Identification of Unknown Target Antigens using PhyNexus Technology

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Today's Presentation

- MacroGenics
- Proprietary Platform Technologies
- Generation of Monoclonal Antibodies
- Screening of Monoclonal Antibodies
- Immunoprecipitation of Target Antigens
- Target Antigen Identification
- Application of PhyNexus Technology
- Results

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MacroGenics' Mission

To discover, develop, and deliver immunotherapeutics, including monoclonal antibodies, to patients with cancer, autoimmune disorders, allergy, or infectious diseases.

A Leader in Novel Biologic Products

- **Multiple clinical-stage programs**
  Teplizumab (anti CD3 mAb) - autoimmunity
  anti-WNV mAb (West Nile Virus) - infectious diseases

- **Highly efficient R&D organization with 1-2 IND's anticipated annually**

- **Acquired and successfully integrated Raven biotechnologies in 2008**
  ➔ tumor derived cell lines and antibody pipeline

http://www.macrogenics.com

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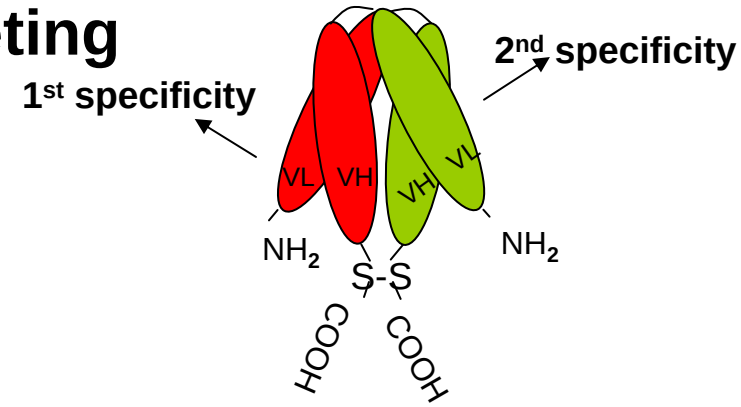
Proprietary Next Generation Platform Technologies

- **Fc engineering: more potent therapeutic mAbs**

- **DART: Dual Affinity Re-Targeting recombinant scaffold binding multiple targets**



- **Homogenous tumor-derived cell lines**
  - ➔ **antibody pipeline**

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mAb Generation

- **mAbs derived from whole cell immunization with homogenous tumor-derived lines**
- **High percentage of surface directed antibodies**
- **Antigens often recognized in their native configuration (sensitive to denaturation / reducing agents)**

