Antibody Selection From Immunoglobulin Libraries Expressed in Mammalian Cells

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Vaccinex Corporate Summary

Headquarters: Rochester, NY

Employees: 51

History:

- Founded in 1997
- Core technology invented by founders at the University of Rochester
- Exclusive world-wide license in all fields of use

Intellectual Property: 70+ U.S. and foreign patents and patent applications.

Funding:

- Closed a $50M Round in 2009
- $33M in previous venture funding
- $10M in research grants

Focus:

- Four antibodies in pre-clinical development
- Two INDs expected to be filed in Q4 2010
- Novel antibody discovery platform

Vaccinex Technology Summary: Discovering Antigens and Therapeutic Antibodies

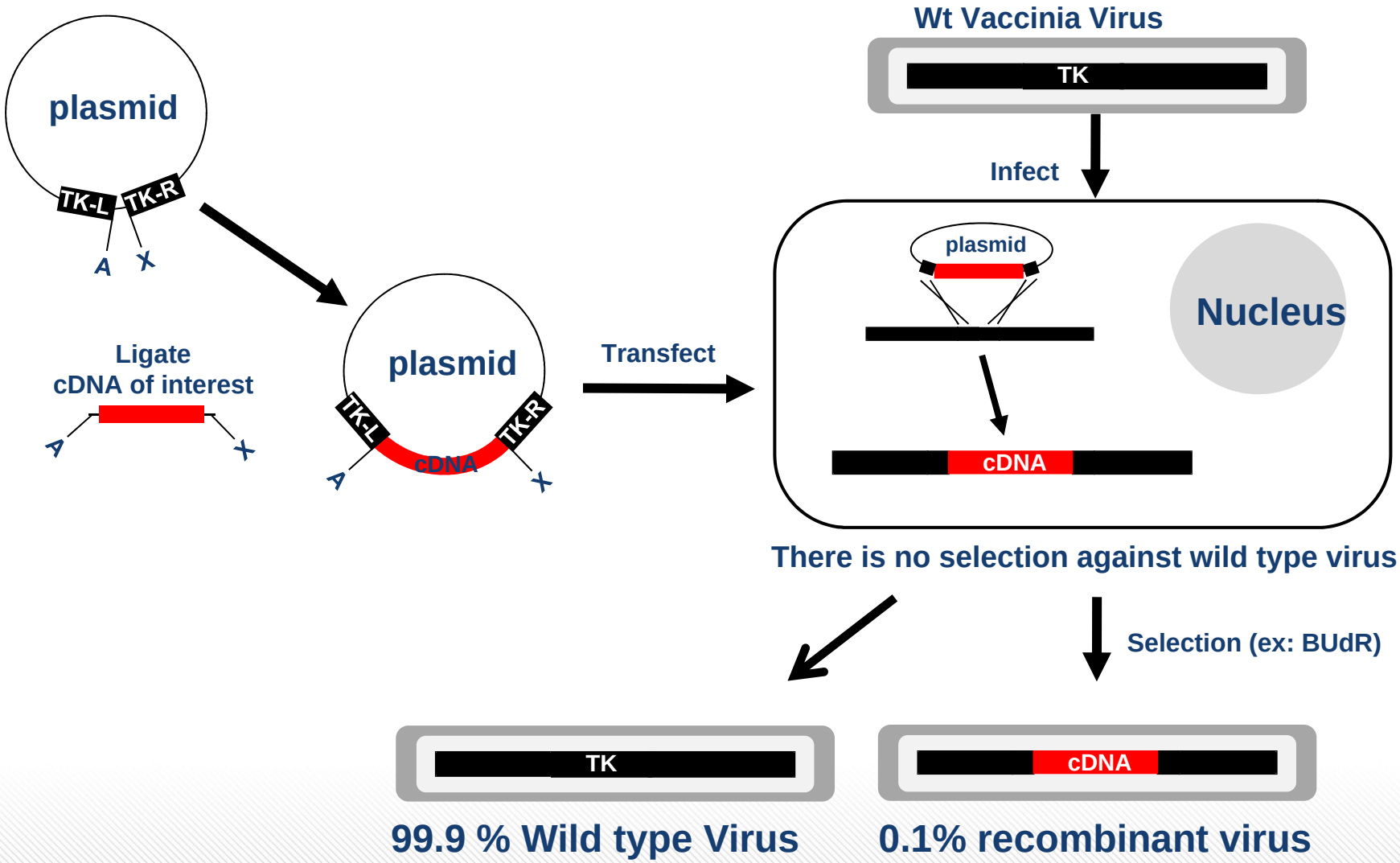
Vaccinia Virus Library Construction

Vaccinia Virus

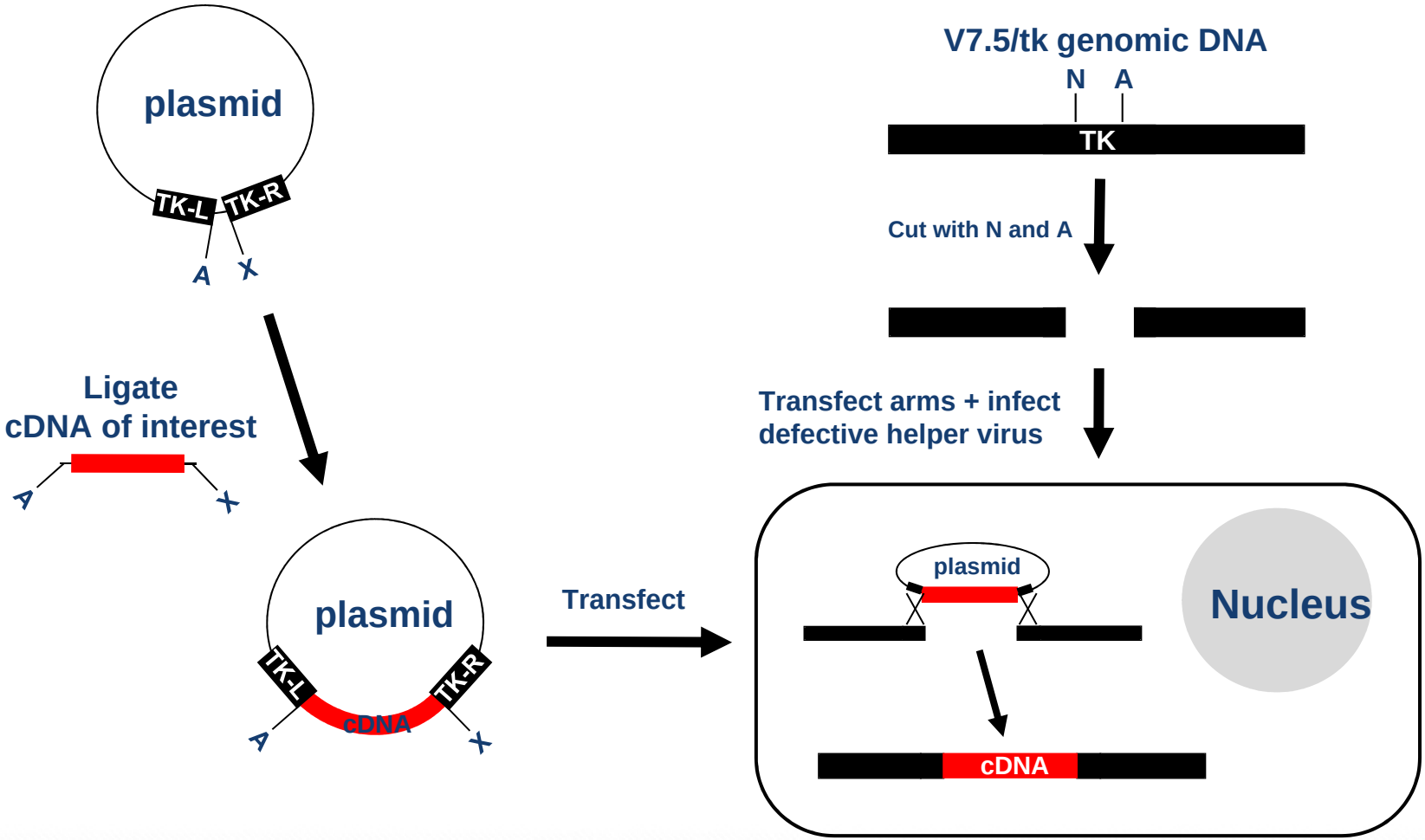
- Vaccinia virus infects most mammalian cells
- Recombinant proteins expressed by vaccinia virus infected cells undergo normal post-translational modifications and trafficking
- Very versatile mammalian expression vector, used for >20 years as a vector for basic scientific and vaccine research
- The 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 library

