Monoclonal Antibody Generated by DNA Immunization: Powerful Tools for Antibody Drug Development

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GenScript
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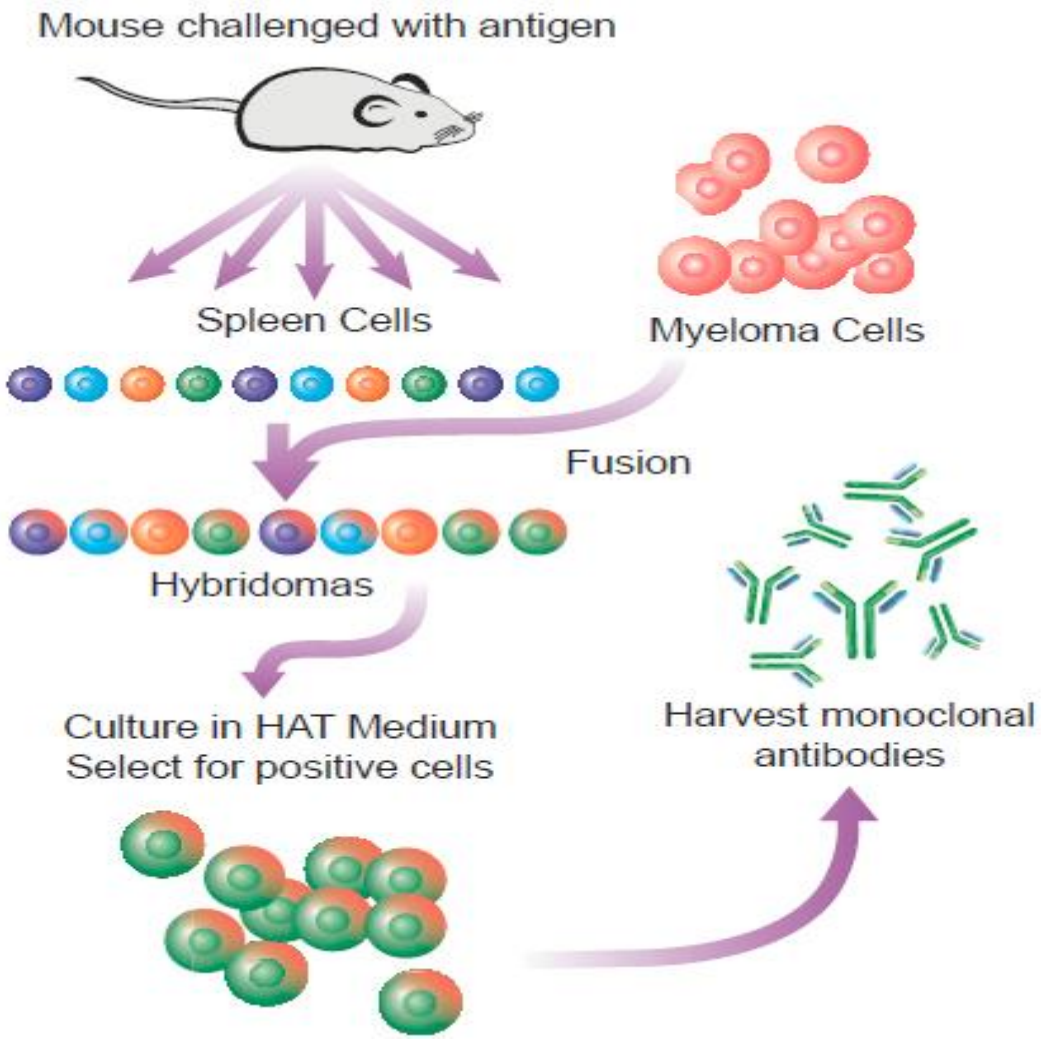
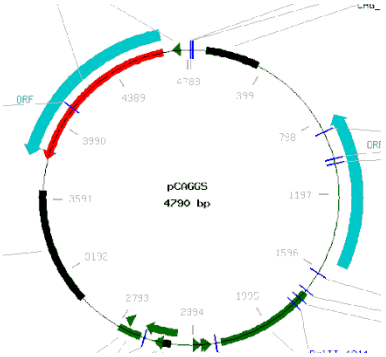
How are Monoclonal Antibodies Made?

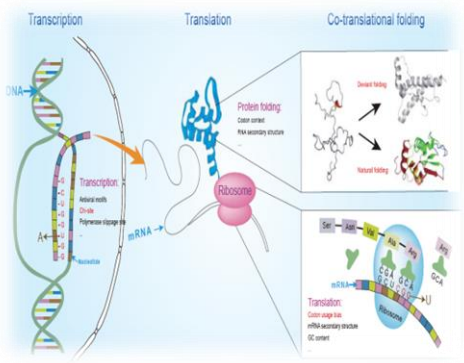


GenScript Multiple Immunogen Designs

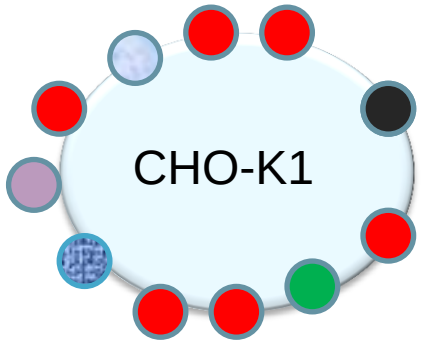
- **Peptides** designed by OptimumAntigenTM design tool
- **Soluble protein** or excellular domain (ECD) recombinant protein production
- **Whole cell** immunization: GenScript develops stable cell line/transient cells for immunization and screening
- **Genetic (DNA)** immunization to deliver the target DNA plasmid into the host animals by Gene Gun technique (today's topic)
- **Virus like particle (VLP)** that contains enriched target protein