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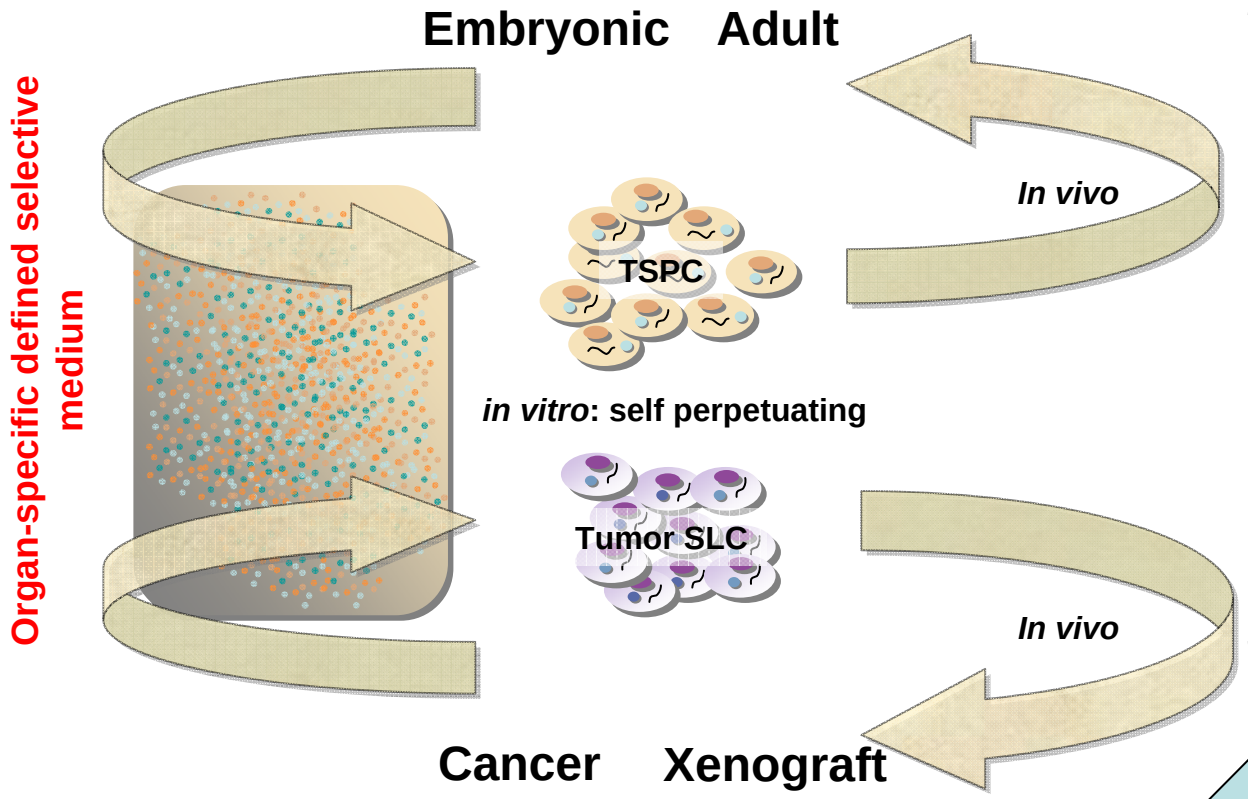
TSPC & Tumor-Derived CSLC Isolation



A selective medium must:

1. Support survival & growth of target cell
2. Inhibit differentiation of target cell
3. Counter-select against growth &/or survival (optimal) of non-target cell types

Optimize:

Nutrients
Growth factors
Matrix/attachment
Cell-cell contact

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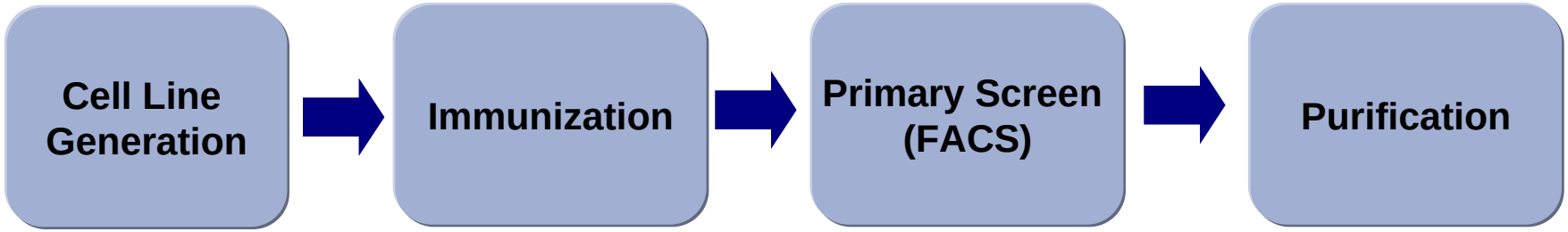
Development of Our Antibody Library



