Anti-Idiotype Antibodies: Powerful Tools for Antibody Drug Development

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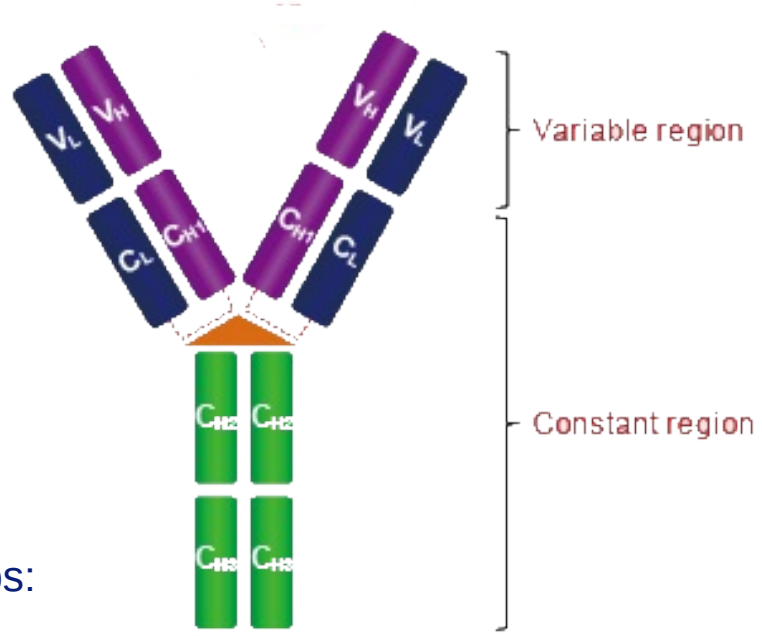
Antibody: Structure and Function

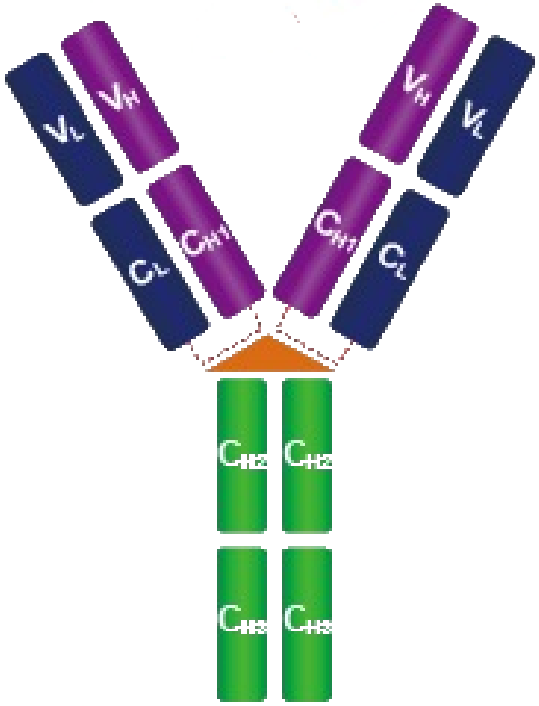
Antibody (Ab): *Recognition proteins found in the serum and other bodily fluids of vertebrates that react specifically with the antigens that induced their formation.*

- Overall structure:
  - **2 identical light chains** (blue)
  - **2 identical heavy chains** (green/purple)
- **Variable regions** and **constant regions**
- 5 classes of Abs:
  - IgG, IgA, IgM, IgD, IgE
  - All contain either λ or κ light chains
  - Biological effector functions are mediated by the C domain
- Chemical structure explains 3 functions of Abs:
  1. **Binding versatility**
  2. **Binding specificity**
  3. **Biological activity**



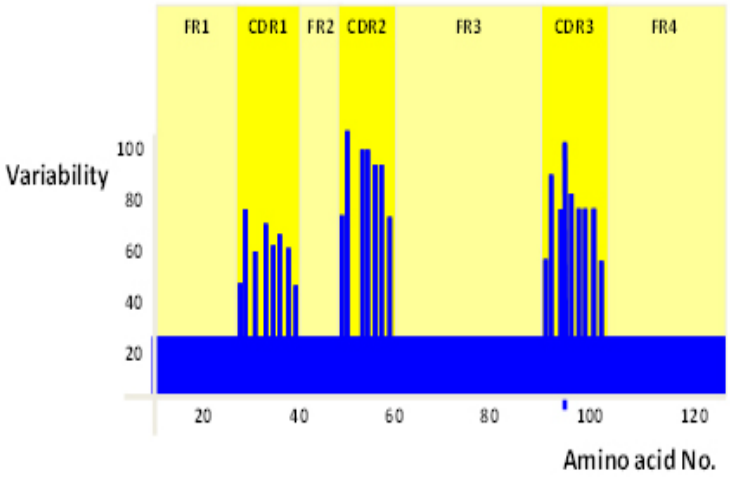
Antibody Binding Regions

- **Idiotope** – the antigenic determinants in or close to the variable portion of an antibody (Ab)
- **Paratope** – the part of an Ab that recognizes an antigen, the antigen-binding site of an Ab or **complementarity determining region** (**CDR**)
- **Epitope** – the part of the antigen to which the paratope binds

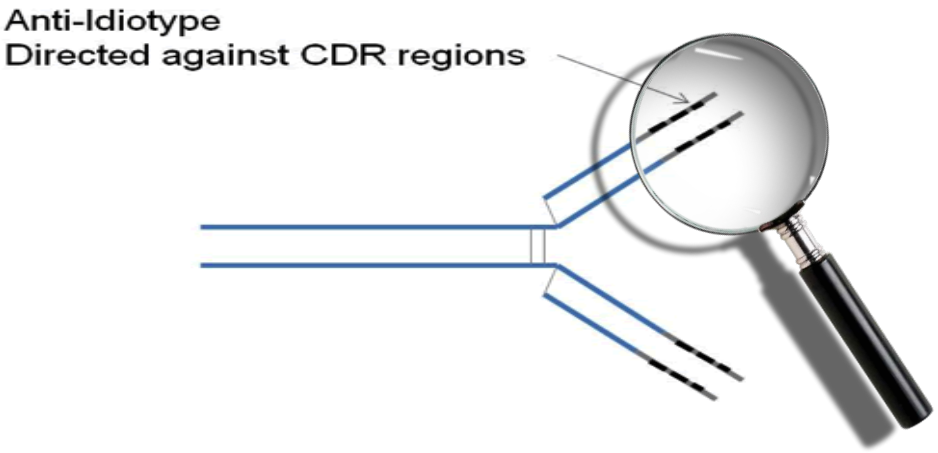


Anti-Idiotype Antibodies

- **Anti-idiotype antibodies (Anti-IDs)** – Abs directed against the paratope (or CDR region) of another Ab

