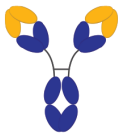
An Introduction to Recombinant Antibody Technology for Diagnostic Applications

White Paper


IgD


IgE


IgM


IgA


Human


IgG1


Mouse


IgG2


Rat


IgG3


Rabbit

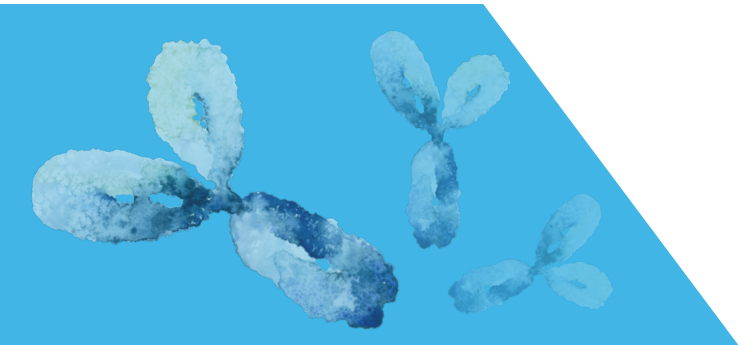

IgG4


Engineered Fc Domain


Fragment


Bispecific


Fc Fusion Protein



absolute antibody

an Absolute Biotech Company

Antibodies have always had the potential to be significant biochemical tools for a range of applications due to their high specificity and selectivity, and they have truly revolutionized the field of biological science over the past 40 years. For research applications, they are excellent tools for the selective detection and/or isolation of antigens and are commonly used in techniques such as Western blots, flow cytometry, enzyme-linked immunosorbent assays (ELISA), and immunohistochemistry (IHC). In addition, the power of antibodies has been harnessed for the treatment of cancer, autoimmune diseases, and other conditions.

Antibodies have also become a critical component of many diagnostic assays used to detect infections, allergies, and other biological markers in the blood. Numerous immunoassays rely on the continual availability and performance of antibodies to ensure accurate results. Without a steady supply of functional antibodies, diagnostic companies are faced with the tough decision to revalidate the immunoassay with a new antibody, or to discontinue it all together. Absolute Antibody appreciates the time, money, and effort committed to developing new and better-performing diagnostic products, and that is why we encourage incorporating recombinant antibodies early in the design process. In this white paper, we will provide an overview of different types of antibodies, introduce recombinant antibody technology, and describe the benefits recombinant antibodies can offer to diagnostic developers.

Antibody Background

Antibody Structure and Isotypes

Antibodies consist of the antigen binding fragment (Fab), which recognizes the antigen, and the crystallizable fragment (Fc), which interacts with other immune system elements to promote removal of the antigen. The basic structure consists of two heavy and two light chains, folded into constant and variable domains (Figure 1).

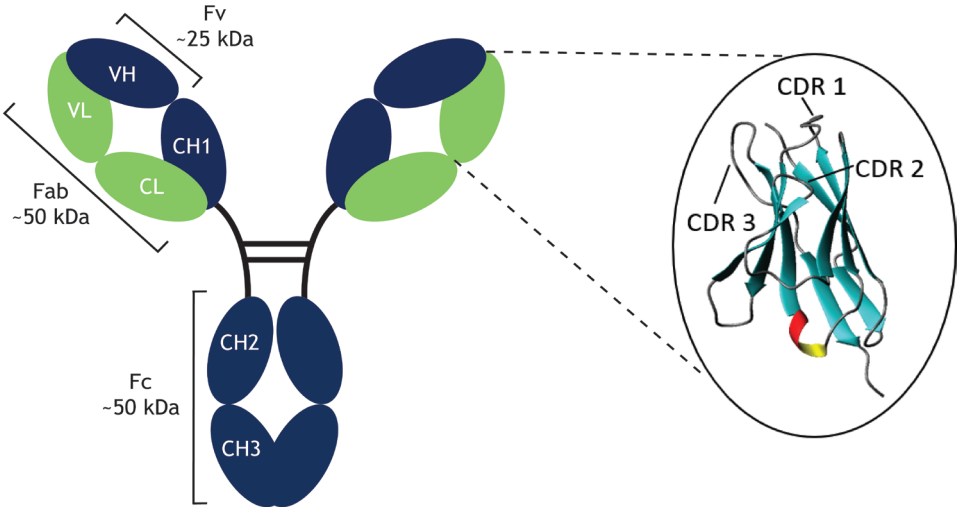


Figure 1. Schematic representation of an IgG. *An antibody consists of two heavy chains (blue) and two light chains (green) folded into constant and variable domains. The enlargement of the variable domain shows a ribbon representation of the β-sheet framework and CDR loops.*

The variable domains (Fv) provide the antigen specificity of the antibody, while the constant domains act as a structural framework (1). Each variable domain contains three hypervariable loops, known as complementarity-determining regions (CDRs), evenly distributed between four less variable framework regions. It is the CDRs that provide a specific antigen recognition site on the surface of the antibody and the hypervariability of these regions enables antibodies to recognize an almost unlimited number of antigens (2).

In mammals, antibodies are classified into five main classes or isotypes – IgA, IgD, IgE, IgG and IgM – (Figure 2) which differ in the sequence and number of constant domains, hinge structure and valency of the antibody (3). IgG is the most abundant antibody in normal human serum, and a common choice for use in immunoassays.