A New Technology was Needed to Enable More Efficient Creation of Recombinant Vaccinia Virus

Standard Homologous Recombination



Strategy to Generate 100% Recombinant Vaccinia Virus



Nature Medicine 7:967-972 (2001)

Vaccinex Technology Summary

→ 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.
- *Nature Medicine 7:967-972 (2001)*

→ Technology for functional cloning in mammalian cells.
- Efficient identification of immune target antigens
- Selection of fully human monoclonal antibodies

Antibody Libraries Expressed in Mammalian Cells

ActivMAb Technology

We have developed a large library-based antibody discovery technology that can efficiently express and select fully functional IgG antibodies in mammalian cells

- Directly expresses complete, bivalent antibodies

- Separate heavy and light chain libraries
  - 10^7 Ig-H X 10^7 Ig-L =10^{14} combinations
    - rapid initial screening of 10^7 to 10^8 combinations
  - Affinity improvement by sequential substitution of heavy and light chains
  - Allows for efficient conversion of mouse MAbs to fully human

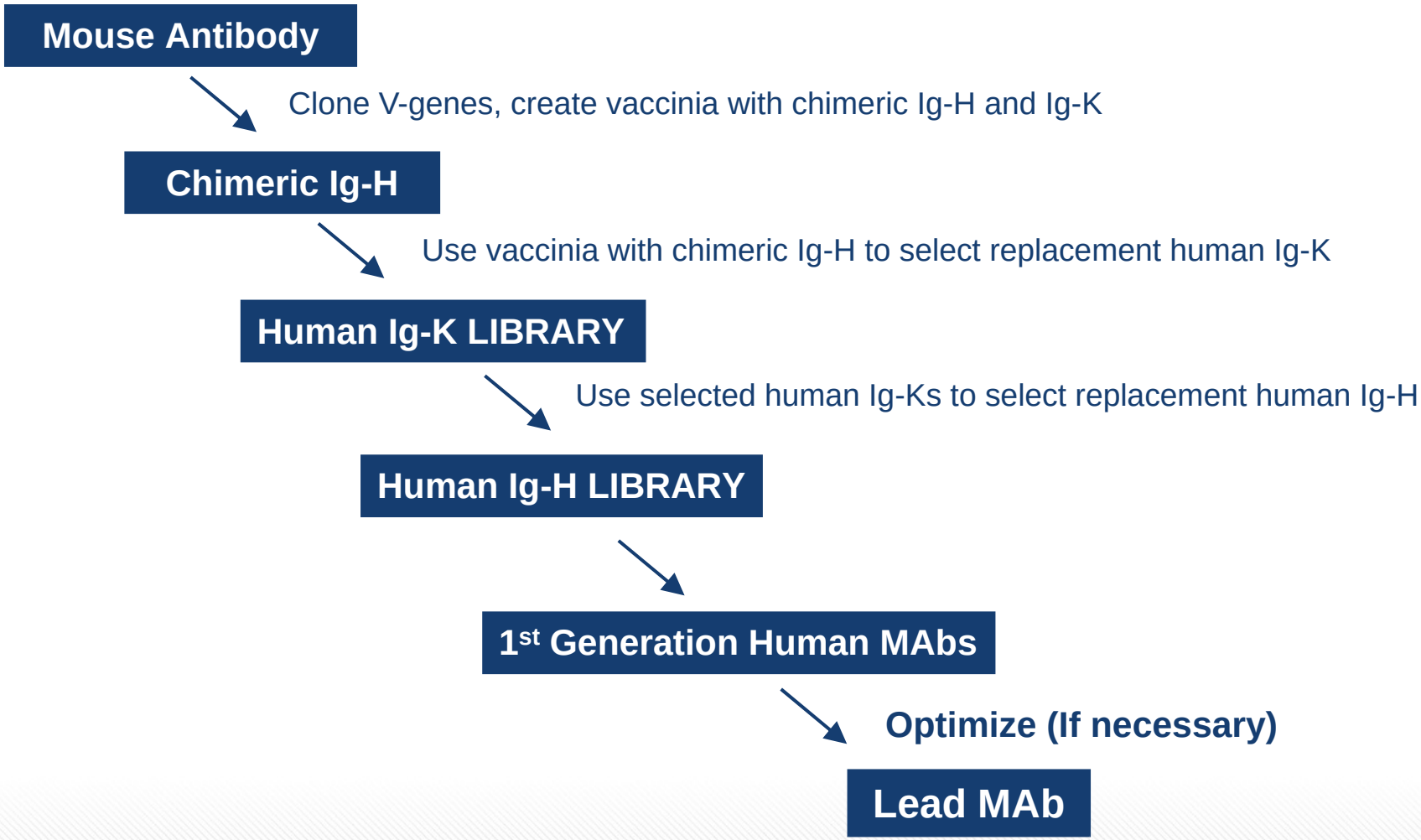
- Expression of antibody libraries in secreted IgG format
  - Ig-H gamma I (no TM domain) + Ig-L = secreted MAb
  - Screen by ELISA (soluble antigen) or FMAT (cell surface antigen)

- Expression of antibody libraries on the surface of mammalian cells
  - Ig-H gamma I (with TM domain) + Ig-L = surface display
  - Isolate specific antibodies by MACS/FACS
  - Isolate specific antibodies following induction of apoptosis