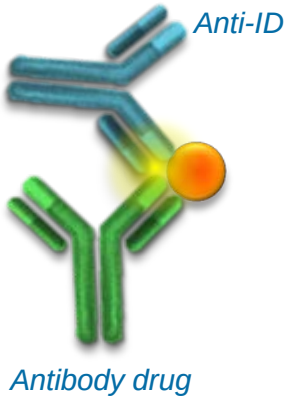


Hypervariable regions (or the idiotype of an Ab) are the antigenic determinants



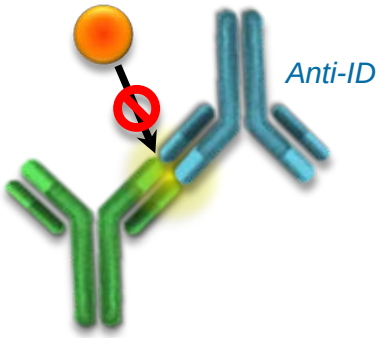
Different types of anti-IDs

Complex-specific



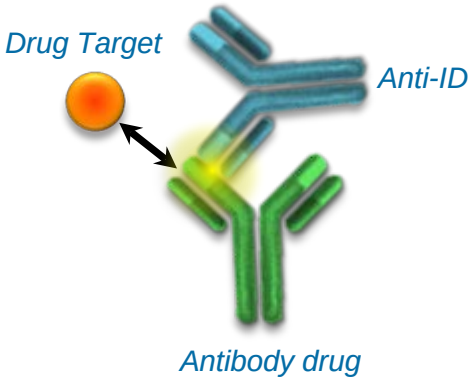
- Drug-target complex specific
- Not inhibitory
- Detects bound drug only

Antigen-blocking



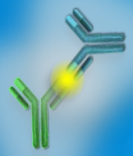
- Paratope-specific
- Inhibitory
- Neutralizing
- Detects free drug

Non-blocking



- Not paratope-specific
- Not inhibitory
- Detects total drug (free, partially bound, fully bound)

Anti-Idiotype Antibodies



Why use anti-IDs?

- Ideal for pre-clinical research and antibody drug development studies
- Allow monitoring of therapeutic Abs in samples
- Allow detection of Ab biotherapeutics that closely resemble circulating human Ig

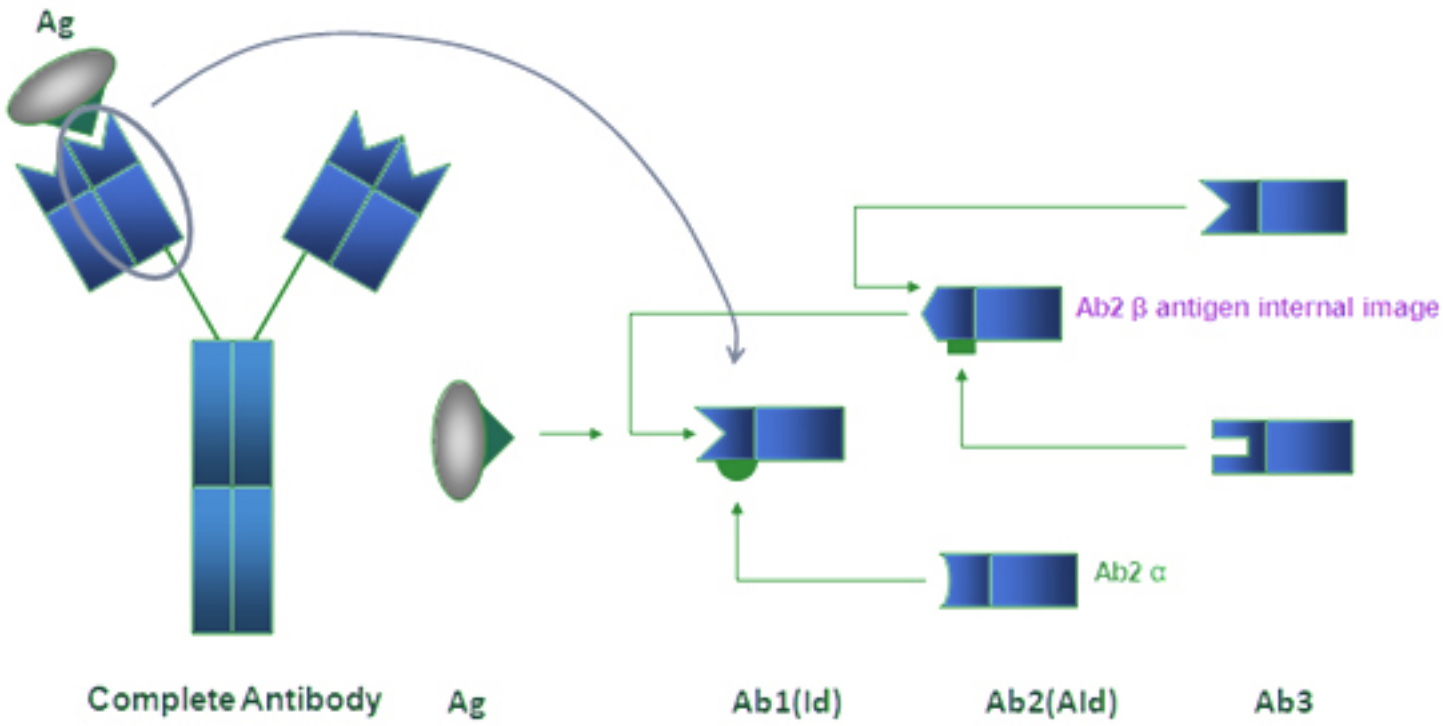
Anti-IDs, specific for the unique variable region of the therapeutic Ab, are ideal for this purpose

Anti-ID Applications

- Anti-IDs can be powerful reagents for the following applications:
  1. **Pharmacokinetic (PK) studies**
     - Used to measure the drug level in patient samples
  2. **Immunogenicity (anti-drug antibody) assays**
     - Used as a positive control or reference standard

