BIO BENCH
Boost Research.

Therapeutic Antibody Discovery Handbook

Support biopharmaceutical for a healthier future

Human Antibody Discovery

Alpaca Antibody Discovery

Single Domain Humanized Antibody Discovery

Common Light Chain Human Antibody Discovery

Mouse/Alpaca Immunized Antibody Discovery

Who is BioBench?

BioBench – A one-stop-shop research service and product provider that specializes in the development of functional antibody biologists in 2013, BioBench has organized research teams which are equipped with leading instruments as well as solid Our passion: accelerate life science research for a healthier future!

BioBench Service Modules and Blocks

Small Scale Protein Expression and Purification

- Multiple expression hosts: E.coli, B.Subtilis, Yeast, Insect cell, Mammalian cell.
- Bioinformatic tool to optimize the codon usage.
- Cell/Bacteria culture volume from mL to XL.
- Protein expression at mini scale – μg to XXmg for preliminary study.
- Combination of purification methods: affinity purification, ion exchange, size exclusive, hydrophobic, etc.. Protein polishing up to 99.9% purity.

Research Stable Cell Line / Cell Pool Establishment

- Mammalian cell line for protein over expression.
- Mammalian cell line and cell pool for cell function, signaling pathway study and/or antibody drug discovery.
- Membrane protein cell line.
- Cell line tested by different methods: flow cytometry, whole cell ELISA, WB, fluorescent microscopy, HCS, etc.
- Tandem protein purification methods to enhance the protein purity.

Antibody Discovery

- Multiple choices for monoclonal antibody hosts: Llama, Mouse, Human, Rabbit and Chicken.
- Multiple choices for polyclonal antibody hosts: Llama, Mouse, Goat, Rabbit and Chicken.
- Multiple immunization methods: peptide, protein and/or gene.
- Monoclonal antibody through hybridoma method and/or phage display based antibody library.
- Antibody production from μg to XXg.
- mAb sequencing and cell line establishment.

Bio Bench Service has helped our clients:

> More than 3000 antibody projects delivered.

> More than 10 000 lots of purified protein delivered.

> More than 200 drug antibody discovery projects delivered.

> More than 200 in vivo study for antibody drug candidates were finalized.

and biologically active protein for drug discovery, IVD kit and life sciences research. Founded by a group of innovation-driven know-how.

Antibody Drug Discovery, in Vitro and in Vivo Study

- Qualified antibody library for drug discovery: 100-billion human antibody library, 10-billion human antibody library, 100-billion humanized alpaca nanobody library, 100-billion humanized common light chain antibody library and mouse antibody library.
- pM to sub nM extreme high affinity binders.
- Antibody engineering to improve drug candidate performance.
- Antibody physico-chemical characterization, in vitro analysis, in vivo animal model.
- Drug candidate cell line establishment according to FDA requirement.

Midi Scale Protein Production

- Large volume fermentation for E.coli, insect cell and Mammalian expression hosts.
- Protein expression at midi scale – XXXmg to XXXg for pilot study and/or production.
- Midi scale purification process: affinity purification, ion exchange, size exclusive, hydrophobic. Protein polishing up to 99.9% purity.
- Protein physico-chemical characterization.
- Protein bioactivity characterization.

Protein Production Bacteria Strain / Cell Line Establishment

- E.coli, Yeast and Mammalian high production strain/cell line establishment.
- Pre-CDMO development for antibody drug development (licensed cell lines).
- PCB/WCB establishment and test.
- Mammalian cell line for high production protein.
- Multiple methods for cell line characterization.
- USP (Up-Stream Process development) for antibody drug projects.

Protein/Antibody Biological Activity Test Platform

- Immunology method based assays, including: PAGE and Western Blotting, ELISA, Immuno-Fluorescence assay, Flow Cytometry, High Content Screening assay, Immunohistochemistry, etc..
- Antibody affinity test, epitope mapping, or other binding/blocking assay by Biacore or ForteBio.
- Imaging based assays, like cell proliferation assay, scratch-wound assay.

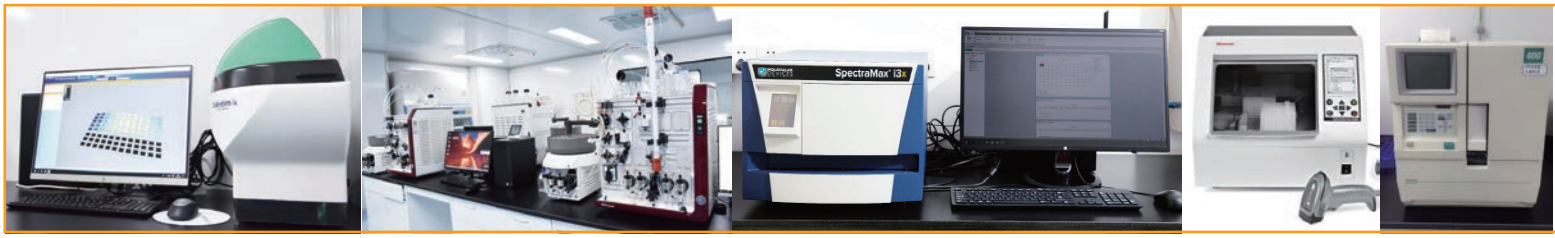
Protein/Antibody Physiochemical Test Platform

- UV, HPLC, LC-MS and MS instruments and methods to test protein concentration, purity, sequence, bonding and residues.
- CEX and iCIEF instruments and methods to test protein charge and isoelectric point.
- UPLC-FLD and MS instruments and methods to test Oligosaccharide and sialic acid.
- DSF instruments and method to test thermostability of the protein.
- HCP analysis by qrt-PCR.

Antibody Engine

- Bioinformatics software, da analyze your antibody sequ 3D.
- Antibody drug proven engi strategy to ensure the maxi
- Plenty of applications supp ization, affinity maturation, pH sensitivity, etc..
- Biacore and/or ForteBio in to validate the result.

BioBench



In Vivo Test Platform

- Mouse model from credited provider and raised in certified lab, SPF or higher.
- Well trained person to operate animal.
- Method from credited source and adjusted by our biopharma industry experience.
- Data analysis by senior scientists team with rich biopharma knowledge.

Cell Line Establishment and Validation Platform

- Flow cytometry cell sorter, single cell imaging instruments and methods.
- ELISA, immuno-fluorescent imaging or function assay to confirm the cell line.
- Cell bank establishment and test.
- 1L – 3L fermenter for medium scale cell culture and upstream process development.

ineering Platform

e, database and method to
sequence in both 2D and

engineering and screening
naximum output.

upported: antibody human-
tion, functionality diversity,

o instruments and method

Phage Display Antibody Discovery Platform

- Pre-established 100-billion and 10-billion human antibody library, qualified for antibody drug project.
- Pre-established 100-billion and 10-billion Alpaca antibody library, qualified for antibody drug project.
- Customizable antibody library for client's specific project: Alpaca, mouse, rabbit and chicken.
- Advanced antibody screening method to ensure maximum output.

In Vitro Test Platform

- ADCC, ATCC, CDC assay.
- Protein/Cell binding, blocking assay.
- Luciferase assay.
- Scratch wound healing assay.
- Cell proliferation assay.
- Cell line purchased from credited source with traceable record.
- Flow cytometer, ELISA, cell imaging and HCS instruments and the methods.

Research Platforms

Elementary Molecular Biology Platform

- Plasmid construction, subcloning and extraction.
- DNA electrophoresis, imaging, quantity test and recovery.
- Protein electrophoresis, quantity test and gel recovery.
- Sample preparation for down-stream assays.
- Plasmid transformation and transfection instruments and reagents.

Protein Production Purification and Modification Platform

- Small to mini scale incubator for culture volume from mL to XL.
- Scale up fermentation volume, 500L for bacteria, 200L for mammalian cell.
- AKTA purification instruments with multiple column selections, from affinity purification, ion exchange purification, size exclusive purification and hydrophobic purification.
- Essential protein operations: tag removal, protein conjugation, endotoxin removal, etc..
- Essential protein modification like FITC.