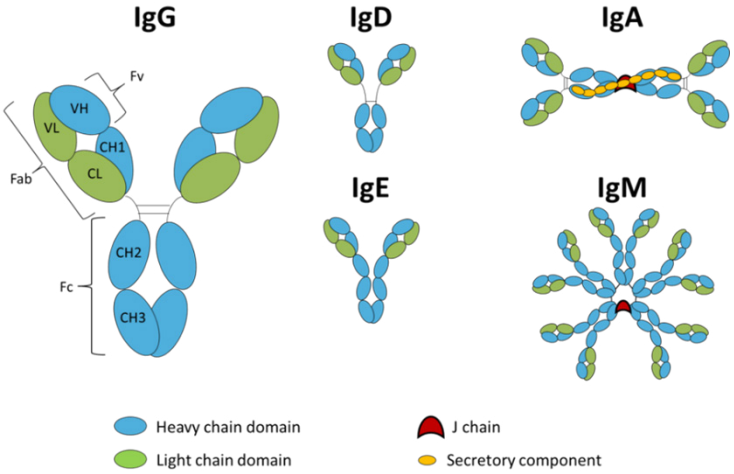


Figure 2. Immunoglobulin isotypes. *Schematic representation of the five immunoglobulin classes or isotypes in mammals.*

Types and Sources of Antibodies

Antibodies can be broken up into two main categories, polyclonal (pAbs) and monoclonal (mAbs). Polyclonal antibodies are a heterogeneous mixture of antibodies directed against various epitopes of the same antigen. Monoclonal antibodies, on the other hand, are a single antibody with specificity for one particular epitope on an antigen (Figure 3). Polyclonals and monoclonals are produced in different ways, and both types of antibodies have been utilized in a variety of diagnostic and research products.

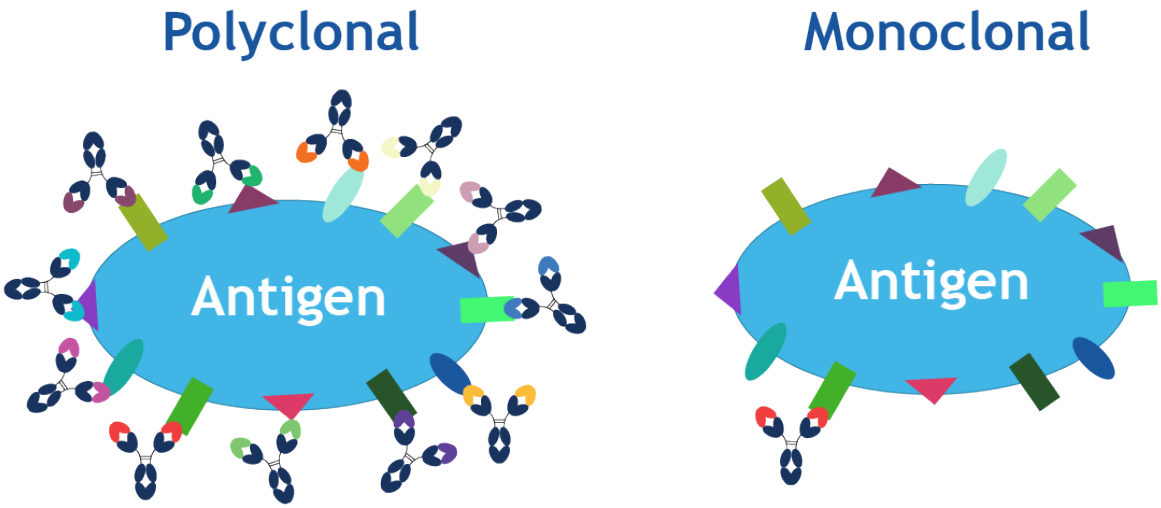


Figure 3. Polyclonal vs. monoclonal antibodies. *Polyclonal antibodies form against multiple epitopes of the same antigen, while monoclonal antibodies form against one specific epitope.*

Polyclonal Antibodies

Polyclonal antibodies are generated by immunizing an animal of choice (rabbit, goat, etc.) with a specific molecule (immunogen) that has been purified and contains the antigen of interest. The animal's immune system will mount a response to the immunogen and begin making antibodies. These antibodies can be obtained by bleeding the animal and collecting the immunoglobulin-rich serum. In most cases, the immunized animal will produce numerous clones of activated plasma cells. Individual clones will produce an antibody that has a different specificity, leading to polyclonal antiserum that is a mixture of antibodies binding to various regions of the antigen. Some of these antibodies may show cross-reactivity with other molecules and will need to be removed through purification steps (4). Once purified, what remains is a heterogeneous mixture of various antibodies with the potential to identify multiple epitopes of the antigen.

The production method of polyclonal antibodies, while cost-effective and requiring less technical expertise, leaves them susceptible to changes in activity and potential loss (5). There is a limit to the amount of viable antibody produced and a finite timeline for its availability. Animals used for polyclonal antibody production typically have short lifespans and, often, an animal producing the polyclonal serum of choice is bled out once the desired antibody is identified. This is done to prevent a shift in antibody expression or risking the loss of the serum. In addition, because of the nature of polyclonal antibodies, in many cases it is difficult to determine which epitope shows the highest binding affinity. This can make purification difficult and complicate the antibody's use in assay development.

Monoclonal Antibodies

Hybridoma Derived

Monoclonal antibodies are a single antibody with specificity for one epitope on an antigen. They were initially produced from hybridomas, which are a culture of cells created by fusing antibody-producing B-cells with immortal myeloma cells (6). A laboratory animal is immunized with an immunogen containing the antigen of choice. After a few weeks, activated B lymphocytes from the spleen are isolated and fused with myeloma cells. Selection occurs for the fused cells and the hybridomas undergo a screening process. The purpose of screening is to select the hybridoma which produces an antibody to the antigen of choice. During the screening process, the hybridoma-producing antibodies to the antigen are identified. Once produced, hybridomas can be cultured *in vitro* allowing the purification of monoclonal antibodies.