Target expression plasmid



Optimized Protein Expression



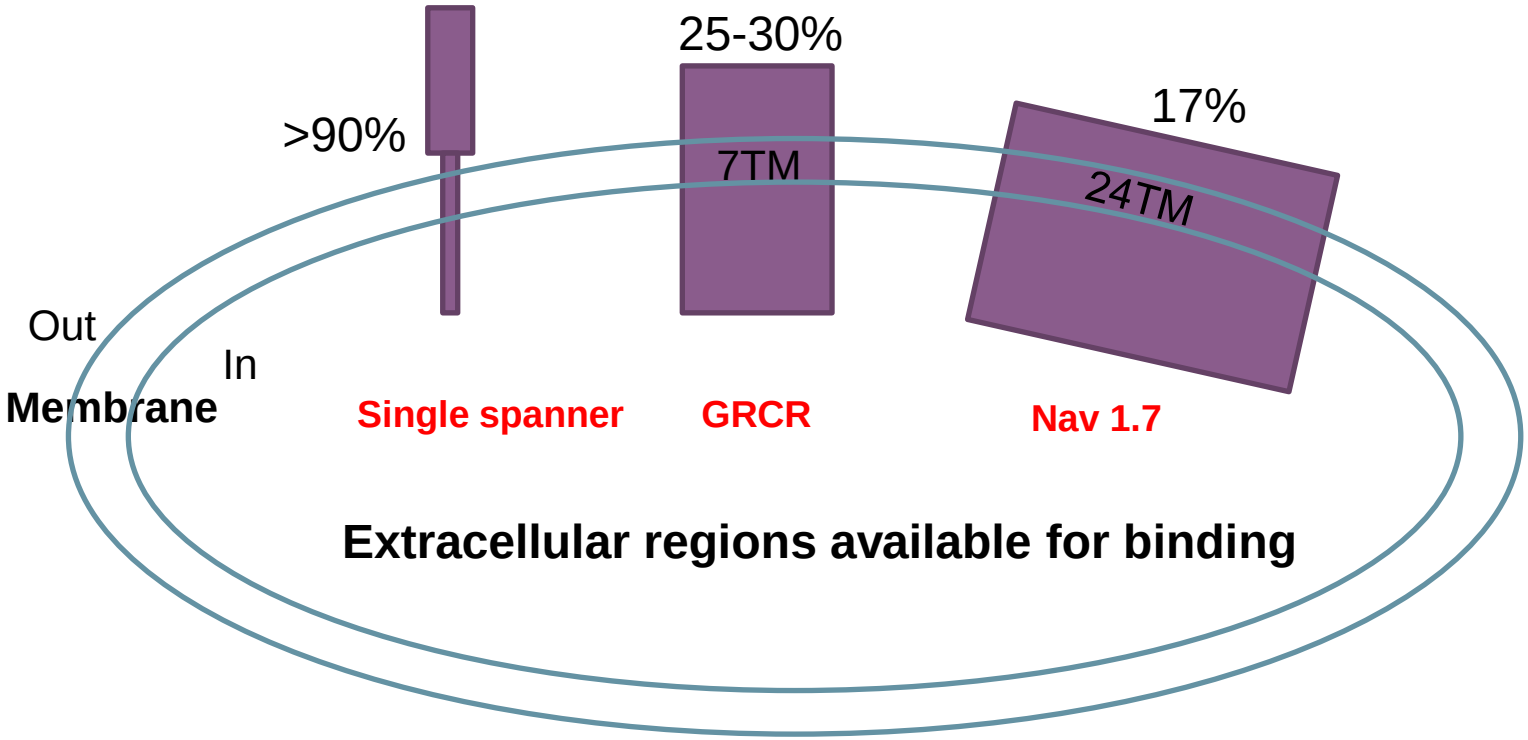
Target transfected cell

Gene Gun

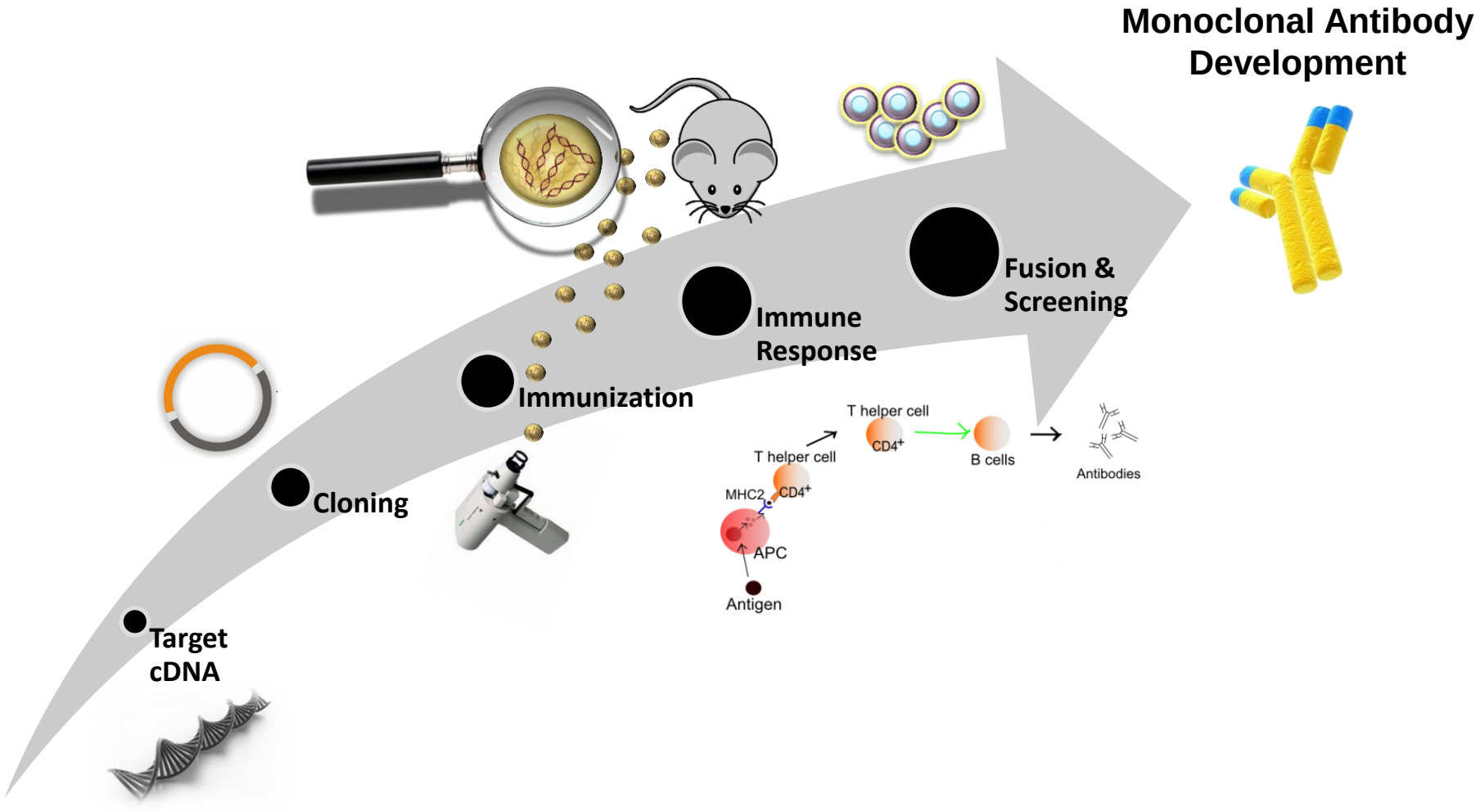
Why MSM-Antibodies are Difficult to Generate?