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CONTENT

Content

Drug Potential Antibody Discovery

Antibody Drug Discovery Platform: An Overview

Background

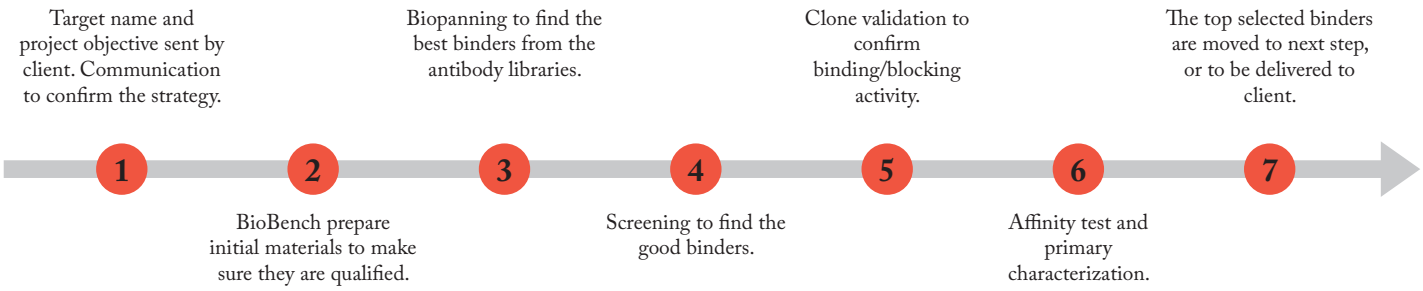
In the process of discovering drug potential antibody, there are plenty of pain points that all biopharmaceutical companies face, such as long project timeline, extremely heavy workload for antibody screening/selection, and a low number of good lead antibodies. When it comes to difficult drug targets or first-in-class drug targets, these problems can be amplified many times over.

BioBench Integrated Solution

BioBench's antibody drug discovery platform is a fully integrated system of industry-qualified tools, deep knowledge in drug discovery, team management and project management. These industry-qualified tools include pre-established antibody libraries, immunized antibody libraries, advanced instruments and reagents, as well as methods. Our knowledge is a collection of experience, solid know-how and the biopharmaceutical industry mindset. Team and project management play a key role in driving the platform to generate the best outcome, as well as shortening the lead time and reducing the cost.

Workflow

One-Stop-Shop service is a benefit for clients who work together with BioBench. You only need to send us the target name and your project objectives, then BioBench will analyze the target according to open publications. The rest of the work will be done by us, and you will receive the top-selected clones.



Achieved Projects at a Glance

BioBench's Antibody Drug Discovery Platform has completed numerous drug discovery projects with high-quality clones delivered. Clients provide us with the target name and project objectives, and BioBench produces the antigen and finds the binders from antibody libraries through several rounds of biopannning. Then, the affinity and binding activity of the positive clones are tested to confirm their basic effectiveness. Below are 2 graphs showing the amount of clones and binder affinity from randomly selected 16 clients' projects. On average, more than 300 clones and more than 600 clones were discovered from the human antibody library and alpaca antibody library, respectively. Of these, more than 60% of the clones perform with high affinity (<10nM).

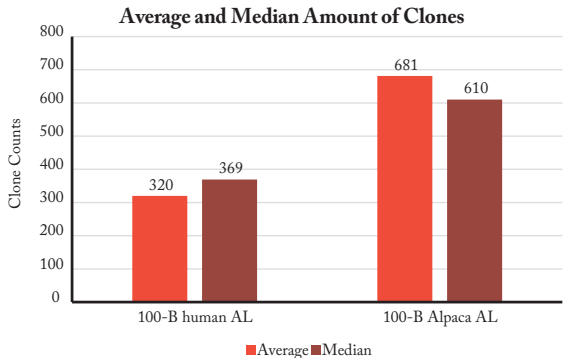


Fig. 1.1 Amount of clones from randomly selected 16 projects.

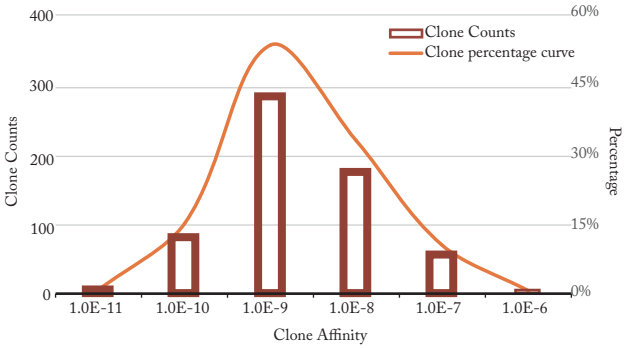


Fig. 1.2 Affinity of the clones from the selected 16 projects.

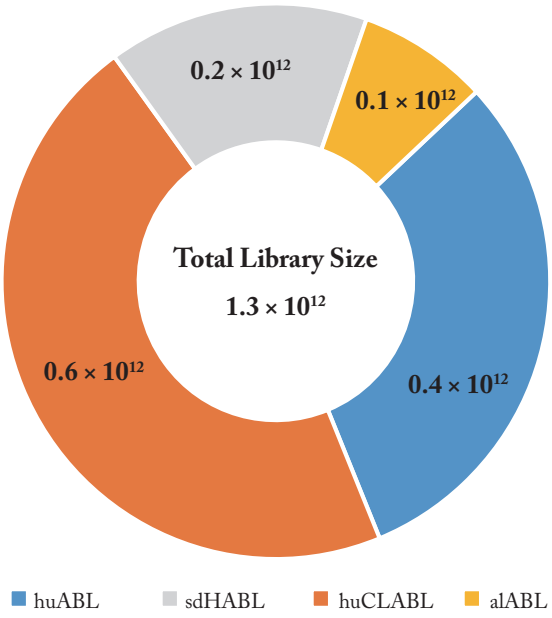
List of Antibody Libraries and Their Main Features

Total library size reached 1.3×10^{12}!

Well begun is half done - There are plenty of features that determine thether a group of initially discovered binders have further drug potential. Amoung these features, the amount of clones and the affinity of these clone are often considered as 2 important factors.

BioBench has several pre-established antibody libraries designed for drug discovery. From these libraries, a large amount of binders with pM to sub-nM affinity can be discovered. Based on our years of data, we learned that a high amount of lead antibodies are able to pass in vitro and in vivo test.

Immunized animal antibody library is a complementary method to harvest more valuable initial binders. BioBench provides customizable service to achieve your task.



100-huABL (100-Billion Human Antibody Library)

- 100 billion (10^{11}) level clones library capacity
- Engineered nature sequence
- ScFv fragment
- Median amount of lead binders: 300+ clones

10-huABL (10-Billion Human Antibody Library)

- 10 billion (10^{10}) level clones per library capacity
- Engineered nature sequence
- ScFv fragment
- Median amount of lead binders: 50+ clones

100-sdHABL (100-Billion Single Domain Humanized Antibody Library)

- 100 billion (10^{11}) level clones library capacity
- Semi-synthetic sequence single domain antibody
- Humanization rate of the sequence: 98%
- Median amount of lead binders: 600+ clones

ImoABL & IalABL (Mouse Immunized Antibody Library) (Alpaca Immunized Antibody Library)

- Hybrid immunization method, antigen including peptide, protein, protein fragment and/or gene
- Customizable Library capacity up to 1 billion (10^9) clones per library
- Magnetic beads array method to find the best binders
- Median amount of lead binders: 30+ clones

100-alABL (Alpaca Antibody Library Semi-synthetic)

- 100 billion (10^{11}) level clones library capacity
- Semi-synthetic sequence
- Single domain antibody
- Median amount of lead binders: 600+ clones

