Creative Biolabs®

Premade Phage Display Antibody/Peptide Libraries

01 About Creative Biolabs

Creative Biolabs is specialized in providing custom services to both academic and biotech/pharmaceutical industries in the field of Antibody development and Production. Based on advanced techniques and enriched experience, we provide elaborate Monoclonal Antibody Production platform to meet your specific demands.

- Monoclonal Antibody Generation in all Species
- Antibody Development against Membrane Proteins
- *De novo* Antibody Sequencing
- Mouse, Rabbit and Chicken Antibody Humanization
- Antibody Murinization, Camelization and Caninization
- Affinity Maturation
- Bispecific Antibody Engineering
- ADC
- Chimeric Antigen Receptor (CAR) Services
- Immunogenicity
- Stable Cell Line Construction

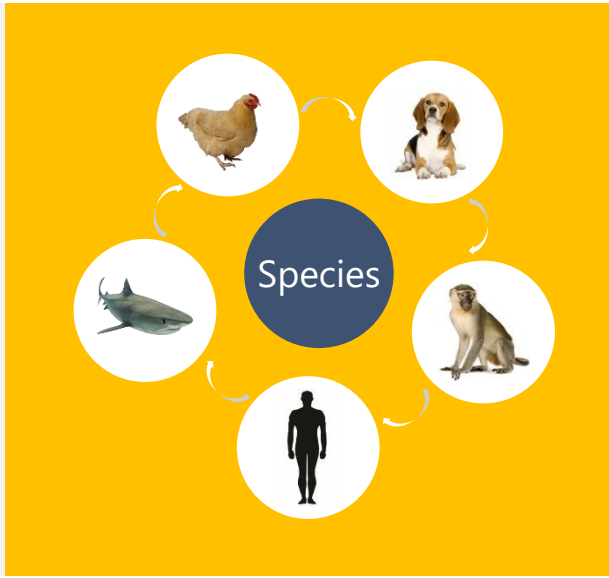
02 Expert in Monoclonal Antibody

Various species available

Creative Biolabs is professional in developing highly specific and affinity monoclonal antibodies from various species (including human, mouse, rat, llama, camel, monkey, chicken, rabbit, shark, monkey, bovine, etc.).

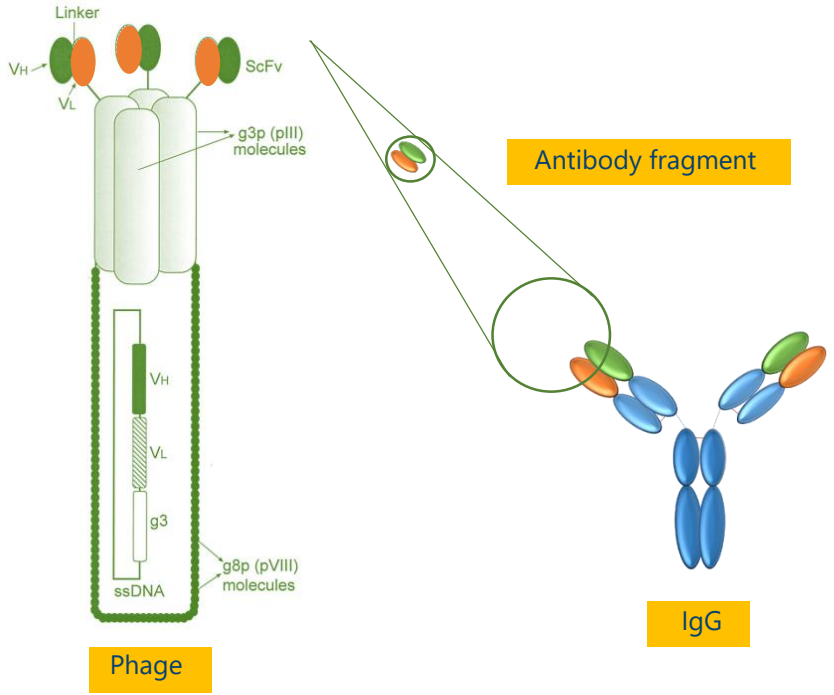
Features

- One-stop, fully customizable solution for monoclonal antibody production
- Different antibody format and display type
- Large library capacity: from 10^7 to over 10^8
- High affinity
- Various phage display systems (M13, T4, T7)
- Tailored biopanning strategies
- Comprehensive QC validation
- State-of-the-art animal facilities
- Capabilities of constructing human Ab libraries from various infectious disease model



Key Advantages

- Phage display generate a linkage between genotype and phenotype.
- Phage display libraries containing several billion variants can be constructed simultaneously.
- Antigen-specific phage particles bearing sequences with desired binding specificities from the non-binding variants.