MSM: Multi-Spanning Membrane Proteins

- Small, constrained, post-translation modified extracellular loops;
- Multi-domain epitopes may be required;
- Native state is required (antibodies against peptides or unfolded proteins fail to recognize native antigen);
- Multiple difficulties in expression, purification, and maintaining the native state.



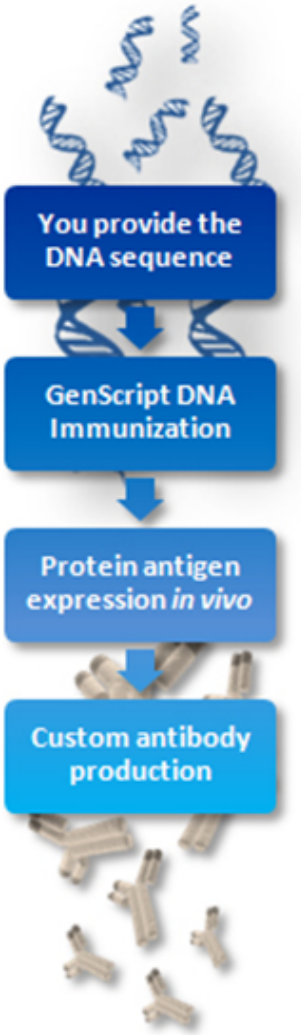
DNA Immunization for Antibody Generation



DNA Immunization Features

Applications and Advantages of DNA Immunization Service

	Antibody Development for Membrane Proteins and Problematic Antigens	Early DNA Vaccine Development
Key Advantages	• Streamlines Ab production against membrane proteins and other problematic antigens • Eliminates need to produce and purify target protein *in vitro* • Abs produced recognize native protein structure • Protein is produced in small quantities *in vivo* driving production of high affinity Abs	• Superior codon optimized gene synthesis technology ensures quality antigen production in vivo • Optimized plasmid vectors and immunization protocols promote transfection efficiency • Specialized adjuvant and immune mediators substantially enhance immune response
	Flexible customization options available Readily integrated downstream applications for antibody drug development	



Advantages

- **Shortened Development and Production Time**
  - Synthesis and purification of proteins no longer necessary
  - Animals can be immunized in as little as 3 days
  - DNA immunization induces a qualitatively superior response in that Abs can be induced after a single vaccination with DNA
- **Affinity and Specificity**
  - Protein is produced in very small quantities, thus it is more likely to drive production of high-affinity Abs
  - Slow, consistent presentation to immune system favors production of high-affinity Abs
  - Enhances quality of Abs produced since those that recognize native folded protein are most useful in proteomics
  - Molecular chaperones are available to help fold proteins that are normally difficult and misfolded proteins are eliminated via the normal proteasome pathway.
  - DNA can be made in highly pure form, reducing the likelihood of generating Abs to contaminants
- **Can circumvent technical issues associated with problematic antigens**
  - Viable method to generate Abs against transmembrane proteins (i.e. **GPCRs and ion channels**), large protein domains, insoluble proteins, toxic proteins, proteins containing disulfide bonds, and PTM-modified proteins

Applications

Solution for Membrane Protein and Problematic Antigens	Early DNA Vaccine Development
• Streamlines Ab production against membrane proteins and other problematic antigens • Eliminates need to produce and purify target protein • Abs produced recognize native protein structure • Protein is produced in small quantities *in vivo* driving production of high affinity Abs	• Superior codon optimized gene synthesis technology ensures quality antigen production in vivo • Optimized plasmid vectors and immunization protocols promote transfection efficiency • Specialized adjuvant and immune mediators substantially enhance immune response

