

Cloning single chain antibody fragments (scFv) from hybridoma cells

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1. INTRODUCTION

The smallest antibody fragment containing a complete antigen binding site is the variable region (Fv), comprised of the antibody V_H and V_L domains. To kinetically stabilize the fragment by generating a very high local concentration for the association of the recombinant V_H and V_L domains, they are usually linked in single-chain Fv (scFv) constructs with a short peptide that bridges the approximately 3.5 nm between the carboxy-terminus of one domain and the amino-terminus of the other (1,2,3). The cloning of these variable domain genes has been well established as a common method for the “immortalization” of valuable mouse or rat hybridoma clones. Furthermore, a large number of protein fusions to the antigen binding variable (Fv) portion of an antibody in *E. coli* has been constructed to add a variety of heterologous functions. The genetic information for the variable heavy and light chain domains (V_H and V_L) is generally amplified from hybridoma cells using the polymerase chain reaction (PCR) with Immunoglobulin specific primers. A variety of primer sets for the amplification of mouse variable domains have been developed (4,5,6). However, several different sequences for each domain may be found in the PCR products amplified from the cDNA of a single hybridoma 'clone'. The fact that one cell line might express more than one heavy and light chain was also observed by other authors (7,8,9,10). Up to nine different V_L and five different V_H sequences with a homology of about 95% have been isolated from a single hybridoma culture of hybridoma clone PA-1 (S. Deyev, personal communication). Various reasons may be responsible for this heterogenous results. Additional antibody variable domain genes may derive from antibody mRNA transcribed from the genes of the parental cells, e.g. the myeloma fusion partner used for the generation of the hybridoma line. These chains, found in older fusion lines, are the easiest to identify by comparison to the known sequences of the original fusion partners.

It also has to be kept in mind that hybridoma cells are very different from the well regulated antibody producing cells in our body. In addition to the distorted ploidy resulting from the cell fusion and the partial cancer cell origin, hybridoma lines grow completely uncoupled from the strict regulation of the immune system, allowing for an abundance of gene expression deviations. Allelic dysregulation may lead to the expression of all V regions available, and pseudogenes with an internal stop codon were found as the most abundant PCR product in some cases. Even tandem-like assemblies of parts of two different V region sequences have been observed, indicating somatic recombination intermediates (J. Görnemann, personal communication). In addition to that, mutations can occur during prolonged cell culture, resulting in a heterogenous population containing a set of highly homologous antibody genes. This is of particular relevance for hybridoma clones which are difficult to propagate, which may indicate a selection pressure against the antibody production. Therefore, it is very important to prepare the cDNA from a freshly subcloned hybridoma clone tested for productivity and antigen binding activity. Additional mutations may be introduced in the PCR amplification step. While the use of polymerase with proofreading activity today allows to eliminate mutations introduced by the polymerase, sequence variation is introduced at the ends of the V regions degenerated mixtures of oligonucleotide primers are used (6). Here, due to the necessity to amplify unknown sequences, the hybridisation conditions during PCR of antibody sequences from cDNA have to be adjusted to allow mismatches. Under these conditions, even amplification of antibody DNA belonging to several different subgroups with the same primer was observed (10). Particular attention has to be paid to the Position 6 of V_H , where a mutational toggling between Gln and Glu has been shown to dramatically influence the stability of the product (11,12). The other primer can be placed in the constant region, eliminating the risk to introduce variation in this sequence. When using this approach, it is necessary to perform a set of PCR reactions with a large set of

different primers, and analyse quite a number of randomly picked V_H and V_L clones after the PCR, in a first screen for heterogeneity. A quick method is to compare the restriction patterns after digestion with BstNI (13). This enzyme has been shown to frequently cleave different sites in V-regions. It is not completely conclusive since it may give a hint for V region genes of completely different genetic origin but will rarely detect point mutations. It is more advisable to sequence the DNA of at least ten to twenty clones of each heavy and light chain PCR reaction product. If different sequences are found, all of their various V_H/V_L combinations should be assembled in an expression vector and tested for antigen binding separately. In case all directly cloned combinations of V_H and V_L fail to bind, the construction of a small phage library from each hybridoma by cloning the PCR products into a surface expression phagemid [e.g. pHAL, see 14] is recommended. This “hybridoma library” can then be screened for binding activity by panning on antigen. Since all the possible combinations of all sequences derived from the hybridoma cDNA are directly screened for antigen binding (15), this allows to functionally exclude wrong sequences, wrong V_H/V_L pairs and ligation artefacts.