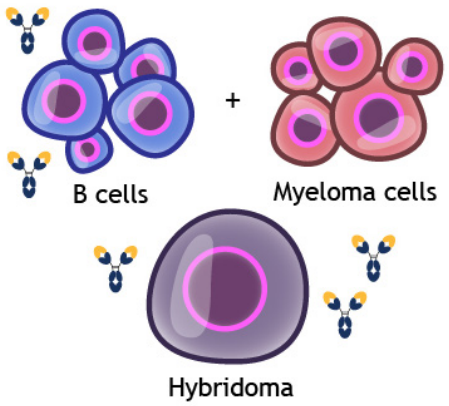


Figure 4. *Fusion of isolated B cells with myeloma cells to create a hybridoma.*

Monoclonal antibodies produced from hybridomas offer more security and longevity than polyclonal antibodies but are still susceptible to loss. Hybridomas can become contaminated or undergo genetic drift, producing antibodies with less specificity and less binding affinity. Cell lines can be struck by catastrophe (freezer failure, fire, etc.) and be permanently lost. They are susceptible to supply chain interruptions and are more difficult to scale up. Additionally, research shows that up to 30% of hybridomas aren't monoclonal (7). Without knowing the genetic sequence there is no way to guarantee the antibodies you are utilizing are truly monoclonal.

Recombinant

A recombinant antibody is a type of monoclonal antibody where the sequence has been identified and then produced synthetically; for example, in human embryonic kidney (HEK) or Chinese hamster ovary (CHO) cells. As recombinant antibodies are defined at the sequence level, you can guarantee production of a truly monoclonal antibody with minimal batch-to-batch variation.

Advantages of Recombinant Antibodies

Recombinant antibodies are monoclonals that are synthetically and consistently produced in serum-free cell lines. Starting with either the hybridoma or antibody protein, the DNA sequence is obtained via Next Generation Sequencing or *de novo* sequencing, respectively. The antibody genes can then be synthesized and cloned into expression vectors using standard molecular biology techniques. This method can be applied to any antibody species and isotype. Once cloning is complete, the antibody can be indefinitely expressed in HEK or CHO cell lines, without fear of genetic drift, contamination, or cell line death.

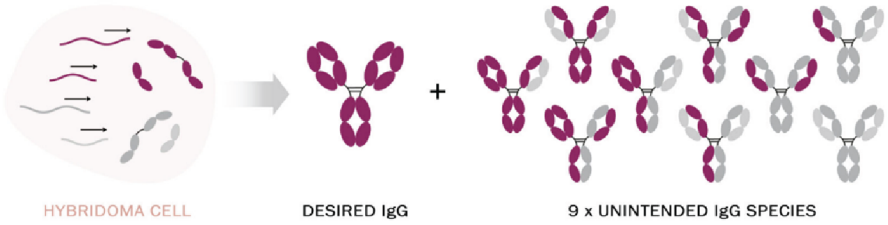
Animal-free recombinant expression of antibodies provides benefits compared to traditionally derived monoclonals from hybridomas, including biological definition, lot-to-lot consistency, and control of supply chain.

Biological Definition

It is a commonly held belief that antibodies produced from hybridomas are always truly monoclonal. In a 2018 paper from Bradbury et. al. (7), researchers sought to put this assumption to the test, sequencing and analyzing 185 randomly selected hybridomas. Of those 185, 59 were identified as containing one or more additional productive heavy or light chains. This means that more than 30% of the hybridomas sequenced were not truly monoclonal (Figure 5). After ELISA and immunohistochemistry analysis, the authors concluded "the expression of additional chains degraded properties of the antibodies, including specificity, binding signal and/or signal-to-noise ration." This degradation can be detrimental for antibodies used in research, therapeutics, and diagnostics. In comparison, recombinant antibodies are defined at the sequence level and production guarantees high purity monoclonal antibodies, without variable biological definition.

Most monoclonal antibodies are produced from hybridoma cells, formed through fusion of an antibody-producing B-cell with an immortal myeloma cell. These cell lines should transcribe one set of antibody heavy and light chains, and secrete one type of antibody.

Unfortunately, almost a third of hybridomas express an additional antibody heavy or light chain transcript. These aberrant transcripts are often derived from the myeloma fusion partner but can also be caused by multiple fusion events.

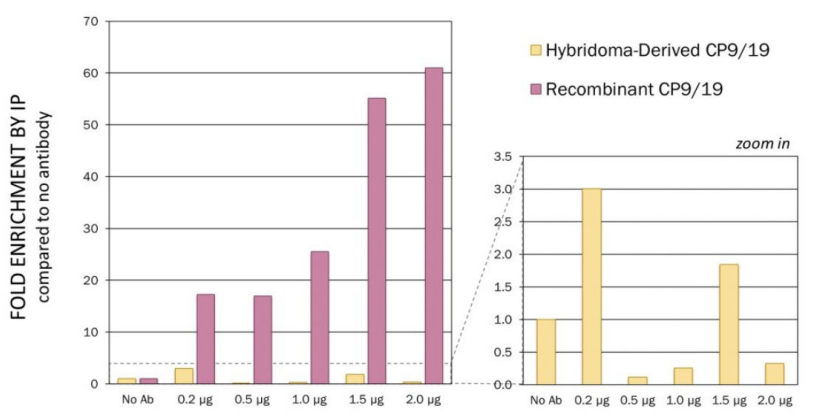


When both an additional heavy and light chain are expressed, the desired IgG becomes one of 10 possible antibody variants – all produced from one 'monoclonal' cell line.

Figure 5. *More than 30% of hybridomas have been shown to not be monoclonal.*

Lot-to-Lot Consistency

Batch-to-batch reproducibility is paramount for antibodies that are used in the development and manufacturing of diagnostic products. Any variation in the antibody/antigen interaction can be detrimental to the function and subsequent results of the test. Monoclonal antibodies produced by hybridomas are susceptible to genetic drift and contamination (Figure 6), which can lead to reduced, or even a complete loss of, antibody production. Recombinant antibodies do not fall victim to these same pitfalls. Because the sequence is genetically defined, there is no risk of genetic drift or change in expression of recombinant antibodies across batches and different lots. Additionally, since the genetic material needed to produce the antibodies is not confined to a specific cell line, contamination and cell line death are not major concerns in recombinant antibody production.