Anti-ID Applications

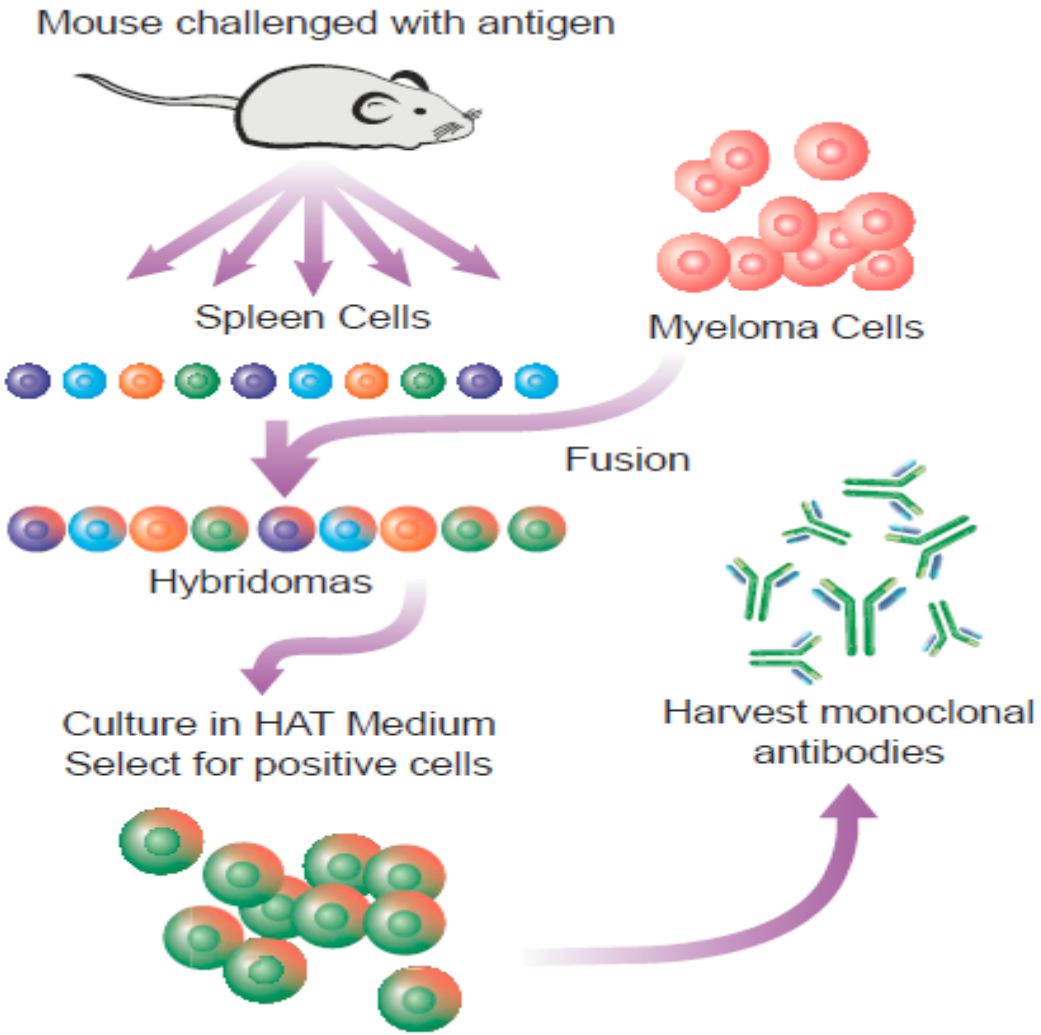
3. Vaccine Development



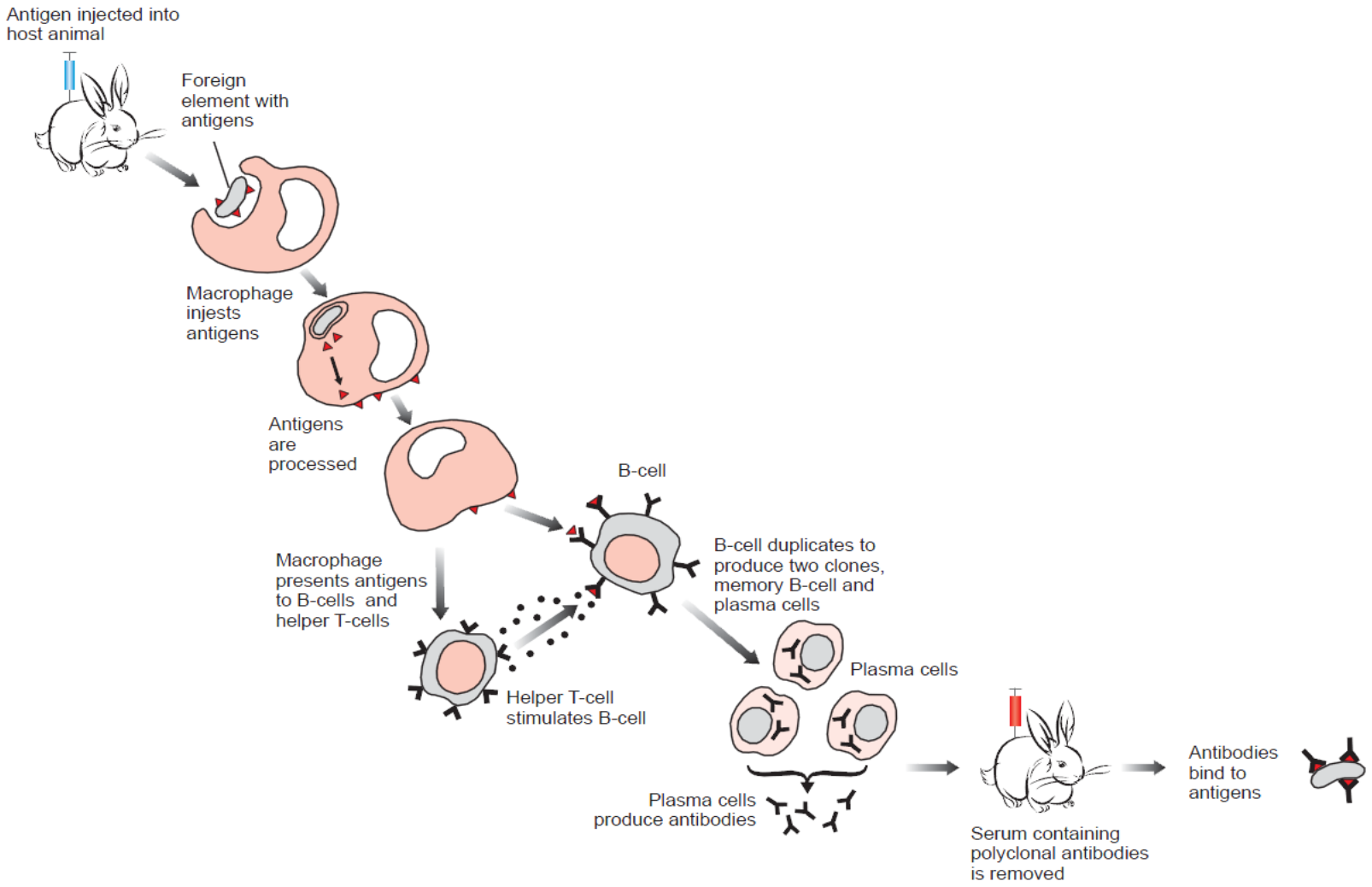
General flow chart of anti-idiotype antibody network

Antibody Production Overview

How are Monoclonal Antibodies Made?

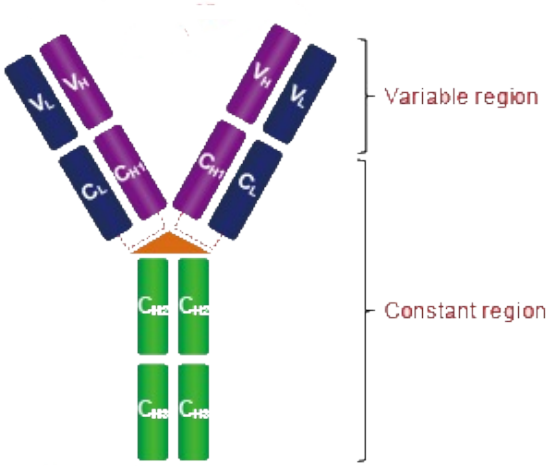


How are Polyclonal Antibodies Made?



