Published in final edited form as:

Methods Mol Biol. 2009 ; 525: 129–xv. doi:10.1007/978-1-59745-554-1_6.

Construction of a Large Naïve Human Phage-Displayed Fab Library Through One-Step Cloning

Zhongyu Zhu and Dimiter S. Dimitrov

Abstract

Antibody-based therapeutics is attracting more attention in the post-genome era, in contrast to a diminution in the initial high expectation for rapid development of gene-based therapeutic modalities. In support to the antibody-based therapeutics, the advent of recent technologies has made human antibody screening and production progressively more economic. Among those technologies, phage-display antibody library has been successfully applied in the antibody-based drug development both as fully human antibody sources and tools for antibody engineering. Building up a high-quality antibody library with a large library size and high diversity has been crucial for successful isolation of antibodies. Here we describe an efficient strategy for the construction of a large naïve phage-display human Fab library with one-step cloning. Optimization of each key step is extensively discussed and simplified protocols for library panning and Fab production are also described.

Keywords

Phage display; Fab; antibody library; IgM; one-step cloning

1. Introduction

Since the invention of phage-display technology, phage-displayed antibody libraries have been successfully used as important sources and tools for development of fully human antibodies and antibody-based therapeutics mostly against cancer, and also against other diseases including infectious diseases and immune disorders. The first phage-display-derived antibody drug (Humira®) against human TNF- α is now in clinical use for treatment of rheumatoid arthritis, Cohen's disease, and ankylosing spondylitis (1–3). Hundreds of other phage-display-derived human antibodies are in preclinical development and dozens in clinical trials. Design and construction of large high-quality phage-displayed antibody libraries, as the one described below, will continue to be of central importance for the successful selection of good high-affinity antibodies. Although the generation of such libraries is a long and labor-intensive process it is still cost-effective in comparison to other methodologies for identification of antigen-specific human monoclonal antibodies like, for example, the generation of transgenic mice with human immunoglobulin genes.

Building a high-quality phage-displayed antibody library requires a source of large number of well-diversified antibody genes. A variety of immunoglobulin gene sources including blood lymphocytes, bone marrow, and spleen have been used for library construction. From these sources IgM-derived antibody genes have been successfully used for library construction and selection of high-affinity antibodies (4–7), even though IgM-specific antibodies are typically of relatively low affinity *in vivo* compared to antibodies from the

IgG isotype. IgM antibody libraries are preferred as non-immune libraries with high diversity, while IgG-based libraries are typically biased to particular antigen(s). An added benefit is that IgM antibody gene repertoire has been also shown to contain self-reactive antibodies (8), which is important in case of targeting cancer cells overexpressing a given membrane protein. Numerous examples have already shown that high-affinity human antibodies against a panel of antigens ranging from viruses to human self-antigens can be isolated from the phage-display antibody library described below which is based on an IgM-derived antibody gene repertoire (9–12). This protocol describes the construction of a large naïve phage-displayed Fab library through one-step cloning: *Sfi*I is used as the cloning enzyme, which can keep almost all the antibody gene repertoire intact during the cloning procedure. Simplified procedures for library panning and Fab preparation are detailed when needed.

2. Materials

2.1. Lymphocyte Isolation

1. Ficoll-Paque Plus reagents (Amersham Bioscience).
2. Eppendorf centrifuge 5804R (Eppendorf).
3. BD Falcon™ Conical Tubes (BD Biosciences).
4. Hemacytometer (Sigma).
5. Qiazol (Qiagen).

2.2. RNA Isolation and cDNA Synthesis

1. RNeasy Mini Kit (Qiagen).
2. SuperScript™ III First-Strand Synthesis SuperMix (Invitrogen).
3. Corning® PCR tubes, free of RNase and DNase (Sigma).
4. Ultra pure water (Quality Biologicals), free of RNase and DNase.
5. Eppendorf centrifuge 5417R (Eppendorf).