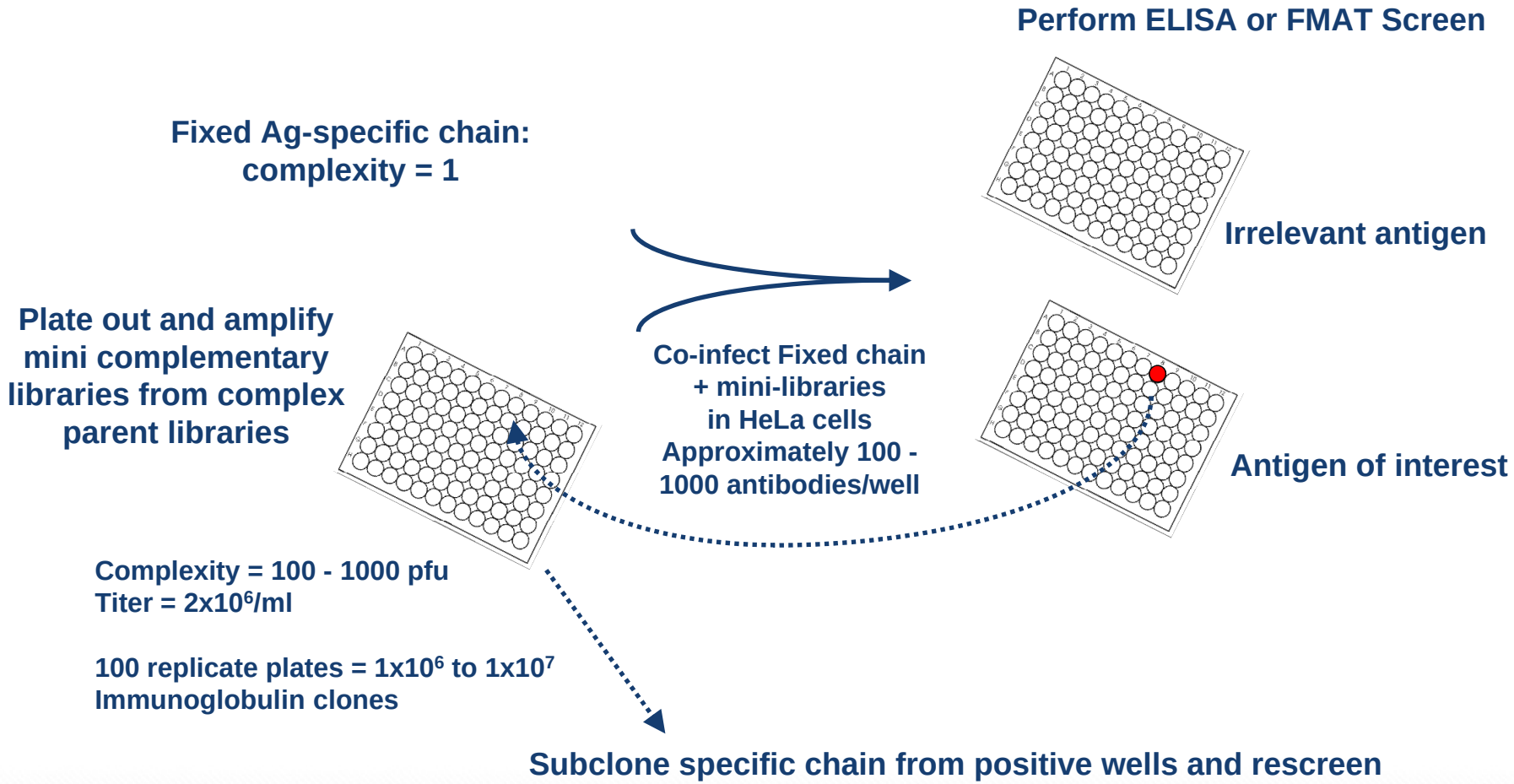
Vaccinex Antibody Selection Platforms

1. **Conversion of Mouse MAb to Human MAb**
   - ➡ **Secreted Antibody Platform**
   - ➡ **Membrane Antibody Platform: Selection by Cell Sorting**
2. ***de novo* Antibody Selection**
3. **Affinity Improvement of Human Antibodies**

Conversion of Mouse MAb to Fully Human MAbs (Flow Chart)



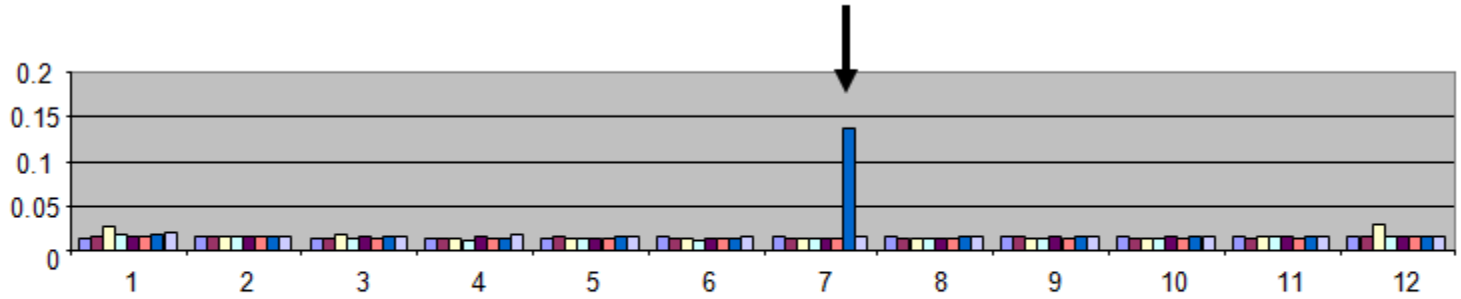
Conversion of Mouse Mab to Human Antibody



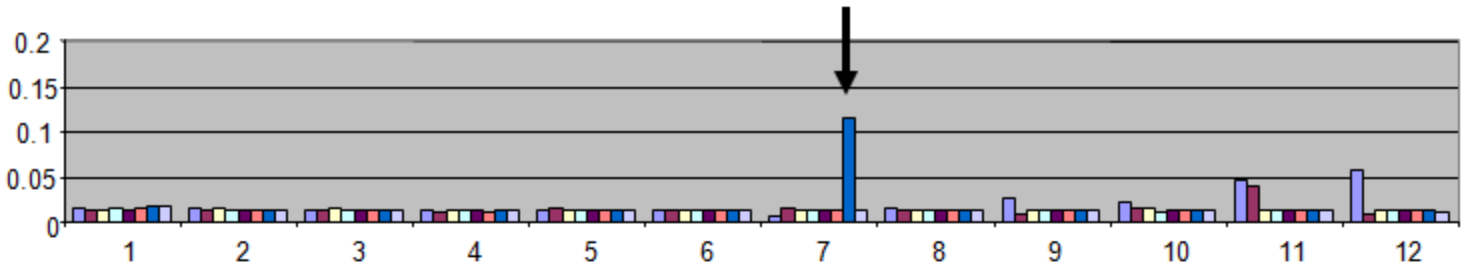
Use Mouse Ig-H to Select Human Ig-K: Round 1

Round 1

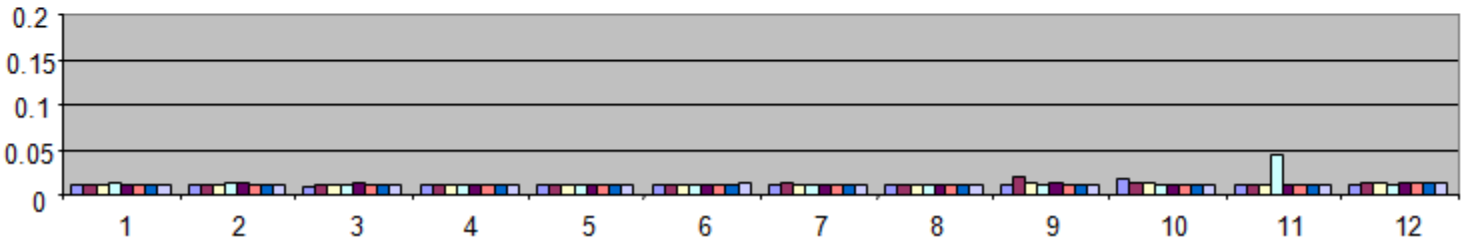
Antigen of interest



Antigen of interest



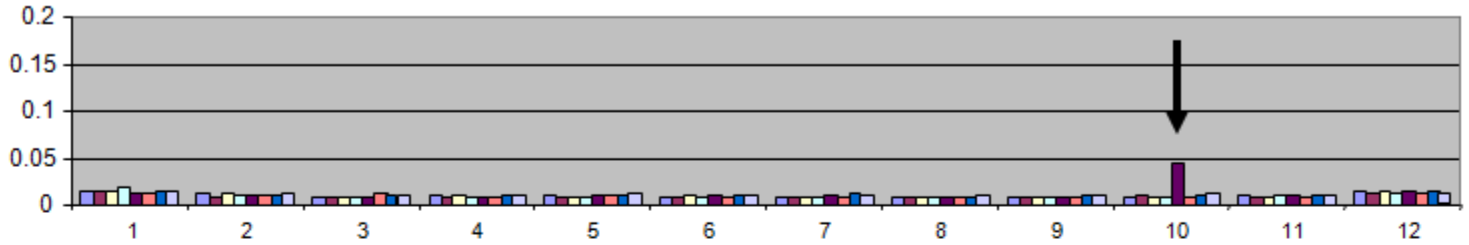
Negative control



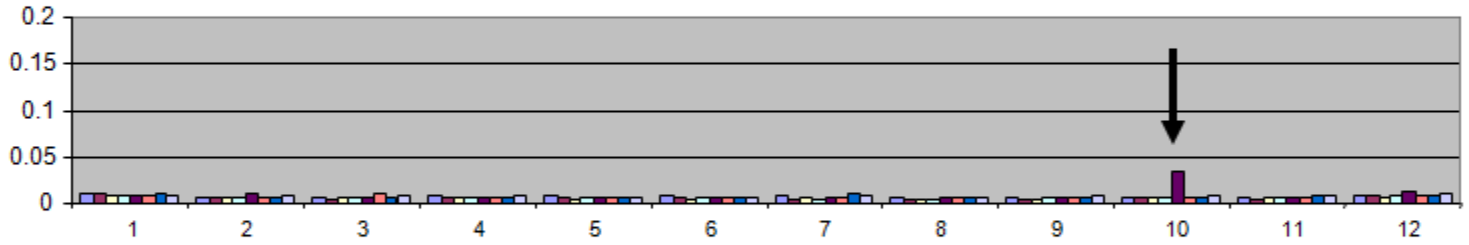
Use Human Ig-K to Select Human Ig-H: Round 1