>30 lines from tumor tissues

High percent of cell surface-directed antibodies

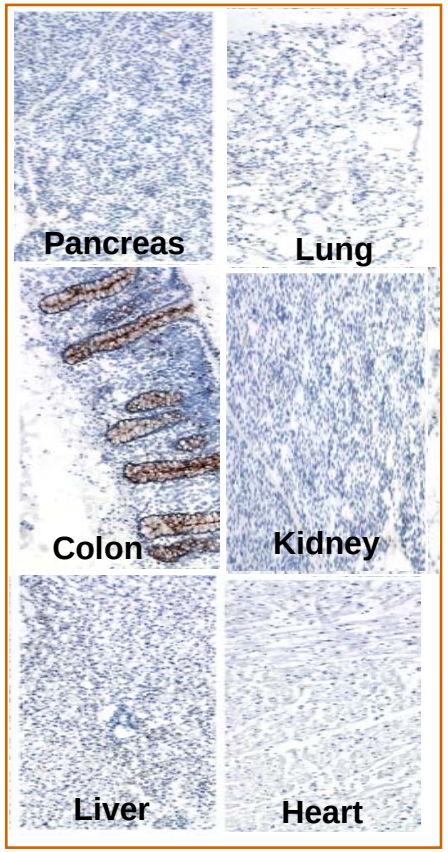
>150,000 mAbs

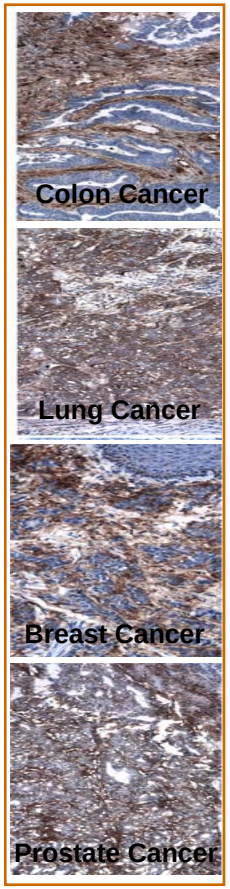
>1,300 mAbs

Extensive Characterization & Functional Screening	
Normal Tissue Screen	Internalization
Tumor Tissue & Cancer Line Screen	ADCC/CTL
Antigen Identification	Xenograft modeling
Growth Inhibition	Redirected Killing

IHC Analyses Identifies Cancer Specific mAb Binding

- **Normal tissue screen**
  - mAbs screened by IHC for binding to panel of 6 critical tissues and tiered based on reactivity.
  - Approximately 400 mAbs with suitable normal tissue reactivity
- **Tumor tissue**
  - Approximately 150 mAbs thus far identified demonstrating expression on tumor specimens
- **Cell Array**
  - mAbs screened for binding to panel of > 30 cancer cell lines by cell array to identify cell types appropriate for functional assays and Xenograft models
  - Cell array platform to be expanded to include CSCs





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Cancer mAb Summary Table

Normal Tissue IHC Profile	mAbs	Tumor Expression by IHC		mAb Antigen ID	
		Cancer Lines	Tumor Tissue	Total	NR
Tier 1 No detectable staining on normal tissue	27	11	3	4	3
Tier 2A 1+ staining on non-crucial organs	162	92	37	28	10
Tier 2b 1+ staining on crucial structures or 2+ staining on non-crucial structures	211	148	55	43	19
Tier 2c 2-3+ staining on crucial structure (1 organ only) or 3+ staining on non-crucial structure	209	153	58	48	19
Tier 3 2-3+ staining on crucial structures (more than 1 organ)	518			48	22
Under Evaluation	206			25	14
Total	1333	404	153	196	61

