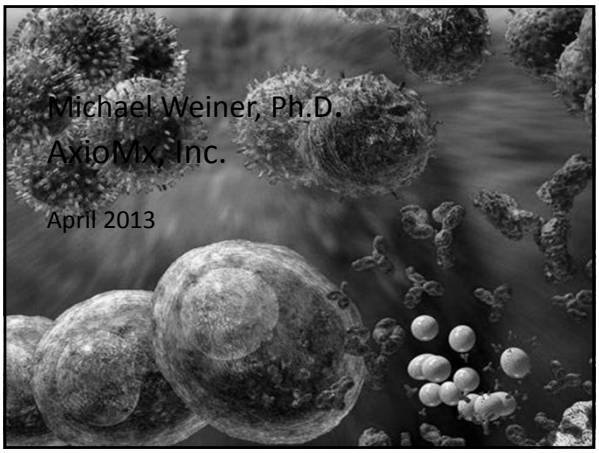


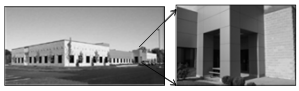
Michael Weiner, Ph.D.

AxioMx, Inc.

April 2013

AxioMx Highlights

- Founded 2012 – Branford, CT
  - Founded by the same scientists who helped start Stratagene (Agilent), 454 Life Sciences (Roche), Raindance Technologies, GnuBio and Affomix (Illumina)
- Vision
  - To enable protein-based research and diagnostics by rapidly generating high-quality, recombinant antibodies
- Funding Status



The Leadership Team

Christopher K. McLeod (CEO), AxioMx, Inc

President and CEO of 454 Life Sciences, EVP of CuraGen Corporation, CEO of Havas Interactive, EVP and Director at CUC International where he was President of the CompuCard division. Mr. McLeod earned his B.S. Magna Cum Laud with a dual major in economics and engineering and applied science from Yale University and his M.S. in management from the Sloan School of Management at Massachusetts Institute of Technology. Mr. McLeod serves on the boards of Sacred Heart University, the CT Yankee Council, Boy Scouts of America and the MIT Sloan School of Management Executive Board.

Michael P. Weiner (CSO), AxioMx, Inc

VP or CSO: Affomix, RainDance Technologies, The Rothberg Institute, 454 Life Sciences. Dept. Head at GlaxoSmithKline, Stratagene. Founder of: Affomix, GnuBio, AxioMx. Dr. Weiner has co-authored over 50 peer-reviewed articles, over 30 patent and patent applications, and has edited 3 books and Journal Supplements in his areas of expertise (cloning vectors, protein cloning and expression, and genomic analysis technology). He received his undergraduate and graduate training at Pennsylvania State University (Microbiology) and Cornell University (Genetics, with minors in Biochemistry and Microbiology), respectively, and did post-doctoral training in the Dept of Physical Chemistry at Cornell University.

Major Advances in DNA Sequencing and Recombinant-Antibody Technologies

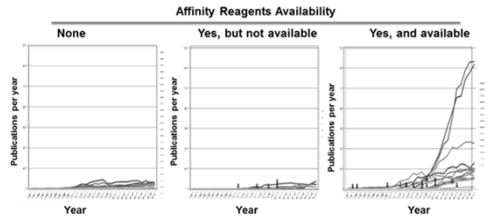
DNA structure determined (1953)	**1950**	
	:	
	1960	(1962) **Antibody structure determined**
	:	
The Asilomar Conference on rDNA (1975)	**1970**	
Maxam & Gilbert and Sanger DNA seq (1977)	:	(1975) **Mouse hybridoma developed**
Sequence of phiX174 (1977)	**1980**	
Semi-automated DNA sequencing (1986)	:	(1985) **Phage display invented**
Haemophilus influenze sequenced (1995)		(1988) Humanized mouse Ab therapeutic
Capillary DNA sequencing (1998)	**1990**	(1990) **Ab phage display published**
Draft sequence of the human genome (2000)	:	(1995) Transgenic mouse
454 founded (2002)	**2000**	
454 Next generation sequencing (2005)	:	
	2010	

- There has been *tremendous* progress in DNA sequencing technology to decrease cost and increase capacity
- There has been no real advance or cost reduction in recombinant antibodies since 1970's; the time is ripe for tech improvements

Why Study Proteins?