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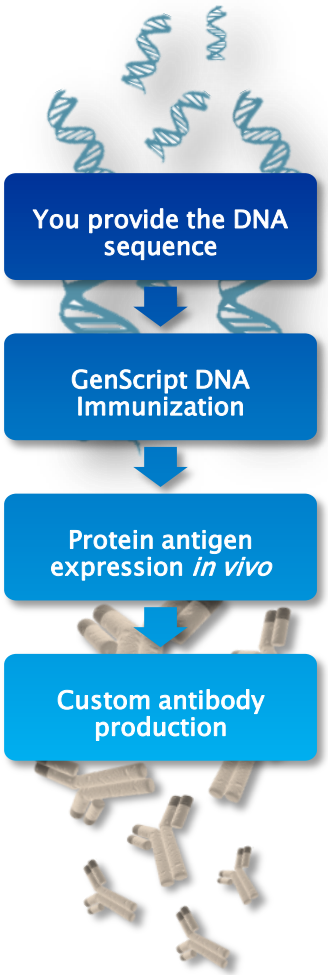


DNA Immunization: A Powerful Solution for…

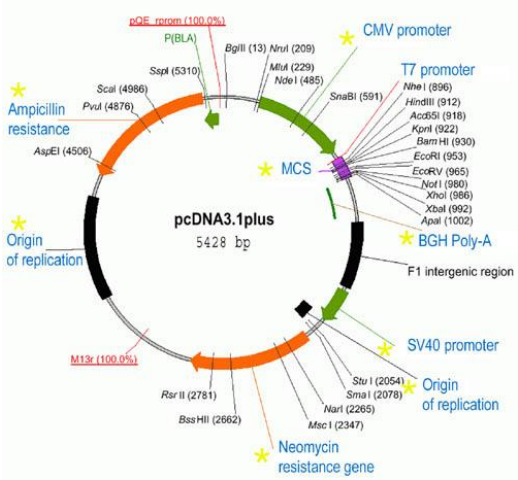
- **Individuals involved in:**
  - Ab drug development
  - Early DNA vaccine development

- **Individuals with problematic antigens:**
  - Transmembrane proteins (i.e. **GPCRs and ion channels**)
  - Large protein domains
  - Insoluble proteins
  - Toxic proteins
  - Proteins containing disulfide bonds
  - PTM-modified proteins
  - Unstable proteins
  - Insufficient amount of protein production *in vitro*

Gene Immunization: DNA design

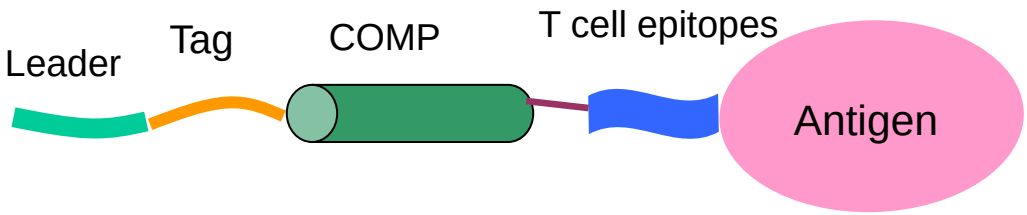
DNA Codon Optimization

- Codon usage bias
- GC content
- Negative CpG islands
- RNA instability motif (ARE)
- Repeat sequences



Expression Vector Selection and Functional Element

- pcDNA3.1 or pCAGGS-plasmid construction
- His/Flag Tag-expression identification
- KOZAK Sequence-Increase protein expression
- ImmnuoPlus Sequence-overcome immune tolerance