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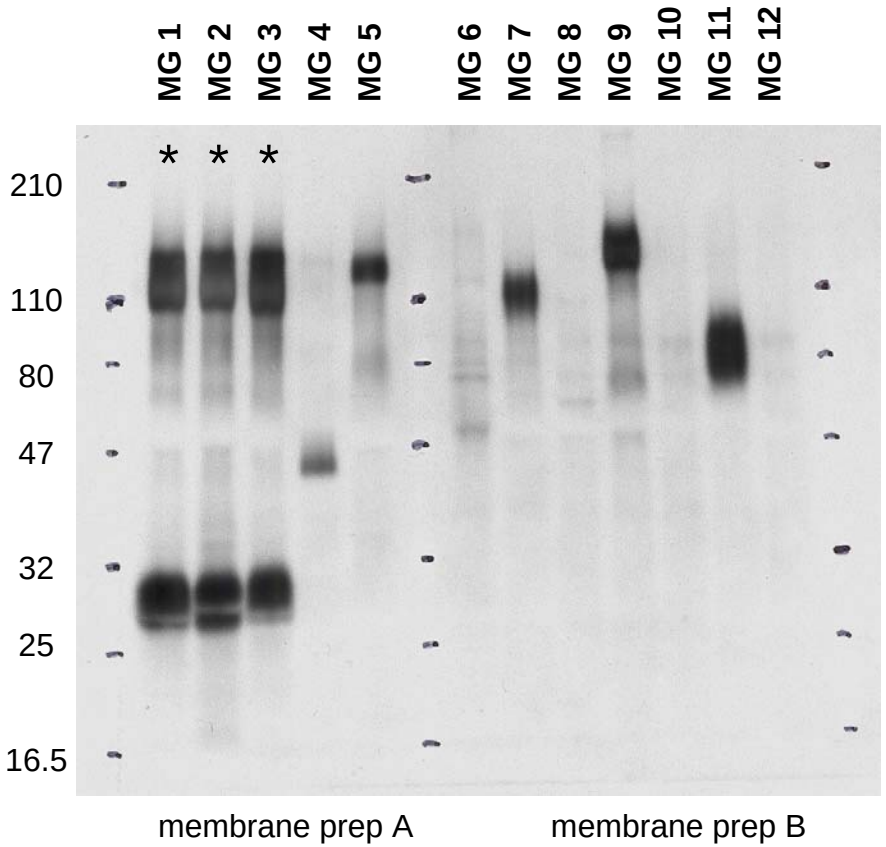
The Antigen Identification Process

- Identify an ATCC cell line that expresses the antigen
- Expand the cell line to multiple T_{175} flasks
- Harvest the cells
- Prepare 2% Triton X-100 cell membrane extracts
- Immobilize mAb of interest onto a resin
- Interact the immobilized mAb with the extracted cell membrane proteins
- Wash, then elute the antigen from the resin
- Subject the eluted antigen to SDS-PAGE
- Stain the gel with coomassie blue
- Excise the eluted antigen band(s)
- Determine antigen identification by mass spectrometry
- Confirm antigen identification