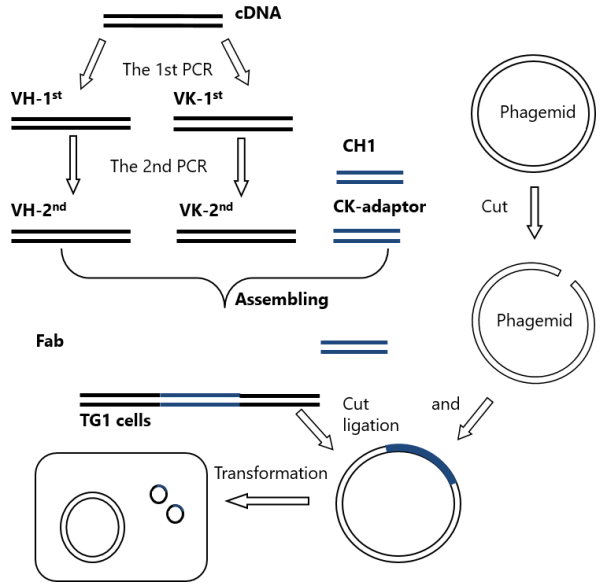


Antibody Formats

Screening Targets

- Specific Antigens: recombinant proteins, peptides, liposomes, polymers, lipoparticles, etc.
- Haptens: inorganic chemical compounds
- Whole Cells

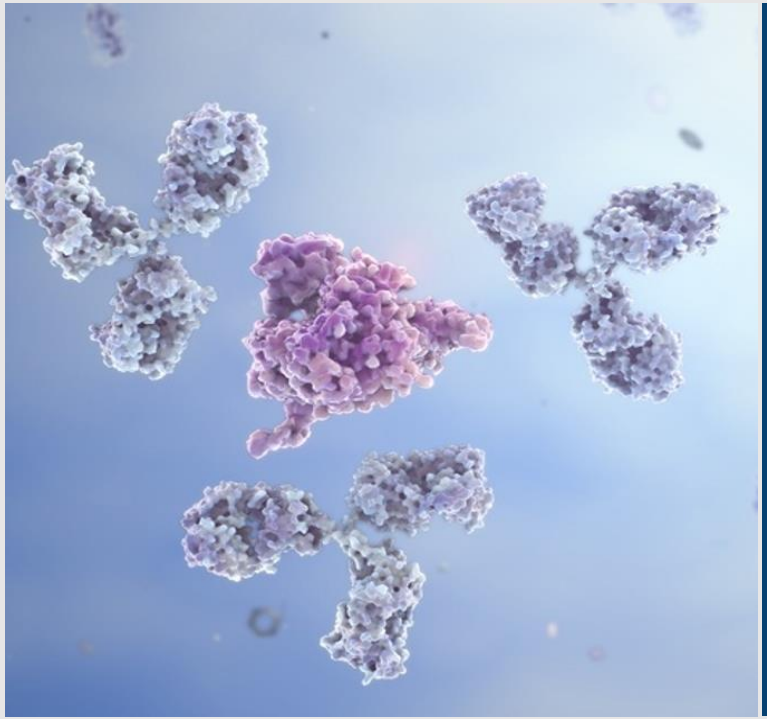
03 Premade Phage display Libraries



Flow chart of the library phagemid library generation

Creative Biolabs has designed and validated a serial of degenerate primers for amplifying rearranged immunoglobulin VH (IgG) and VL chains. We have used these primers to generate many superior scFv/Fab libraries with overwhelming capacity and diversity.

03-1 Premade Antibody Phage display Libraries



Creative Biolabs has following human antibody libraries with great diversity to be able to derive high-affinity antibodies with affinity ranging from 10pM to 10nM. A large number of reputable references are available, for whom we have generated good human antibodies for therapeutic use using these human antibody libraries. Quite a few of our customers have moved their antibody candidates into preclinical studies and 3 antibody candidates isolated from the libraries have entered into phase I and II clinical trials.

03-1 Premade Antibody Phage display Libraries

Library Source

- **Native libraries**: constructed from non-immunized host (human, mouse, rat, llama, camel, shark...)
- **Synthetic libraries**: constructed from antibody framework with randomized CDR regions.
- **Immunized libraries**: constructed from antigen-immunized hosts (human patients, mice, etc.)