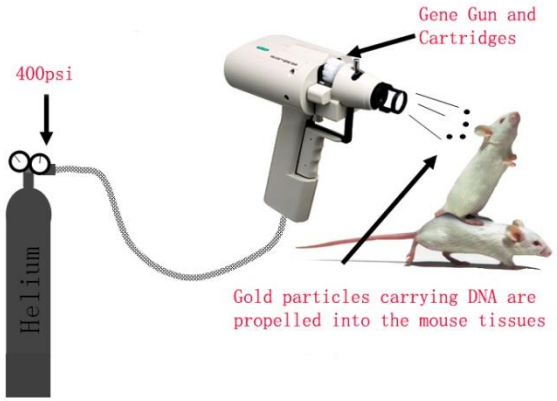


Plasmid construction

Gene Immunization Schedule

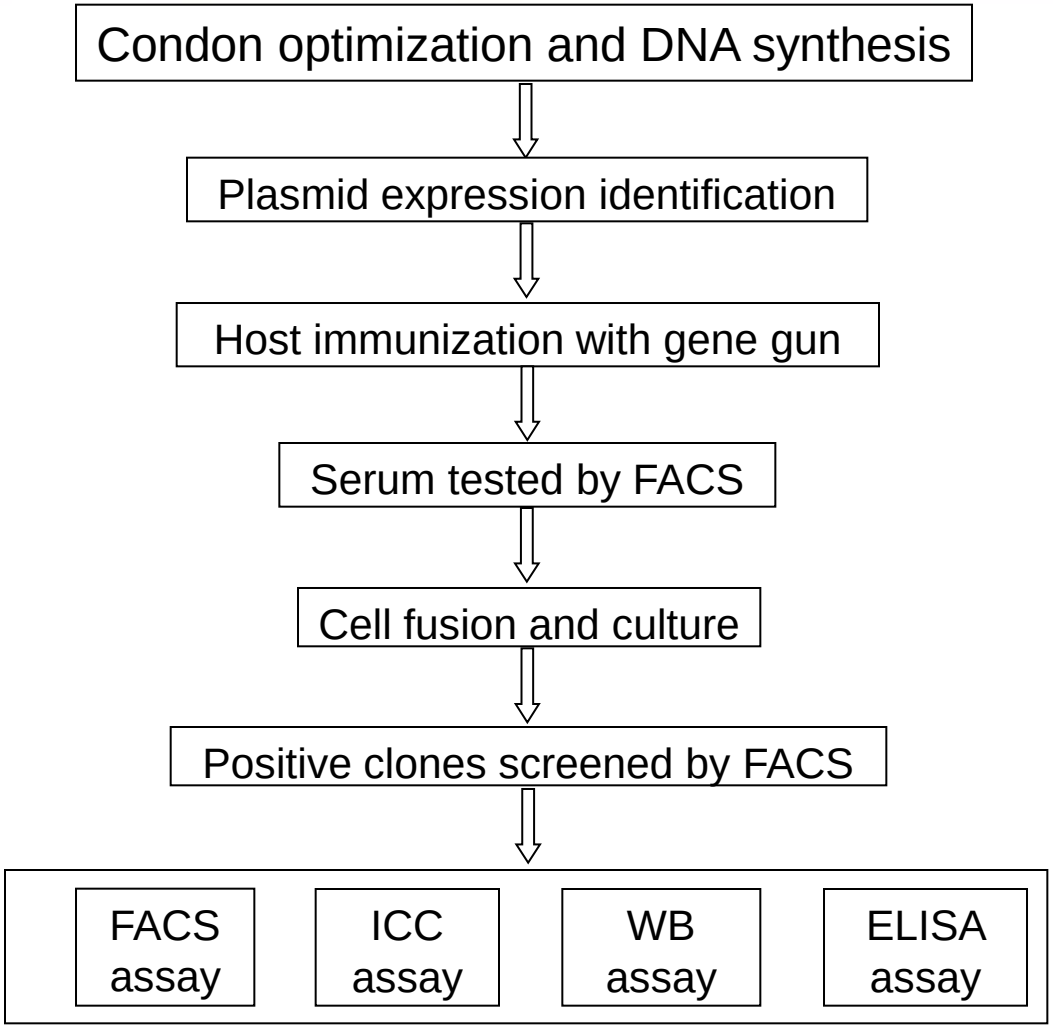
DNA Immunization

- Gene Gun to deliver plasmid DNA
- Immunization target tissue
  - abdominal skin and muscle cell
- DNA immunization + protein/cell/membrane boost
- Pressure of helium
- Optimized Adjuvant



Date	Immunization and bleed
0 day	Bleed 0.2 ml (yields 0.1 ml pre-immune serum) 1st immunization
14th day	2nd immunization
21st day	Test bleed by FACS or ICC
28th day	3rd immunization
35th day	Test bleed by FACS or ICC
42nd day	4th immunization
49th day	Test bleed by FACS or ICC

DNA Immunization Flowchart



Comparison of Immunogen Preparations

Immunogen	Native Conformation	Immunogen Concentration	Immunogen Purity	Success Rate
Purified Recombinant Protein/ECD	-	+++	+++	None
Reconstituted Protein in Vesicles/Membrane	+/-	+++	+++	Low
Peptides	+/-	+++	+++	Low
Over-expressing cells	+	+	+	Yes
DNA	+	+	+++	Yes
Lipoparticles	+	++	+	Yes

Approaches used for generation of antibodies against MSM

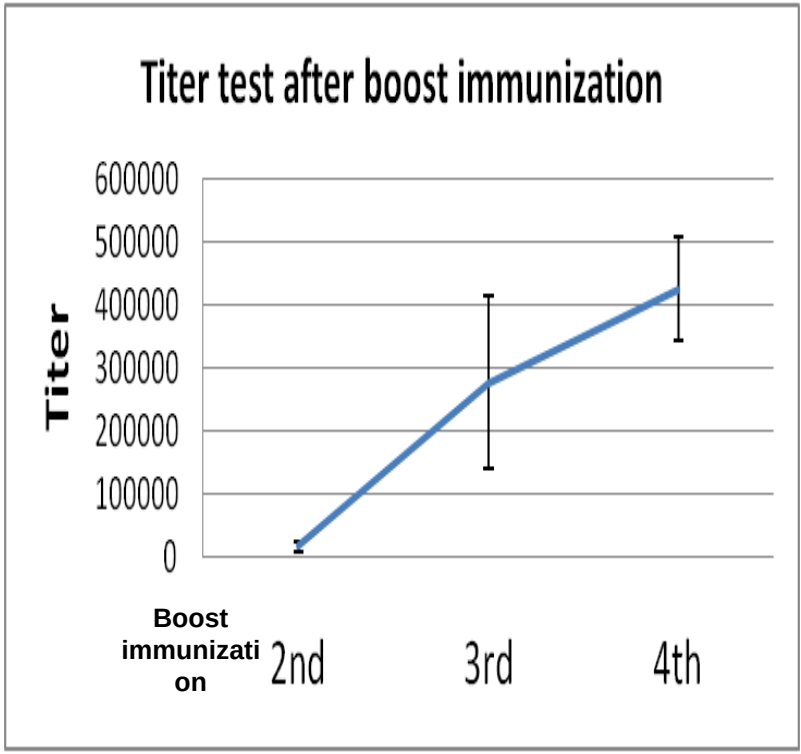
Advantages of the GenScript DNA Immunization Service

Advantages in Gene (DNA) immunization:

1. Benefit for evoking immune response on **natural** epitopes, e.g. GPCR
2. Bypassing protein preparation steps, time saving and cost effective