Size Determination of Unknown Antigens



***band pattern indicates antigen is potentially an integrin; later confirmed by IP and MS**

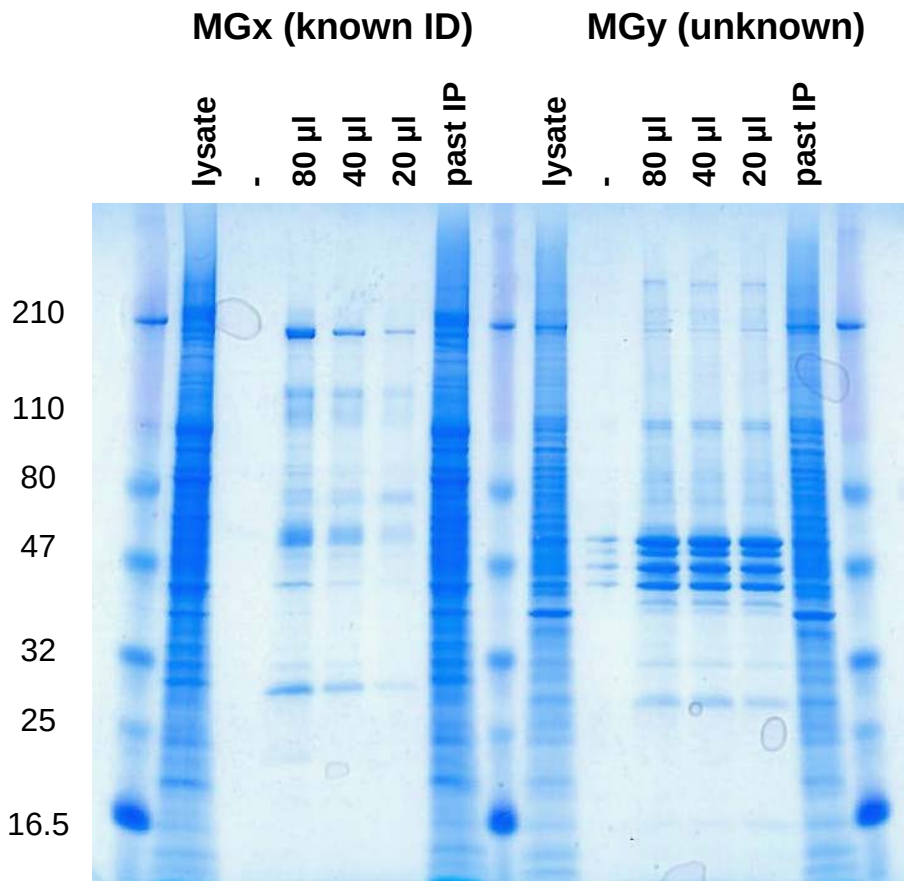
Phynexus 200

1. MG monoclonal antibodies bind to ProtG or ProPlus 5µL columns
2. Antigens from surface biotinylated membrane preparations bind to mAb on column
3. Elution, separation on SDS-PAGE gels, Western blot

Advantage:

* low background
* signal amplification
* size determination facilitates identification of proper protein band in SDS-PAGE gels for mass spec

IP of Unknown Antigens with Different Size Columns



Phynexus 1000

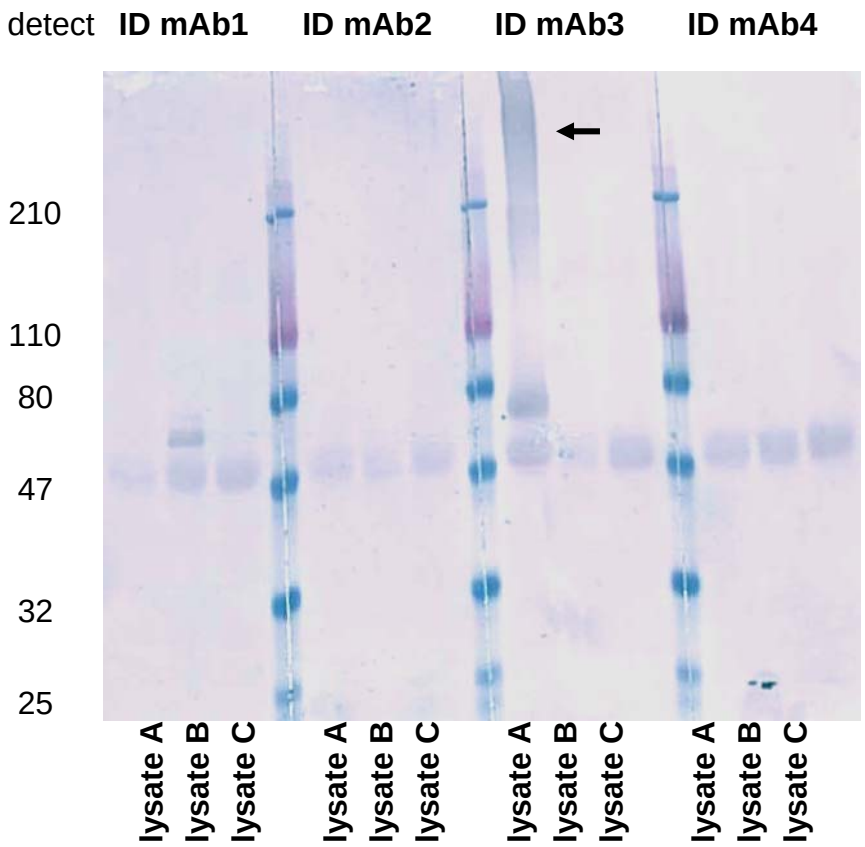
1. Biotinylated antibodies bind to Streptavidin columns of different bed volume (20-80 µL)
2. Antigens from membrane preparations (1mL) bind to mAb on column
3. Elution, separation on SDS-PAGE gels
4. Band isolation and mass spectrometry

Summary/ Conclusion:

- Columns bound a minimum of 2 µg of biotinylated antibody per µL of bed volume
- Amount of antigen retrieval depends on antibody/antigen pair

ID Confirmation - Verifying ID and Antigen Expression

Immuno precipitation MGx



Example:

- Mass spectrometry does not result in definite ID (conserved peptide)
- Potential IDs show differences in expression (depending on cell type, differences in glycosylation, etc.)