Challenges During Anti-ID Production

1) **CDR region of Ab may not be very immunogenic**
   - ***Antigen immunogenicity:*** *The ability of a particular molecule to elicit an immune response determined by whether the immune system can recognize the antigen*
2) **Percentage in antiserum very low**
   - Most Abs raised target the Fc region
   - Anti-ID clone may be missed if conventional screening methods are used
3) **Must optimize clone selection from numerous anti-IDs**

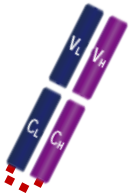


Overcoming the Obstacles to Anti-ID Production

1. CDR Region Not Immunogenic

Solution

- Use antibody drug-KLH conjugate as immunogen
- Use F(ab)$_2$ (fragment, antigen binding) as immunogen
  - Constant heavy chain determinants are absent



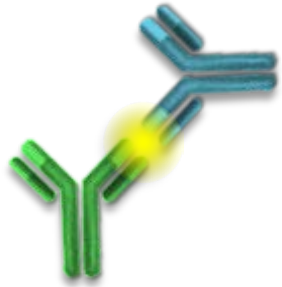
- Adjust immunization schedule

- Consider different animal host

2. Low Percentage of Anti-IDs in Serum

Solution

- Use proper secondary Ab (anti-mouse Fc to prevent cross-reaction with human Ig)
- HTP binding screening
- Capture ELISA to select natural epitope recognized by Ab

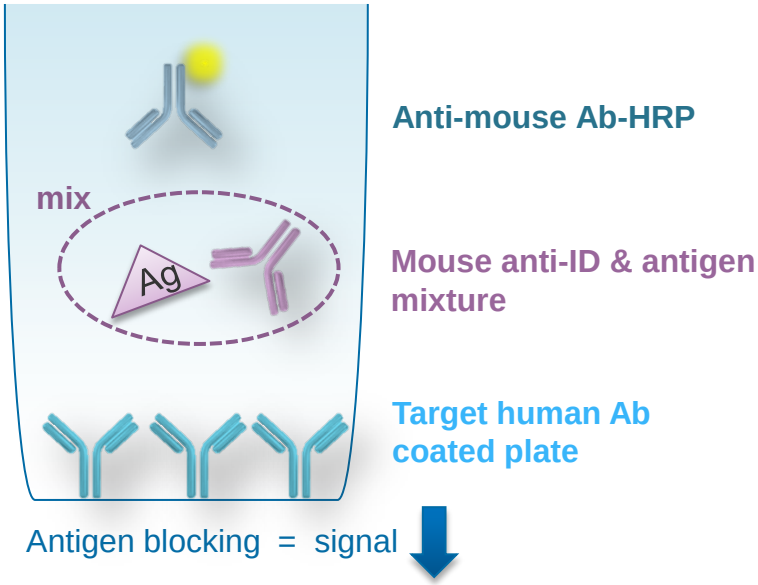


3. Optimize Clone Selection from Numerous Anti-IDs

Solution

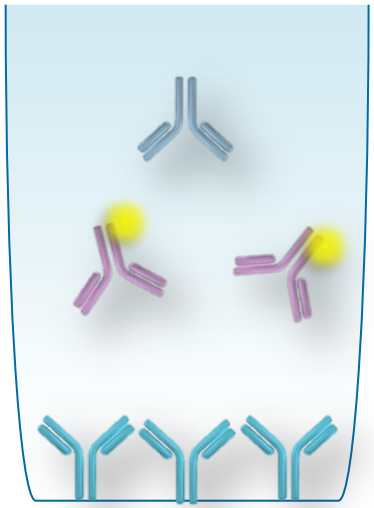
- Antibody pairing
  (for PK study)
- Affinity ranking
  (K_{off} and K_d ranking)
- Epitope binning
  (ELISA or SPR-based)
- Antigen blocking

Antigen blocking assay



Optimizing clone selection: Epitope binning

- Abs tested in a pairwise combinatorial manner
- Abs that compete for binding to the same binding region are grouped into the same epitope bin.



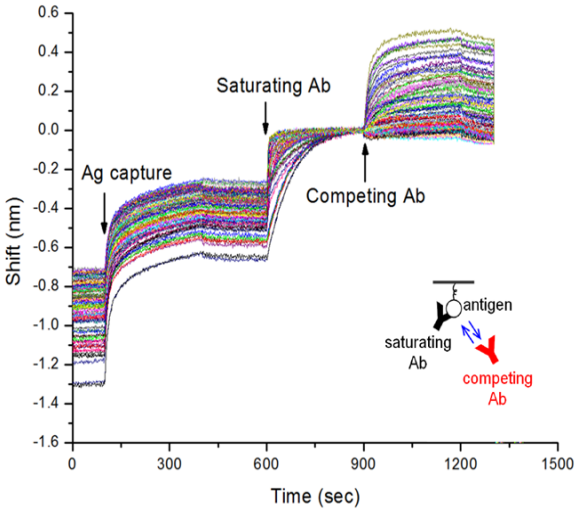
Mouse anti-ID (against epitope 2)

Mouse anti-ID-HRP (against epitope 1)

Target human Ab coated plate

Same epitope results in ↓ signal

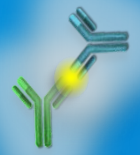
Epitope binning by Octet RED 96



Case Study:

	Epitope			
Assess relative affinity rank	Epitope 1	Epitope 2	Epitope 3	Epitope 4
1	4F5	5A7	12A7	9A2
2		5B11	3E5	19C12
3		3H9		
4		3B3		
5		5G7		

GenScript Anti-ID Development Protocol



Animals are immunized with antibody drug candidates

Titer tested for reactivity with species-specific antibody

Hybridoma screening for specific reactivity to immunogen antibody by ELISA or Fab expressing cells by FACS; multiple closely related counter proteins

Optimal clone selection: epitope binning/antibody pairing, antigen blocking and affinity ranking

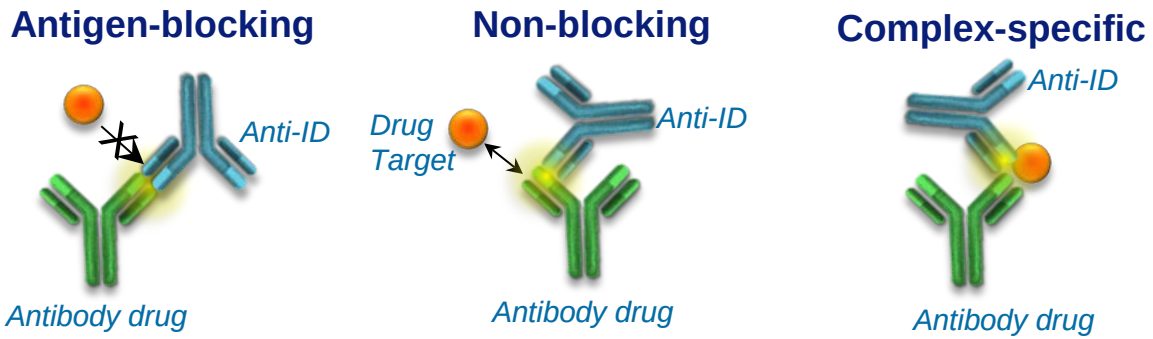
Antibody candidates subcloning and antibody production