A popular CP9/19 hybridoma, originally a rat IgG2a, had historical contamination with mouse antibody. Absolute Antibody identified the correct sequence and the antibody was recombinantly expressed in our proprietary platform. The recombinant version showed significantly improved performance compared to a hybridoma-derived version from a competitor.

Figure 6. Saving a Contaminated CP9/19 Hybridoma. *Left: Recombinant CP9/19 shows a higher fold enrichment compared to hybridoma-derived CP9/19. Right: Scaled to show poor performance of hybridoma-derived CP9/19.*

Control of Supply Chain

For diagnostic companies, having a secure supply chain of high-quality reagents is critical for withstanding unforeseen circumstances. Recombinant antibody technology has historically been reserved for therapeutic companies in their development of novel biologics; however, the technology at its core mitigates manufacturing risk by "digitizing" antibody assets and guaranteeing long-term reproducible production. Implementing this technology offers supply security in several ways:

- **Decreased reliance on a physical asset.** Sequencing the hybridoma or antibody protein results in the antibody no longer being physically or geographically constrained to one facility. Digital antibody sequences can be easily accessed by multiple stakeholders around the world, allowing companies to exploit the flexibility of regional supply chains. The same exact antibody can be manufactured anywhere in the world, even if the original antibody is lost, mutated, or contaminated.
- **Flexibility to make or buy.** Make versus buy is a question faced by every manager from large international companies to small startups. Laboratory resources should be focused on core competencies in order to maximize output. Recombinant antibody technology allows for the outsourcing of antibody manufacturing with ensured bath-to-batch reproducibility.
- **Agile response to reagent requirements.** One of the biggest benefits of recombinant antibody technology is the ability to quickly change antibody formats, isotypes, subtypes, and species with minimal effect on binding specificity. This opens up possibilities never before considered when working with hybridoma technology and allows diagnostic developers to reformat antibodies as needed for optimal assay performance.
- **Manufacturability and ability to scale up.** Manufacturability of an antibody, and the ability to scale up production, are important components to consider early in the development phase. In some instances, developability concerns can potentially be engineered out during the early-stage research phase. Manufacturability encompasses a range of properties, such as expression titer, aggregation, long-term stability, and solubility, and must be optimized for large-scale production.

Recombinant Antibody Technology Overview

Recombinant antibody technology has been utilized by the therapeutic and biotechnology industries for decades. The ability to engineer custom antibodies and consistently produce them indefinitely has proven to be invaluable. Diagnostic developers have taken notice of the benefits of recombinant antibodies and the importance of utilizing them early in the research and design process. This next section will provide an overview of the three main areas of recombinant antibody technology, sequencing, engineering, and expression, and demonstrate how recombinant antibody reagents can be used to further diagnostic capabilities.

Antibody Sequencing

The first step in utilizing recombinant antibody technology begins with identifying the genetic sequence of the antibody. This process not only lays the foundation for future antibody engineering and expression opportunities; it also protects against antibody loss and allows for control of antibody supply. Once the sequence is known, the antibody can be recombinantly produced indefinitely. Sequencing of hybridomas can be completed using PCR or NGS methods, while *de novo* sequencing is used for monoclonal and polyclonal antibody protein.

Hybridoma Sequencing

Many common hybridoma sequencing services use traditional PCR and Sanger sequencing that provide quick turnaround for a low cost. These methods amplify sequences using PCR primers designed on the assumed species and isotype of the antibody. As a result, these methods can only provide one heavy and one light chain. If those are not the correct variable domains, the problem would not be recognized until the expressed antibody did not perform as expected. In order to combat these shortfalls, a few clones from each antibody will be sequenced for confirmation. This means that after running a PCR reaction, and subcloning into sequencing vectors before transformation into bacteria, a few colonies will be sequenced to confirm the correct sequence is identified. This is to exclude any single nucleotide polymorphisms introduced by the PCR and to also exclude any occurrence of non-clonality from additional transcripts. When this method is used, it is a gamble on the relative abundance of the truly active transcript versus any aberrant transcripts. The more aberrant transcripts present, the likelihood decreases that the active transcript is identified.

Next Generation Sequencing (NGS) involves sampling millions of reads with hundreds of thousands mapping to the antibody transcripts. This allows for the delivery of all variable heavy (VH) and variable light (VL) domains of the hybridoma cell lines, regardless of species or isotype. A full report characterizing the hybridoma's sequences and heterogenicity or other antibody isotypes that may be present is generated, which allows for the correct variable domains to be selected prior to moving forward with an antibody candidate.

Additionally, NGS offers several key advantages compared to traditional methods, including high-throughput sequencing of more than 50 hybridomas concurrently, ability to sequence any species or isotype, requiring less cells (100,000) than PCR (>500,000), and the capability to rescue contaminated or non-viable cells.

Figure 7 below represents a hypothetical scenario where each yellow line represents a colony with the truly active transcript. Next Generation Sequencing is the superior option for ensuring the correct sequence is identified and recombinantly expressed.