As a result, the most recommended strategy is to start with a protein sequencing step. This both allows to design the optimal primers representing the aminoterminal regions of the V regions for perfect fit, and helps to verify the amplification of the correct V regions if part of the CDRs are covered in the sequencing runs.

However, even if the correct sequences have been cloned, an scFv may fail to bind in an assay optimised for the original monoclonal for several reasons. First, expression levels of individual scFvs derived from hybridomas can vary dramatically depending on the individual V region sequences, mostly as a result their folding efficiency, with the consequence of impractically low yields in some cases (16). The periplasmic preparation used for the specificity test should therefore always been analysed for its content of scFvs, most conveniently by immunoblot using an anti-tag reagent. Second, despite

many scFvs are quite rigid molecules, others may be unstable in solution, their activity decaying within a few hours. In contrast to scFvs derived from phage display libraries which during the panning rounds in addition to specificity are co-selected for efficient functional expression in *E. coli*, hybridoma derived scFv fragments have not undergone this selection. The use of freshly prepared periplasmic fractions can help to prove specificity of such a sequence prior to a genetic stabilisation by subcloning the V regions into vectors allowing expression as Fab or IgG. Finally, scFvs designed in the usual regime (with an 15-18 aa linker connecting the two regions) may fail to bind in comparison to their monoclonal parent due to the loss of the avidity effect allowed by densely coated antigen. Having two (in case of IgG) or 10 (in case of IgM) binding arms, the apparent affinity of in ELISA or on an Immunoblot can be increased by orders of magnitude when compared to a single binding domain. The best way to check this would be to use an enzymatically prepared Fab fragment of the parental mAb for positive control. However, these are not always easy to be prepared, but the reverse approach can be tried by dimerising/multimerising the scFv. A straightforward approach to achieve this is the reduction of the linker length between the V_H and V_L, thus forcing the scFv to assemble in the diabody/triabody/tetrabody format (17, 18). Alternatively, one of various vectors encoding multimerisation domains fused downstream to the scFv gene can be used (19).

Finally, a number of scFv with posttranslational modifications in the CDR regions have been described. Unpaired Cysteines in a CDR may affect activity or stability in long term storage, since they are prone to oxidation (20). Glycosylation is not unfrequently found in various CDRs, but does not necessarily affect binding (21). In some cases, the glycosylation decreases the affinity and the antibody benefits from a mutation removing the glycosylation site (22,23). However, in cases where the sugars contribute to the binding, the scFvs may not be functionally produced in *E. coli*. Mammalian cell lines (e.g.

CHO) are the expression host of choice in these cases. The most easy way to test this factor is by subcloning into a scFv-Fc expression vector (14). The contribution of sugars to the binding may also be assessed prior to cloning by glycosidase digestion of the maternal hybridoma antibody.

A large variety of expression vectors for scFv fragments have been developed. They all allow a secretion of the scFv fragment into the periplasmic space of *E. coli*, where the biochemical milieu promotes correct folding and formation of the intrachain disulphide bonds. This is achieved by employing an aminoterminal bacterial leader sequence (24) which is removed during secretion. Most vectors introduce additional motifs suited for identification and/or purification of the scFv fragments. Most common is the His-tag, allowing both IMAC purification and detection on Immunoblots, in ELISA etc. An example is the vector pOPE101 (25). Some vectors encode an unpaired Cys residue in the tag region of the expressed polypeptide. These vectors should only be employed if the cysteine residue is required afterwards for chemical conjugation since unpaired Cys residues have a negative influence on the yield (26). A collection of complementing protocols on various recombinant antibody selection, expression and analysis systems as well as alternative hybridoma cloning methods can be found in (27).