Immunogenecity and PK assay development and sample test

Downstream Assay Development

Anti-IDs Offer Flexibility for Specialized Assay Development

- **Due to different binding modes and levels of affinity**
- **Using a combination of anti-IDs one can obtain:**
  1. PK profile
  2. Degree of target saturation by the antibody drug
- **Possible binding modes of anti-IDs:**
  - **Complex-specific** anti-IDs detect **bound** Ab drugs directly
  - **Antigen-blocking** (neutralizing) anti-IDs detect **free** drug
  - **Non-blocking** (non-inhibitory) anti-IDs detect **free and bound** drug



Guide for how to use concentration data

Concentration data	Intended use
mAb_{free} or mAb_{total}	To characterize in vivo PK behavior and to project human PK To assess PK and PD response relationships in animal models To calculate the safety margin and determine the safe starting dose or efficacious dose
L_{free}	To assess the inhibition of L_{free} following drug treatment; understand the dynamics of L_{free}
L_{total}	To assess the redistribution/modulation of L_{total} following drug treatment; understand the dynamics of L_{total}
L_{free}/L_{total} ratio	To compare in vivo and in vitro binding affinities
mAb and L dynamic relationship	To understand the underlying dynamic relationship between mAb and L to facilitate dose and dosing schedule selection

L = target ligand in circulation
mAb = monoclonal antibody therapeutic

Table adapted from The AAPS Journal, Vol.13 (1), 99-110, March 2011.

Downstream applications of anti-IDs

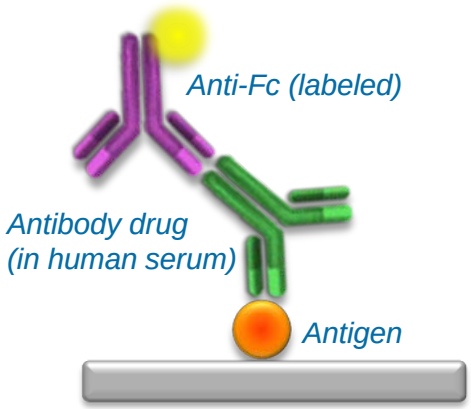
1. Pharmacokinetic Assays
2. Immune Response Assays

Pharmacokinetic (PK) Assays

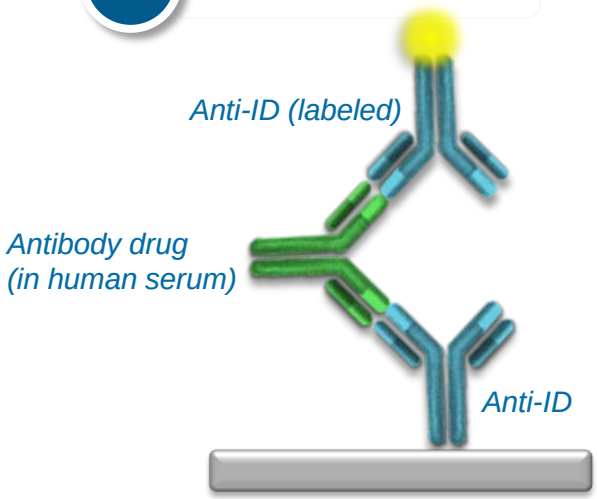
- **Purpose:** **Detect and quantitate human Ab drugs in serum**
- Human serum contains **5-9.5 mg/ml IgG1**
- Required sensitivity for Ab drug is in the **ng/ml range**
- Thus, assays must accurately detect Ab drug despite a million-fold excess of similar molecules
- Possible agents for detection include:
  - Antigen (i.e. drug target)
  - **Anti-ID antibodies**

Typical PK Assay Formats

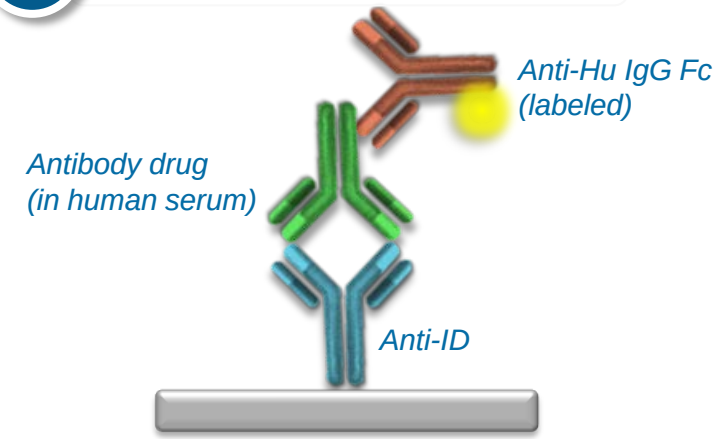
1 Antigen Capture



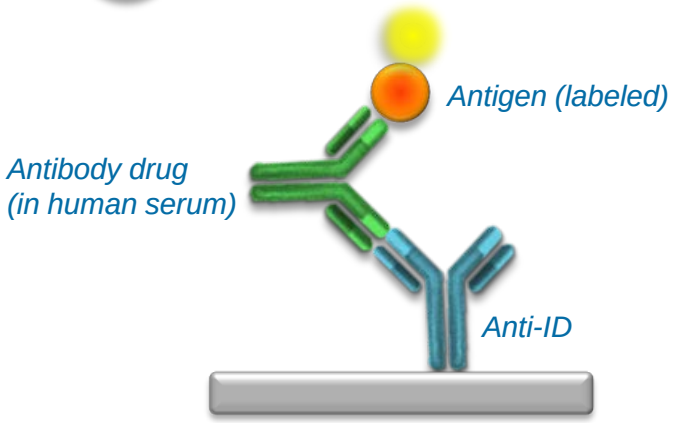
2 Anti-ID-Bridging



3 Anti-ID Capture Sandwich



4 Anti-ID-Antigen-Bridging



Immune Response (IR) Assays

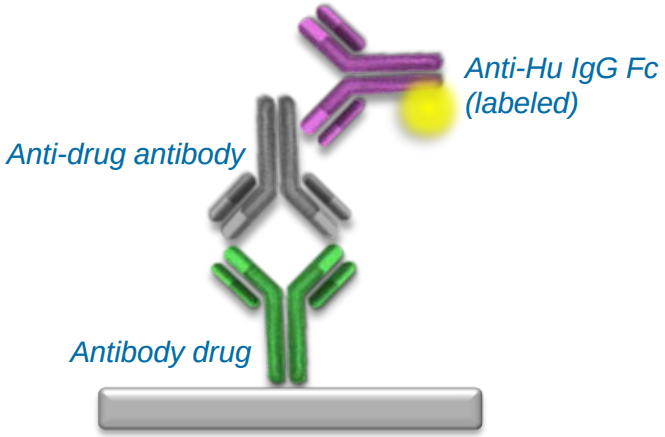
Overview of Design Elements

1. **FDA recommends a multi-tiered approach**
   - **Tier 1:** A rapid, sensitive screening assay
   - **Tier 2:** Positive should then be subjected to a confirmatory assay, such as ligand or antigen competition assay
   - **Tier 3:** Further characterization: neutralization/class/isotype/titer