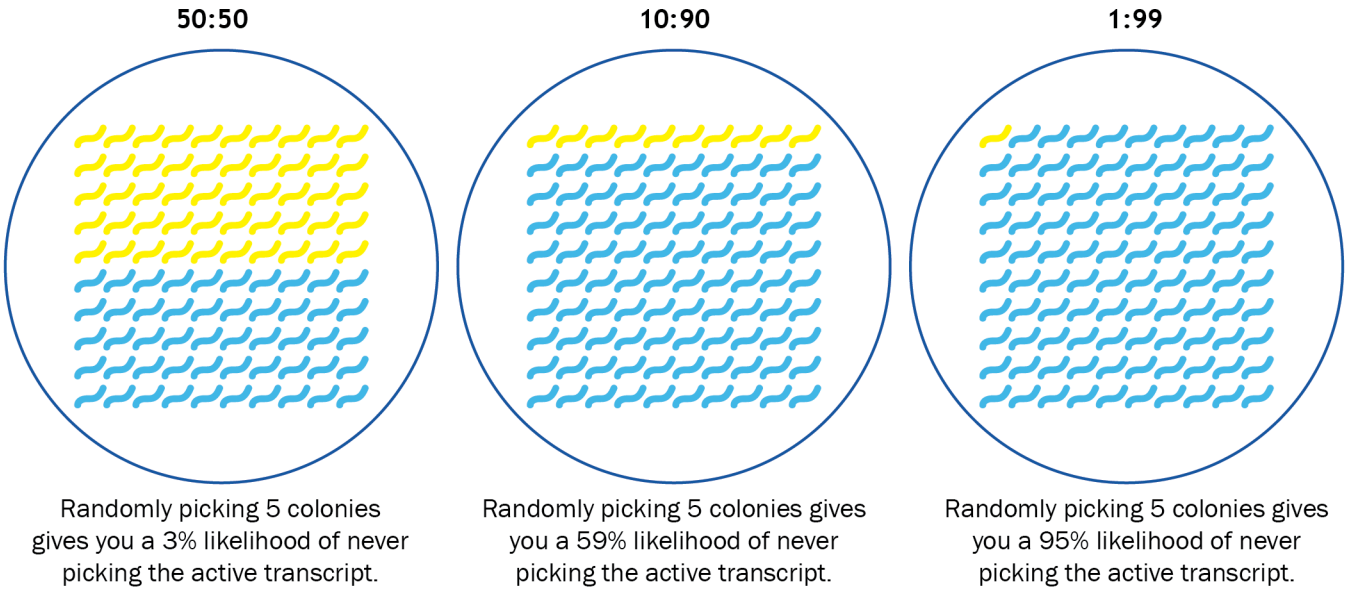


***Figure 7.* Hypothetical hybridoma sequencing scenario.** *With PCR and Sanger sequencing, the typical process is to sequence a few clones for each antibody to confirm the sequence. This method does not account for aberrant transcripts; the more aberrant transcripts present in the sample, the more likely you are to miss the active transcript entirely.*

Antibody Protein Sequencing

De novo sequencing technology is used to sequence both monoclonal and polyclonal antibody proteins and delivers complete antibody sequences, including CDRs. The process involves:

1. Starting with the purified antibody, the sample undergoes multi-enzyme digestions.
2. The fragments are measured with LC-MS/MS to derive the *de novo* peptide sequences.
3. These fragments are then analyzed to assemble full-length antibody sequences of any isotype.

Sequencing monoclonal and polyclonal antibody protein can allow diagnostic and reagent companies to rescue invaluable antibodies. Antibody-producing hybridomas can become contaminated or be lost due to genetic drift, cell death, or even a freezer failure. Polyclonal antibodies are susceptible to many of these same risks in addition to the loss of the antibody-producing animal. A case study from Absolute Antibody demonstrates the *de novo* sequencing, expression, and characterization of two monoclonal antibodies from a popular goat polyclonal antibody from protein only (Case Study page 15).

Antibody Engineering

After the antibody sequence has been obtained, it can then be used in antibody engineering. Antibody engineering allows for the modification of antibodies to build a reagent better suited for use in diagnostic applications. Researchers and scientists are utilizing a variety of engineering options in the early development stages to improve the overall behavior of their antibodies, specifically:

- Isotype Switching
- Species Switching
- Humanization
- Antibody Reformatting

Isotype Switching

Isotype switching, also referred to as class switching, allows you to alter the isotype of any antibody (Figure 8). Since some isotypes are prone to aggregation, class switching can help overcome this obstacle and even improve stability. Converting an IgG to an IgM is used to increase an antibody's avidity, while converting an IgM to an IgG can increase manufacturability and stability. Isotype switching can also reduce the number of needed controls in an experiment.

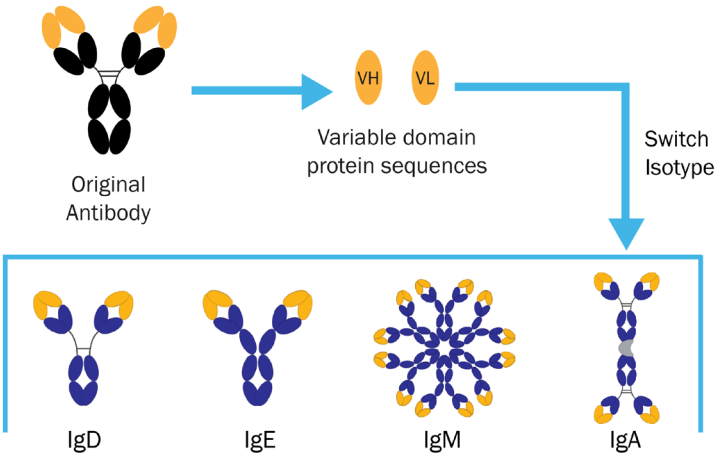
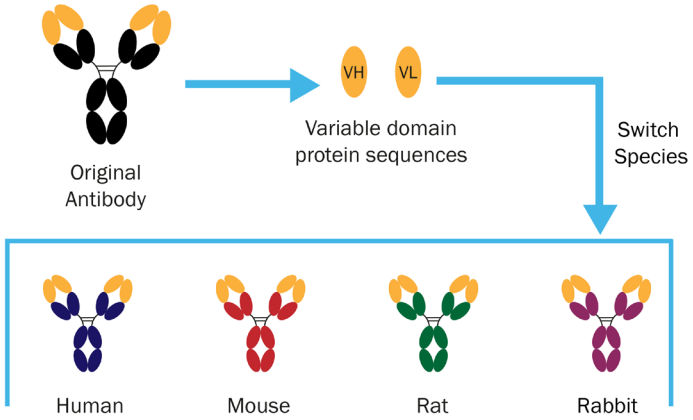