2.3. Amplification of Heavy Chains, Light Chains, and Linker

1. Bio-Rad PTC-100 thermal cycler (Bio-Rad).
2. High-Fidelity PCR Master (Roche).
3. Primers shown in Table 6.1.

2.4. Preparation of Fab Fragment Insert Through Overlapping PCR

1. Plasmid extraction Kit (Qiagen).
2. Gel purification Kit (Qiagen).
3. Primers shown in Table 6.1.

2.5. Construction of Fab Library

1. Micron Ultracel YM-50 (Millipore).
2. TG1 electroporation-competent cells (Stratagene).
3. Gene Pulser/MicroPulser Cuvettes (Bio-Rad).
4. Gene Pulser (Bio-Rad).

5. Restriction enzymes: *Sfi*I, 20,000 units/ml (New England BioLabs).
6. T4 DNA Ligase, 400,000 units/ml (New England BioLabs).

2.6. Preparation of Library Stock in Three Different Formats

1. *2YT medium*: 0.5% (w/v) NaCl; 1% (w/v) Yeast extract (BD Biosciences); 1.6% (w/v) Tryptone (BD Biosciences). Dissolve in distilled water and autoclave.
2. 20% (w/v) glucose (Sigma) in distilled water. Sterilize by filter (Nalgene).
3. M13KO7 helper phage (Invitrogen).
4. *Antibiotics*: 100 mg/ml ampicillin (Sigma); 100 mg/ml kanamycin (Sigma).
5. PEG8000 (Sigma).
6. NaCl (Sigma).

2.7. Library Panning Against Antigens Conjugated on Magnetic Beads

2.7.1. Conjugation of Proteins to Magnetic Beads

1. Dynabeads M-270 Epoxy (Invitrogen)
2. 0.1 M sodium phosphate buffer, pH7.4 (Quality Biological Inc.).
3. 3 M ammonium sulfate (Sigma).

2.7.2. Phage Library Panning

1. *PBST*: 1 × PBS containing 0.05% Tween 20 (Sigma).
2. MagnaBind Magnet for 1.5-ml Microcentrifuge Tube (Pierce Inc.).

2.7.3. Phage ELISA Screening

1. 96-well ELISA plate (Corning).
2. HRP-conjugated anti-M13 phage antibodies (Amersham Bioscience).

2.7.4. Fab Expression and Purification

1. HB2151 bacteria strain.
2. Polymixin B (Sigma).

3. Methods

Full coverage of the antibody gene repertoire and large size are crucial for a high-quality phage-display antibody library. To cover each subfamily of the human antibody genes, carefully selected primers matching the conserved N-terminal and C-terminal region of heavy-chain and light-chain were used for the amplification of each family separately. To avoid the loss of diversity of the gene repertoire, enough of the DNA template input should be used at each overlapping PCR; also limited cycles of amplification should be avoided to prevent from introducing bias in the final library. The efficacy of DNA transformation into bacteria has always been the limiting step for constructing large libraries, electroporation has been frequently used to make large bacterial libraries due to its high efficacy; still the DNA preparation for electroporation should be always optimized to reach high transformation efficiency.

3.1. RNA Isolation

1. As the easiest source for antibody gene cloning, peripheral blood lymphocytes from healthy donors can be used. Isolate B lymphocytes from total of 2 l of blood from 10 donors on a Ficoll-Paque gradient following the Manual of the manufacturer.
2. Dissolve the cell pellet immediately in 50 mL of Qiazol (*see Note 1*). Isolate total RNA following the instructions from RNeasy Mini Kit with optional step to eliminate the genomic DNA (*see Note 2*).
3. Freeze the total RNA in water at -80°C for short-term storage. Vacuum-dried RNA should be prepared for long-term storage.

3.2. cDNA Synthesis

1. In order to completely recover the entire antibody gene repertoire, prepare cDNA with both random hexamer and Oligo dT as primer; 250 μg of total RNA for each primer with SuperscriptTM III first-strand Synthesis System from Invitrogen can be used.
2. Denature the total RNA for 5 min at 65°C in the presence of 20 μg of random primer or Oligo dT; subsequently add dithiothreitol according to the supplier's instructions, as well as 250 μM dNTP, 800 units of RNaseOUT (40 units/ μl), and 2000 units of Moloney murine leukemia virus reverse transcriptase (200 units/ μl) in a total volume of 500 μl . After 50 min at 50°C , terminate the incubation reaction by heating at 85°C for 5 min.
3. Mix and concentrate the cDNA from the two reactions by filtering (filter with molecular mass cutoff size 10 kDa) and store in 100 μl of water at -80°C freezer (*see Note 3*).