Library Format

- scFv libraries
- VHH libraries

- Fab libraries
- VNAR libraries

03-1 Premade Antibody Phage display Libraries (selected list)

Library ID	Library Format	Library Source	Library Capacity
HuScL-3	Recombinant naïve scFv	Human	1.0×10^{16}
HuScL-5	Synthetic scFv	Human	2.0×10^{10}
HuFabL-1	Naïve Fab	Human	2.0×10^{9}
HuFabL-2	Naïve & synthetic Fab	Human	2.0×10^{10}
MuScL-1	Naïve scFv	Mouse	8.0×10^{8}
MuFabL-1	Naïve Fab	Mouse	6.0×10^{9}
RaFabL-1	Naïve Fab	Rat	7.5×10^{9}
Chicken-ScL-1	Naïve scFv	Chicken	1.2×10^{9}
CaVHHL	Naïve VHH	Camel	1.5×10^{9}
LlaVHH-1	Naïve VHH	Llama	2.0×10^{9}
HuSdL-1	Camelized synthetic VHH	Human	1.5×10^{9}
HuSdL-2	Camelized synthetic VHH	Human	2.5×10^{10}

03-2 Premade Peptide Phage display Libraries

Creative Biolabs currently provides a broad range of phage display peptide/scaffold libraries of diverse length and format.

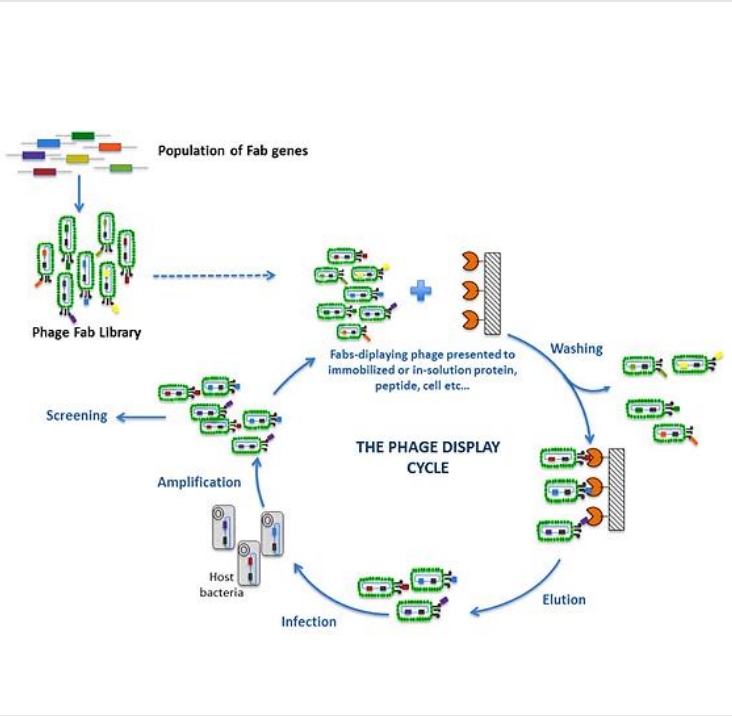
Of note, we carry two good linear peptide libraries: 16-mer and 20-mer, which are constructed using Trimer Codon technology. These random peptide libraries are best-in-class random 16-mer and 20-mer peptide libraries in terms of their quality and diversity.

04 Various Biopanning Methods

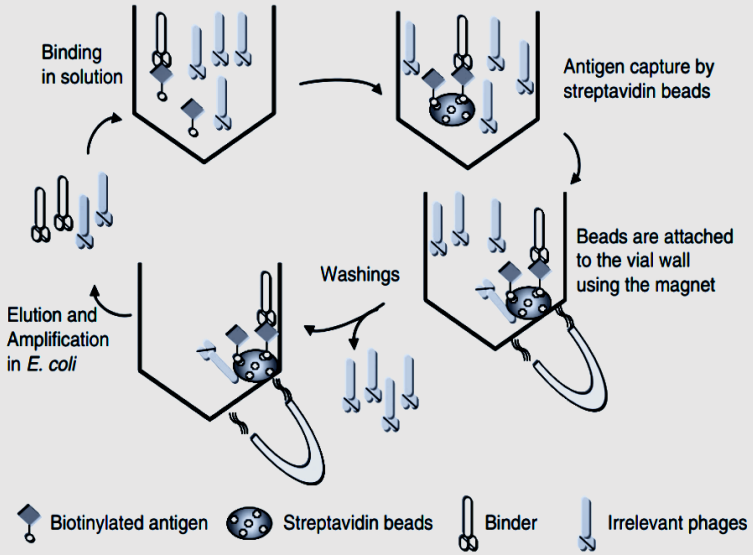
Solid-phase screening

Solid-phase screening indicates an effective selection approach that can apply to most of binder selection projects.

Phage libraries are selected by flowing through an affinity column with the immobilized antigen of interest. Following washing of the column to remove nonspecific clones, specific binders are eluted and amplified in *E. coli*.