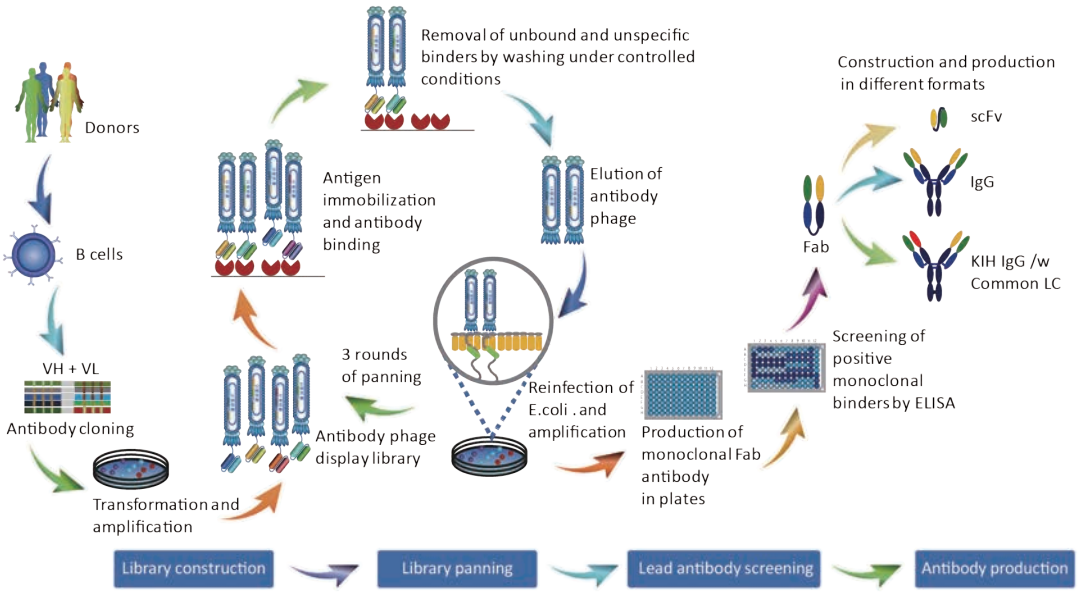
100-huCLABL (100-Billion Common Light Chain Human Antibody Library)

- 100 billion (10^{11}) level clones library capacity
- Designed for bi-specific antibody drug discovery
- Selected and engineered nature sequence, containing optimized light chains and diversified heavy chains
- Median amount of lead binders: 300+ clones

Human Antibody Library - Features and Case Study

Previous generations of antibody drugs were either murine-sourced or murine-human chimeric antibodies, which leds to a series of side effects in clinical treatment due to the HAMA reaction. It was difficult to avoid the side effect in the early years since there were no alternative tools to raise a large amount of drug-potential antibodies. As every scientist understood, finding human antibody directly from human host is the most ideal direction, and the method development continued since the early years. Scientists tried to isolate antibodies from patients and tested their cancer/tumor inhibition function. However, these antibodies were unreproducible and could only be used for protein/cell level research purposes. Furthermore, their performance was far from that of the murine-sourced or chimeric antibodies.

Phage display technology changed the game and made it possible to raise antibodies directly from human host. Immune cells are collected from healthy doners/patients, and then the antibody sequences are reverse transcribed into DNA. These DNA fragments are subcloned into phagemids, and later each antibody is displayed on the phage. Following biopanning, the high affinity binders are harvested.



BioBench initially established the human antibody libraries based on the phage display technology, followed by sequence engineering aimed at enhancing the performance of the final antibodies. Compared with the major antibody libraries from other service suppliers, BioBench's human antibody library has several advantages: 10× more individual clones, higher sequence diversity comparable to nature antibody repertoire, 5× more binders after panning, and 5× higher affinity of the binders.

Key Features of huABL

Large library capacity

BioBench human antibody library is established based on large amount of hosts as well as sequence engineering, which ensure the libraries reach a total capacity of 4×10^{11}. Theoretically, such a large number of clones is capable of covering all of the epitopes.

huABL Capacity: 4×10^{11}

Key Features of huABL continue

High Sequence Diversity

Sequence diversity is one of the most important indicators of the quality of an antibody library. Due to the limited availability and quality of the original sample, reverse transcription efficiency, and the skills involved in library establishment, the diversity of the final antibody library can often be negatively impacted. At BioBench, we examine the entire process from sample to the end library, with the aim of maximizing the sequence repertoire. Based on our test result, the diversity of BioBench human antibody library is comparable to that of natural antibody repertoire.

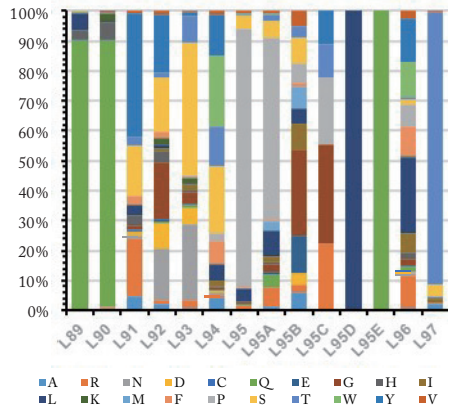


Fig. 1.3A CDR-L3 AA frequency

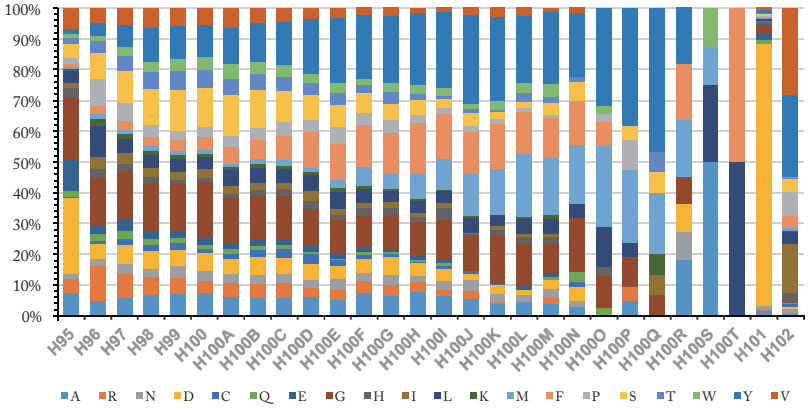


Fig. 1.3B CDR-H3 AA frequency

Fig. 1.3 AA frequency in 2 complementarity-determining regions (CDRs)

These 2 images are showing the amino acid ratios of different sites on the light chain and heavy chain of CDR3.
regions of the antibodies from the Sanyou trillion fully human antibody library are shown in figure 1, which indicates the extremely high abundance of antibody sequences in the Sanyou trillion fully human antibody library, and its consistency with amnio acid ratios in the native antibody library.

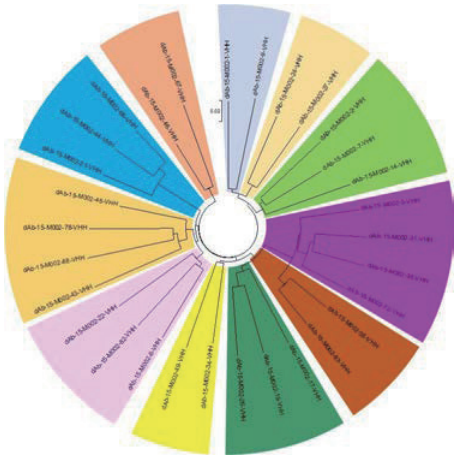


Fig. 1.4 Evolution tree of the antibody sequences

Antibody sequences from 1 project (out of the 16) were analyzed. These sequences were harvested after biopanning and the phagemids were send for sequencing and analyzed from evolution aspect. these sequences showing rich diversity.

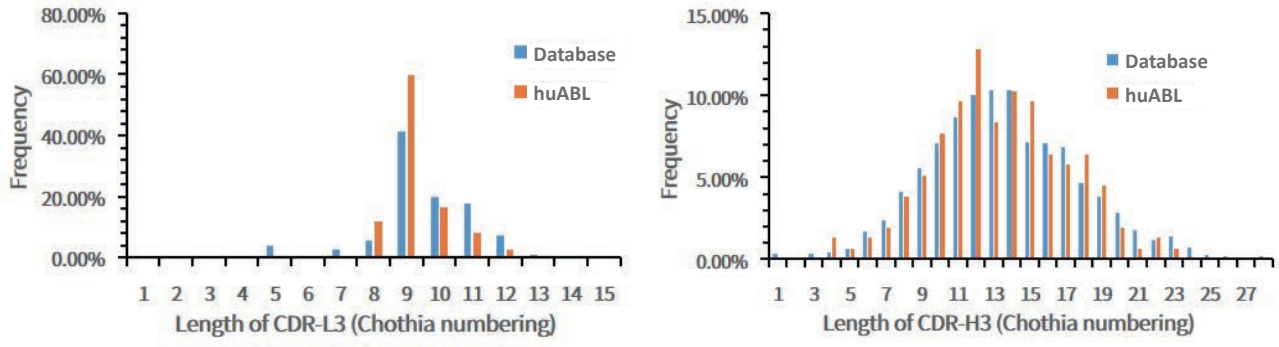


Fig. 1.5 Complementarity-determining regions (CDRs) length diversity

The aa length of CDRs are variable, this feature also plays a key role in improving the binding ability with diverse epitopes. In nature antibodies, the antibody counts of each aa length shall form a typical normal distribution curve (blue bar from international ImMunoGeneTics informationsystem, IMGT).The antibody sequences from the selected 16 projects were analyzed their aa length and compared with database. Result showing that the harvested binders have good aa length diversity.