Phynexus 200

1. MGx monoclonal antibody bound to 5µL ProPlus column
2. Antigen binding from different cell lysates
3. Elution, separation on SDS-PAGE gel
4. Western blot with antibodies against 4 potential antigens

Advantage:

- Quick elimination of false hits

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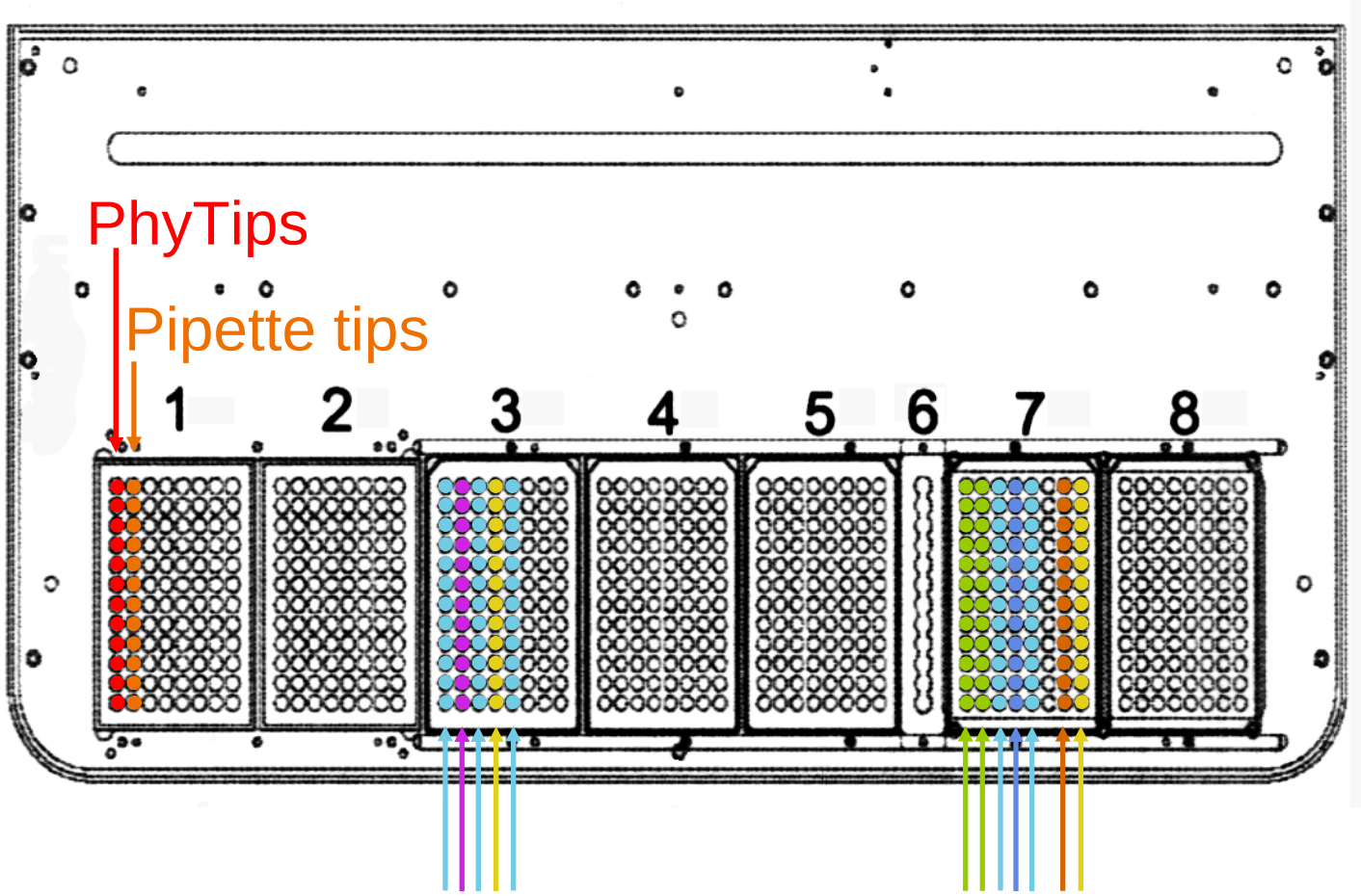
PhyNexus MEA Layout