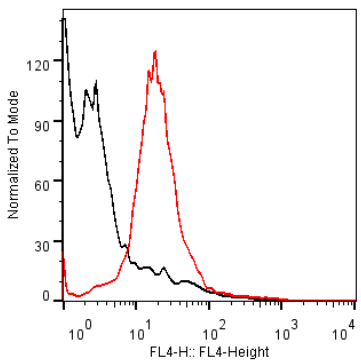
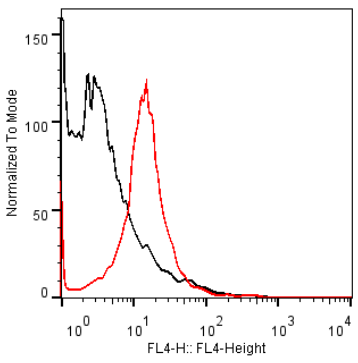
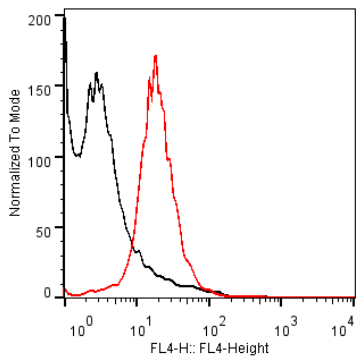
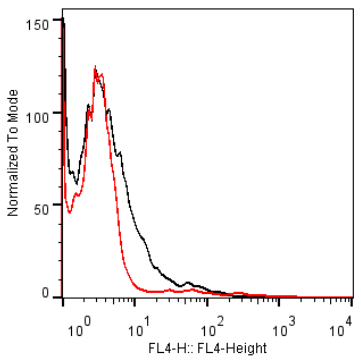
GenScript Experience:

1. Optimized plasmid vectors, codon optimization, and immunization protocol to promote transfection efficiency
2. Special adjuvant and immune mediators
3. Gene gun to deliver DNA cartridge
4. Successfully delivered cases (finish >20 projects, like GLP1R)
5. Our experienced team includes renowned expert and GenScript consultant, Dr. Shan Lu, a pioneer in the field of DNA vaccination.



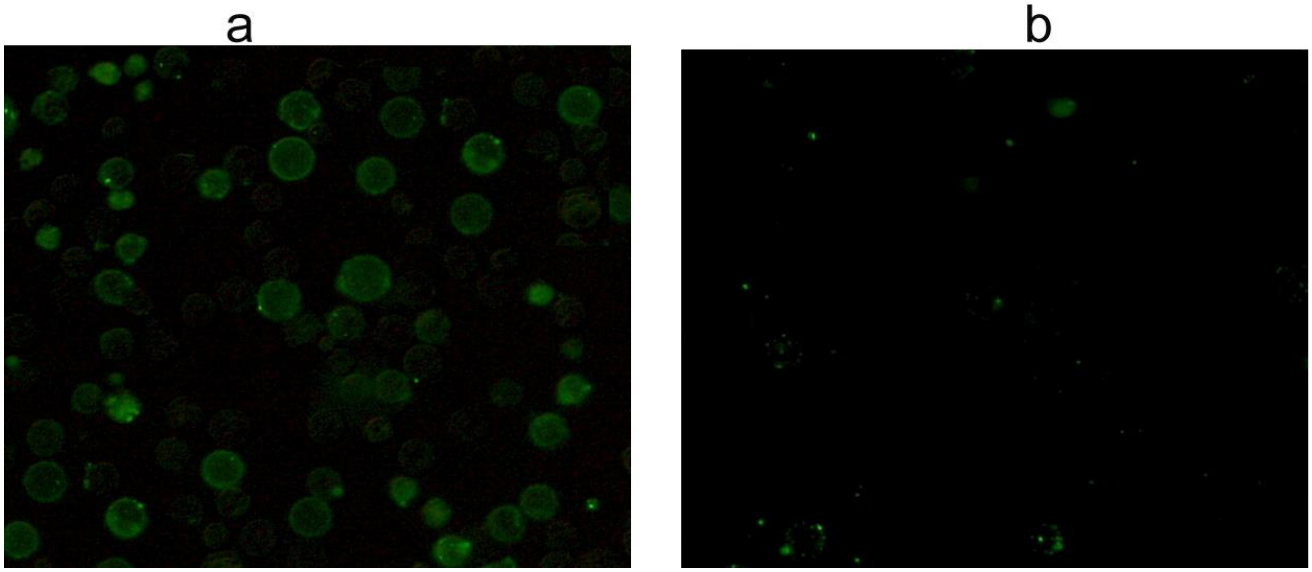
Case Study (1)

Anti-GLP1R mAbs generated by gene immunization

10F7E3	5F11F9	1F1E7	Mouse IgG control
			

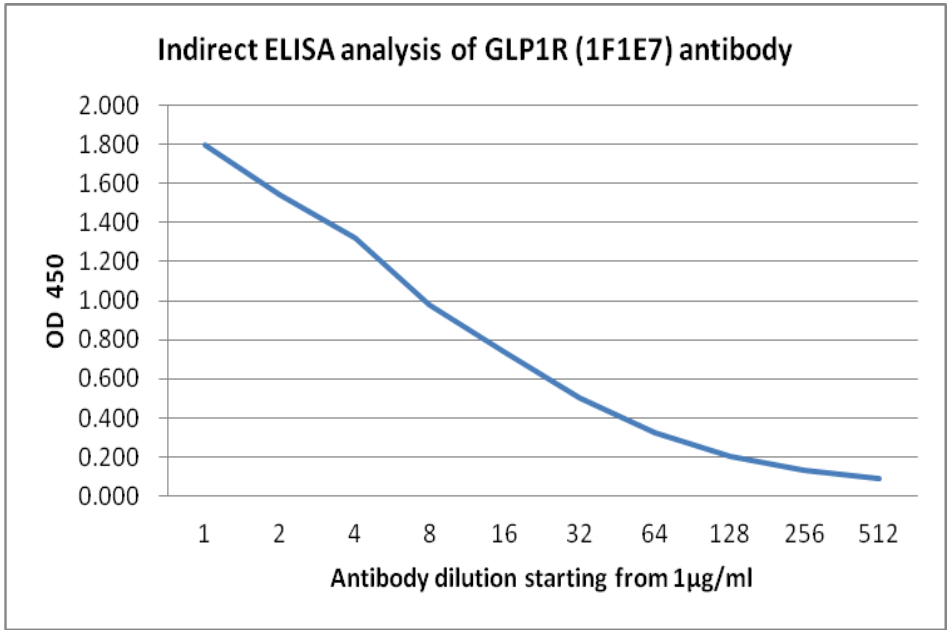
Flow cytometric analysis of CHO-K1/GLP1/Gα15 stable cells expressing GLP1R (GenScript, M00451) and CHO negative control cells with three mouse anti GLP1R monoclonal antibodies(red and black respectively).
The signal was developed with iFluor647 conjugated Goat Anti-Mouse IgG.