In-solution Panning

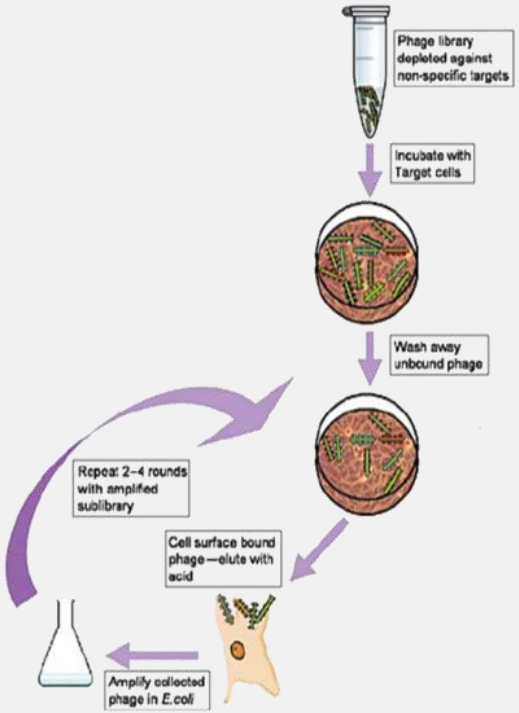


This technique allows solution binding and overcomes issues with conformational changes that are encountered upon coating antigens on solid surfaces.

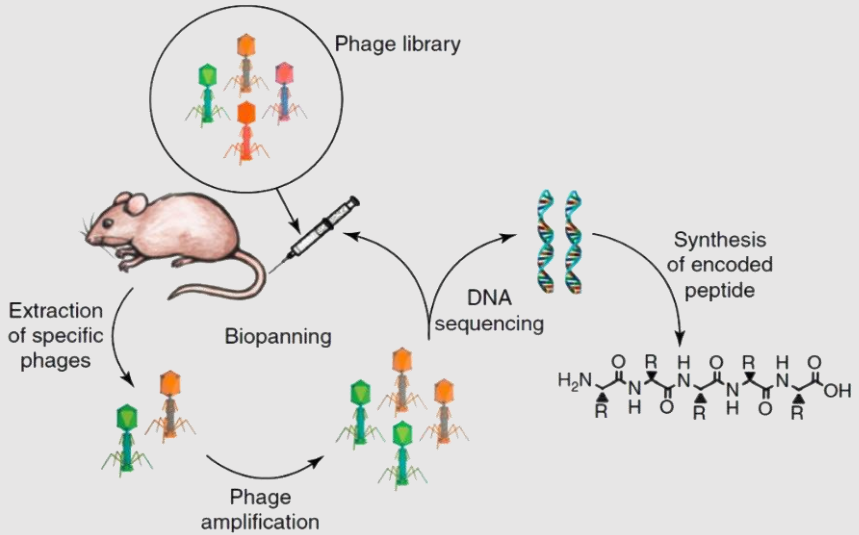
The use of labeled soluble antigens also allows a more accurate quantification of the antigen used during selection and consequently enhances the ability to use lower concentrations of the Ag to favor selection of high-affinity phage Ab

Cell-based Screening

- Direct selection of antibodies against markers on cell surfaces may be carried out on either mono-layers of adherent cells or on cells in suspension.
- It circumvents the disadvantages of solid phase selection by displaying the antigens in their native forms.
- Cell-based selection could target the cell surface proteins that are overexpressed or modified by disease, which brings in the novel epitopes.
- The best application of this method is to select antibodies for cell surface receptors, such as GPCR, ion channel-linked receptors and enzyme-linked receptors.



In vivo Screening



- The isolated phage-displayed peptides home selectively to “intact” targets of interest.
- An inherent blocking step is included where most of the phage-displayed peptides that recognize ubiquitous plasma and cell surface proteins are eliminated.
- These peptides may be useful for the functional analysis of new receptors and potential identification of novel drug target candidates because some of the isolated peptides have been found to bind to endothelial receptors expressed in the vasculature of specific tissues

05 Antibody Identification Strategies

Phage ELISA

1. Phage amplification of 80 binder phage clones obtained from enriched phage library obtained in phase
2. Verify the specific binders, including:
   - Phage amplification
   - Phage ELISA against the target
   - Phage DNA extraction
   - Phage DNA sequencing

Magic™ Platform

Magic™ Antibody Discovery Platform provides information of **all the antibody variants** in the library.

- Identification of hundreds of binders.
- Minimization of the selection rounds.
- Information of the sequence frequency.
- Annotated DNA, protein sequence and CDRs.

06 Premade Phage Display Human scFv Library In-Solution Panning Sample Report

Creative Biolabs
The client would like to obtain antibodies which have cross-reactivity against the human and mouse targets. We are contracted to identify such antibodies by screening premade phage display human scFv library.