Round 1

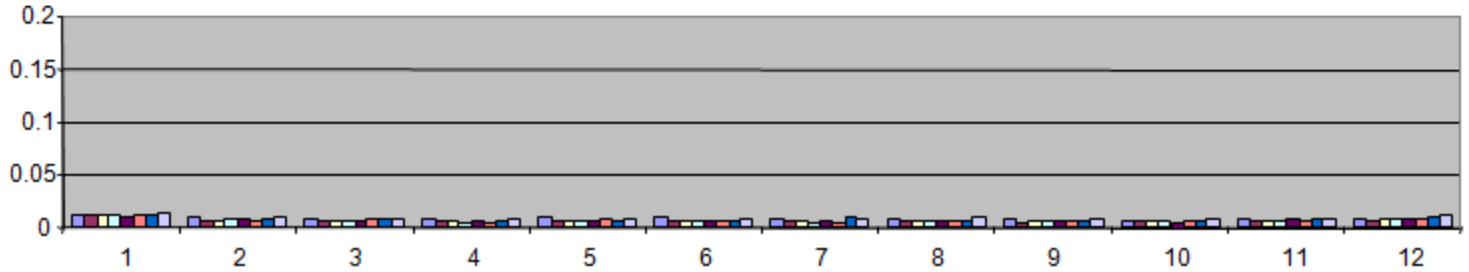
Antigen of interest



Antigen of interest



Negative control

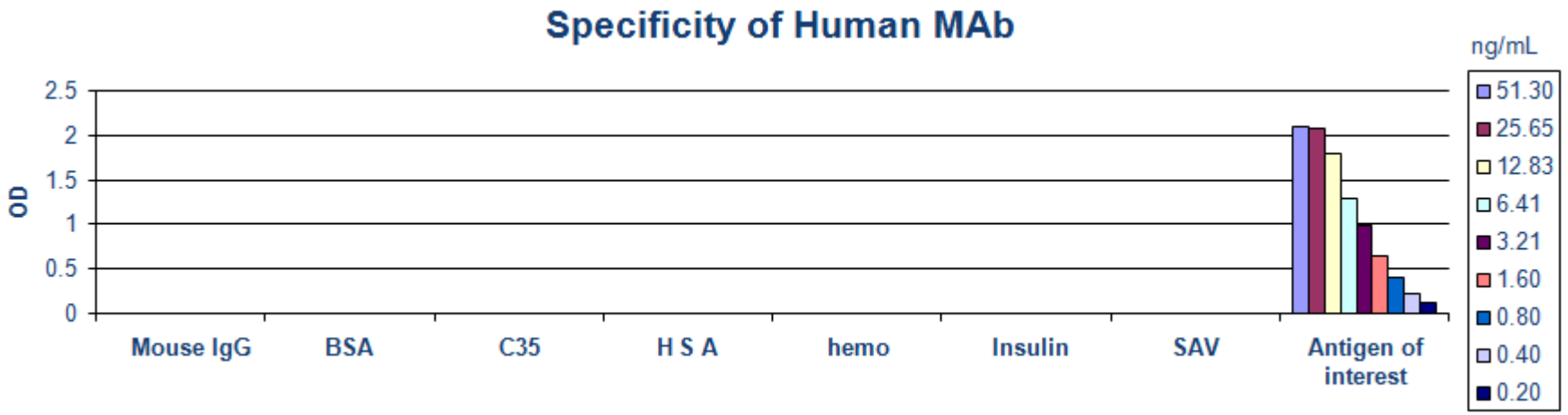


Expression and Characterization of Selected MAbs

- The V genes in the clonal Ig-Hγ chain and Ig-L chain vaccinia recombinants are cloned from vaccinia virus into mammalian expression plasmids.
- Monoclonal antibody is expressed as full length IgG1/Kappa by transfection of the plasmids into CHO cells. IgG is purified using Protein A, and each selected MAb is tested for specificity, affinity and function.
- As shown on the following slide, purified IgG was tested against an antigen of interest as well as a panel of control antigens.

Specificity of Antigen-Specific MAb

The V Genes From Selected MAbs are Transferred into Mammalian Expression Vectors and expressed as soluble IgG



Affinity of Antigen-Specific Antibodies

A large panel of human antigen-specific MAbs were selected including the two leads MAb 2071 and MAb 2090:

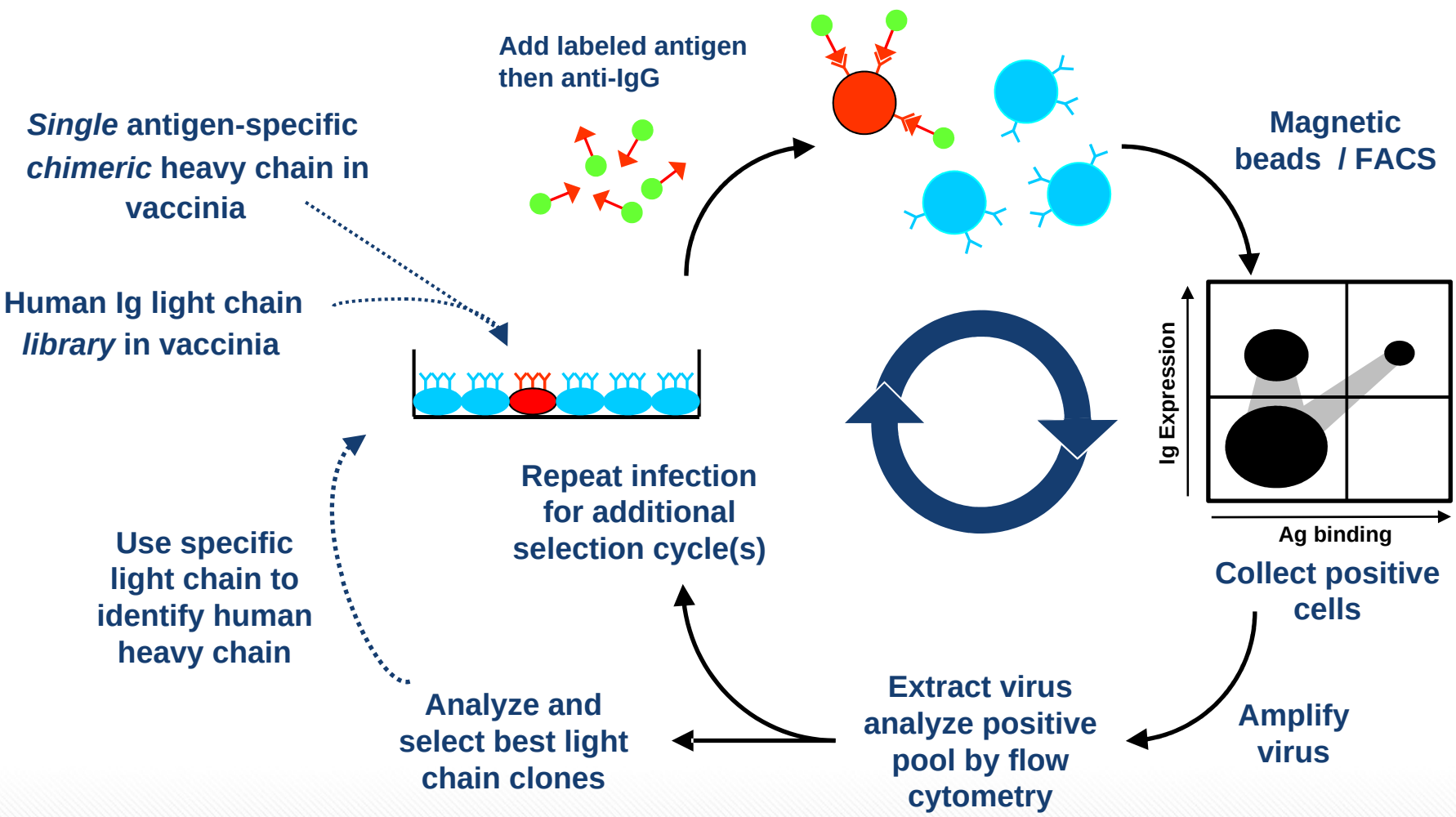
MAb	Biacore affinity (nM)
Chimeric	0.08
MAb 2071	0.03
MAb 2090	0.03

Selected Antibodies compete with the mouse antibody for binding to the antigen of interest and demonstrate functional activity that is superior to that of the chimeric antibody