- Gene expression doesn't always correlate with protein abundance levels
- Post-translational modification (esp important in cell signaling) can not be studied at the nucleic acid level
- Nucleic acids are just a surrogate for studying proteins because good tools for proteins are not available. "The best material model of a cat is another, or preferably the same, cat." Norbert Wiener. Philosophy of Science (1945)

The Harlow-Knapp* Effect
Or why good antibodies are needed



- **Ca. 20,000 human genes identified in human genome project. Sequence alone has been insufficient to develop genomic based medicine**
- **75% of papers published today focus on proteins known (and popular) prior to the 2000 publication of the human genome**
- **To increase the study of ignored genes, good probes/antibodies are required**

Edwards et al., Nature 470: 163-165
* Fedorov et al., 2010; Gruenberg et al., 2008

Why Recombinant Antibodies?

- Consistent quality
- Recognize proteins, peptides, and non-peptide antigens (unlike aptamers, synbodies, DARPINS, etc., which *can not*)
- Renewable, inexpensive and fast to produce
- Ability to genetically modify for improved biophysical properties, including Dx and Rx applications: enzyme fusions, DNA fusions, thermal-stability, ligand attachment, increased affinity, etc.

Current methods for creating recombinant antibodies take too long and often do not result in a consistent, renewable, quality reagent!

Generating Affinity Reagents Today

Cell, protein analysis	Protein expression and purification	Antibody generation	Final reagent

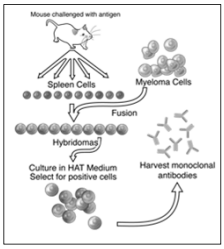
Current paradigm takes too long and may not result in a consistent, renewable, quality reagent and doesn't leverage improvements in 'omics technologies

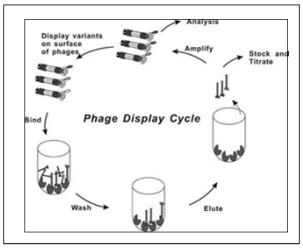
Generating Affinity Reagents Tomorrow

Sequence analysis	Epitope expression and purification	Recombinant Ab generation	Final reagent

- We will leverage advances in DNA sequencing technologies
- We will build a rapid-response custom affinity reagent pipeline using technology being developed today

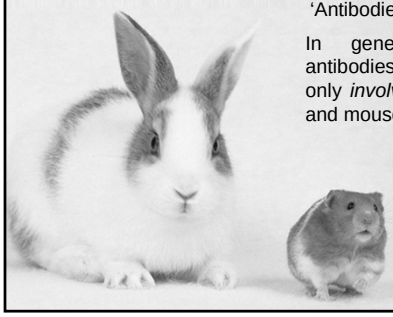
Current rAb Technologies





Current technologies are low throughput and expensive to set up

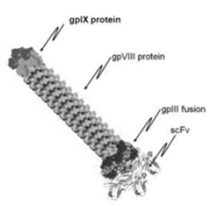
Recombinant Affinity Reagents

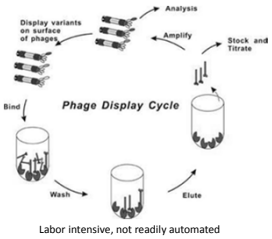


'Antibodies without Animals'

In generating functional antibodies, the bacteria are only *involved*, but the rabbit and mouse are *committed*.