3.3. Amplification of Heavy Chains, Light Chains, and Linker

1. Oligonucleotides used for primary PCR amplification of human heavy- and light-chains are described in Table 6.1 and can be synthesized by any service provider.
2. First obtain IgM-derived heavy-chain variable regions by a primary PCR with a HuIgM Reverse primer and separate VH FOR primers.
3. Re-amplify the VH fragments with a combination of JHR primers, annealing to the 3' end of VH, and separate VH FOR primers, annealing to the 5' end of corresponding purified PCR product; 150 ng of purified DNA fragment can be used as template in a 100- μl reaction volume (*see Note 4*).
4. Amplify CH1 fragment of IgG1 for the overlap PCR from vector pComb3X, using primer CH1F and primer FlagR in the PCR.
5. To obtain the Fd fragment, perform overlap PCR with 100 ng of purified VH and CH1 fragments in $5 \times 100 \mu\text{l}$ PCR reactions (7 cycles of 30 s at 94°C , 30 s at 50°C , and 2 min at 72°C without primer and another 15 cycles with primer VHF and FlagR) (*see Note 5*).

¹Total RNA in peripheral blood lymphocytes dissolved in Qiazol can be stored at -80°C for several months without losing diversity and quality, which allows collecting enough B cells for a longer time if it is difficult to collect liters of blood at one time.

²To make sure that there is no contamination of genomic DNA in the RNA product, which is essential for high-quality PCR amplification of antibody gene fragments later on.

³Avoiding phenol-chloroform extraction and ethanol-salt precipitation through filtering-dialysis at this step can increase the recovery of cDNA from the reaction mixture and improve the performance of PCR later.

⁴The large amount of input and small number of PCR cycles are important for the maintenance of the diversity of the antibody gene repertoire in the library.

6. Amplify light chains (kappa and lambda families) by PCR with a set of C κ reverse or C λ reverse primer annealing to the 3' end of the constant domain and *Sfi*-tagged FOR primers, priming at the 5' end of the VL-regions.
7. Perform PCR in a volume of 100 μ l using High-Fidelity PCR MasterTM and 500 pM of each primer for 28 cycles (1 min at 94°C, 1 min at 55°C, and 2 min at 72°C); nine separate IgM-derived VH amplifications can be generated with 3 μ l cDNA (equivalent to 6 μ g of PBL RNA) as template for each reaction.
8. For the light-chain families, six different kappa-chain products and 11 lambda-chain products can be obtained and mixed from each family and gel-purified.
9. For the preparation of linker between light chain and heavy chain, primer linkerF and primer linkerR should be used. Purify all amplified products from agarose gel with the QIAquick Gel Extraction Kit and determine the DNA concentration through spectrophotometer measurement.

3.4. Preparation of Fab Fragment Insert Through Overlapping PCR

1. Fuse the heavy-chain and light-chain by three-fragment overlapping PCR using 100 ng of each gel-purified light chain and heavy chain and the same molar amount of linker in 5 $\times$ 100 μ l reactions.
2. Pre-amplify the full-length Fab insert without flanking primers for nine cycles of 30 s at 94°C, 30 s at 50°C, and 3 min at 72°C, and then amplify the full-length Fab insert for another 15 cycles with the flanking primers SfiF and FlagR under the same conditions (*see Note 5*).

3.5. Construction of Fab Library

1. For the construction of the Fab library, gel-purify the overlapped PCR products containing the light chains and heavy chains prior to and after digestion with *Sfi*I.
2. Digest the vector with *Sfi*I restriction enzyme and gel-purify it following the instructions of the manufacturers (*see Note 6*).
3. Ligate gel-purified fragments (8 μ g with kappa light-chain and 6 μ g with lambda light-chain) into 8 μ g and 6 μ g of *Sfi*I-digested and gel-purified phagemid vector pComb3X in 500- μ l reactions, respectively.
4. Change the DNA buffer system to pure water using Micron filter with a 50 kDaD cutoff size, and further concentrate to appropriate volume through DNA vacuum concentrator (*see Note 7*).
5. Electroporate the two desalted ligation mixtures into competent TG1 bacteria with the Gene Pulser System II; mix 100 μ l of the total DNA-ligated preparation with 1.5 ml of TG1-competent cells, distribute into 60 aliquots, and put each one into 0.1-cm cuvette, then electroporate using the pre-set program with setting at 1.8 kV/200 ohms/25 μ F.