Vaccinex Antibody Selection Platforms

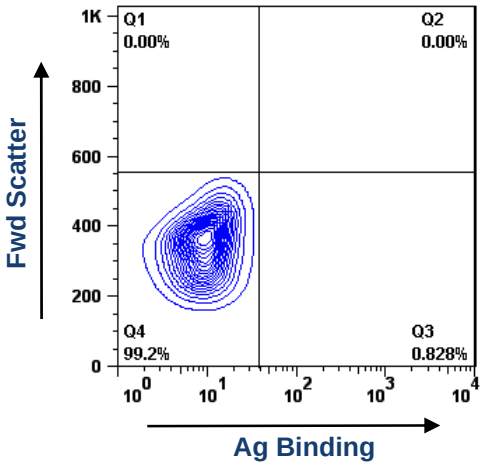
1. **Conversion of Mouse MAb to Human MAb**
   - ➡ **Secreted Antibody Platform**
   - ➡ **Membrane Antibody Platform: Selection by Cell Sorting**
2. ***de novo* Antibody Selection**
3. **Affinity Improvement of Human Antibodies**

Vaccinex Antibody Platform Transformation of Mouse MAb to Human MAb

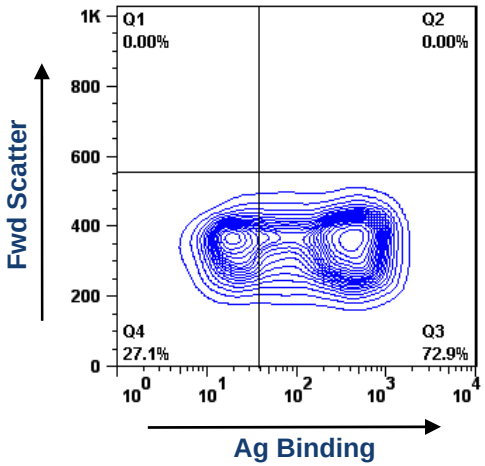


Transformation of Mouse MAb into Human MAbs

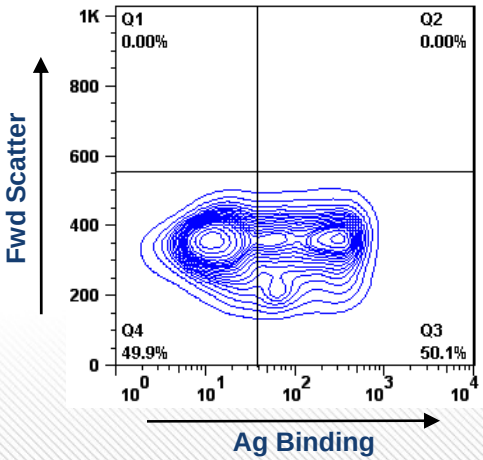
Wild Type



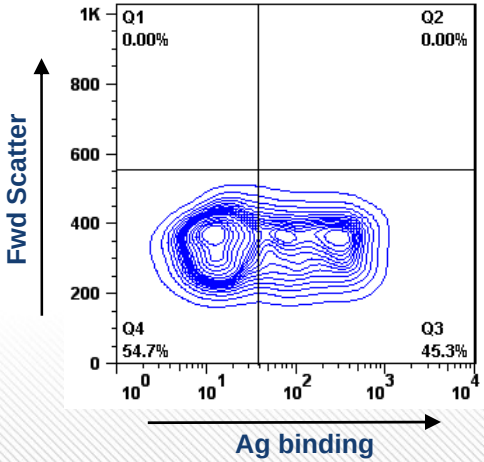
Chimeric Ig-H + Chimeric Ig-L



Chimeric Ig-H +Human Ig-L



Human Ig-H + Human Ig-L



Relative affinity of fully human antibodies and chimeric MAb

- The Ig-H and Ig-L chain genes are cloned from recombinant vaccinia virus into mammalian expression plasmids. Antibody is produced as full length soluble IgG1/Kappa by transfection of the plasmids into CHO cells.
- IgG is purified using Protein A, and each selected MAb is tested for specificity, affinity and function.

MAb	Biacore affinity (nM)
chimeric	*0.05*
Human Mab 416	0.27
Human Mab 926	0.15
Human Mab 1156	0.09
Human Mab 1338	0.07
Human Mab 1339	0.05
Human Mab 1259	0.09

All MAbs compete for binding to antigen with the original mouse MAb and demonstrate strong functional activity

Advantages of ActivMAb

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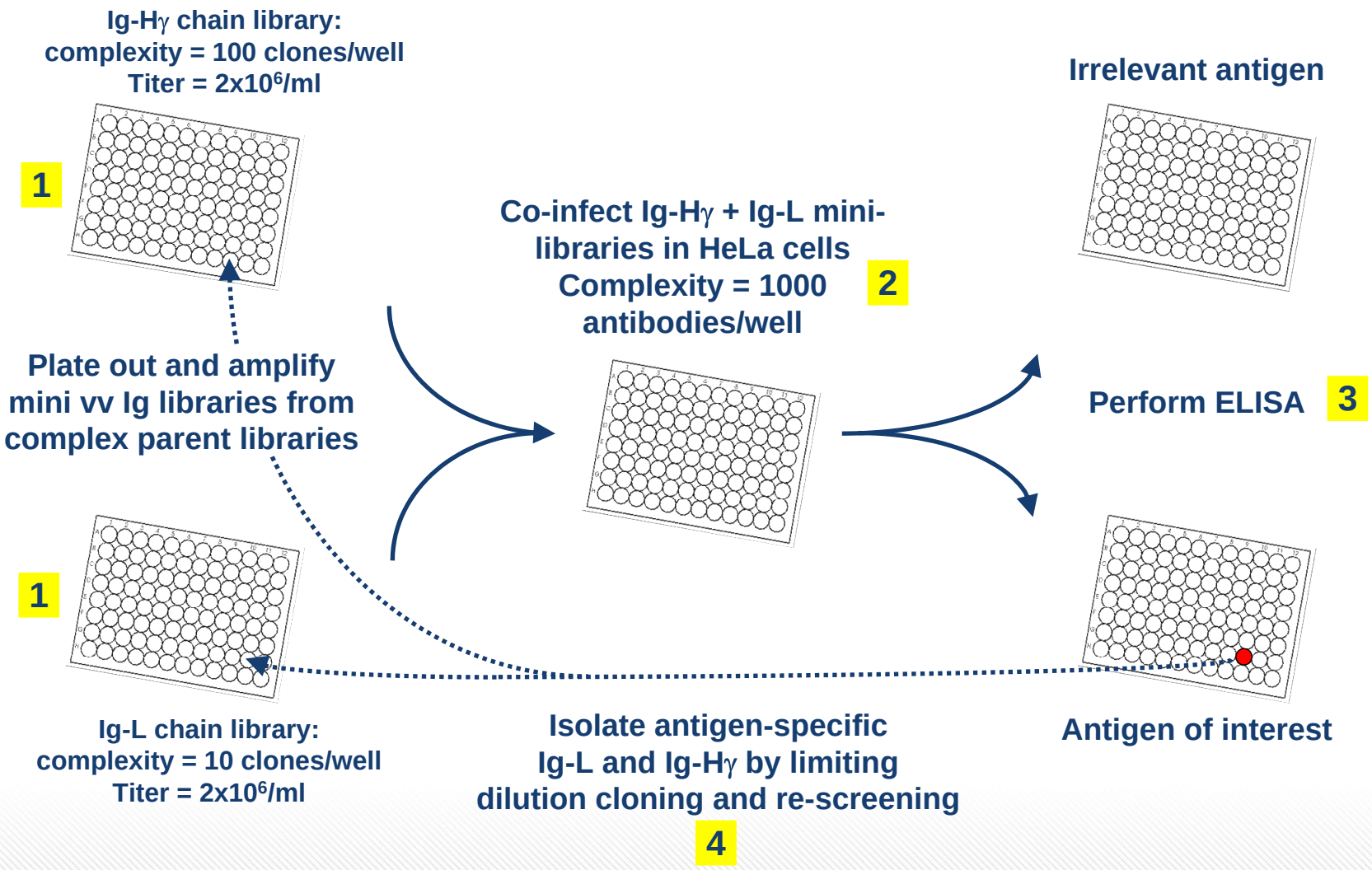
For the conversion of mouse MAbs into human MAbs, our library technology allows for:

- **The selection of multiple candidate lead antibodies derived from distinct VH and VL germ line genes**