M13-based Phage Display





Labor intensive, not readily automated
Requires multiple rounds of selection

- It is a validated method for deriving high-affinity rAbs
- There are opportunities for more advanced development

Types of Screening Rounds

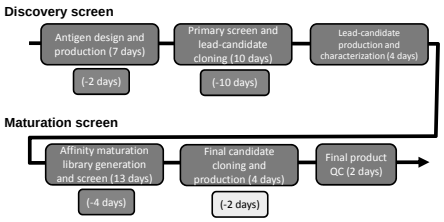
Discovery Screening:

- Uses a large (>10^{10}) naïve library
- Excess antigen during screen used for *enrichment* of antigen-binders
- Subtraction with excess 2°-protein in solution used for increasing specificity

Affinity Maturation Screening (empirical replacement for 3D-QSAR):

- Uses a smaller (>10^{8}) AXM^{2}-mutagenized, focused library (pools or singlets)
- Limiting antigen during screen used to select molecules with increased affinity
- Competition with free antigen during screen used to enrich for increased affinity

Discovery & Maturation Process



Process improvements are focused on reducing the time needed to generate an antibody *from 40 days to <3 weeks*

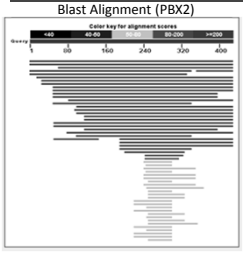
Requirements for Antigen

We have focused on methods that reduce the need for purified antigen

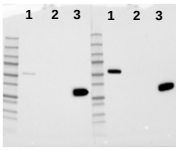
We have focused on peptides to initiate a project thereby reducing the need for native antigen for screening

Antigen Design
Transcription Factors

Blast Alignment (PBX2)



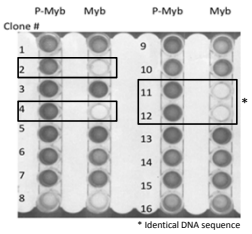
Western blot
Abs against fragment of PBX2



Peptides can be used when proteins are difficult to produce or have many close homologs in the proteome

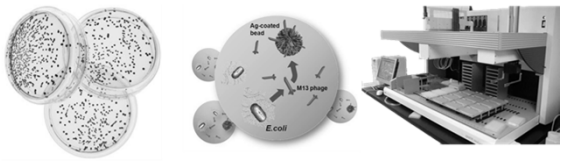
Post-translationally Modified Antigens

P-Myb$_{(1\text{-}20,\ pSer11)}$ Phospho-peptide Screening



Peptides needed for phospho-specific Abs when kinase not available

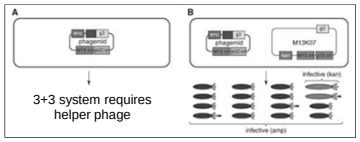
Biopanning Technologies



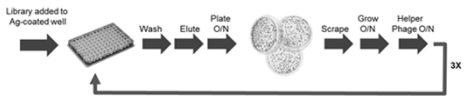
'Traditional' Emulsion-based Automated

We have experience in all three technologies

Traditional Technology



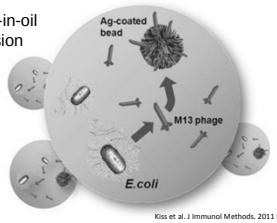
3+3 system requires helper phage



Traditional method requires 3-4 days per round

Emulsion Screening

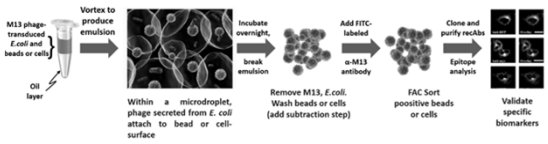
water-in-oil emulsion



Kiss et al. J Immunol Methods. 2011 Mar 31;367(1-2):17-26.
Buhr et al. Methods 2012 Sep;58(1):28-33.