Case Study (2)



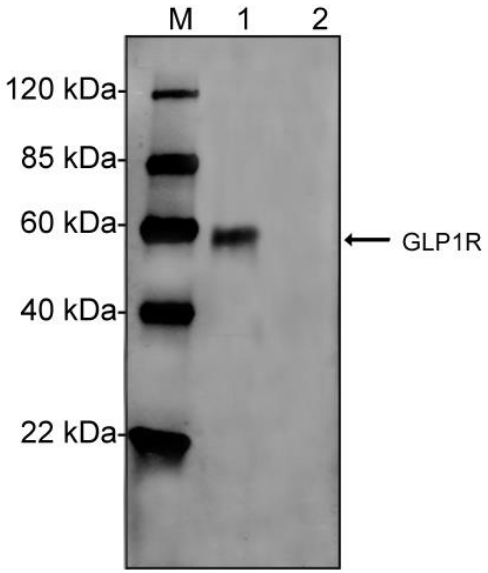
Immunocytochemistry/Immunofluenrescence analysis of HEK293 cell transfected with GLP1R plasmid (a) and non-transfected HEK293 cells (b) using mouse anti GLP1R (1F1E7) monoclonal antibody (4 µg/ml).
The signal was developed with iFluor488 conjugated Goat Anti-Mouse IgG.

Case Study (3)



Indirect ELISA analysis of Virus like particle (VLP) expressing GLP1R by **mouse anti GLP1R (1F1E7) monoclonal antibody prepared by DNA immunization**.

Case Study (4)



Western blot analysis of Virus like particle expressing GLP1R with **mouse anti GLP1R (1F1E7) monoclonal antibody**.

Lane 1. GLP1R Antibody, mAb, Mouse (1 µg/ml)

Lane 2. Mouse IgG control (1 µg/ml)

Predicted size: 57 kDa

Observed size: 57 kDa

The signal was developed with IRDyeTM800 Conjugated affinity Purified Goat Anti-Mouse IgG.

Service Package

DNA Immunization Protocol Details* (SC1693)

Step	Specification	Timeline
Gene Synthesis & Validation	• Codon optimization • Gene synthesis & plasmid preparation • In vitro cell transfection for expression validation	2-3 weeks
DNA Immunization	• DNA immunization via gold particle bombardment with gene gun • Test bleed by ELISA	6-10 weeks
Cell Fusion & Screening	• Animals selected for fusion based on titer • Primary screening by whole cell based ELISA • Customer can evaluate hybridoma supernatants and select the top clones for their application • Additional screening options available	4-6 weeks
Subcloning, Expansion & cryopreservation	• Hybridomas are subcloned by limiting dilution according to the evaluation result from the customer, then expanded & frozen	
Monoclonal Antibody Production (optional)	• Production of mAbs for each cell line with roller bottle culture • Purification • ELISA results	Based on project

GenScript's DNA immunization service has been designed to seamlessly combine with several of our popular custom Ab services including:

- Anti-ID Ab development services – Powerful tools for antibody drug PK/PD and immunogenicity studies
- Custom mAb services – Fully customizable mAb development packages tailored to meet your specific needs
- MamPower™ recombinant mAb services – Guaranteed production service provides 50 mg purified Ab
- Ab scale-up services – High throughput, large-scale Ab production suitable for industrial-size yields

Quotation and Ordering

Get a Quote Now
Via email, phone, or fax

Advantages of GenScript Custom Abs

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

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- Optimized immunization using our OptimumAntigen™ design tool and intelligent Antigen Strategy increasing specificity and affinity of antibodies

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- Guaranteed results: quantity of antibodies or hybridoma, ELISA titer, and WB guarantee (varies with specific package)

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- Optimized immunization using our OptimumAntigen™ design tool and intelligent Antigen Strategy increasing specificity and affinity of antibodies
- 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 600 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)*

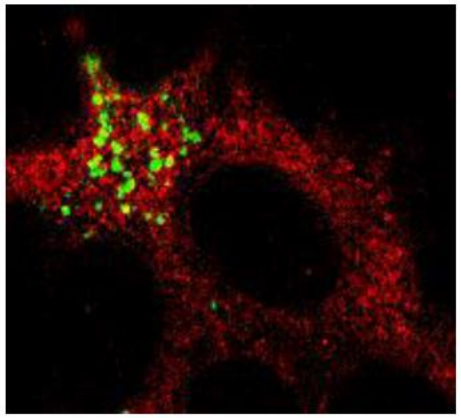
➢**HYPERSENSITIVE TO HIGH LIGHT1 Interacts with LOW QUANTUM YIELD OF PHOTOSYSTEM II1 and Functions in Protection of Photosystem II from Photodamage in Arabidopsis.**
Wang HB, Wang J, Qi K, etc.
***Plant Cell,** (Mar 2014)*