Figure 8. *The binding specificity of the variable heavy (VH) and variable light (VL) chains of the antibody are maintained and engineered onto the backbone of a different isotype.*

For example, an IgG antibody, the major antibody of the secondary immune response, can be reformatted to an IgM antibody, the predominant antibody of the primary immune response, to aid in infectious disease research and diagnostic assay development. During the early stages of the COVID-19 pandemic, Absolute Antibody was able to rapidly reformat our anti-coronavirus spike glycoprotein CR3022 antibody into human IgG, IgA, and IgM versions. These antibodies were widely used for research and as serological controls in diagnostic assays, leading to the WHO including them in their guidelines for secondary standards (8).

Species Switching

Species switching, also called chimerization, involves reformatting an antibody's variable regions to the antibody backbone of a different species, while maintaining the original specificity and affinity (Figure 9). Chimeric antibodies have grown in popularity for *in vitro* research due to the ability to increase compatibility with a secondary antibody and enable easier co-labeling studies. For diagnostic applications, chimeric antibodies reduce the risk of non-specific binding to heterophilic antibodies, such as human anti-mouse antibodies (HAMA), that can cause false positive assay results.



Additionally, species switching prevents unwanted antibody interactions in serological assays, allows for the development of serological controls for human or veterinary use, and permits the standardization of Fc domains to streamline conjugation and immobilization protocols. Chimerization also enables the creation of a panel of antibodies from different species to increase options for multiplexing.

Figure 9. *The binding specificity of the variable heavy (VH) and variable light (VL) chains of the antibody are maintained and engineered onto the backbone of a different species.*

Humanization

Antibody humanization is an engineering process that involves transferring critical non-human amino acids onto a human antibody framework (Figure 10). This primarily includes the grafting of amino acids in the complementarity-determining regions (CDRs) but also other framework amino acids critical for the variable heavy:variable light (VH:VL) interface and CDR orientation (9).

Humanized antibodies have shown promising potential for use as serological standards for diagnostic applications. For example, researchers in the UK sought to compare 16 different humanized antibody variants targeting Crimean-Congo hemorrhagic fever virus (CCHFV) and assess their function (10). Indirect ELISA was performed to compare the binding of fully humanized antibodies to that of the chimeric control, with four of the humanized variants showing activity within two-fold of the chimeric version. Serological assays utilizing humanized antibodies could help "...address a shortfall in available tests that meet regulatory diagnostic standards...". Since humanized antibodies are recombinantly expressed, they can be consistently produced and aid the development of standardized serology tests.

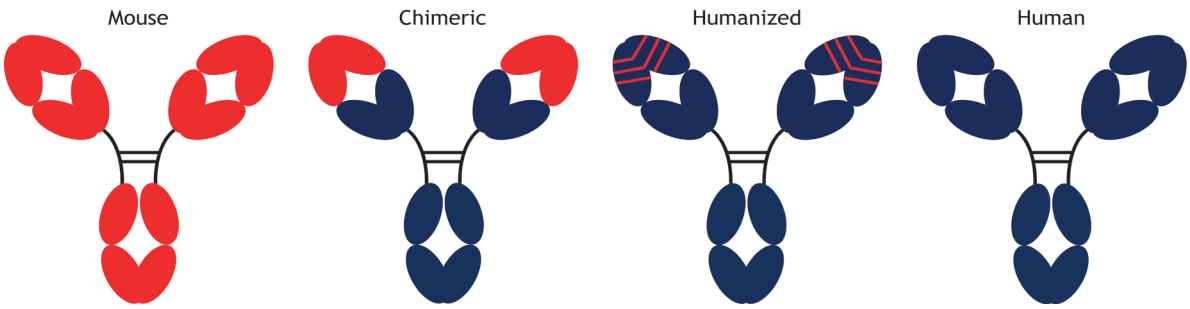


Figure 10. Progressive humanization of antibodies. *A schematic representation of the advancement from fully mouse antibodies, represented by red domains, to fully human antibodies, represented by blue domains. Note these images are somewhat misrepresentative, as immunoglobulin sequences are highly homologous across species, meaning a so-called fully mouse antibody is still relatively close in sequence to a fully human one.*

Antibody Reformatting

There are a variety of engineering capabilities available when it comes to reformatting an antibody (Figure 11). Common options for diagnostics developers include:

- **Fc silencing**: Fc silencing involves engineering the Fc domain of an antibody to have minimal to no binding to Fc receptors. This is beneficial for *in vitro* applications to remove background staining.
- **Fragments**: Antibody fragments are produced by converting IgG into smaller, non-Fc containing fragments. The original variable domains can be used to create formats with a range of valencies, including Fab2, Fab3, and Fab4, which can lead to greatly enhanced assay sensitivity. These multivalent formats show high avidity to the antigen and can see 1 to 4-log increases in binding.
- **Fc fusion proteins**: Fc fusion proteins are composed of the Fc domain of an IgG genetically linked to a peptide or protein of interest. *In vitro* applications of Fc fusion proteins include, among others, ELISA, flow cytometry (FC), protein binding assays, and use as microarray baits. In these applications, the Fc domain behaves as a supporting module to which proteins can be attached while retaining their native biological activity.
- **Multispecifics**: Bispecific and multispecific antibodies are a class of engineered antibody that combines two or more different specific antigen-binding elements in a single construct. These antibodies can be used in diagnostic applications to dual target, which would allow the antibody to detect or bind to a target cell type with much higher specificity than monospecific antibodies.