2. MATERIALS

2.1. Isolation of Antibody DNA

1. TRIzol Reagent (Invitrogen, Carlsbad, CA)
2. DEPC (Carl-Roth, Karlsruhe, Germany)
3. optional: Oligotex mRNA Mini Kit for isolation of mRNA from total RNA (QIAGEN, Hilden, Germany)

4. SuperScript[™] III First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA)
5. DNA Polymerase with proof reading function, e.g. Phusion[™] High-Fidelity DNA Polymerase (New England Biolabs, Ipswich, MA) or Expand High Fidelity PCR System (Roche, Mannheim, Germany)
6. dNTP Mix (MBI Fermentas, St.Leon-Rot, Germany)
7. Thermal cycler
8. Agarose gel electrophoresis equipment
9. QIAquick PCR Purification Kit and QIAquick Gel Extraction Kit (QIAGEN, Hilden, Germany)

2.2. Cloning into an Expression Vector

1. Restriction endonucleases HindIII, MluI, NcoI and NotI with appropriate buffers (New England Biolabs, Ipswich, MA)
2. Alkaline phosphatase (CIP) for DNA dephosphorylation (New England Biolabs, Ipswich, MA)
3. pOPE101 expression vector (24)
4. Agarose gel electrophoresis equipment
5. QIAquick PCR Purification Kit and QIAquick Gel Extraction Kit (QIAGEN, Hilden, Germany)
6. T4 DNA ligase and buffer (Promega, Madison, WI)
7. *E. coli* XL1-Blue -competent cells (Agilent, Santa Clara, CA)
8. LB medium containing 100 mM glucose and 100 µg/mL ampicillin
9. LB agar plates containing 100 mM glucose and 100 µg/mL ampicillin
10. QIAprep Spin Miniprep Kit (QIAGEN, Hilden, Germany)
11. Glycerol

2.3. Small Scale Expression Test

1. 2 x YT medium containing 100 mM glucose and 100 µg/mL ampicillin
2. 100 mM solution of isopropyl-β-D-thiogalactopyranoside (IPTG). Store in aliquots at –20°C in the dark.
3. SDS-PAGE and immunoblot equipment
4. anti-c-myc monoclonal antibody Myc1-9E10 (Invitrogen, Carlsbad, CA) and/or anti-penta-His monoclonal antibody (QIAGEN, Hilden, Germany).
5. HRP-conjugated goat-anti-mouse IgG antibody (Invitrogen, Carlsbad, CA)
6. TMB stabilized substrate for HRP (Progen, Heidelberg, Germany)

3. METHODS

Methods which are not specifically described step by step can be done according to the manufacturers protocol (Kits) or to Sambrook et al., 1989 (27)

3.1. Isolation of Antibody DNA

1. Collect up to 5×10^6 hybridoma cells by centrifugation.
2. Isolate the total RNA from the hybridoma cells using TRIzol™ reagent (see Note 1).
3. optional: Isolate the mRNA from the total RNA using a kit (see Note 2).
4. Prepare the first strand cDNA from the mRNA using a kit (see Note 3). A second strand synthesis is not necessary.

First PCR for the amplification of antibody DNA:

5. For each reaction combine the primer specific for the constant domain and a primer specific for the variable domain (see Tab. 1). Perform each reaction in a

total volume of 50 µl. Each 50 µl reaction contains 25 pmol of each primer, dNTP mix (10 mM of each) and polymerase buffer as described by the supplier. Use 1 µl of the prepared cDNA for each 50 µl reaction. Add 1 µl (2,5 U) of Phusion™ High Fidelity DNA Polymerase per 50 µl reaction (see Note 4).

6. Place the PCR tubes in a thermal cycler (see Note 5). Denature at 95 °C for 5 minutes (see Note 6).
7. Perform 30 cycles with the following cycling program: 45 seconds denaturation at 94 °C, 45 seconds annealing at the appropriate primer hybridization temperature (see Note 7) and 90 seconds extension at 72 °C. After 30 cycles perform a final extension step for 5 minutes at 72 °C.
8. Take out 1/10 volume of each PCR reaction for analytical gel electrophoresis on a 1 % agarose gel. Purify the PCR products using a kit. Elute DNA in H₂O (see Note 8).