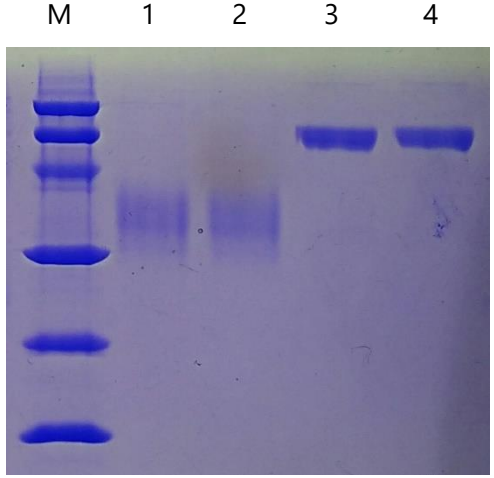
- QC of target preparation
- Process monitoring of biopanning
- Process monitoring of in-solution panning with Dynabeads
- QC polyclonal phage ELISA
- ELISA validation of the candidates
- QC of the selected binders
- Conclusions

06-1 QC of Target Preparation

- Two targets named as human-1 protein and mouse-1 protein were first biotinylated and verified by SDS-PAGE.
- After biotinylation, the two targets were tested by ELISA. The two targets were successfully biotinylated and have good binding capacities to HRP-streptavidin.

Coating Protein	OD490
Bio-human-1 protein	2.971
Bio-mouse-1 protein	3.102
P : 2.155	

Primary antibody: HRP-streptavidin



SDS-PAGE

M: molecular weight standard
Lane 1: human-1-His Tag (2.24 μg)
Lane 2: human-1-His Tag-biotin (2.24 μg)
Lane 3: mouse-1-Fc Tag (2 μg)
Lane 4: mouse-1-Fc Tag-biotin (2 μg)

06-2 Process Monitoring of Biopanning

Round	Conditions	Input	Output	Enriching factor
1st	Target protein: 30 μg/mL Bio-human-1 protein Washing: 0.1% Tween20 PBST, 9 times Elution: Trypsin digestion Pre counter select: 2% M-PBS	2.00×10^{11}	1.42×10^{5}	1.41×10^{6}
2nd-P	Target protein: 30 μg/mL Bio-human-1 protein Washing: 0.2% Tween20 PBST, 9 times Elution: Trypsin digestion Pre counter select: 2% M-PBS	2.41×10^{11}	5.80×10^{5}	4.16×10^{5}
2nd-N	Target protein: no coating Washing: 0.2% Tween20 PBST, 9 times Elution: Trypsin digestion Pre counter select: 2% M-PBS	3.01×10^{10}	6.98×10^{4}	4.31×10^{5}
3rd-P	Target protein: 20 μg/mL Bio-human-1 protein Washing: 0.3% Tween20 PBST, 9 times Elution: Trypsin digestion Pre counter select: 2% M-PBS	2.43×10^{11}	2.34×10^{8}	1.04×10^{3}
3rd-N	Target protein: no coating Washing: 0.3% Tween20 PBST, 9 times Elution: Trypsin digestion Pre counter select: 2% M-PBS	3.05×10^{10}	4.48×10^{7}	6.81×10^{2}

06-2 Process Monitoring of Biopanning

Round	Conditions	Input	Output	Enriching factor
4th-P	Target protein: 30 μg/mL Bio-mouse-1 protein Washing: 0.3% Tween20 PBST, 9 times Elution: Trypsin digestion Pre counter select: 2% M-PBS	2.55×10^{11}	3.64×10^{8}	7.0×10^{2}
4th-N	Target protein: no coating Washing: 0.3% Tween20 PBST, 9 times Elution: Trypsin digestion Pre counter select: 2% M-PBS	3.19×10^{10}	6.29×10^{7}	5.07×10^{2}
4th-C	Target protein: 30 μg/mL Bio-human-1 protein Washing: 0.3% Tween20 PBST, 9 times Elution: Trypsin digestion Pre counter select: 2% M-PBS	3.19×10^{10}	3.58×10^{7}	8.91×10^{2}

Enriching factor=input/output

We performed four rounds screening for bio-human-1 protein and got the good enriching effect. On the basis of three rounds screening for bio-human-1 protein, we performed one round screening for bio-mouse-1 protein (together with the fourth round screening for bio-human-1 protein). As shown in the table above, we got good elution output of bio-mouse-1 protein panning result, and an obvious change of enriching factor was also observed. However, heavier backgrounds were found, which may due to the binding of biotin to streptavidin.