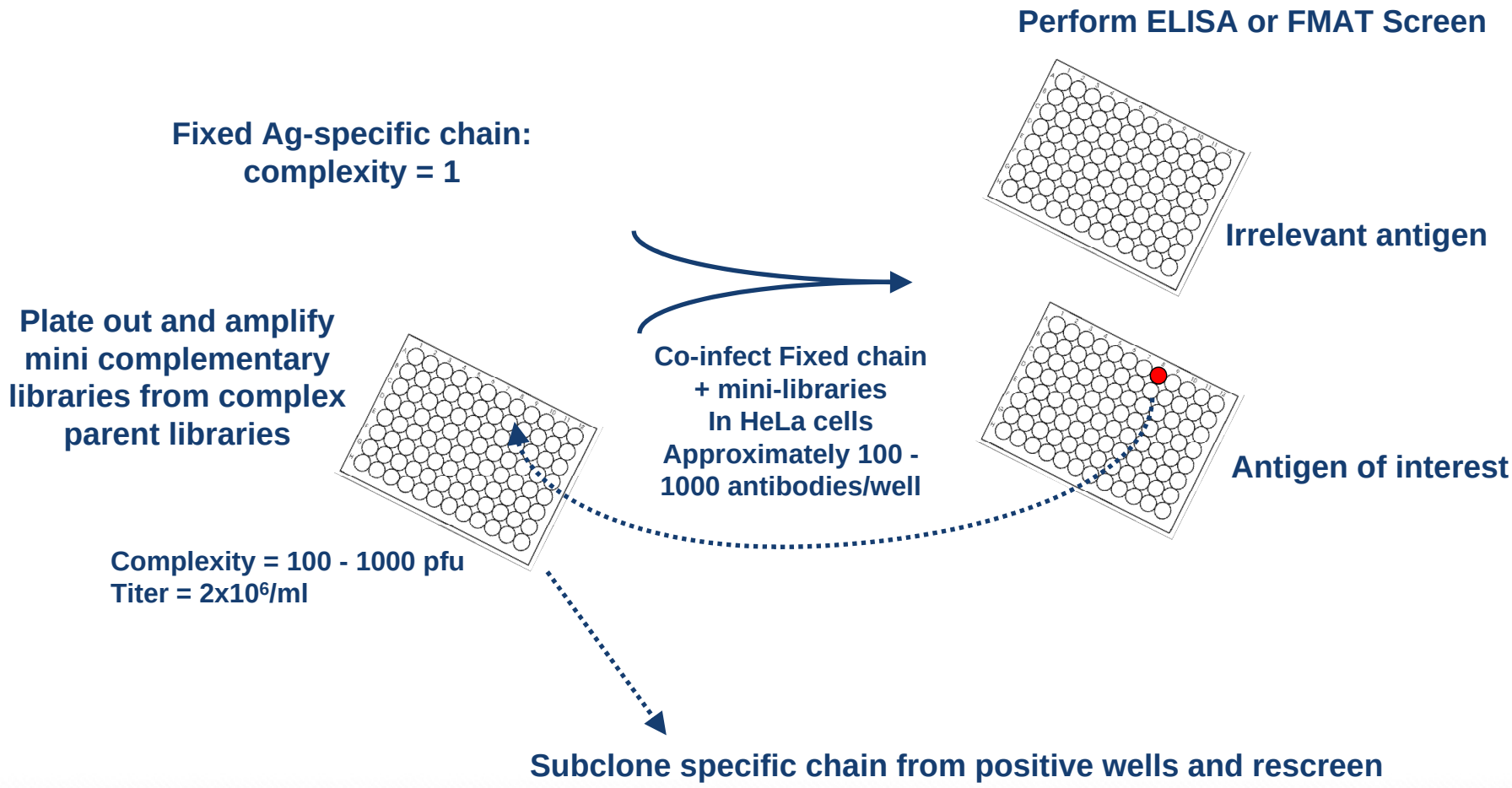
  → **Multiple distinct Leads and backups**

- **Conservation of epitope specificity**
- **Affinity improvement from the original MAb**
- **Built in selection for good expression levels in mammalian cells**

Screening for Human MAbs in Secreted IgG Format



Affinity Improvement



Affinity Improvement of Human MAbs by V-Gene Replacement

- The V genes from positive clones are isolated and cloned into mammalian expression plasmids. Antibody is produced as full length soluble IgG1/Kappa by transfection of the plasmids into CHO cells.
- IgG is purified using Protein A, and each selected MAb is tested for specificity, affinity and function

Antibody	Biacore affinity (nM)
Parental	***40***
Replacement #1	**27**
Replacement #2	**6**
Replacement #3	**4**

Summary of Selected Projects

Project	Approximate number of Primary MAbs selected	Selection type
"A"	10	*De novo*
"B"	60	mouse to human
"X"	10	mouse to human
"I"	20	*De novo*
"P"	20	*De novo*
IL6	60	mouse to human
C35	90	*De novo* and mouse to human
VX5	60	mouse to human
VX70	20	mouse to human
VX90	30	mouse to human

Development of New Antibody Selection Strategies

Chimeric Receptor Based Selection Strategies

Because of their size, the throughput of MACS/FACS with mammalian cells is still lower than what can be achieved with yeast or Phage

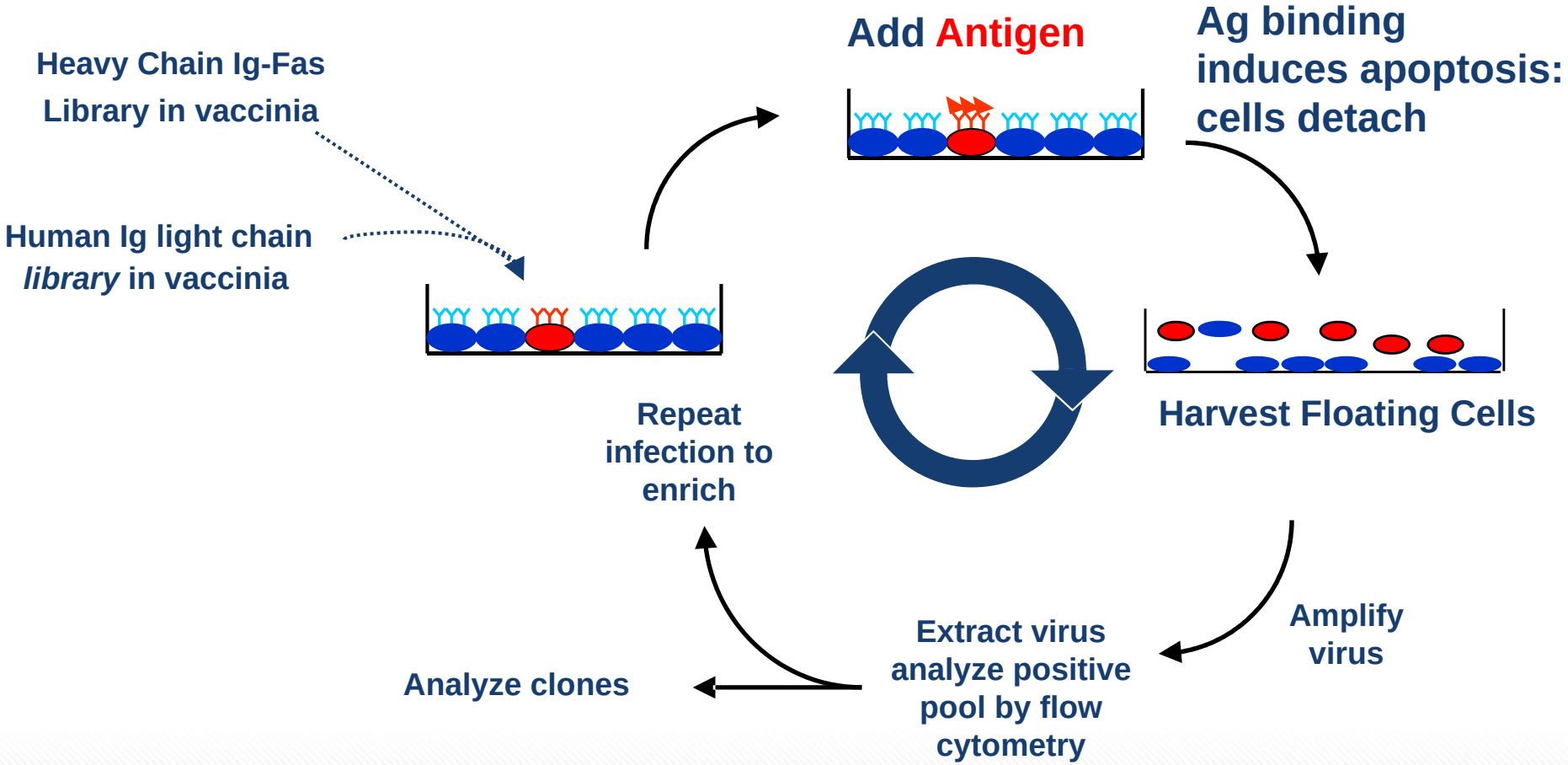
Employ hybrid receptors whose signaling results in the induction of apoptosis and loss of cell adherence (recombinant virus can be recovered from floating apoptotic cells)

