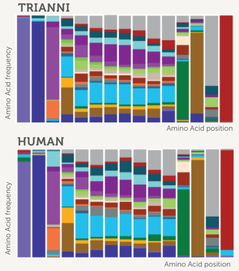
TRIANNI

Exceptional Human Antibody Discovery Technology



CDR-H3 residue utilization in antibodies derived from human samples and the Trianni transgenic Ig Mouse. In the naive Trianni Mouse, heavy chain CDR3 (CDR-H3) aa utilization frequency is effectively the same in humans and in The Trianni Mouse.

Discover Your Next Biologic Therapeutic With The Trianni Mouse™

- Novel chimeric antibody gene segments – each comprised of human coding sequences combined with mouse regulatory genomic sequences
- Expression of a complete repertoire of human heavy and light chain variable domains
- Multiple enhancements to antibody gene segments to improve V(D)J recombination and expression
- Retention of mouse constant domain exons for optimal platform performance
- Designs allow for facile future modifications to loci
- Robust class switching and somatic hypermutation lead to superior antibody diversity

TRIANNI, INC. IS A BIOTECH COMPANY SPECIALIZING IN ANTIBODY DISCOVERY TECHNOLOGY. TRIANNI'S LEAD TECHNOLOGY, THE TRIANNI MOUSE™, IS A POWERFUL, NEXT-GENERATION PLATFORM ENABLING EFFICIENT GENERATION OF FULLY-HUMAN MONOCLONAL ANTIBODIES. TRIANNI'S TRANSGENIC PLATFORM LEVERAGES A NOVEL APPROACH TO DESIGN MADE POSSIBLE BY ADVANCES IN DNA SYNTHESIS AND GENOMIC MODIFICATION TECHNOLOGY MAKING IT A POWERFUL THERAPEUTIC ANTIBODY DISCOVERY PLATFORM.

TRIANNI

MOUSE POWERED PROGRESS

PRODUCING HUMANIZED MONOCLONAL ANTIBODIES WITH TRANSGENIC MICE

The Best Antibody Discovery Technology Is Now at Your Fingertips

Trianni Mouse Antibodies are a Match for Humans

The Trianni Mouse™ platform is the only transgenic antibody discovery platform ever developed that offers the entirety of human antibody variable gene diversity in a single organism.

The V-gene segments in The Trianni Mouse are chimeric, but the variable domains of antibodies made by the mouse are entirely human. The result is human antibody leads generated from antibody genes optimized for function in the mouse. Or, in the simplest terms, The Trianni Mouse is a more human mouse.

To learn more about this innovative platform and how it can help you leave your mark on therapeutic antibody discovery and development, visit **Trianni.com.**

MOUSE-POWERED PROGRESS: PRODUCING HUMANIZED MONOCLONAL ANTIBODIES WITH TRANSGENIC MICE

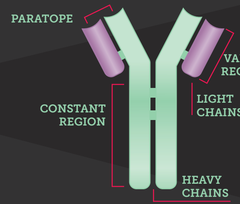
SPONSORED BY: TRIANNI

CUSTOM PUBLISHING FROM: The Scientist — EXPLORING LIFE, INSPIRING INNOVATION

Antibodies drive the human adaptive immune response, allowing for the recognition and elimination of a tremendous range of external threats. Immunotherapy has harnessed the innate properties of the antibody, converting the protein from a threat-recognition agent into a disease-fighting one. New immunotherapeutic strategies such as monoclonal antibody therapy demand new high-throughput production of selective and non-autoimmunogenic custom-engineered antibodies for both research and clinical purposes. Transgenic humanized mice have ascended to the forefront of antibody production, presenting an adaptive research model and a powerful bioproduction host.

Antigen Seekers: ANTIBODY 101

Antibodies (a.k.a. immunoglobulins) are large proteins designed to identify and eliminate pathogens using a "lock-and-key" mechanism to bind specific epitopes expressed by antigens. Antibodies can neutralize foreign pathogenic agents directly (e.g., by impeding mechanisms essential for survival) or label them for removal by other immune components (e.g., macrophages, cytotoxic T cells).



Why All the Fuss? ANTIBODY APPLICATIONS

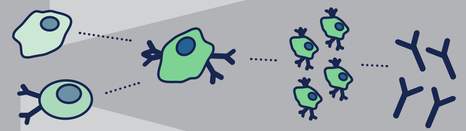
Antibody presence is usually indicative of immune activation, and antibody levels are therefore used as biomarkers in disease diagnosis. Antibodies are also used extensively in laboratories as tags and labels. Lastly, variable region-encoding genes reorganize to generate unique paratopes in a process called V(D)J recombination. By harnessing this, scientists can control antibody specificity and build therapeutic approaches using antibodies targeting markers expressed only by pathogenic cells (e.g., cancer).