Use pipette tips

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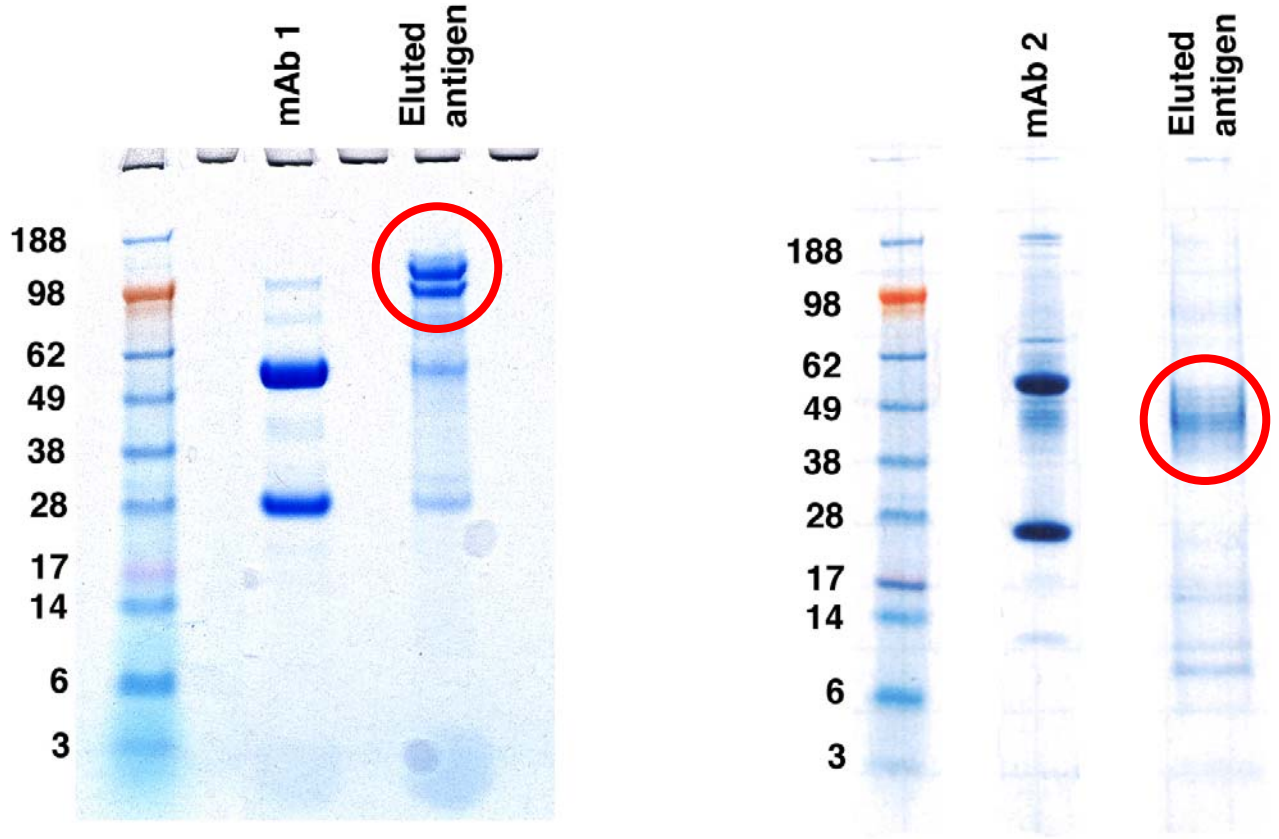
Examples of PhyNexus Results



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Summary

- Very adaptable to all kinds of applications
  matrix, volume, repeats, velocity
- Consistent sample handling - reduced variability
- No contaminations from matrix carry-over
- No loss of matrix (+ antigen) compared to spin methods
- Elimination of tedious sample handling (centrifuge steps)
- Frees up a block of time
- Sample sizes (amount of antibody/cell lysates = material usage)
  same or less than conventional methods
- We haven’t reached capacity yet - Phynexus not the limiting factor

- Excellent customer service!