Second PCR for reamplification and introduction of appropriate restriction sites into the antibody DNA:

9. Each 50 µl reaction contains 25 pmol of each primer, dNTP mix (10 mM each) and polymerase buffer as described by the supplier. Use up to 50 ng of purified PCR product from the first PCR for each 50 µl reaction. Add 1 µl (2,5 U) of Phusion™ High-Fidelity DNA Polymerase per 50 µl reaction (see Note 4). Use the primer pairs (with the introduced restriction sites, see Tab. 2) that gave the desired PCR product in the first PCR.
10. Place the PCR tubes in a thermal cycler (see Note 5). Denature at 95 °C for 5 minutes (see Note 6).
11. Perform 20 cycles with the following cycling program: 45 seconds denaturation at 94 °C, 45 seconds annealing at the appropriate primer hybridization temperature

(see Note 9) and 45 seconds extension at 72 °C. After 20 cycles perform a final extension step for 5 minutes at 72 °C.

12. Take out 1/10 volume of each PCR reaction for analytical agarose gel electrophoresis on a 1.5 % agarose gel. Purify the PCR products using the kit. Elute DNA in H₂O (see Note 8).

3.2. Cloning into an expression vector (pOPE101)

Cloning of the two V region gene fragments is done in two subsequent steps. First, the purified PCR product of the light chain is cloned into pOPE101:

1. Digest both the PCR product of the light chain and pOPE101-215(Yol) with MluI and NotI (see Note 10). To prevent self-ligation, the vector should be treated with CIP.
2. Run the digests on an agarose gel and gel purify the vector backbone from the original V_L and gel- or PCR purify the digested light chain. Elute plasmid DNA in H₂O.
3. Estimate DNA concentrations of vector and insert by measurement of the Absorbance at 260 nm. Prepare the ligation mix with an approximate molar ratio vector:insert of 1:3 in a reaction volume of 20 µL. The use of 50 ng of vector DNA is recommended (see Note 11).
4. Incubate 30 min at 22°C.
5. Transform *E.coli* XL1-Blue cells (or any other suitable strain) and plate on LB agar plates containing 100 mM glucose and 100 µg/mL ampicillin (LB_{GA}). Incubate overnight at 37°C (see Note 12).
6. Pick 5 single colonies and grow in 5 mL LB medium containing 100 mM glucose and 100 µg/mL ampicillin. Shake overnight at 230 rpm, 37°C.
7. Miniprep the plasmid DNA and make glycerol stocks.

8. To confirm the presence of the appropriate insert, an analytical digest (run on agarose gel electrophoresis) with MluI and NotI as well as sequencing should be performed.

In a second step, the heavy chain is cloned:

9. Digest the PCR product of the heavy chain and the ligation product from step 9 with NcoI and HindIII. The vector should be CIP-treated.
10. From here you can follow steps 2-8 as given for the light chain DNA cloning.
11. Confirm the presence of the correct insert by digesting the construct with NcoI and HindIII as well as by sequencing.

3.3. Small Scale Expression Test

In order to confirm the expression and correct size of the scFv fragment, a small scale expression followed by western blotting should be performed (see Note 13)

1. Prepare an overnight culture of *E. coli* cells transformed with the appropriate pOPE vector construct in 5 mL of 2 x YT_{GA} medium (see Note 14).
2. Dilute 300 µL of the overnight culture into 6 mL (1/20) of 2 x YT_{GA} and shake at 37°C and 230 rpm to an OD₆₀₀ of 0.6-0.8 (see Note 15).
3. Separate the culture into two equal aliquots. To one of them add IPTG to a final concentration of 50 µM. (see Note 16).
4. Incubate the induced and the control culture for 3 hours of vigorous shaking at 270-280 rpm at 25°C (see Note 17).
5. Incubate the cultures for 10 min on ice.
6. To check the production of the antibody fragment take out 1 mL of each culture for immunoblotting. Centrifuge at 5000 x g for 5 min at 4°C and resuspend the pellet in 100 µL 2x SDS sample buffer. Boil at 95 °C for 5 min and spin down for 3 min at 12000 x g before loading the supernatant onto the SDS gel (see Note 18).