⁵Pre-amplification of the two fragments without flanking primers to fuse the full-length heavy-chain fragment is crucial for the specific amplification with primers later on. And also longer cycles than 15 after the adding of primers could create a lot of unspecific amplification; optimization of the PCR conditions such as DNA input amount and number of cycles at each stage is strongly suggested at this step.

⁶Plasmid or gel-purified DNA with high quality is very important for complete digestion with *Sfi*I. Preparation of the vector using plasmid containing full-length Fab insert is essential to monitor the complete digestion on agarose gel and purify the complete cut vector from the uncut. Longer flanking sequences beside the *Sfi*I site on the insert are necessary for efficient digestion and better differentiation of the digested product from the uncut fragment on the agarose gel.

⁷Avoiding the regular phenol-chloroform extraction and ethanol-salt precipitation at this step can dramatically improve the electroporation efficacy.

6. Spread transformed bacteria on 16 large 2YT agar plates (24 cm × 24 cm) supplied with 100 µg/ml ampicillin and 1% glucose, and incubate at 37°C overnight.

3.6. Preparation of Library Stock in Three Different Formats

3.6.1. Bacterial Glycerol Stock

1. Scrape the bacteria from the plates with 2YT-Amp-Glu medium (2YT medium containing 100 µg/ml ampicillin and 1% glucose) to get 250 ml of suspension and divide into three parts for library stock preparation (*see* Note 8).
2. Add glycerol to a final concentration of 25% to one part of the bacteria and freeze aliquots of 2 ml at –80°C as bacterial library stock.

3.6.2. Phage Glycerol Stock Preparation

1. Grow the second part of the bacteria to 100-fold larger number of bacteria in 2 l of the 2YT-AMP-GLU medium at 37°C for several hours until OD at 600 nm reaches 0.8.
2. Add M13KO7 helper phage at multiplicity of infection (MOI) of 10 to the bacteria and incubate at 37°C for another 30 min.
3. Spin down the bacteria infected with helper phage, resuspend the bacterial pellet into 2 l of 2YT medium supplied with 100 µg/ml ampicillin and 25 µg/ml of kanamycin, and grow the bacteria at 30°C overnight with shaking at 250 rpm.
4. Spin down the bacteria at 8000 *g* for 15 min at 4°C, add ¼ volume of PEG/NaCl solution into the collected supernatant and incubate the mixture on ice for 1 h. Use 50 ml of PBS per liter of culture to resuspend the phage pellet after centrifugation at 10,000 *g* for 15 min. Perform another round of centrifugation at 10,000 *g* for 10 min to eliminate the bacterial contamination in the phage pellet.
5. Repeat the phage precipitation with PEG/NaCl to further purify the phage and resuspend in the same volume of PBS (*see* Note 9), add glycerol to a final concentration of 50% and freeze at –80°C 1-ml aliquots for long-term storage as phage library stock.

3.6.3. Plasmid Stock

1. Grow the third part transformed bacteria in 1 l of 2YT-Amp-Glu medium at 37°C with shaking for 1–2 h to revive the bacteria.
2. Spin down the bacteria and extract the plasmid using the Maxi plasmid kit for plasmid library stock preparation.
3. Freeze aliquots containing 50-µg plasmid DNA library stock at –80°C for long-term storage.

⁸Storing the library in different formats is important to preserve the valuable antibody library source for long term. The plasmid stock is the most stable format while the phage library stock can be directly and easily recovered for library panning.

⁹Two-time PEG/NaCl precipitations are needed to further purify the phage library from bacterial contaminants and also soluble antibody fragment or bacterial proteins and possible proteinase contamination.