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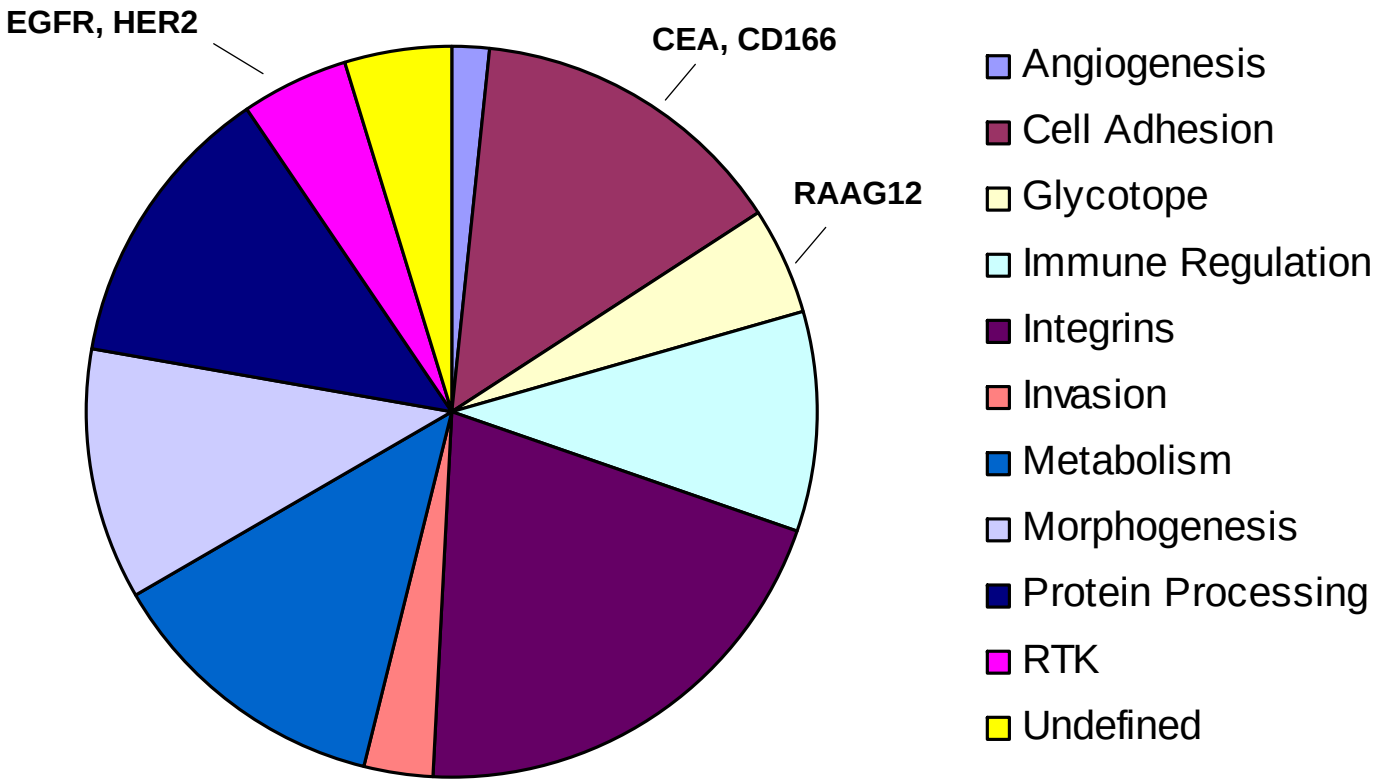
Cancer mAb by Functional Category

> 60 Independent Antigens Identified



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