Light Chain Heavy Chain? ANTIBODY ANATOMY

The antibody is a Y-shaped protein consisting of two identical light and two identical heavy polypeptide chains, with each chain comprised of a variable and a constant region. The variable regions of the light and heavy chains form a unique antigen-binding site (a.k.a. paratope) for each molecule targeted. This gives the immune system the flexibility to recognize and respond to a wide range of threats.

Harnessing Nature: ANIMAL ANTIBODY

The utility and popularity of antibodies for both research and their therapeutic applications necessitates mass production, and laboratory animals have filled this need since the 1970s.[1] Monoclonal antibody production commonly uses hybridomas. These cells are B lymphocytes that have been conditioned in vivo against the target antigen, then extracted for in vitro immortalization, clonal selection, and culture.



AUTOIMMUNITY and ANTIBODIES

Mice have been the model of choice for monoclonal antibody production owing to their compatibility with myeloma cell lines commonly used for fusion and the subsequently generated hybridomas.[1,2] However, murine antibodies are unsuitable for therapeutics as they trigger immune responses and the creation of "human anti-mouse antibodies (HAMAs)".[3] HAMA formation effectively immunizes the individual against all subsequent therapeutics using murine antibodies.

Bypassing Nature: ANTIBODY HUMANIZATION

To circumvent immunogenicity, scientists have developed "humanized" antibodies. In the beginning, this process entailed the in vitro replacement of antigenic murine framework domains with human equivalents, creating chimeric antibodies with murine paratopes and human structures.[4] Technological advancements have since facilitated the generation of fully human antibodies using transgenic mice.[5]

The TRANSGENIC MOUSE

Transgenic mice allow researchers to create "humanized" mice with human equivalents of murine antibody-coding genes. These mice are fully capable of V(D)J recombination, allowing them to produce antigen-specific antibodies upon challenge.[4] However, the first generation of humanized mice could not produce the entire human antibody repertoire,[6,7] and differences between human and murine constant regions limited antibody class switching/affinity and impeded B-cell differentiation into plasma cells.[7]

The Best of BOTH WORLDS

Transgenic mice that produce chimeric antibodies with murine constant regions and human variable regions combine the production throughput of the mouse with the epitope coverage necessary for human research and therapeutic needs. New transgenic strains possessing longer CDR3 domains offer increased antibody diversity compared to their predecessors. With the usage of transgenic animal-produced human antibodies currently both popular and rapidly growing, these models and others represent powerful and versatile platforms for the generation of new research tools and revolutionary therapeutic agents.[6,7]

Unlocking the HUMAN ANTIBODY REPERTOIRE

To create a mouse capable of producing the full human antibody repertoire, researchers introduced the full complement of human variable region-coding genes from all three loci into their corresponding murine counterparts. In doing this upstream of murine constant region genes, they generated a mouse that produces antibodies with mouse constant and human variable regions – combining proper responses to endogenous immune responses with broad epitope coverage.[7]

1. P.K.K. Liu, "The history of monoclonal antibody development – Progress, remaining challenges and future innovations," *Ann Med Surg (Lond)* 3(4):113-116, 2014. 2. M. Leenaars and C.F. Hendriksen, "Critical steps in the production of polyclonal and monoclonal antibodies: evaluation and recommendations," *ILAR J* 46(3):269-279, 2005. 3. J.J. Tjandra, et al., "Development of human anti-murine antibody (HAMA) response in patients," *Immunol Cell Biol* 68(Pt 6):367-376, 1990. 4. N. Lonberg, "Human antibodies from transgenic animals," *Nat Biotechnol* 23(9):1117-1125, 2005. 5. F.A. Harding, et al., "The immunogenicity of humanized and fully human antibodies: residual immunogenicity resides in the CDR regions," *MAbs* 2(3):256-265, 2010. 6. M. Brüggemann, et al., "Human antibody production in transgenic animals," *Arch Immunol Ther Exp (Warsz)* 63(2):101-108, 2015. 7. L-C. Lee, et al., "Complete humanization of the mouse immunoglobulin loci enables efficient therapeutic antibody discovery," *Nat Biotechnol* 32:356-363, 2014.