Case study - anti-hCD40 human antibody discovery

1. Project Background

In 2018, the Nobel Prize in Physiology or Medicine was awarded to James P. Allison and Tasuku Honjo, who discovered Immune Checkpoint Blockade therapy (ICB) for cancer treatment. However, only a subset of patients show favorable response. It was discovered that, solid tumors in patients can be classfied into "hot tumor" and "cold tumor", based on the level of T cell infiltration. Cold tumor has low or no T cell infiltration, which causes the tumor to escape from immunotherapy. In contrast, the hot tumor has high T cell infiltration, and is showing response while being treated by immunotherapy. Therefore, finding ways to turn "cold tumor" into "hot tumor" has became a key area of focus for ICB therapy. Among the many possible solutions, the activation of immune cells is considered an efficient pathway to turn cold tumor hot.

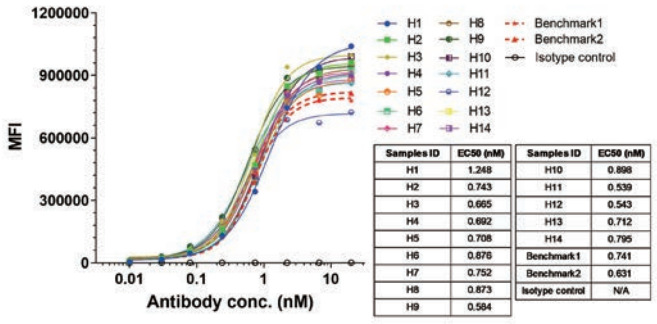
CD40 is a type I transmembrane protein found on antigen-presenting cells, and it plays a role in their activation. CD40 can interact with dendritic cells (DCs) surface,which in turn promotes the production of cytokines and chmokines, upregulates the expression of costimulation moleculars, and improves the cross-presentation of antigens. CD40 can also activate DC cells, which triggers T cell activation. Consequently, the membrane protein CD54 and CD86 are upregulated, leading to an increased interaction between CD cells and T cells. This results in T cell proliferation and activation, like the chain reaction.

The agonistic CD40 antibody can trigger CD40 signaling, thus bypass the need of CD4+ helper T cells to activate T cells and DC cells. This activation can turn the cold tumor into hot tumor, making it responsive to ICB therapy. This discovery has made CD40 antibody a new potential therapeutic tool and is currently being investigated.

2. Key Project Outcomes

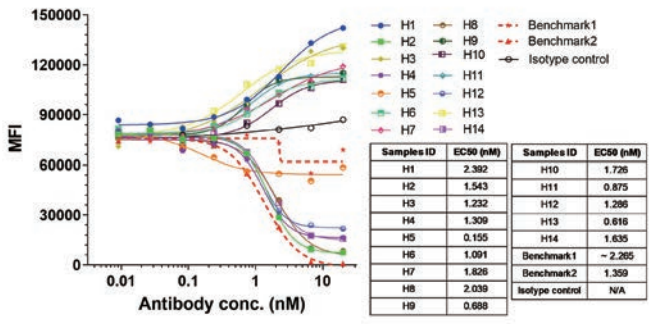
2.1 Binding assay, blocking assay and activation assay

After biopanning, totally more than 100 clones were discovered (data not shown). Client selected 14 top clones, and BioBench expressed these clones into full lengh IgG antibodies. These 14 reconstructed IgG antibodies were tested for binding, blocking, and activation assays on cell samples using flow cytometry. Fig.1.6 shows that all 14 clones have good binding ability, compare to the benchmark antibody. Fig.1.7 shows that these 14 clones can be devided into 2 groups based on their function: blocking group has 6 clones, and the activation group has 8 clones. According to client's request, the ideal candidate antibodies should show blocking activity as indicated by benchmark2 antibody. However, the activation group is valuble for other types of projects.



Samples ID	EC50 (nM)	Samples ID	EC50 (nM)
H1	1.248	H10	0.898
H2	0.743	H11	0.539
H3	0.665	H12	0.543
H4	0.692	H13	0.712
H5	0.708	H14	0.795
H6	0.876	Benchmark1	0.741
H7	0.752	Benchmark2	0.631
H8	0.873	Isotype control	N/A
H9	0.584		

Fig.1.6 Cell binding activity test (by flow cytometry)



Samples ID	EC50 (nM)	Samples ID	EC50 (nM)
H1	2.392	H10	1.726
H2	1.543	H11	0.875
H3	1.232	H12	1.286
H4	1.309	H13	0.616
H5	0.155	H14	1.635
H6	1.091	Benchmark1	~ 2.265
H7	1.826	Benchmark2	1.359
H8	2.039	Isotype control	N/A
H9	0.688		

Fig.1.7 Cell blockong/activation test (by flow cytometry)

Case study continue - anti-hCD40 human antibody discovery

2.2 In vitro efficacy cell model evaluation

CD40L (CD40 Ligand) can bind to CD40. Then the complex will activate DC cells, leading to the promotion of IL-12 secretion and CD83 upregulation. Agonistic anti-CD40 mAb has a similar effect to CD40L in that it binds to CD40 and activate DC cells. The function of the activation group Abs were analyzed by monitoring IL-12 and CD83 secretion from monocytes-induced DC cells (iDC). The result of IL-12 and CD83 are shown in images Fig.1.8 and Fig.1.9, respectively. We can see that 7 out of 8 candidate Abs show activation function compared to the benchmark Ab.

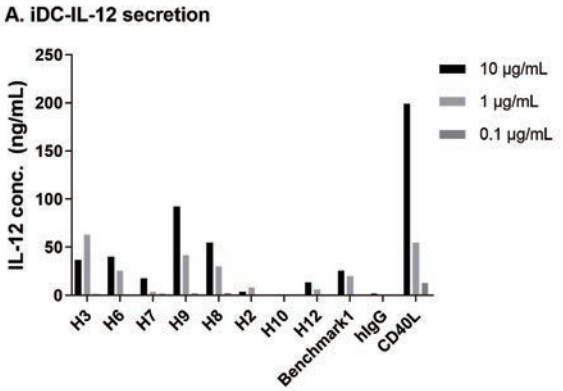


Fig.1.8 iDC cell activation assay - IL-12.

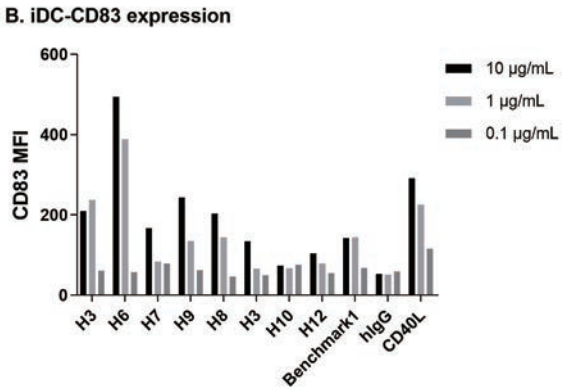


Fig.1.9 iDC cell activation assay - CD83.

2.3 In vivo efficacy animal model evaluation

Clone H9 was selected to perform in vivo efficacy animal model evaluation. Ramos cell line derived xenograft nude mouse was used as animal model. Both candidate antibody H9 and Benchmark were tested at 3 different doses (0.2mpk, 1mpk and 5 mpk). PBS was used as control. Both of the Abs were administrated 3 times per week, totally 2 weeks. Results are illustrated in Fig.1.10. Compare to the benchmark antibody, candidate clone H9 demonstrates better tumor inhibition efficiency at doses 0.2mpk and 1mpk, with equal inhibition efficiency at 5mpk dose.