06-3 Process Monitoring of In-Solution Panning with Dynabeads

Round	Conditions	Input	Output	Enriching factor
1st-P	Target protein: 500 μL library phages+50 μg(30 μg/mL) Bio-human-1 Protein Beads: 500 μg Dynabeads MyOne Streptavidin T1 Blocking: 5% BSA-PBS Washing: 0.1% Tween20 PBST, 9 times Elution: Trypsin digestion Pre counter select: pre-blocked 500 μg Dynabeads MyOne Streptavidin T1	5.00×10^{11}	3.16×10^{4}	1.58×10^{7}
1st-N	Target protein: 500 μL library phages+1.5 mL PBS Beads: 500 μg Dynabeads MyOne Streptavidin T1 Blocking: 5% BSA-PBS Washing: 0.1% Tween20 PBST, 9 times Elution: Trypsin digestion Pre counter select: pre-blocked 500 μg Dynabeads MyOne Streptavidin T1	5.00×10^{11}	1.98×10^{4}	2.53×10^{7}
2nd-P	Target protein: 500 μL library phages+50 μg(30 μg/mL) Bio-human-1 Protein Beads: 500 μg Dynabeads MyOne Streptavidin T1 Blocking: 5% BSA-PBS Washing: 0.2% Tween20 PBST, 9 times Elution: Trypsin digestion Pre counter select: pre-blocked 500 μg Dynabeads MyOne Streptavidin T1	5.15×10^{11}	1.40×10^{6}	3.68×10^{5}

06-3 Process Monitoring of In-Solution Panning with Dynabeads

Round	Conditions	Input	Output	Enriching factor
2nd-N	Target protein: 500 μL library phages+1.5 mL PBS Beads: 500 μg Dynabeads MyOne Streptavidin T1 Blocking: 5% BSA-PBS Washing: 0.2% Tween20 PBST, 9 times Elution: Trypsin digestion Pre counter select: pre-blocked 500 μg Dynabeads MyOne Streptavidin T1	5.15×10^{11}	1.04×10^{4}	4.95×10^{7}
3rd-P	Target protein: 500 μL library phages+50 μg(30 μg/mL) Bio-human-1 Protein Beads: 500 μg Dynabeads MyOne Streptavidin T1 Blocking: 5% BSA-PBS Washing: 0.3% Tween20 PBST, 9 times Elution: Trypsin digestion Pre counter select: pre-blocked 500 μg Dynabeads MyOne Streptavidin T1	5.60×10^{11}	2.80×10^{10}	20
3rd-N	Target protein: 500 μL library phages+1.5 mL PBS Beads: 500 μg Dynabeads MyOne Streptavidin T1 Blocking: 5% BSA-PBS Washing: 0.3% Tween20 PBST, 9 times Elution: Trypsin digestion Pre counter select: pre-blocked 500 μg Dynabeads MyOne Streptavidin T1	5.60×10^{11}	1.24×10^{5}	4.52×10^{6}

06-3 Process Monitoring of In-Solution Panning with Dynabeads

Round	Conditions	Input	Output	Enriching factor
4^{th}-P	Target protein: 500 μL library phages+50 μg(30 μg/mL) Bio-mouse-1 Protein Beads: 500 μg Dynabeads MyOne Streptavidin T1 Blocking: 5% BSA-PBS Washing: 0.3% Tween20 PBST, 9 times Elution: Trypsin digestion Pre counter select: pre-blocked 500 μg Dynabeads MyOne Streptavidin T1	5.00×10^{11}	2.00×10^{10}	25
4^{th}-N	Target protein: 500 μL library+1.5 mL PBS Beads: 500 μg Dynabeads MyOne Streptavidin T1 Blocking: 5% BSA-PBS Washing: 0.3% Tween20 PBST, 9 times Elution: Trypsin digestion Pre counter select: pre-blocked 500 μg Dynabeads MyOne Streptavidin T1	5.00×10^{11}	1.94×10^{5}	2.58×10^{6}

Enriching factor=input/output

In order to remove the background binding, we performed four rounds of In-Solution Panning with Dynabeads. A prominent enriching effect for the human-1 protein was observed. The difference between the target screening and the no-coating screening was obvious. The Bio-mouse-1 protein was also used as target in the 4^{th} round of biopanning, no heavy background binding was found.

06-4 QC Polyclonal Phage ELISA

QC polyclonal phage ELISA (solid phase):

Clones	Coating: Human-1 Protein	Coating: Mouse-1 Protein	No coating BSA blocking
1st	0.190	0.166	0.114
2nd-P	0.267	0.185	0.121
3rd-P	0.443	0.224	0.114
4th-P	0.420	0.310	0.113
M13KO7	0.132	0.130	0.126
1% M-PBS	0.107	0.112	0.115
Direct coating of M13K07: 3.033			

From polyclonal phage ELISA for the all rounds elution outputs from the solid phase panning, no significant difference was observed between the target protein and the negative control (BSA blocked no coating well), which is consistent with the enriching factor.