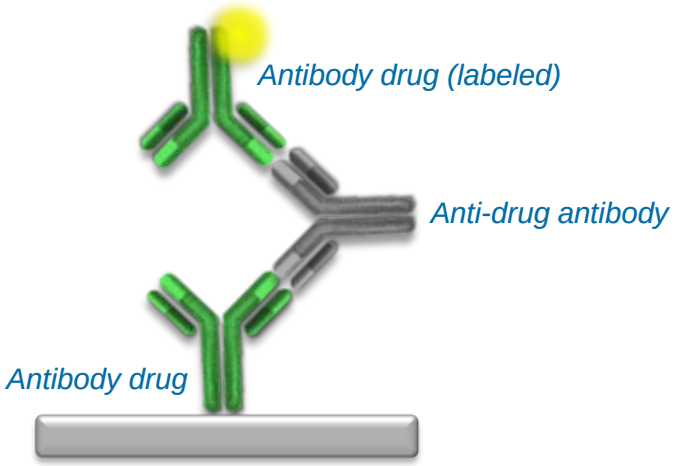
2. **Aspects of Assay Development**:
   - Highly sensitive
   - Able to detect all isotypes (IgM, IgE and IgG subtypes); carefully consider the avidity of control used to evaluate the assay; Should conduct assay performance test in the same concentration of matrix as that used to assess patient samples.

Direct and Bridging ELISAs

Direct Binding ELISA



Bridging ELISA



Anti-ID (mAb or pAb) serve as the best **positive controls** for IR assay development

Typical IR Assay Formats

- Direct and Bridging ELISA assays
  - Advantages: Sensitive, inexpensive, equipment readily available
  - Disadvantages: May not detect early immune response and may be influenced by high levels of circulating drug
- Radio-immuno precipitation assay (RIPA)
  - Advantages: sensitive, inexpensive, equipment readily available
  - Disadvantages: May not detect early immune response and may be influenced by high levels of circulating drug
- Surface Plasmon Resonance (SPR)
  - Advantages: Method of choice for detecting early immune response and has Ab characterization capabilities
  - Disadvantages: Expensive equipment, generally less sensitive than RIP or ELISA/ECL
- Electrochemiluminescence (ECL) assay
  - Advantages: Sensitive, can be modified to respond in the presence of high levels of circulating drug
  - Disadvantages: Equipment can be expensive, may not easily detect rapidly dissociating Abs

Successful GenScript Case Studies

Case study 1: Anti-ID mAb binds specifically to idiotype Ab, but not other negative controls

Clones	Antibody drug	Human IgG1	Normal IgG	Human serum
No.1	2.632	0.086	0.285	0.124
No.2	2.691	0.19	0.186	0.285
No.3	1.576	0.094	0.084	0.253
No.4	2.133	0.21	0.085	0.111
No.5	2.469	0.093	0.116	0.483
No.6	2.546	0.122	0.091	0.176
No.7	1.803	0.084	0.153	0.311
No.8	2.149	0.09	0.081	0.133
No.9	2.498	0.094	0.182	0.082
No.10	2.399	0.195	0.084	0.195

Figure 1: Final sub-clones with high specificity to humanized Ab when screened against different counter screenings.

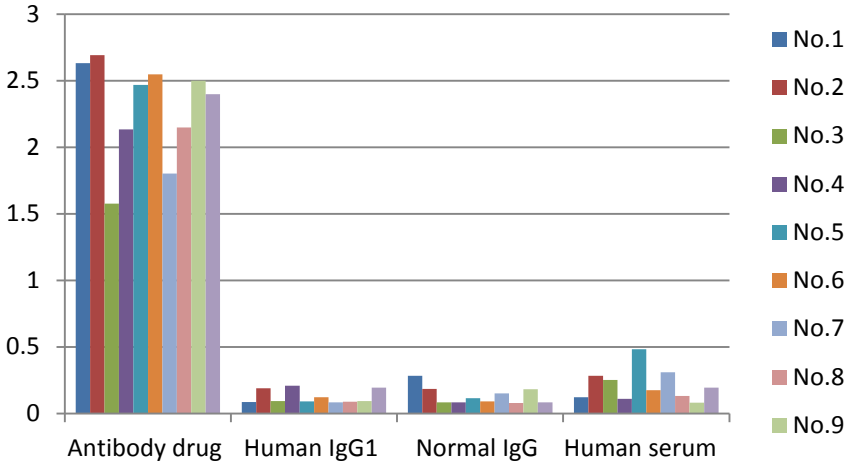


Figure 2: Final sub-clones with high specificity to humanized Ab when screened against isotype control, normal human IgG and human serum.

Successful GenScript Case Studies

Case study 2 : GenScript can generates high affinity anti-idiotype antibody with sub-nanomolar level

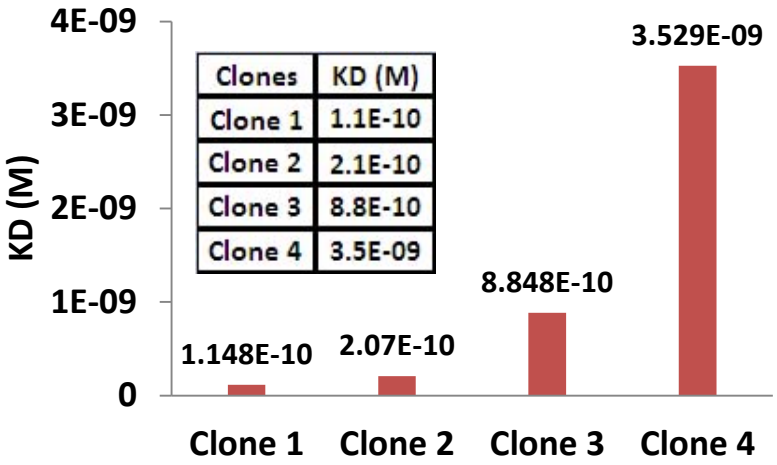


Figure 3: Affinity Ranking of antibodies by using BIAcore; clones with sub-nM affinity

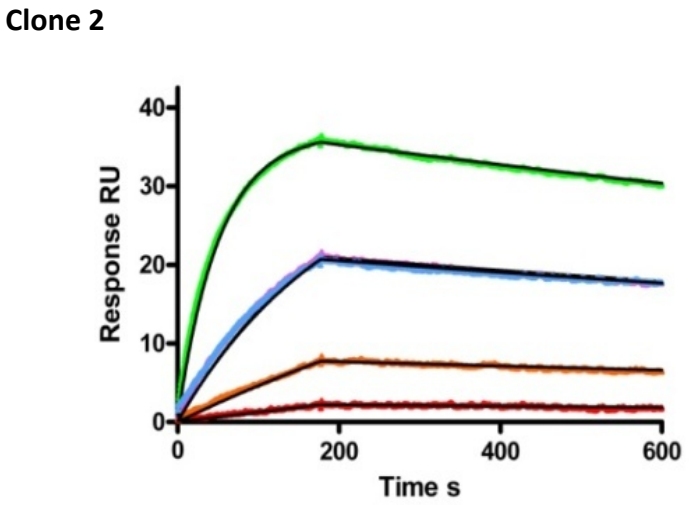


Figure 4: Affinity determination of Clone 2 antibodies by using BIAcore

Successful GenScript Case Studies

Case study 3 :