3.7. Library Panning Against Antigens Conjugated on Magnetic Beads

3.7.1. Conjugation of Proteins to Magnetic Beads

1. Weigh out the required quantity of beads (15 mg beads is equivalent to 10^9 beads) and wash the beads three times with 0.1 M sodium phosphate buffer, pH 7.4. Make a homogeneous suspension of the washed beads in the same buffer.
2. Add the calculated amount of ligand solution to the bead suspension. Mix the suspension well before adding the calculated ammonium sulfate stock solution (3 M ammonium sulfate).
3. Incubate for 16–24 h at 37°C with slow tilt rotation (*see* Note 10).
4. Place the tube on the magnet for 4 min for magnetic separation. Carefully turn the magnet (with the tube in place) upside-down twice, to ensure collection of any beads that might remain in the cap. Remove the supernatant.
5. Wash the coated beads four times with PBS or PBS with blocking protein. Blocking protein like BSA or skimmed milk powder should be added at 0.1%–0.5% when this does not interfere with your downstream application.
6. If the downstream application involves elution steps, physically adsorbed ligand can be removed by washing for 10 min in 0.5%–1% (w/v) Tween 20/Triton X-100 or similar nonionic detergent.
7. Resuspend the coated beads to the desired concentration in PBS or PBS with blocking protein. The beads are now ready for use.

3.7.2. Phage Library Panning

1. Pick single colony from the TG1 bacterial plate and grow the bacteria in 3 ml 2YT medium at 37°C until $OD_{600} \approx 0.9$ and store at room temperature for phage infection later.
2. At the same time, thaw a phage library aliquot. Precipitate the phage library with 250 μ l (1/4 of the phage suspension volume) PEG/NaCl solution (20% PEG8000 and 2.5 M NaCl) and incubate on ice for 30 min followed by spinning the precipitated phage for 10 min at 10,000 g. Discard supernatant and resuspend the phage pellet in 200 μ l PBS.
3. Add 100 μ l 4% MPBS to the 200 μ l phage library and then add 150 μ l suspension of antigen conjugated to magnetic beads ($\sim 3 \times 10^7$ beads with about 7 μ g pure antigen).
4. Incubate on a rotator at room temperature for 2 h followed by washing the beads 10 times with 1 ml 0.05% PBST (0.05% Tween-20 in PBS).
5. Resuspend the final pellet of magnetic beads in 100 μ l PBS and keep 10 μ l of beads suspension from the panning for long-term storage and monoclonal phage preparation for ELISA screening later on (*see* Note 11). Directly mix the rest 90 μ l of the suspension into 2 ml TG1 cells prepared earlier, incubate the mixture at 37°C

¹⁰Incubation at temperatures down to 4°C may be used for temperature-sensitive ligands, but be aware that the bond formation is slower and less efficient at low temperatures, and an additional 24 h should be used to ensure covalent coupling. Do not let the beads settle during the incubation period.

¹¹One of the advantages for panning on beads but not on plates or in immune tubes is that there is no need to elute the phage from the bound antigen on beads; phage bound to beads can stably stay in PBS for long-time storage and also the beads do not interfere with the infection of the bacteria by the phage.

with shaking for 1 h and then dilute into 10 ml 2YT medium containing 25 µg/ml ampicillin and 1 % glucose (*see* Notes 12, 13).

6. After two more hours of incubation at 37°C with shaking at 250 rpm, add ampicillin to a final concentration of 100 µg/ml. Let the bacteria grow until OD at 600 nm reaches 0.1, add calculated M13KO7 helper phage into the amplified TG1 cells with MOI of 10 and incubate at 37°C for another 30 min.
7. Spin down the helper phage-infected bacteria, resuspend the mixture pellet in 1 ml 2YT medium and remove the beads through magnetic absorption. Dilute the resuspended bacteria into 50 ml of 2YT medium supplemented with 100 µg/ml ampicillin and 25 µg/ml kanamycin and incubate at 30°C with shaking at 250 rpm overnight for phage amplification.
8. Refer to Section 3.6.3 for phage preparation; two times PEG/NaCl-precipitated phage is ready for the second round of panning.