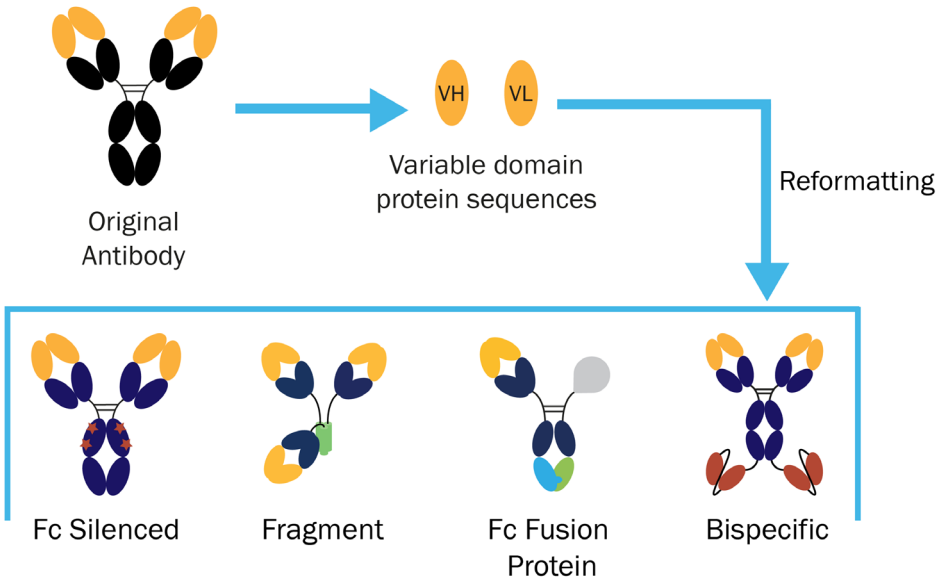


Figure 11. *The binding specificity of the variable heavy (VH) and variable light (VL) chains of the antibody are maintained and engineered onto various formats.*

Antibody Expression

The antibody has been sequenced and engineered to improve functionality, and now it is time to express it. The production of recombinant antibodies involves cloning the antibody sequence into vectors, generating plasmid DNA for transfection, transiently transfecting cells, and finally expressing and purifying the antibody. Absolute Antibody recombinantly expresses antibodies by utilizing a serum-free mammalian expression system that generates high purity antibodies in an absolutely defined protein concentration, with all production occurring in our ISO 9001:2015-certified manufacturing facility in the UK. This animal-free method allows for the consistent and continual production of antibodies without the risk of genetic drift, contamination, or cell line death.

There are two main mammalian cell line options for transient expression, CHO and HEK293. Transient expression is a more affordable and rapid alternative to stable cell line generation and is ideal for early-stage testing. While stable cell line generation typically takes six months to a year, transient expression platforms can produce milligram-to-gram quantities of high-quality recombinant antibodies in engineered formats within a month. Once the ideal antibody variant is identified, production can then be scaled up to stable cell lines which can yield several grams of antibody protein per liter.

CHO (Chinese hamster ovary) cell lines are the preferred platform for therapeutic antibody expression. CHO expression is ideal for research projects in which minor differences between HEK and CHO-made antibodies may be significant, such as half-life, potency, and glycosylation studies. Transient CHO production, at milligram-to-gram scales, is particularly useful for bridging the gap to stable CHO production, or for tackling challenging constructs.

HEK293 (human embryonic kidney) cell lines are relatively easy to work with and have historically produced higher protein production yields than CHO. Expression in a HEK platform is a cost-effective option for early-stage antibody development, offering high-throughput recombinant production ideal for screening antibody candidates. Additionally, HEK293 platforms may provide improved expression for antibodies that are difficult to produce in CHO (11). Becasue of these attributes, HEK293 is the expression system of choice for reagent and diagnostic antibodies.

Recombinant Reagents

At Absolute Antibody, we have applied our antibody expertise to create a rapidly growing catalog of unique engineered antibodies and Fc Fusion proteins, all recombinantly produced to ensure batch-to-batch reproducibility. Of particular interest to assay developers are our recombinant free light chains; recombinant polyclonal anti-IgG antibodies; serological controls as an alternative for rare sera; and a wide variety of antibodies for allergy, virology, and autoimmunity research. All antibodies are available off-the-shelf in a variety of species, isotypes, and formats.

Free antibody light chains are important diagnostic markers for a range of disorders including amyloidosis, various myelomas, and lymphomas. Absolute levels as well as imbalances in the ratio of free kappa and lambda chains are used as diagnostic and prognostic markers. The recombinant expression platform used by Absolute Antibody allows the direct expression of free antibody light chains, overcoming the common problems faced in purifying these proteins. Our serum-free culturing system and affinity purification result in ultra-pure products that may be used as calibrators and positive controls for diagnostic assays (Figure 12).