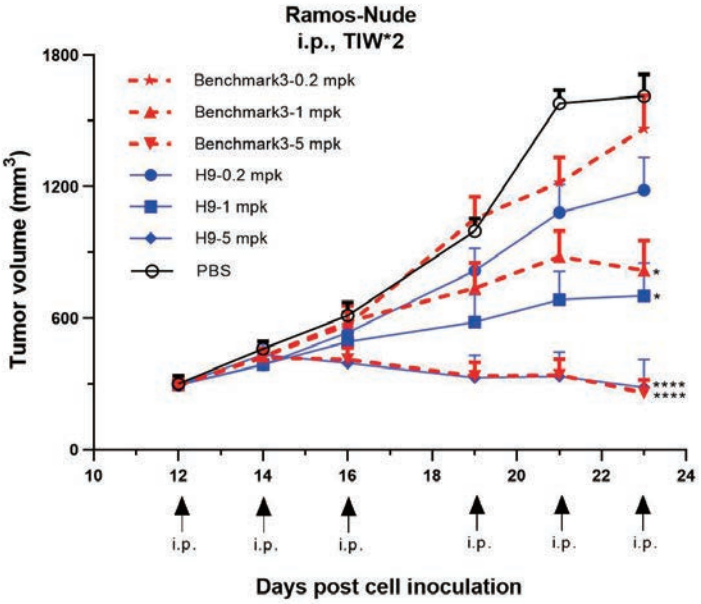


Fig.1.10 In vivo tumor inhibition efficacy assay.

Ramos-Nude mouse was selected as in vivo animal model and 3 doses at 0.2mpk, 1mpk and 5mpk were administrated TIW*2.

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Your candidate antibody discovery work flow

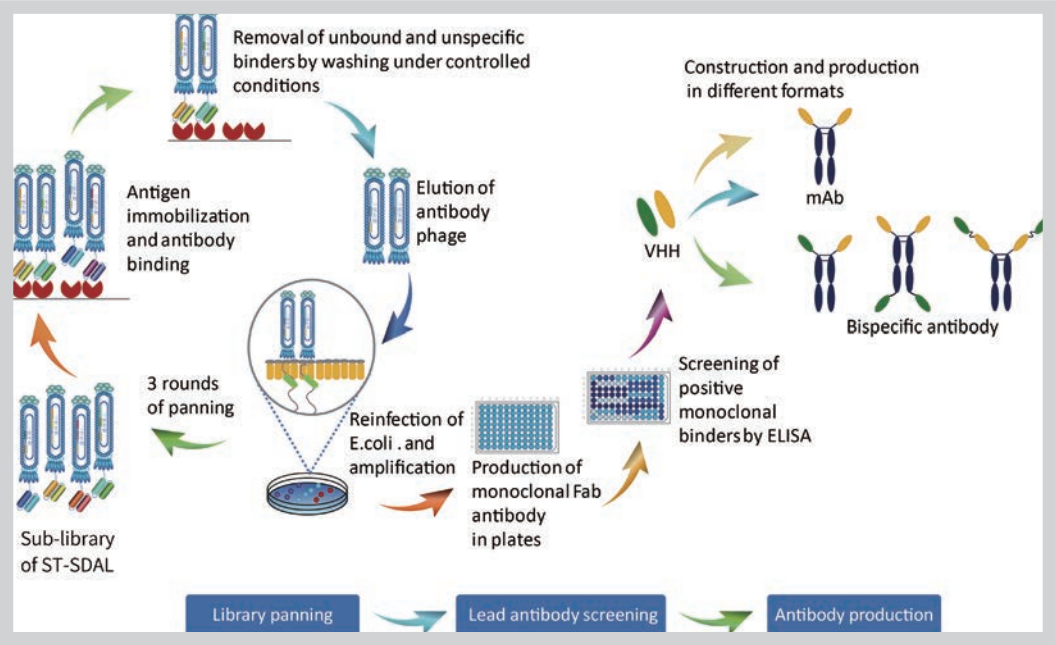
Service content - human antibody discovery

Service	Service Content	Client Provides	Deliverables	Lead Time
D01301 Drug potential antibody discovery from 100-huABL (100-billion human antibody library)	1. Antigen identification and quality test. 2. Antigen labeling and quality test. 3. Biopanning from the human antibody library to find the top binders. 4. Antibody full length IgG expression and purification. 5. Affinity test and preliminary physicochemical characteristic test.	Optimum quantity of purified antigen protein.	1. At least 50 top selected binders. 2. Optional to receive 100ug of selected binder to test in house. 3. Service report.	6 - 8 weeks
D01302 Drug potential antibody discovery from 10-huABL (100-billion human antibody library)			1. At least 30 top selected binders. 2. Optional to receive 100ug of selected binder to test in house. 3. Service report.	

Single Domain Humanized Antibody Library (sdHABL) - Features and Case Study

Heavy-chain antibody is a type of antibody which consists of only two heavy chains and lacks the two light chains usually found in antibodies. Heavy-chain antibody was discovered naturelly exists in camelid and in cartilaginous fishes. After sequence engineering, single domain antibodies obtained from heavy-chain antibodies found in camelids are called V_HH fragments; and single domain antibodies obtained from heavy-chain antibodies (also called IgNAR, Immunoglobulin New Antigen Receptor) found in cartilaginous fishes are called V_{NAR} fragments.

Compare to cartilaginous fish, raising and operating on camelid is far easier. Therefore, current single domain antibody libraries are often derived from camelids. Initially, BioBench established the alABL (Alpaca Antibody Library) and tested the performance in many projects. According the result of each project, alABL achieved good performance: large amount of clones harvested with high affinity. However, clients often prefer to receive humanized sequences directly to avoid addtionial cost associated with antibody humanization.



BioBench Single Domain Humanized Antibody Library (sdHABL) is established based on the previous alABL, with humanization of all of the sequences. sdHABL has good humanization rate of 98%, at the same time retain the high affinity of its origin sequences.

Key Features of sdHABL

Large library capacity

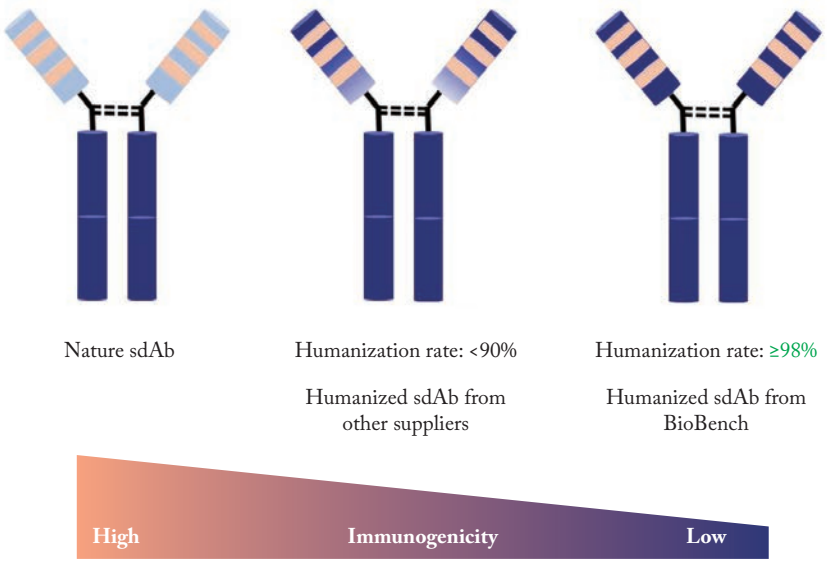
BioBench sdHABL human antibody library is established based on the alABL, which contains engineered antibodies derived from more than 200 alpacas. Both sdHABL and alABL have a capacity of 1.2×10^{11}.

sdHABL Capacity: 1.2×10^{11}

Key Features of sdHABL continue

High level of humanization 98%

BioBench engineered the entire Framework Region (FR) to ensure that each antibody discovered from sdHABL has humanization rate of at least 98%. This not only helps clients save on the cost of humanization, but also ensure that the harvested sequences have good drug developability for bioprocessing.