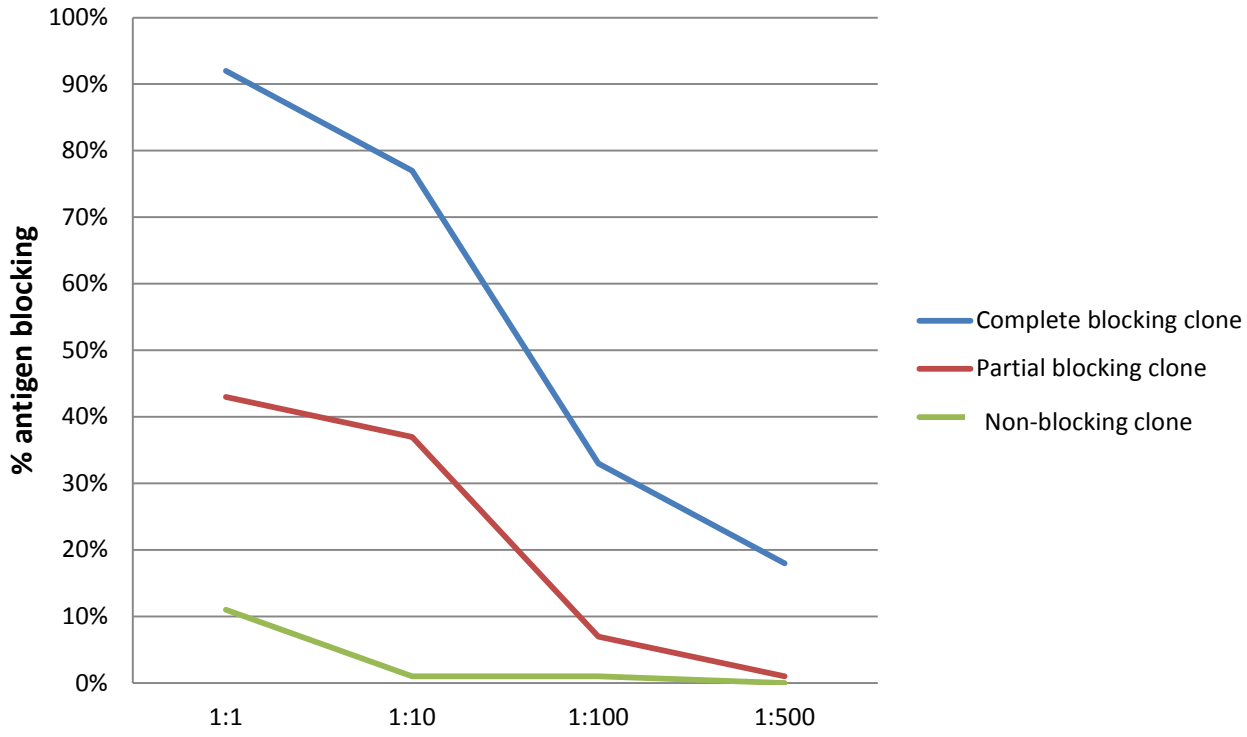


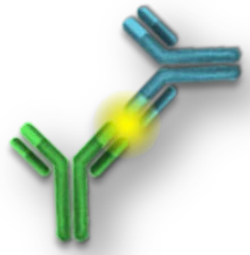
Figure 2: Specific inhibition of anti-Idiotype mAbs in an antigen ligand blocking assay.

GenScript Full Anti-ID Development Services

GenScript has developed a panel of high affinity, high specificity anti-IDs for use in PK and IR assays

Features of GenScript Anti-ID Platform:

- High specificity and high affinity
- High speed production: **2-3 months**
- Antigen ligand blocking, epitope binning, and antibody pairing
- **Anti-ID polyclonal antibody packages** (rabbit & goat)
- **Anti-ID monoclonal antibody packages** (mouse & rat)
- Proven track record: **100% success rate**
- **PK** and **IR assay** development



GenScript Anti-ID Packages

Anti-Idiotype Antibody Packages		
Services	**Anti-idiotype mAb (*SC1184*)**	**Anti-idiotype pAb (*SC1185*)**
Starting material	Target antibody drug 2-3 mg	Target antibody drug 20 mg or more
QC	Cross-reactivity with control IgG <10%	Cross-reactivity with control IgG <10%
Deliverables	Hybridoma cell lines, supernatants and purified anti-ID antibody (optional)	0.5-3 mg purified anti-ID antibody/rabbit

Ordering is easy:

http://www.genscript.com/anti-idiotype-antibody.html

Advantages of GenScript Custom Abs

- Sequence to purified antibody service with no need to provide an antigen

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- Guaranteed results: quantity of antibodies or hybridoma, ELISA titer, and WB guarantee (varies with specific package)
- Fast turnaround time: delivery of purified pAb or development of specific hybridoma in 45 days.
- Certified facility: AAALAC International accreditation and OLAW certification, demonstrating our commitment to responsible animal care and use.

Variety of GenScript Antibody Services

Polyclonal Antibody Services

FAST pAb Services-PolyExpress™, Standard pAb Services

Monoclonal Antibody Services

FAST mAb Services-MonoExpress™, Custom mAb Services, Premium Hybridoma Services

Phospho-Specific Antibody Services

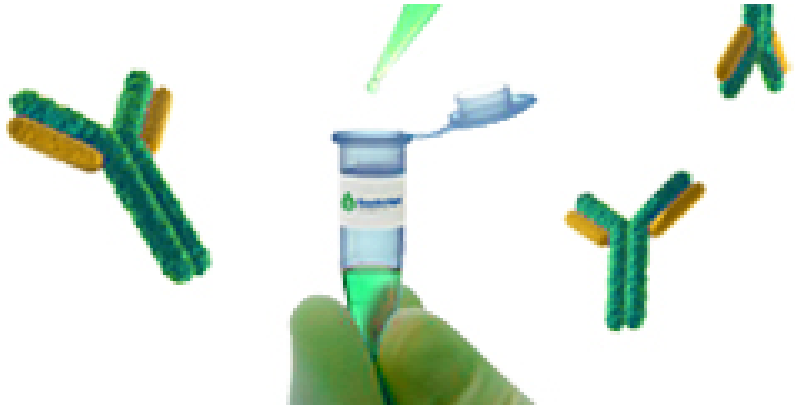
Phospho-Specific pAb and mAb Services

Scale-up Antibody Services

Scale-up pAb and mAb Services, *In vivo* Ascite production, *In vitro* Roller bottle production

Specialized Antibody Services

Antibody Drug Development, Immunoassay, Purification, Modifications, Conjugation



Over 500 Publications Citing Our Ab Services

- **Methylation protects microRNAs from an AGO1-associated activity that uridylates 5' RNA fragments generated by AGO1 cleavage.**
  Yu B, Chen X, Vinovskis C, etc.
  ***PNAS,** (Apr 2014)*