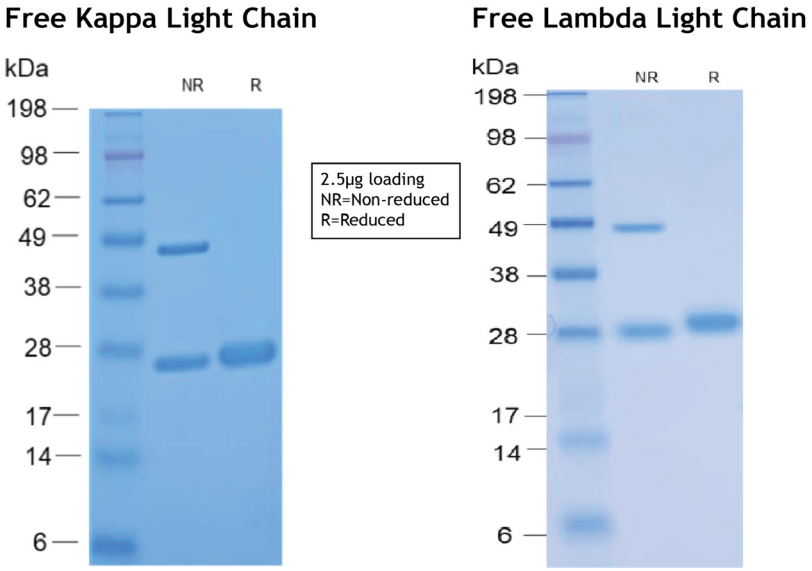


Figure 12. *Coomassie-stained SDS-PAGE gel of human recombinant free kappa (left) and lambda (right) light chains. Starting from the tissue culture supernatant, a single purification step was perfomed to enrich for recombinant free light chains, both as monomer and dimer (at around 28 and 50 kDa, respectively.)*

In addition, engineered recombinant antibodies can help overcome issues obtaining serological controls. Calibrators and control sera in diagnostics are generally formulated using untreated human sera from patients, but these samples may contain pathogens, pose a biohazard risk, can vary greatly across lots, are finite, and be difficult to obtain. Instead, controls can be formulated using artificial or treated human sera and recombinantly produced antibodies, which minimizes the need to work with potentially infectious materials and enhances the lot-to-lot standardization of the controls. These artificial controls have high batch-to-batch reproducibility which provides absolute definition of the standards and can quantify the results of the assay.

Conclusion

As the popularity of recombinant antibody technology grows in the diagnostics industry, so do the opportunities to enhance antibody functionality. Knowing the genetic sequence of an antibody, whether that is through NGS or *de novo* sequencing, is crucial to the protection and longevity of the antibody and subsequent assets. The sequence can be safeguarded should catastrophe strike, or used to engineer an improved antibody format that demonstrates better performance and manufacturability. Expressing the antibody in a serum-free mammalian cell line allows for large-scale and indefinite production of the antibody without risk due to genetic drift, cell death, or contamination. Absolute Antibody has been the industry leader in recombinant antibody technology for more than a decade and can provide expert consultation for your next antibody project.

Case Study: Sequencing and expression of monoclonal antibodies from a polyclonal goat antibody

Methods

The goat antibody was an anti-AIF1 polyclonal antibody (GT-1) that was raised against AIF1 peptide in Nepalese goats. The protein sample underwent digestion using multiple enzymes and different peptides were *de novo* sequenced by LC-MS. Heavy and light chains were assembled, paired and isoleucine/leucine amino acids were identified. This resulted in the full-length sequences for two monoclonal antibodies, which were distinct in both framework and CDR regions. Both monoclonal antibodies were recombinantly expressed in HEK293 cells, and purified by Protein A chromatography.

Results

The two recombinant monoclonal antibodies were tested against the peptide antigen by ELISA and showed activity comparable to the original polyclonal. Irrelevant monoclonal or polyclonal antibodies of the same isotypes showed no activity (Fig 13A). The two monoclonal antibodies were shown to be non-competitive with each other, and their binding was efficiently blocked by the original polyclonal antibody, proving that they bind distinct epitopes and that they capitulate binding characteristics of the input polyclonal antibody (Figure 13B).

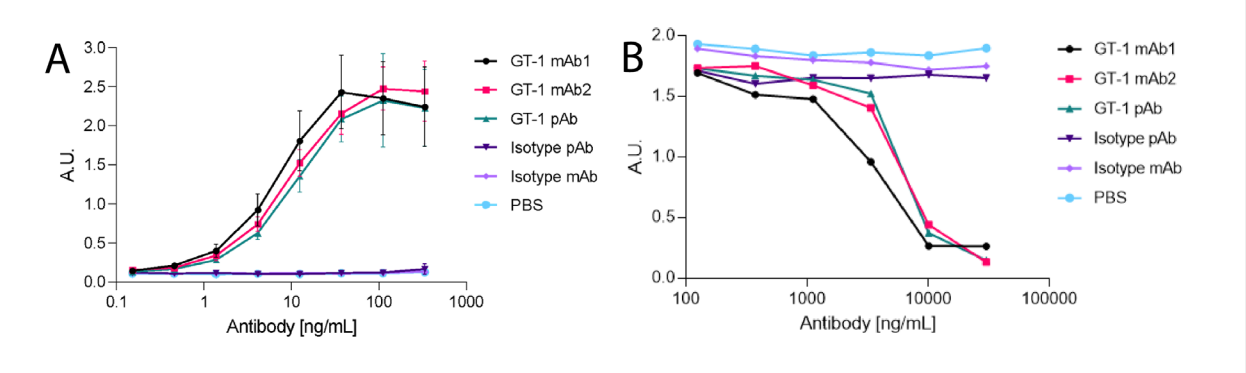


Figure 13. *A) Indirect chromogenic ELISA of monoclonal and polyclonal antibodies titrated against AIF-1 peptide (2.5 ug/ml) and detection by HRP-conjugated anti-goat secondary antibody. B) Competition ELISA of directly HRP-conjugated GT-1 against unconjugated versions, and polyclonal antibody.*

Conclusion

The data demonstrate that advances in antibody protein sequencing technology have made it possible to sequence a polyclonal antibody protein sample and obtain highly functional recombinant monoclonal antibodies from the sequence. This will allow the conversion of important polyclonal antibodies used in research and diagnostics into infinitely reproducible, reliable, and animal-free recombinant antibodies. This work will allow reagent manufacturers and diagnostic developers to transition their reagents into a more reproducible and ethical era, without sacrificing reagent performance.

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