ex: Selection of FZD4-specific Mabs

- FZD4 used as target antigen for FZD4 selection.
- Use Fc fusion protein.
- No tags required; direct coating of antigen onto the beads.

Vaccinex Selection of Mabs

- If necessary, the selected IgG is amplified and targeted to murine or other species.

Additional Bioanalytical Characterization of Clones

- IgG affinity is determined by ELISA.
- IgG concentration is determined by BCA assay.

Affinity Improvement of a FZD4-specific Mab by Selecting Optimal Light Chains

- Example 1
- Example 2
- Example 3

Species cross-reactivity

- CTLA4 Selection Round 2

Partial Summary of Selection Projects

- Antigen titration studies allows for a preliminary estimate of affinity.
- Sorting at low antigen concentration allows for rapid selection of high affinity antibodies.

Vaccinex Manufacturing

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