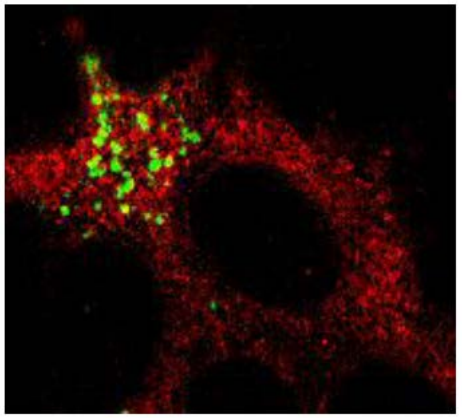
- **HYPERSENSITIVE TO HIGH LIGHT1 Interacts with LOW QUANTUM YIELD OF PHOTOSYSTEM II1 and Functions in Protection of Photosystem II from Photodamage in Arabidopsis.**
  Wang HB, Wang J, Qi K, etc.
  ***Plant Cell,** (Mar 2014)*

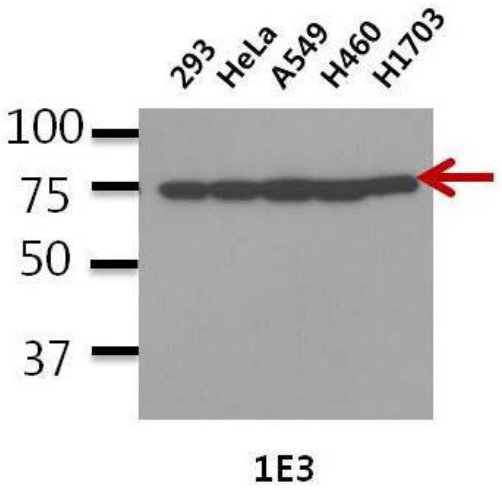
- **Tousled-like kinases phosphorylate Asf1 to promote histone supply during DNA replication.**
  Groth A, Jensen ON, Nielsen ML, etc.
  ***Nature Communications,** (Mar 2014)*

- **Dirigent domain-containing protein is part of the machinery required for formation of the lignin-based Casparian strip in the root.**
  Hosmani PS, Kamiya T, Danku J, *etc.*
  ***PNAS,** (August 2013)*

- **PfSETvs methylation of histone H3K36 represses virulence genes in Plasmodium falciparum**
  Jiang L, Mu J, Zhang Q, Ni T, etc.
  ***Nature,** (July 2013)*

- **Wheat Mds-1 encodes a heat-shock protein and governs susceptibility towards the Hessian fly gall midge.**
  Liu X, Khajuria C, Li J, Trick HN, Huang L, *etc*
  ***Nature Communications,** (Jun 2013)*





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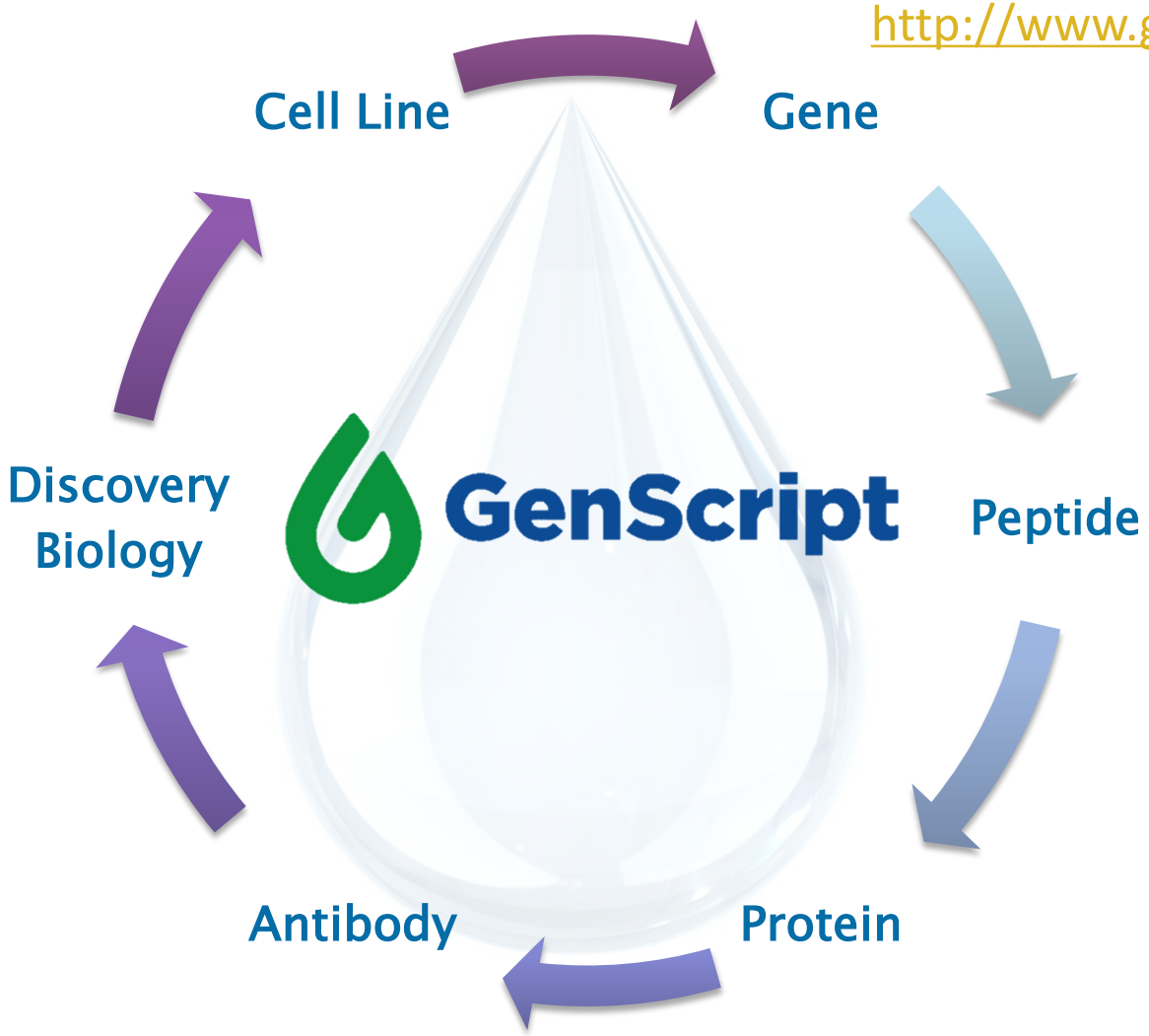

Q & A

Thank you for your participation

We wish you all success in your research

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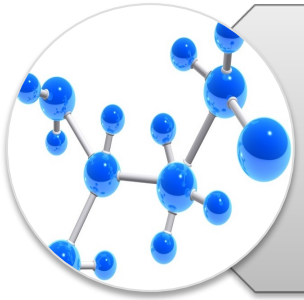

November 25, 2014/8:00 am and 2:00 pm EST

Peptide design strategy: basics, optimization, and application – *Tiffany Gupton Campolongo, PH.D.*

December 3, 2014/8:00 am and 2:00 pm EST

Fusion partner for recombinant soluble protein production in *E. Coli* – *Keshav Vasanthavada*

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