3.7.3. Phage ELISA Screening

1. Prepare 2YT medium with 0.2% glucose and 100 µg/ml ampicillin and add 100 µl medium to each well of 96-well plate.
2. Pick single colony and add to each well from TG1 cells infected with bead-bound phage after panning. Incubate the 96-well plates at 37°C with shaking at 250 rpm until OD₆₀₀ reaches 0.5. It usually takes about 2 h.
3. Add 10⁹ helper phage in 25 µl 2YT medium per well. For one 96-well plate prepare 10¹¹ phages in 2.5 ml 2YT medium containing only 100 µg/ml ampicillin (Do not add glucose at this stage).
4. After adding the helper phage, incubate for 30 min at 37°C for the infection to complete. Add 50 µl of kanamycin and ampicillin in 2YT medium to make a final concentration of 100 µg/ml for ampicillin and 25 µg/ml for kanamycin. Do not add glucose at this stage.
5. Grow the phage at 30°C in a shaker overnight – from 12 h to 15 h.
6. Spin the plates at 3000 rpm for 15 min. Transfer the phage in the supernatant to target antigen-coated ELISA plates for phage ELISA screening; positive binding can be detected by HRP-conjugated anti-m13 phage antibodies.
7. Select positive clones for plasmid extraction and DNA sequencing. Select clones with unique sequences for soluble Fab preparation and characterization.

3.7.4. Fab Expression and Purification

1. Transform *E. coli* HB2151 competent cells with plasmid DNA from unique clones by heat-shock for 90 s at 42°C. Plate the transformed cells onto 2YT+ 100 µg/ml ampicillin and 1% glucose plates and incubate at 37°C overnight (*see* Note 14).
2. Inoculate 200 ml of 2xYT medium containing 100 µg/ml of ampicillin and 0.2% glucose with single colony from the freshly transformed plate. Incubate at 37°C in a shaker (250 rpm) until OD at 600 nm reaches about 0.5–0.8 (*see* Note 15).

¹²A volume of 2 µl of the infected bacteria at this step can be diluted and plated on 2YT agar plate supplemented with ampicillin and glucose to calculate the output number for monitoring the panning enrichment later on.

¹³Low ampicillin concentration at this stage allows the antibiotic activity of the infected bacteria to recover while inhibiting the growth of the uninfected bacterial.

¹⁴Whenever possible, always use freshly transformed bacteria for Fab expression, since even overnight storage of transformed bacterial colony at 4°C can dramatically decrease the production yield.

3. Induce the culture by adding IPTG to a final concentration of 0.5 mM and incubate at 30°C in a shaker (250 rpm) overnight.
4. Spin down the bacteria at 9000 *g* for 15 min at 4°C. Resuspend the bacterial pellet in 50 ml of PBS per liter culture supplemented with 0.5 million units polymixin B (see Note 16) and incubate at room temperature for 30 min with rotation.
5. Spin down the cellular debris at 20,000 *g* for 30 min at 4°C. Transfer the supernatant to a clean tube.
6. Purify the 6-histidine-tagged Fab by using Ni-NTA resin: wash the Ni-NTA with at least 10 ml PBS to eliminate the ethanol. Add 0.3 M NaCl and 5 mM imidazol to the supernatant. Mix well and incubate at room temperature for 10 min before loading onto column. Wash the column with at least 10 ml of PBS containing 0.3 M NaCl and 5 mM imidazol (washing buffer). Elute the Fab with two portions of 1 ml PBS containing 200 mM imidazol from the Ni-NTA resin.

Acknowledgments

This project has been funded in whole or in part with federal funds from the National Cancer Institute, National Institutes of Health, under contract N01-CO-12400. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the US Government. This project is also funded by the NIH Biodefense Program (D.S.D.).