06-4 QC Polyclonal Phage ELISA

The 2nd QC polyclonal phage ELISA (in-solution):

Clones	Coating: Human-1 Protein	Coating: Mouse-1 Protein	No coating BSA blocking
1st-P	0.217	0.137	0.125
2nd-P	2.938	0.207	0.104
3rd-P	2.779	0.221	0.111
4th-P	1.132	0.522	0.118
M13KO7	0.164	0.124	0.137
1%M-PBS	0.097	0.116	0.114
Direct coating of M13K07: 2.699			

From polyclonal phage ELISA for the all rounds elution outputs from the in-solution panning, a difference was observed between the target protein and the negative control (BSA blocked no coating well). The difference between the mouse-1 protein and the negative control (BSA blocked no coating well) was also significant.

06-5 ELISA Validation of The Candidates

QC monoclonal phage ELISA:

Clones	Coating: human protein	Coating: mouse protein	No coating BSA blocking
1	2.4940	0.1020	0.1240
2	2.6530	0.3970	0.0990
8	2.8820	0.1120	0.1010
10	1.7210	0.3770	0.0900
12	2.8820	0.1180	0.2990
14	2.8310	0.0830	0.0990
15	2.7870	0.4820	0.0900
17	2.7730	0.0760	0.0780
20	2.6020	0.1720	0.1010
21	2.6210	0.3890	0.0950
22	2.5770	0.0810	0.0780
25	2.7600	0.1910	0.0650
27	0.5040	0.3880	0.1380
28	2.4670	0.1890	0.2530
31	2.4040	0.0740	0.0730
32	2.5540	0.0610	0.0660
34	0.4420	0.3980	0.1190
35	2.5210	0.1020	0.0920
36	2.7970	0.4010	0.1010
37	2.7970	0.0940	0.1070
38	2.5460	0.3950	0.0970
39	2.3800	0.1000	0.1050

We picked up 40 clones from the 4rd round phage pool for phage amplification and validation using monoclonal phage ELISA. As shown in table, we found 19 out of 40 clones bind to the human target positively, while 8 clones of them can also bind to the mouse target.

06-6 QC of the Selected Binders

By DNA sequencing for all the 8 positive clones, 2 unique scFv were identified, named as 10 and 27. The amino acids sequences of clone 10 and clone 27 were as shown.

Sequence summary of the positive clones:

Sequence	Clones
1	2,**27**,34
2	**10**,15,21,36,38

➢ Clone 10:

Protein: VH-linker-VL, CDRs are framed

EVQLLESGGGLVQPGGSLRLSCAASGFTFS[ ]WVRQAPGKGLEWVS[ ]ISRDNSKNTL
YLQMNSLRAEDTAVYYCAK[ ]WGQGTLVTVSSGGGGSGGGGSGGGGSTDIQMTQSPSSLSASVGDRVTITC[ ]
[ ]WYQQKPGKAPKLLIY[ ]GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC[ ]FGQGTKVE
IKR

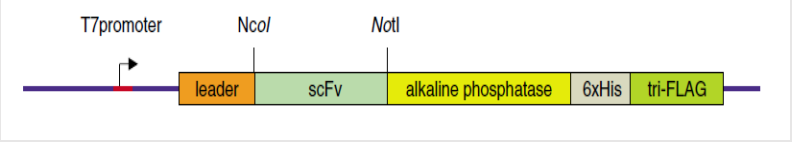
➢ Clone 27:

Protein: VH-linker-VL, CDRs are framed

EVQLLESGGGLVQPGGSLRLSCAASGFTFS[ ]WVRQAPGKGLEWVS[ ]ISRDNSKNTL
YLQMNSLRAEDTAVYYCAK[ ]WGQGTLVTVSSGGGGSGGGGSGGGGSTDIQMTQSPSSLSASVGDRVTITC[ ]
[ ]WYQQKPGKAPKLLIY[ ]GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC[ ]FGQGTKVE
IKR

06-6 QC of the Selected Binders

- The schematic map of pExpress-1™



- QC soluble scFv-AP ELISA [coat the cell lysate directly]

Clones	Induce at 30℃	Induce at 37℃
10-①	2.022	1.561
10-②	2.944	1.578
27-①	2.005	1.632
27-②	1.324	0.821
TG1 lysate	0.146	
TBS	0.141	

- QC soluble monoclonal soluble scFv-AP ELISA [coat the target]

Clones	Induce Temp	Coating: Human	Coating: Mouse	No coating
10-①	30℃	2.891	0.532	0.180
	37℃	3.610	0.630	0.177
10-②	30℃	3.414	0.489	0.155
	37℃	2.102	0.458	0.148
27-①	30℃	3.404	0.463	0.153
	37℃	3.595	0.496	0.125
27-②	30℃	2.799	0.483	0.115
	37℃	3.541	0.492	0.120
TG1 lysate		0.257	0.120	0.154
TBS		0.140	0.123	0.128

- We cloned clones 10 and 27 into soluble scFv-AP expression vector and they were expressed as soluble scFv-AP successfully.
- From the two ELISA assay, two selected clones can bind to the human-1 protein positively.
- Consistent with phage ELISA results, the two clones also showed weakly binding to the mouse target.