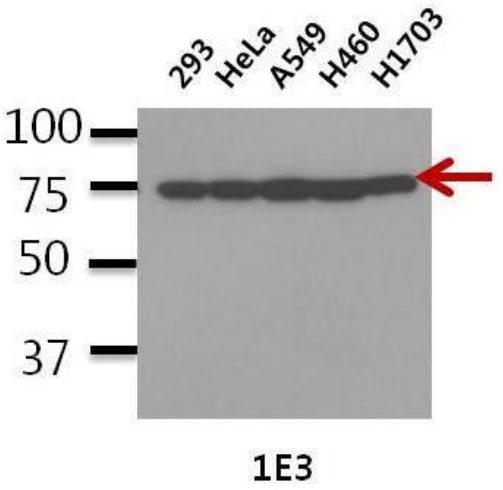
➢**Tousled-like kinases phosphorylate Asf1 to promote histone supply during DNA replication.**
Groth A, Jensen ON, Nielsen ML, etc.
***Nature Communications,** (Mar 2014)*

➢**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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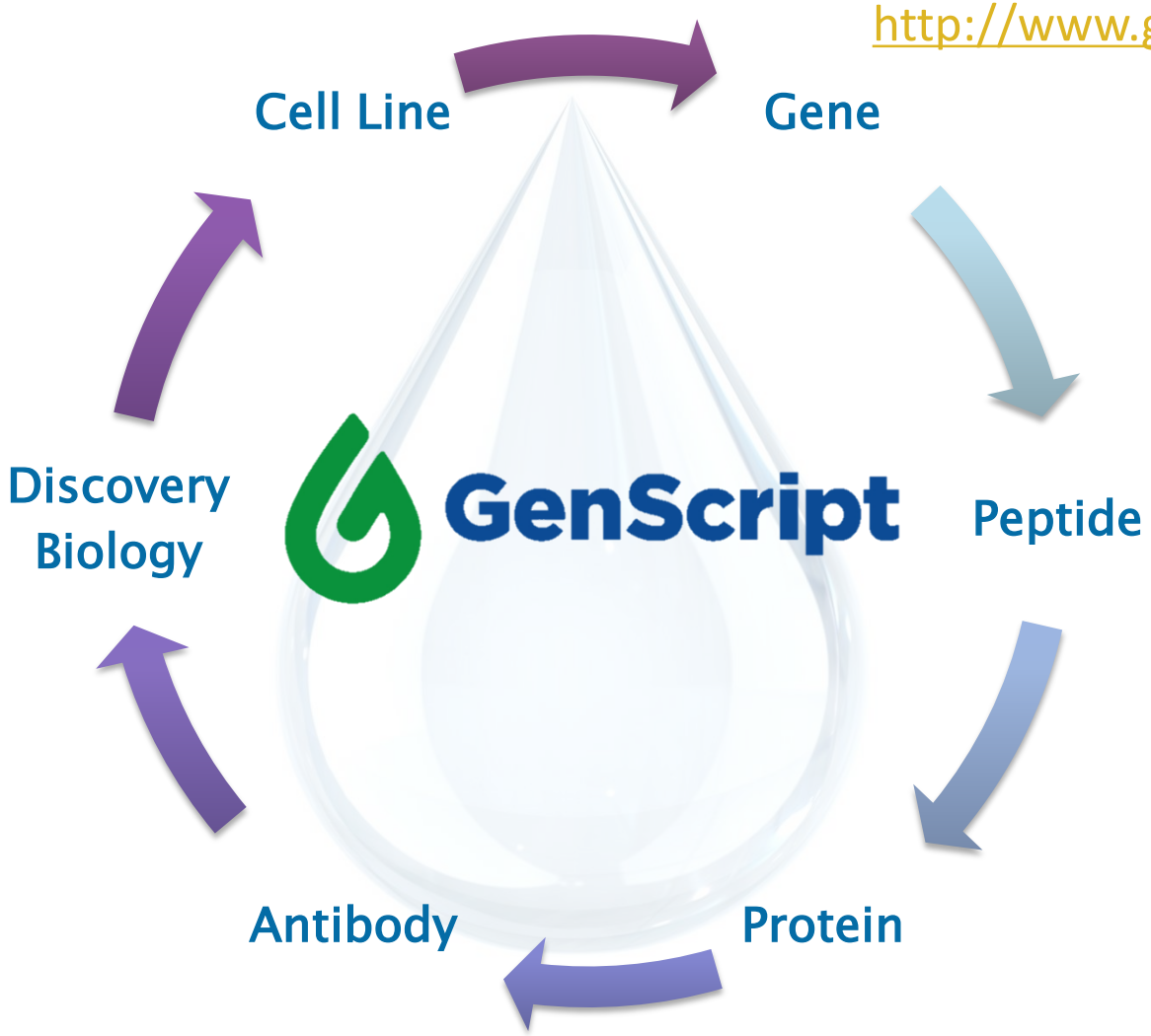
http://www.genscript.com



Q & A

Thank you for your participation

We wish you all success in your research

Email me: Dawei.Sun@GenScript.com

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April 16, 2015/2:00 pm EST

Testing chemotherapy related cognitive dysfunction in animals with GenScript– *Amy Mendenhall, PH.D.*

April 30, 2015/2:00 pm EST

Clone less, know more: efficient expression optimization of proteins and pathways using the RBS calculator – *Prof. Howard Salis, Penn State University*

References

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2. Bertrand Allard, Fabienne Priam, Fre´de´ rique Deshayes, Fre´de´ ric Ducancel, Didier Boquet, Anne Wijkhuisen, and Jean-Yves Couraud(2011). **"Electroporation-Aided DNA Immunization Generates Polyclonal Antibodies Against the Native Conformation of Human Endothelin B Receptor"** DNA AND CELL BIOLOGY : Pp. 727–737.
3. Coralie Alexandrenne, Anne Wijkhuisen, Fatima Dkhissi, Vincent Hanoux , Christophe Créminon, Didier Boquet, Jean-Yves Couraud ((2009) **Generating antibodies against the native form of the human prion protein (hPrP) in wild-type animals: A comparison between DNA and protein immunizations** Journal of Immunological Methods 341 41–49