- Candidate: Fas
  - → Anti-Fas antibody induce apoptosis
- Create Ig-Fas Chimeric molecules (ECD = Ig; ICD = Fas DD):
  - → Infect Hela in monolayer
  - → Add Ag
  - → Harvest apoptotic cells (floaters) after overnight incubation

Advantages:

- Selection system
- No FACS/Beads needed, so throughput is very large

Selection of Human MAbs using Hybrid Ig-Fas Receptor Libraries

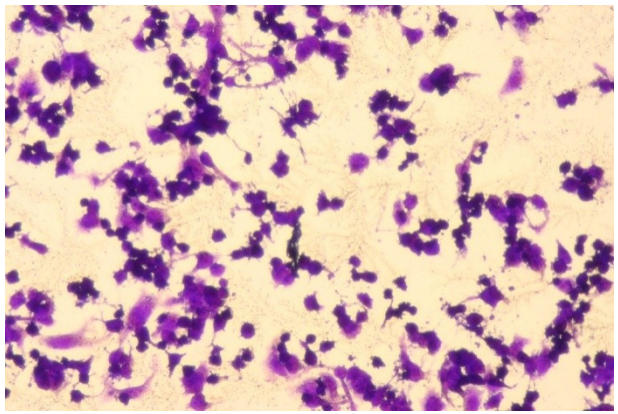


Test Cell Detachment with Ig-Fas Recombinant Vaccinia Virus

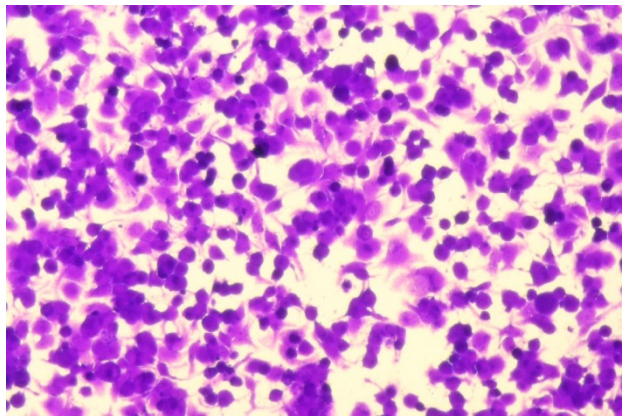
- → **Confluent monolayers of HeLa cells were infected at moi = 1 each of Ig-Fas and Ig-L**
  - Antigen specific and control Ig-Fas used
- → **6 hours after infection, Antigen is added**
- → **Cells allowed to detach for 24 hours**
- → **Supernatant was harvested, the wells were washed two times with PBS**
- → **Remaining cells were stained with crystal Violet**
  - Take Pictures
  - Solubilize with Acetic Acid and read OD570

Ig-Fas Recombinant Vaccinia Virus Mediates Antigen-Dependent Cell Detachment

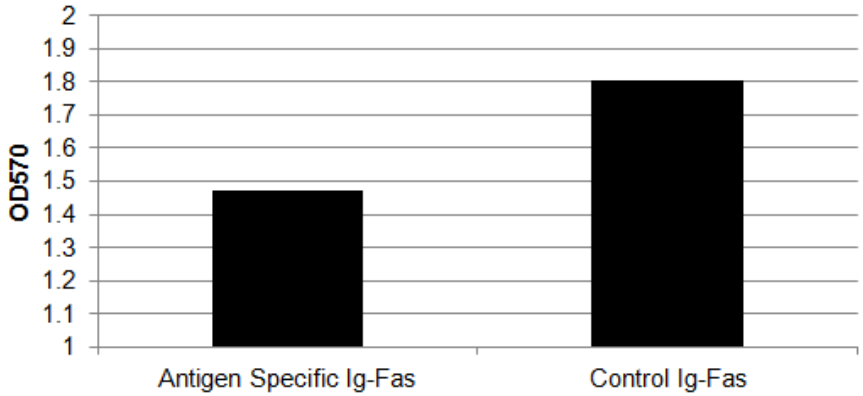
Antigen Specific Ig-Fas



Control Ig-Fas



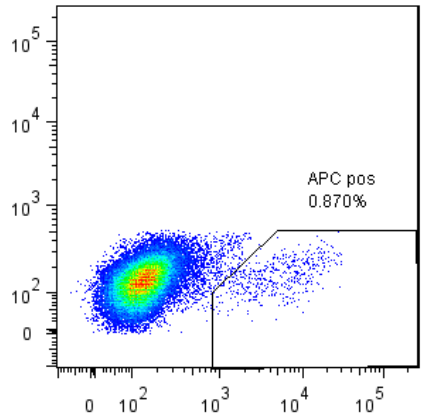
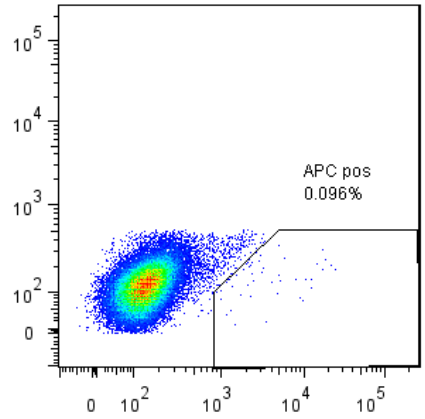
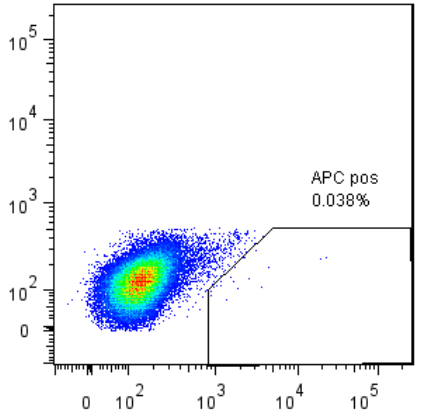
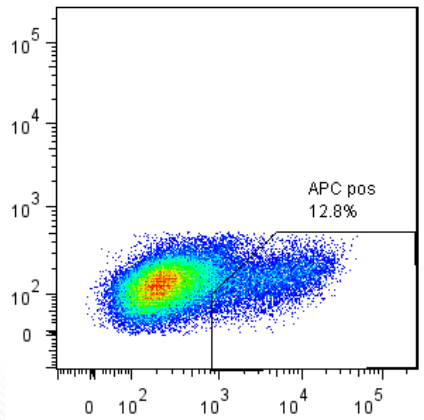
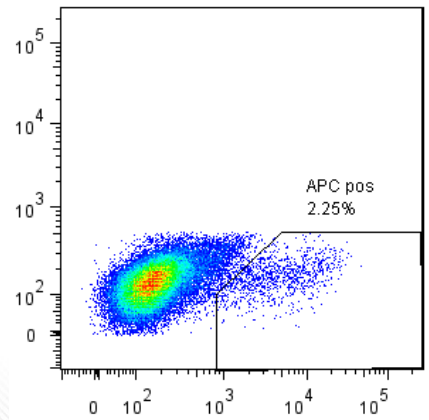
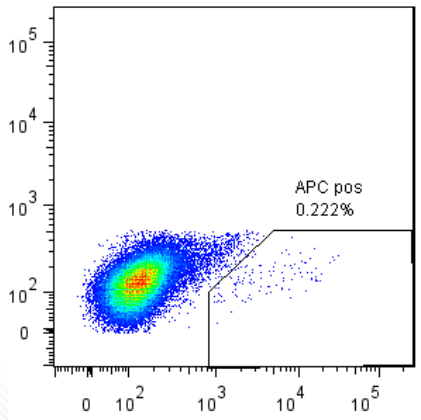
Cells remaining after detachment