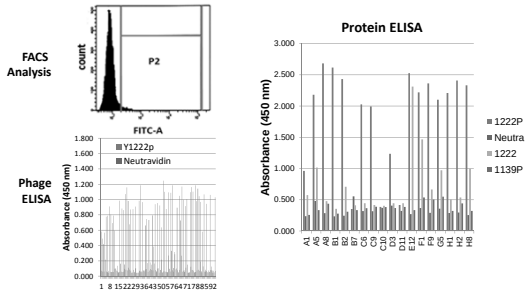
Stable emulsion is analogous to 10^7 wells
More than one cell and antigen-coated bead per droplet
Clonal amplification of each phage within droplet

Emulsion Screening



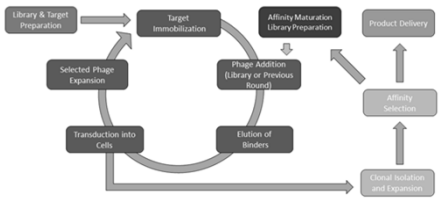
Combine screening with biophysical characterization

FACS Results – Emulsion Selection



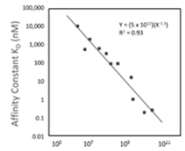
Libraries *can* be sorted without biopanning

AxioMx's Key Improvements to Affinity Screening - An All Liquid Approach -

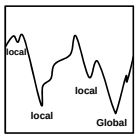


Eliminating plating between rounds decreases costs and FTE

Large Antibody Display Libraries
For the Isolation of High-Affinity Antibodies



Library size*



Multiple Minima Problem

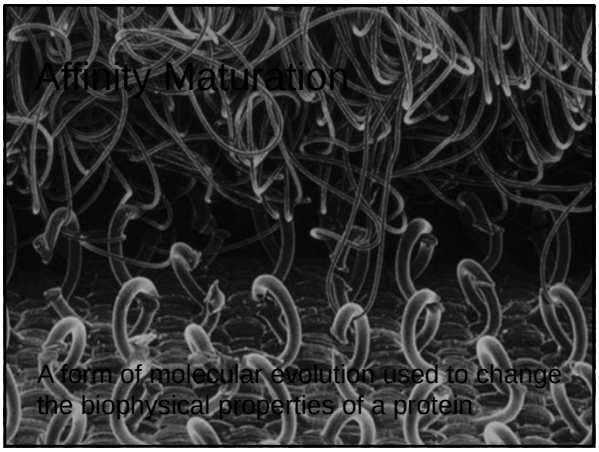
There is a direct relationship between the size of a library and the best affinity reagent isolated from the library

To obtain the best biophysical characteristic (stability, affinity, specificity, etc) use either a: i) large naïve-library (w/o affinity maturation) or ii) affinity-mature discovery clones

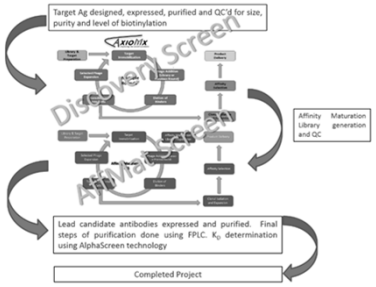
**Ling. Combinatorial Chemistry & High Throughput Screening,* 2003, 6, 421-432

AXIOMX

Proprietary 25



Affinity Maturation



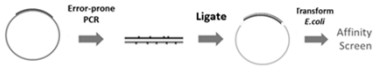
- AffMat doubles the time through the pipeline

AXIOMX

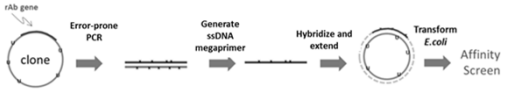
Proprietary 27

Affinity Maturation

Standard error-prone PCR and subcloning:



AXM mutagenesis approach:



Holland et al. 2013 (submitted)

- Common set of mutagenic primers can be used for all antibodies
- Eliminating cloning step saves time and money, and increases throughput

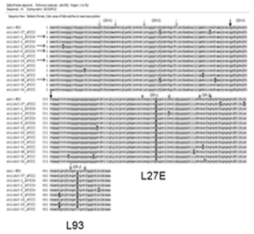
Library Diversity Analysis

Mutagenesis method	Transformants per electroporation	Library diversity	Mutation rate
Std Error-prone PCR	1.00E+05	7.50E+04	1.1
AXM mutagenesis	2.80E+08	1.29E+08	1.5