Case study - Anti-CLDN18.2 Nanobodies

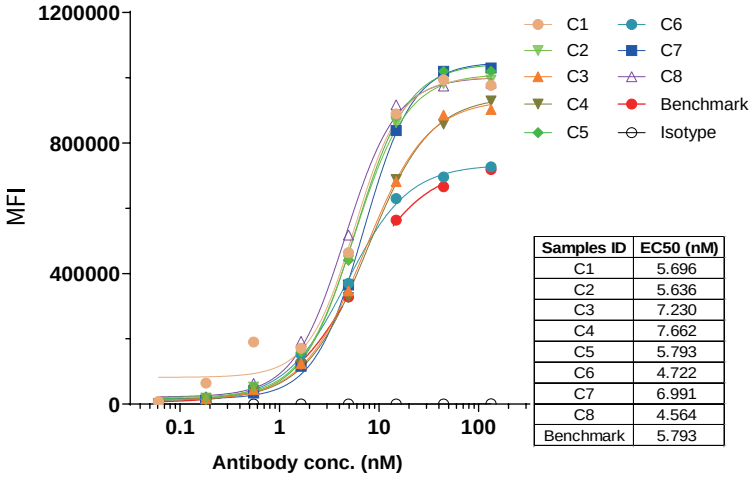
1. Project Background

The claudin18.2 (CLDN18.2) is an isoform of claudin18, a member of the tight junction protein family, is a highly selective biomarker with limited expression in normal tissues and often abnormal expression during the occurrence and development of various primary malignant tumors, such as gastric cancer/gastroesophageal junction (GC/GEJ) cancer, breast cancer, colon cancer, liver cancer, head and neck cancer, bronchial cancer and non-small-cell lung cancer. CLDN18.2 participates in the proliferation, differentiation and migration of tumor cells. Several studies have identified CLDN18.2 expression as a potential specific marker for the diagnosis and treatment of these tumors.

With its specific expression pattern, CLDN18.2 has become a unique molecule for targeted therapy in different cancers, especially in GC; for example, agents such as zolbetuximab (claudiximab, IMAB362), a monoclonal antibody (mAb) against CLDN18.2, have been developed. Its clinical result from Phase II study, showing that when combine IMAB362 together with paclitaxel, the overall survival of patients with GC/GEJ cancer increased from 9 months to 16.7 months.

2. Key Project Outcomes

2.1 Cell binding activity assay



Samples ID	EC50 (nM)
C1	5.696
C2	5.636
C3	7.230
C4	7.662
C5	5.793
C6	4.722
C7	6.991
C8	4.564
Benchmark	5.793

Fig.1.11 CLDN18.2 candidate Ab binding activity assay

After biopanning, totally more than 300 clones were discovered (data not shown). Client initially selected 8 top clones to for cell binding activity test. These 8 clones were firstly expressed into full human IgG1 form. Then the candidate Abs together with benchmark Ab and isotype Ab were incubated with CLDN18.2 expressing HEK293T cell line and tested by flow cytometry.

Result shows that all 8 candidate Abs have good binding activity, with C1, C2, C6 and C8 showing higher binding activity compare to benchmark Ab.

Case study continue - CLDN18.2 nanobody discovery

2.2 ADCC assay and CDC assay

The candidate Abs were expressed in human IgG1 format, which containing the full function of antibody. It binds to claudin 18.2 on the tumor cell surface to stimulate cellular and soluble immune effectors that activate antibody-dependent cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC). Therefore HEK293-hCLDN18.2 stable cell line was established to examine these 2 effects.

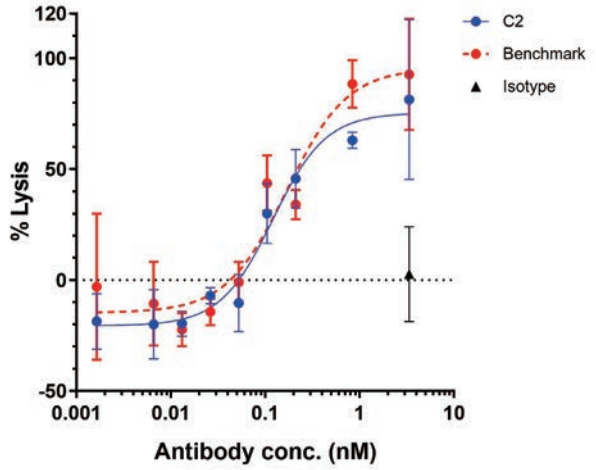


Fig.1.12 CLDN18.2 candidate Ab C2 ADCC assay

Result shows that clone C2 has equilent effect compare with benchmark antibody.

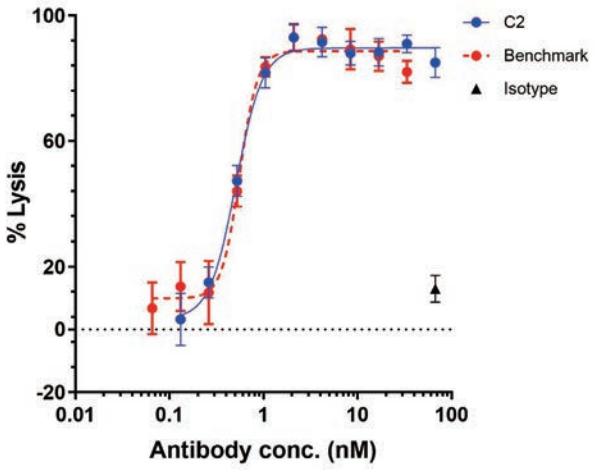


Fig.1.13 CLDN18.2 candidate Ab C2 CDC assay

Result shows that clone C2 has equilent effect compare with benchmark antibody.

2.3 Endocytosis assay to analyze the potential of usage as antibody drug conjugate (ADC)

Antibody can carry a small molecular and enter the cell through endocytosis to perfom the tumor/cancer cell killing function. CLDN18.2 is only expressed in tumor cell and never been found on normal cell, this specialty makes it possible to be developed into ADC drug. Clone C2, benchmark Ab and isotype Ab are all conjugated with a small molecular. HEK293T-hCLDN18.2 stable cell line was used to study the endocytosis effect. Result shows that C2 has better endocytosis-killing activity compare with benchmark Ab.

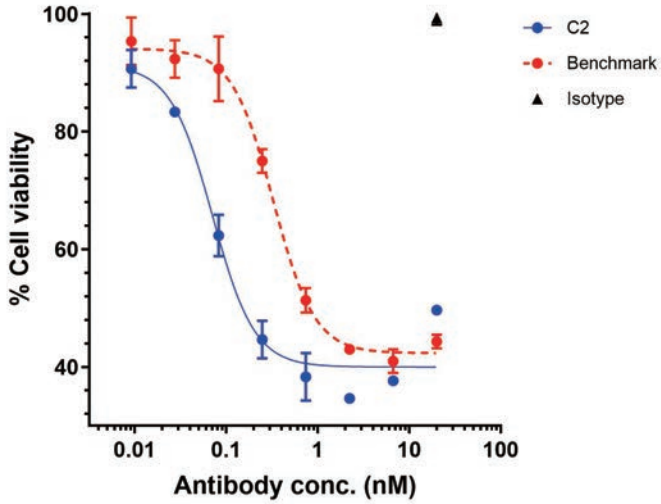


Fig.1.14 Endocytosis assay to test the clone C2 and benchmark Ab

Result shows that clone C2 has better endocytosis-kill effect compare with benchmark antibody.

Case study continue - CLDN18.2 nanobody discovery

2.4 In vivo animal model efficacy assay

To achieve the best outcome, a humanized mouse together with MC38 cell line was used to evaluate the efficacy of the candidate Abs. After 6 days of tumor bearing, the mice were administrated clone C2 with dose at 0.4MPK, twice per week for totally 3 weeks (BIW*3). Result shows that the candiate Ab C2 has better efficacy compare with benchmark Ab.