Reference

1. Mease PJ. Adalimumab an anti-TNF agent for the treatment of psoriatic arthritis. *Expert Opin. Biol. Ther.* 2005; 5:1491–1504. [PubMed: 16255652]
2. Machold KP, Smolen JS. Adalimumab – a new TNF-alpha antibody for treatment of inflammatory joint disease. *Expert Opin. Biol. Ther.* 2003; 3(2):351–360. [PubMed: 12662147]
3. Plosker GL, Lyseng-Williamson KA. Adalimumab in Crohn's disease. *Bio Drugs.* 2007; 21:125–132.
4. De Haard HJ, van NN, Reurs A, et al. A large non-immunized human Fab fragment phage library that permits rapid isolation and kinetic analysis of high affinity antibodies. *J. Biol. Chem.* 1999; 274:18218–18230. [PubMed: 10373423]
5. Loiset GA, Lobersli I, Kavlie A, et al. Construction, evaluation and refinement of a large human antibody phage library based on the IgD and IgM variable gene repertoire. *J. Immunol. Methods.* 2005; 299:47–62. [PubMed: 15914190]
6. Dorsam H, Rohrbach P, Kurschner T, et al. Antibodies to steroids from a small human naive IgM library. *FEBS Lett.* 1997; 414:7–13. [PubMed: 9305722]
7. Gram H, Marconi LA, Barbas CF III, Collet TA, Lerner RA, Kang AS. In vitro selection and affinity maturation of antibodies from a naive combinatorial immunoglobulin library. *Proc. Natl. Acad. Sci. USA.* 1992; 89:3576–3580. [PubMed: 1565653]
8. Tsuiji M, Yurasov S, Velinzon K, Thomas S, Nussenzweig MC, Wardemann H. A checkpoint for autoreactivity in human IgM+ memory B cell development. *J. Exp. Med.* 2006; 203:393–400. [PubMed: 16446381]
9. Zhu Z, Chakraborti S, He Y, et al. Potent cross-reactive neutralization of SARS coronavirus isolates by human monoclonal antibodies. *Proc. Natl. Acad. Sci. USA.* 2007; 104:12123–12128. [PubMed: 17620608]

¹⁵Adding 0.2% glucose in the growth medium is necessary for shutting down the Fab expression at the early-stage growth of the bacterial culture before adding the IPTG for Fab expression induction.

¹⁶Polymixin B, as an antibiotic with bactericidal action on *E. coli*, has been successfully used for extraction of periplasm proteins from bacteria because of its simplicity and high efficiency compared to other procedures; it works through interfering with the permeability of bacterial cytoplasmic membrane.

10. Prabakaran P, Gan J, Feng Y, et al. Structure of severe acute respiratory syndrome coronavirus receptor-binding domain complexed with neutralizing antibody. *J. Biol. Chem.* 2006; 281:15829–15836. [PubMed: 16597622]
11. Feng Y, Zhu Z, Xiao X, Choudhry V, Barrett JC, Dimitrov DS. Novel human monoclonal antibodies to insulin-like growth factor (IGF)-II that potently inhibit the IGF receptor type I signal transduction function. *Mol. Cancer Ther.* 2006; 5:114–120. [PubMed: 16432169]
12. Zhu Z, Dimitrov AS, Bossart KN, et al. Potent neutralization of Hendra and Nipah viruses by human monoclonal antibodies. *J. Virol.* 2006; 80:891–899. [PubMed: 16378991]