06-6 QC of the Selected Binders

- We performed competitive ELISA assay to test the inhibition of the soluble scFv antibodies to the interaction between Protein-1 and its ligand L1 .
- Both of the two clones could inhibit the binding of Protein-1 to its ligand L1.

QC monoclonal phage competitive ELISA

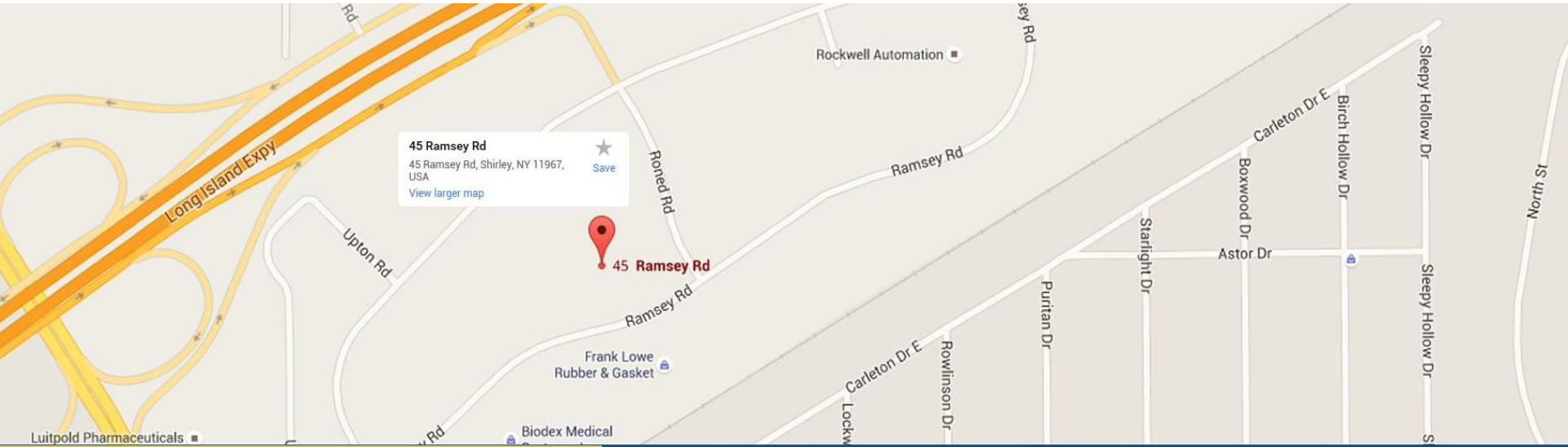
	Phage (Diluted in PBS)	OD_{490}	
TBS + Ligand	-	2.069	2.077
Clone 10 in TBS+ Ligand	1x	0.485	0.398
	10x	1.912	1.923
Clone 27 in TBS+ Ligand	1x	1.432	1.337
	10x	1.809	1.812

Coating: Protein-1; Ligand: L1-Biotin (0.6 μg/well);
Secondary antibody: HRP-streptavidin; Phage titers: ~10^{13} cfu/mL

06-7 Conclusions

- The client provided two target proteins named as human-1 protein and mouse-1 protein, respectively. We were contracted to identify antibodies which have cross-reactivity to both targets and could inhibit the interaction between the protein target and its ligand.
- We used our premade phage display human scFv library to select the scFv clones to the target proteins.
- We first performed four rounds of solid phase biopanning for human-1 protein. However, although good enriching effect was observed, heavier background binding also appeared.
- We then performed 4 rounds of in-solution panning and successfully removed the background binding.
- From the validation of 40 clones, we found 8 positive clones and finally identified two unique clones: clone 10 and clone 27.
- The two clones were expressed in soluble protein form. They can bind to the human-1 protein positively and weakly bind to the mouse-1 target.
- Both of two clones could inhibit the binding of protein-1 to its ligand L1.

Contact Us



Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-871-5806 **Fax:** 1-631-207-8356

Email: info@creative-biolabs.com

Web: www.creative-biolabs.com