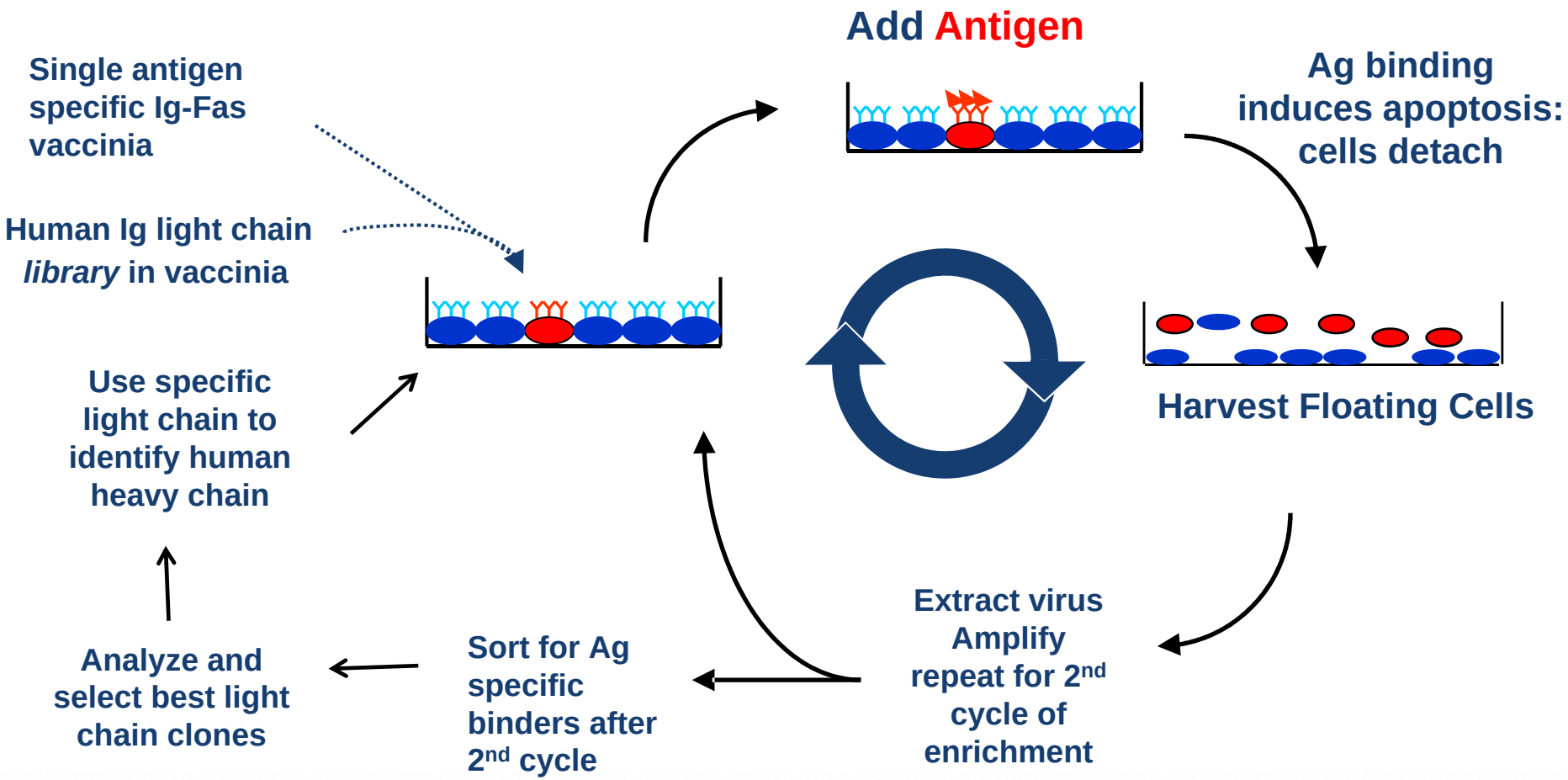
Test Enrichment with Ig-Fas Recombinant Vaccinia Virus

- **Confluent monolayers of HeLa cells were infected at moi = 1 with antigen specific Ig-Fas and co-infected with admixtures of Ig-L virus**
  - 1.0% Antigen specific Ig-L + 99% Control Ig-L
  - 0.1% Antigen specific Ig-L + 99.9% Control Ig-L
  - 0.01% Antigen specific Ig-L + 99.99% Control Ig-L
- **6 hours after infection, Antigen was added**
- **Cells allowed to detach for 24 hours**
- **Harvest Floating cells, extract and amplify virus**
- **Use this virus for a second round of enrichment.**
- **After the second round, take the virus and test for enrichment by staining for Antigen specific binders by flow cytometry.**

Enrichment for Antigen specific Ig-L

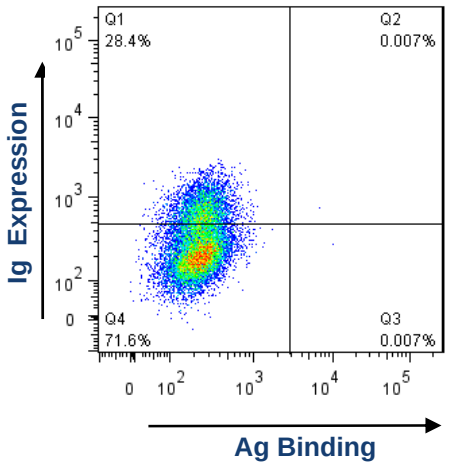
	1.0%	0.1%	0.01%
Starting Sample			
After Enrichment			

Transformation of Mouse MAb to Human MAb Using Ig-Fas Hybrid Libraries

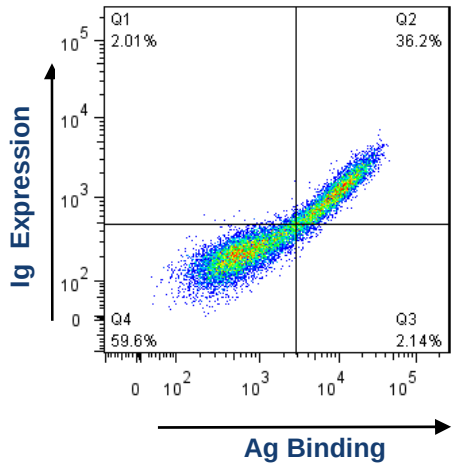


Transformation of Mouse MAb into Human MAbs

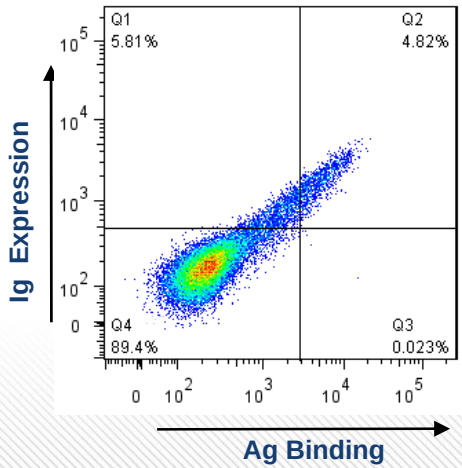
Control Ig-Fas



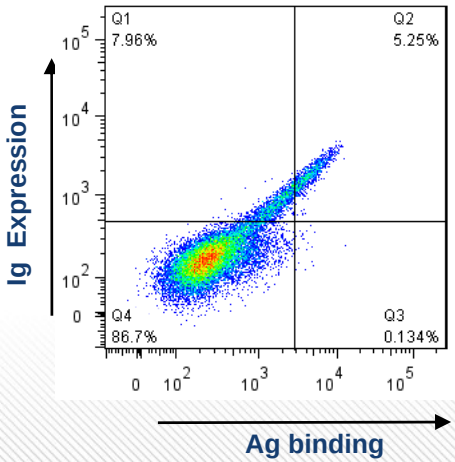
Antigen Specific Control Ig-Fas



Clone 6



Clone 11



ActivMAb Competitive Advantages

VACCINEX

Challenge	Vaccinex Technology Advantage
Conversion of Non-human Antibodies to Fully Human and Affinity Improvement of Existing Human Antibodies	• Selection of antibodies with conserved epitope specificity and similar or even improved affinity and functional activity. • Selection of multiple antibodies derived from distinct VH and VL germ line genes with different biochemical properties.
Manufacturing	Intrinsic selection for high expression in mammalian cell lines, easily adaptable to manufacturing.
De novo Antibody Selection	• Broader target range than mouse-based platforms • Very Large Throughput
Re-engineering	Vaccinex expresses complete MAbs that do not require re-engineering into IgG format.