Table 6.1

Oligonucleotide primers used for construction of the library

HuIgM Reverse	5'-TGG AAG AGG CAC GTT CTT TTC TTT-3'
Cκ Reverse	5'-CTC CTA ATT AAT TAT CTA GAA TTA ACA CTC TCC CCT GTT GAA GCT CTT-3'
CA Reverse	5'-CTC CTA ATT AAT TAT CTA GAA TTA TGA ACA TTC TGT AGG GGC CAC TG-3'
V_H Forward	
HuVH1B/7A-FOR	5'-GCT GCC CAA CCA GCC ATG GCC CAG RTG CAG CTG GTG CAR TCT GG-3'
HuVH1C-FOR	5'-GCT GCC CAA CCA GCC ATG GCC SAG GTC CAG CTG GTR CAG TCT GG-3'
HuVH2B- FOR	5'-GCT GCC CAA CCA GCC ATG GCC CAG RTC ACC TTG AAG GAG TCT GG-3'
HuVH3B- FOR	5'-GCT GCC CAA CCA GCC ATG GCC SAG GTG CAG CTG GTG GAG TCT GG-3'
HuVH3C- FOR	5'-GCT GCC CAA CCA GCC ATG GCC GAG GTG CAG CTG GTG GAG WCY GG-3'
HuVH4B- FOR	5'-GCT GCC CAA CCA GCC ATG GCC CAG GTG CAG CTA CAG CAG TGG GG-3'
HuVH4C- FOR	5'-GCT GCC CAA CCA GCC ATG GCC CAG STG CAG CTG CAG GAG TCS GG-3'
HuVH5B- FOR	5'-GCT GCC CAA CCA GCC ATG GCC GAR GTG CAG CTG GTG CAG TCT GG-3'
HuVH6A- FOR	5'-GCT GCC CAA CCA GCC ATG GCC CAG GTA CAG CTG CAG CAG TCA GG-3'
Vκ Forward	
HuV κ 1B- FOR	5'-GGG CCC AGG CGG CC GAC ATC CAG WTG ACC CAG TCT CC-3'
HuV κ 2- FOR	5'-GGG CCC AGG CGG CC GAT GTT GTG ATG ACT CAG TCT CC-3'
HuV κ 3B- FOR	5'-GGG CCC AGG CGG CC GAA ATT GTG WTG ACR CAG TCT CC-3'
HuV κ 4B- FOR	5'-GGG CCC AGG CGG CC GAT ATT GTG ATG ACC CAC ACT CC-3'
HuV κ 5- FOR	5'-GGG CCC AGG CGG CC GAA ACG ACA CTC ACG CAG TCT CC-3'
HuV κ 6- FOR	5'-GGG CCC AGG CGG CC GAA ATT GTG CTG ACT CAG TCT CC-3'
Vλ Forward	
HuV λ 1A- FOR	5'-GGG CCC AGG CGG CC CAG TCT GTG CTG ACT CAG CCA CC-3'
HuV λ 1B- FOR	5'-GGG CCC AGG CGG CC CAG TCT GTG YTG ACG CAG CCG CC-3'
HuV λ 1C- FOR	5'-GGG CCC AGG CGG CC CAG TCT GTC GTG ACG CAG CCG CC-3'
HuV λ 2- FOR	5'-GGG CCC AGG CGG CC CAR TCT GCC CTG ACT CAG CCT-3'
HuV λ 3A FOR	5'-GGG CCC AGG CGG CC TCC TAT GWG CTG ACT CAG CCA CC-3'
HuV λ 3B- FOR	5'-GGG CCC AGG CGG CC TCT TCT GAG CTG ACT CAG GAC CC-3'
HuV λ 4- FOR	5'-GGG CCC AGG CGG CC CAC GTT ATA CTG ACT CAA CCG CC-3'
HuV λ 5- FOR	5'-GGG CCC AGG CGG CC CAG GCT GTG CTG ACT CAG CCG TC-3'
HuV λ 6- FOR	5'-GGG CCC AGG CGG CC AAT TTT ATG CTG ACT CAG CCC CA-3'
HuV λ 7/8- FOR	5'-GGG CCC AGG CGG CC CAG RCT GTG GTG ACY CAG GAG CC-3'
HuV λ 9- FOR	5'-GGG CCC AGG CGG CC CWG CCT GTG CTG ACT CAG CCM CC-3'
V_H Reverse	
HuJH1/2-R	5'-CGA TGG GCC CTT GGT GGA GGC TGA GGA GAC GGT GAC CAG GGT GCC-3'
HuJH3-R	5'-CGA TGG GCC CTT GGT GGA GGC TGA AGA GAC GGT GAC CAT TGT CCC-3'
HuJH4/5-R	5'-CGA TGG GCC CTT GGT GGA GGC TGA GGA GAC GGT GAC CAG GGT TCC-3'
HuJH6-R	5'-CGA TGG GCC CTT GGT GGA GGC TGA GGA GAC GGT GAC CGT GGT CCC-3'
SfiF	5'-GAG GAG GAG GAG GAG GAG GCG GGG CCC AGG CGG CC-3'

CH1F	5'-GCC TCC ACC AAG GGC CCA TCG GTC-3'
FlagR	5'-CTG GGG ACT GCC CTT ATC GTC ATC GTC CTT G-3'
linkerF	5'-TAA TTC TAG ATA ATT AAT TAG GAG-3'
linkerR	5'-GGC CAT GGC TGG TTG GGC AGC-3'
VHFor	5'-GCT GCC CAA CCA GCC ATG GCC-3'