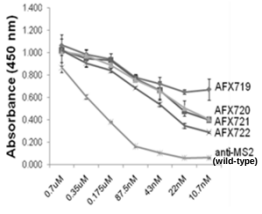
AXM mutagenesis approach is >1000x more efficient

Affinity Enrichment of Mutations

Mutations (in red) of clones displaying higher affinity



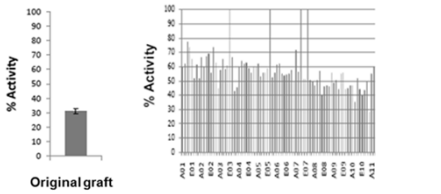
Increased affinity in ELISA format



Hot-spot for mutations at Kabat positions L27e and L93 in affinity matured scFvs

Library Screening under High Temperature

% Activity in ELISA after heating at 70ºC

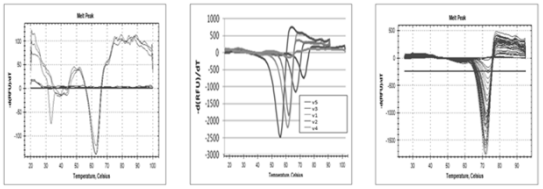


Clones selected from mutagenized library screened under high temperature

Identified clones with improved heat tolerance

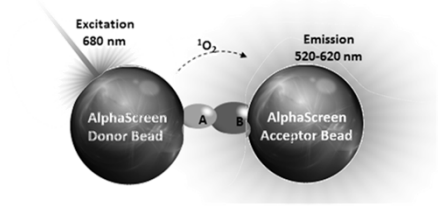
SYPRO Orange Melt Test

Wild-type scFv **CDR Grafts** **AXM704**



Original scFv melts at around 63 ^{0}C, AXM704 melts at around 72 ^{0}C

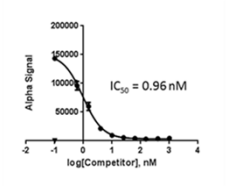
Antibody Characterization
AlphaScreen



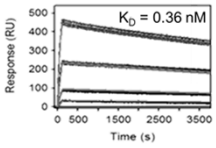
AlphaScreen Technology is used in our pipeline for ranking and measurement of antibody affinities.

Competitive AlphaScreen for Measuring K_D

Competitive AlphaScreen

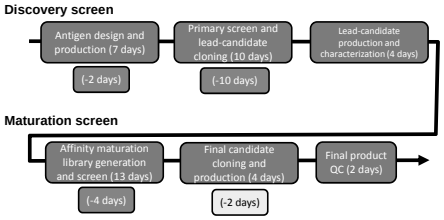


SPR



Similar affinities obtained using AlphaScreen and SPR

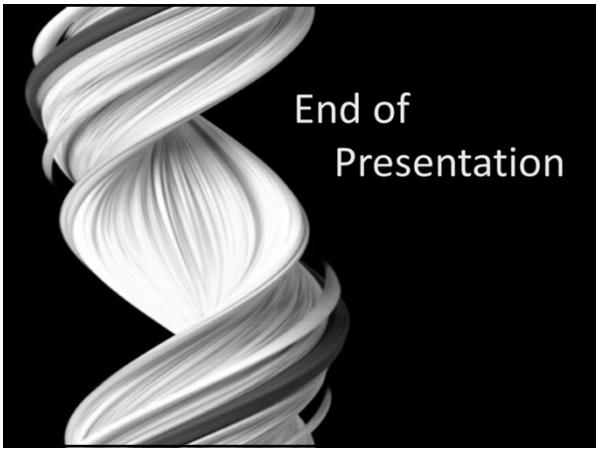
Discovery & Maturation Process



Process improvements are focused on reducing the time needed to generate an antibody *from 40 days to <3 weeks*

Highlights

- Peptides can be used when full length antigen is unavailable or highly homologous to other proteins in proteome
- Emulsions and RLS can be used to increase screening efficiency
- AXM mutagenesis increases efficiency of affinity maturation
- AlphaScreen can be used in place of SPR
- BioPlex (Luminex) can determine clonal uniqueness in constant framework libraries


End of
Presentation