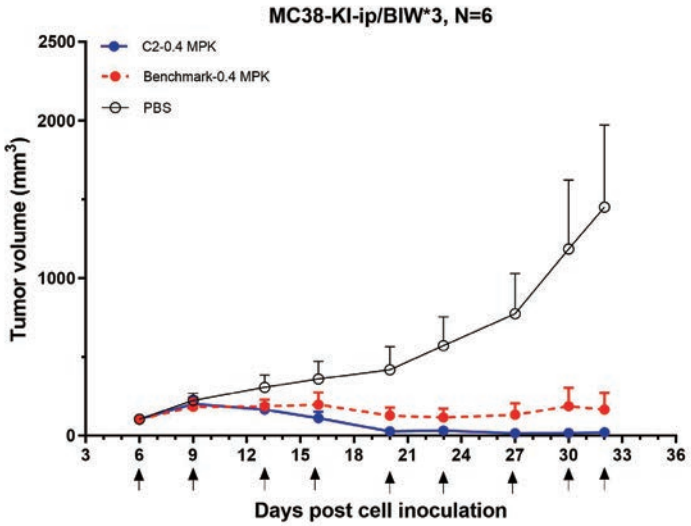


Fig.1.15 CLDN18.2 candidate Ab C2 in vivo efficacy study

Result shows that clone C2 has better efficacy compare with benchmark antibody.

Your candidate antibody discovery work flow

Service content - single domain humanized antibody discovery

Service	Service Content	Client Provides	Deliverables	Lead Time
D01311 Drug potential antibody discovery from sdHABL (Single Domain Humanized Antibody Library)	1. Antigen identification and quality test. 2. Antigen labeling and quality test. 3. Biopanning from sdHABL antibody library to find the top binders. 4. Antibody full length IgG expression and purification. 5. Affinity test and preliminary physicochemical characteristic test.	Optimum quantity of antigen protein.	1. At least 50 top selected binders. 2. Optional to receive 100ug of selected binder to test in house. 3. Service report.	6 – 8 weeks

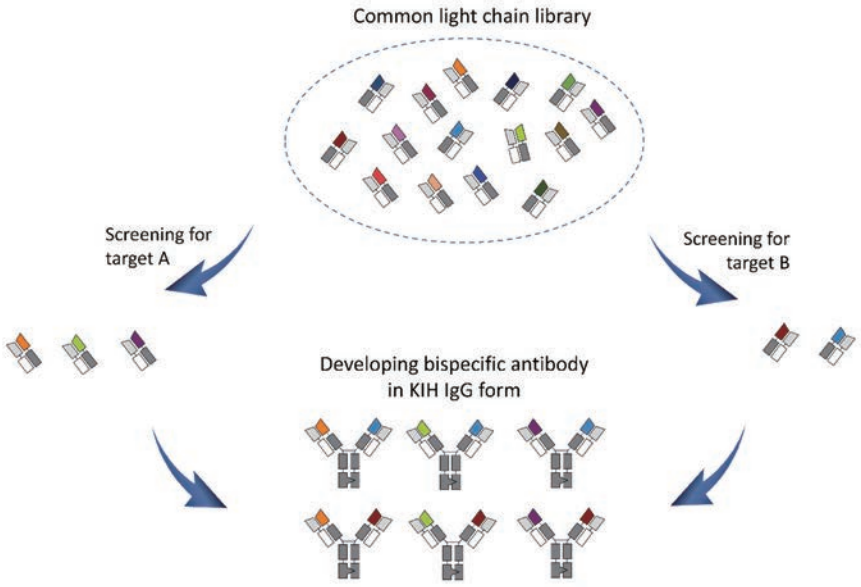
Common Light Chain Human Antibody Library (100-huCLABL) - Features and Case Study

Bispecific antibodies combine two different antigen-binding sites (to be simple: 2 antibodies) in a single molecule, enabling more specific targeting, novel mechanisms of action, and higher clinical efficacies. Although they have the potential to outperform conventional monoclonal antibodies, many bispecific antibodies have issues regarding production, stability, and pharmacokinetic properties. One of the solutions is to combine these 2 antigen-binding sites antibodies by an identical common light chain, which comes from chosen sequence or from existing therapeutic antibody.

Common light chain antibody has some significant advantages compare to other type of bispecific Ab, such as easy assembly of full IgG antibody, better expression level, better stability, better drug developability and lower immunogenicity. These advantages make common light chain antibody an ideal solution for developing the next generation of bispecific Ab.

Compare to snythesizing a bispecific antibody from 2 drug-potential Abs, finding antibodies from a well-established common light chain antibody library is a much more reliable method. The antibody library has already been engineered to minimize known risk, while retaining affinity and specificity of final clones.

BioBench established the 100-huCLABL based on our human antibody library, which includes several addtional engineering features: the common light chain fragment is derived from a well-characterized high drug developability sequence; the antibody library is composed of Fab format clones; and the final IgG antibody can be easily assembled into knob-into-Hole (KIH) format.



Key Features of 100-huCLABL

Large library capacity

BioBench 100-huCLABL is established based on 100-huABL, therefore both 100-huABL and 100-huCLABL have a capacity of 2.2×10^{11}.

huCLABL Capacity: 2.2×10^{11}

High sequence diversity, comparable to huABL

100-huCLABL is established based on 100-huABL and after engineering, it retaines the high sequence diversity of its orginal antibody library. Fig.1.16, 1.17, 1.18 and 1.19 demonstrate the high diversity of different antibody fragments.

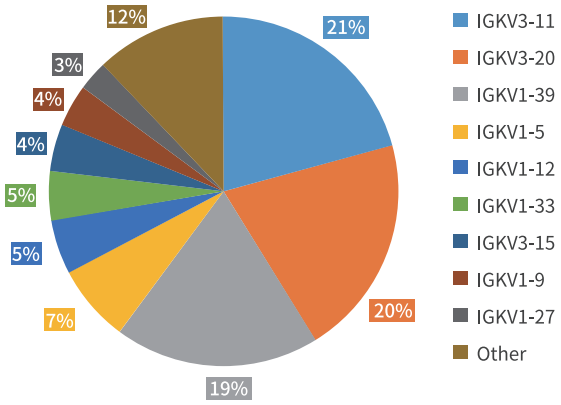


Fig.1.16 VL germline diversity

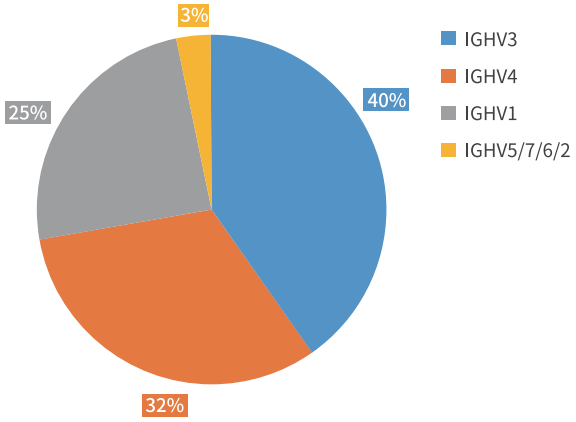


Fig.1.17 VH germline diversity

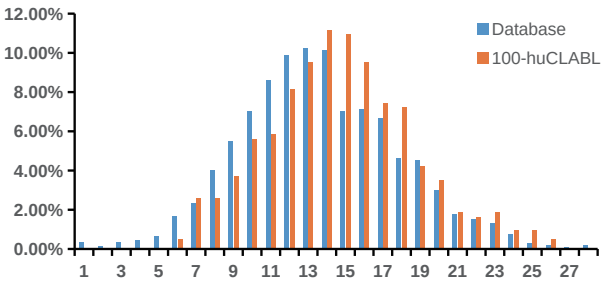


Fig.1.18 CDR-H3 length diversity and comparison with nature antibody

Fig.1.19 Candidate Abs evolution tree analysis of heavy chain sequence

High expression level in mammalian cell line

An important advantage of common light chain antibody is the better drug developability compare to other types of bispecific antibodies. We analyzed 68 randomly selected clones by testing their expression level in CHO cell line. These antibodies were expressed in IgG format. Results show that more than 95% of clones have an expression ability of >10mg/mL, and the median expression level is 45mg/L.

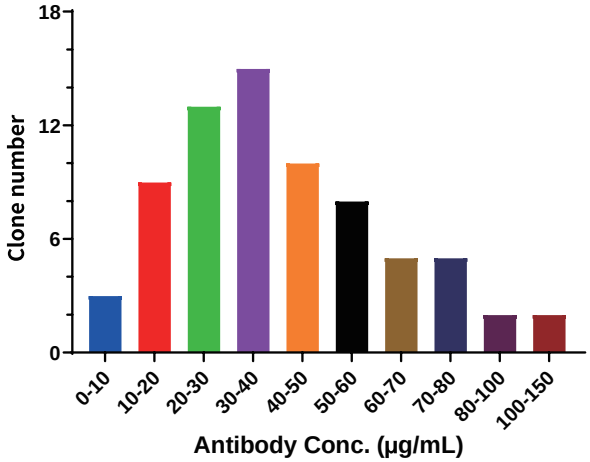


Fig.1.20 huCLABL antibody CHO expression level test

Case study - huCLABL antibody

1. Project Background

A bispecific antibody project is accomplished by screening the huCLABL. After antibody validation, a full human IgG format antibody was expressed by CHO cell line to test in vivo efficacy. NCI-N87 gastric carcinoma cell line and nude mice were used to establish the animal model.

Results show that BsAb groups and Ab1+Ab2 groups both demonstrated better effcacy compare to the group where only Ab1 was administrated. when CLC BsAb was administrated at 20mpk, the tumor inhibition effect was better than the BsAb BM group, which was also administrated at the same dose.

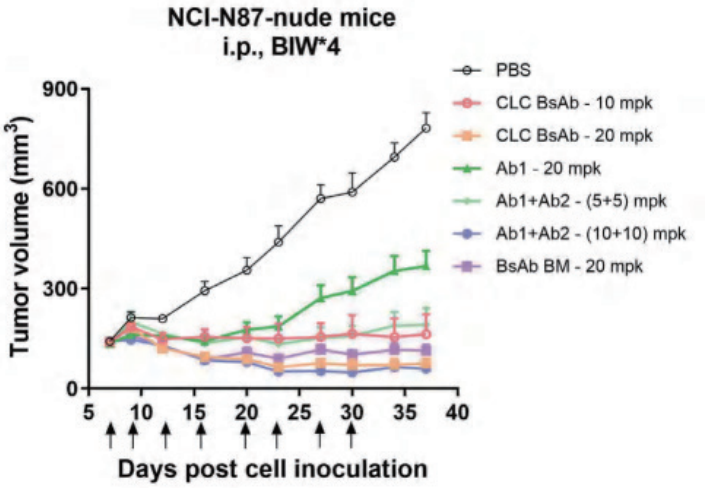


Fig.1.21 huCLABL discovered bispecific antibody in vivo efficacy study.

CLC BsAb: common light chain bispecific antibody
Ab1: monoclonal antibody 1 benchmark
Ab2: monoclonal antibody 2 benchmark
Ab1 + Ab2: 2 benchmark mAb mixed together
BsAb BM: bispecific antibody benchmark
PBS: PBS control group

Your candidate antibody discovery work flow

Service content - single domain humanized antibody discovery

Service	Service Content	Client Provides	Deliverables	Lead Time
D01321 Drug potential common light chain antibodies discovery from huCLABL (Human Common Light Chain Antibody Library)	1. Antigen identification and quality test. 2. Antigen labeling and quality test. 3. Biopanning from huCLABL antibody library to find the top binders. 4. Antibody full length IgG expression and purification. 5. Affinity test and preliminary physicochemical characteristic test.	Optimum quantity of antigen protein.	1. 50 top selected binders for each antigen. 2. Optional to receive 100ug of selected binder to test in house. 3. Service report.	8 weeks

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ImoABL & IalABL - Features and Case Study
(Mouse Immunized Antibody Library)
(Alpaca Immunized Antibody Library)

To obtain effective monoclonal antibodyies, immunizing animals is the conventional method. However, this method has a high rate of clone loss during monoclonalization and a lengthy workflow, necessitating a more efficient strategy for antibody discovery. The phage display method can preserve a high percentage of antibody sequences from animal immune cells, significantly increasing the antibody pool and reducing the number of animals sacrificed. As a result, the entire antibody discovery work flow can be shortened and simplfied.

BioBench has established a research line to facilitate your project from animal immunization to phage display antibody library, then finalize with tested antibodies. Hereunder we will show some result from our past projects.

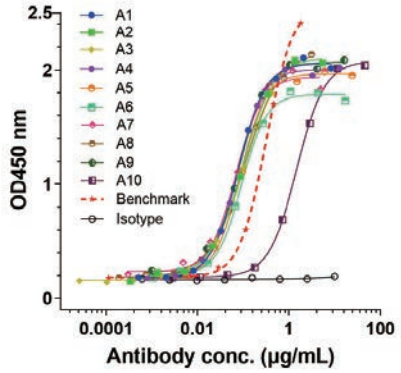


Fig.1.22 Immunized mouse antibody library project M056, candidate antibody affinity test

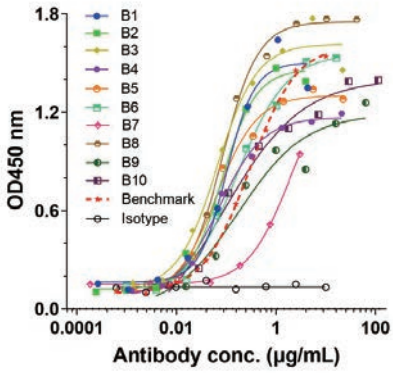
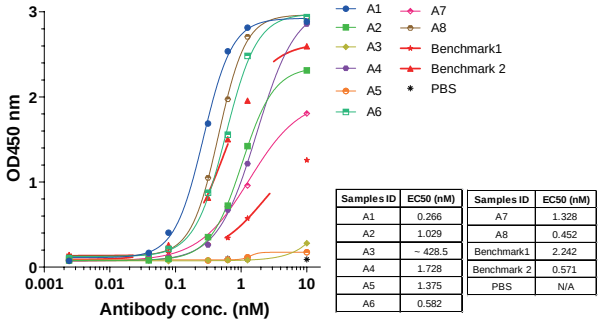
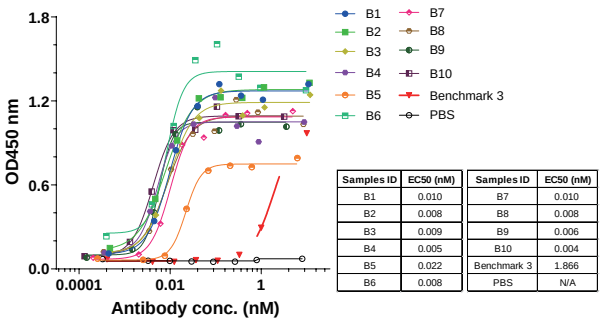


Fig.1.23 Immunized mouse antibody library project M051, candidate antibody affinity test



Samples ID	EC50 (nM)	Samples ID	EC50 (nM)
A1	0.266	A7	1.328
A2	1.029	A8	0.452
A3	~ 428.5	Benchmark1	2.242
A4	1.728	Benchmark 2	0.571
A5	1.375	PBS	N/A
A6	0.582		

Fig.1.23 Immunized alpaca antibody library project SD010, candidate antibody affinity test



Samples ID	EC50 (nM)	Samples ID	EC50 (nM)
B1	0.010	B7	0.010
B2	0.008	B8	0.008
B3	0.009	B9	0.006
B4	0.005	B10	0.004
B5	0.022	Benchmark 3	1.866
B6	0.008	PBS	N/A

Fig.1.24 Immunized mouse antibody library project SD041, candidate antibody affinity test

Service content - single domain humanized antibody discovery

Service	Service Content	Client Provides	Deliverables	Lead Time
D01331 Drug potential ImoABL (Mouse Immunized Antibody Library)	1. Antigen identification and quality test; antigen labeling and quality test. 2. Animal immunization and titre test. 3. Antibody library construction through phage display technology. Antibody library quality test. 4. Biopanning from the antibody library to find the top binders. 5. Antibody full length IgG expression and purification. 6. Affinity test and preliminary physicochemical characteristic test.	Optimum quantity of antigen protein.	1. 20 top selected binders for each antigen. 2. Optional to receive 100ug of selected binder to test in house. 3. Service report.	14 weeks
D01332 Drug potential IalABL (Alpaca Immunized Antibody Library)		Optimum quantity of antigen protein.	1. 20 top selected binders for each antigen. 2. Optional to receive 100ug of selected binder to test in house. 3. Service report.	14 weeks

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