7. In addition, a periplasmic fraction can be prepared from the induced culture to directly test for antigen binding affinity in ELISA (see Note 19).

4. Notes

1. Use the TRIzolTM Reagent protocol for isolation of total RNA from animal cells. Special additional homogenization of the cells is not necessary.
2. Optional: Use the Oligotex mRNA Spin-Column protocol for isolation of poly A⁺ mRNA from total RNA. Use up to 250 µg total RNA for the “miniprep” protocol. The concentration and purity of poly A⁺ mRNA can be determined by measuring the absorbance at 260 nm and 280 nm in a spectrophotometer. If using oligo(dT) primer for cDNA synthesis, mRNA extraction is not necessary.
3. Use the protocol for First-Strand Synthesis Using oligo(dT) for the isolated RNA instead of total RNA, as given.
4. The PCR setup with the PhusionTM High-Fidelity DNA polymerase can be done at room temperature. For detailed information see the PhusionTM High-Fidelity DNA polymerase manual. Alternatively it is possible to use other proofreading polymerases, e.g. the Expand High Fidelity PCR System (Roche, Mannheim, Germany). Only a few primer combinations will give a PCR product. Please note that due to the conditions of the reaction, the same primary sequence may be amplified with several different primer combinations, depending on the individual sequence. The size of the resulting PCR product is approximately 350 bp.
5. If using a thermal cycler with a heated lid, do not use mineral oil. Otherwise, overlay the reaction with approximately 50 µl mineral oil to prevent evaporation.

6. If using a hot-start DNA polymerase the enzyme is activated by this initial heating step.
7. For the oligonucleotide primers described in Tab. 1, an annealing temperature of 54 °C should be tried initially. If no PCR products are found, decrease the annealing temperature by steps of 2°.
8. Use a PCR Purification Kit. If there are multiple bands found on the gel per reaction, use a Gel Extraction Kit for purification of the correct PCR product (size of approximately 350 bp).
9. An annealing temperature of 56 °C is recommended.
10. Per µg vector use 3U NotI and 3U MluI, 2h, 37°C. Per µg insert apply 30U NotI and 30U MluI, 2h, 37°C.
11. A critical factor for the proper function of the T4-DNA ligase is ATP. It is recommended to prepare aliquots of 10x reaction buffer which has been supplemented with ATP to 100 mM and store frozen until used. Caution: This (high) ATP concentration inhibits blunt end ligation reactions.
12. Chemical transformation using a heat shock can be performed and leads in most cases to a sufficient amount of clones. Alternatively, electroporation can be performed using half of the ligation and 3-5 µl electrocompetent cells (Agilent) and bringing to a final volume of 50 µL with H₂O. For a 2 mm diameter cuvette use 2.5 kV/25 µF at 200 Ohm.
13. To save time, this test can be combined with the generation of cells for DNA “minipreps” and preparation of glycerol stocks by using the remainder of the overnight starter culture.
14. If possible use glycerol stocks for the preparation of overnight cultures since clones on agar plates can mutate easily after prolonged storage even at 4°C. Glucose must always be present in the bacterial growth medium since it is necessary for the tight

suppression of the synthetic promotor of the pOPE vector family and thus for the genetic stability of the insert.

15. Protein production from pOPE101 cannot be induced in bacteria grown to stationary phase.

16. With pOPE-vectors in *E.coli* XL1-Blue, we achieved optimal protein secretion with 20 μ M IPTG at 25°C. This optimal IPTG concentration can vary between different Fv-sequences by a factor of about two. Higher IPTG concentrations lead to dramatically increased amounts of total recombinant protein, but in this case most of the scFv fragments still carry the bacterial leader sequence and form aggregates. However, for the immunoblot analysis of total cellular SDS extracts, it is not necessary to discriminate between unprocessed and processed protein. Therefore, a higher IPTG concentration is used simply to increase the intensity of the protein band on the blot. To optimize expression conditions, IPTG concentrations between 10 and 100 μ M should be tested for each individual fusion protein. Be aware that IPTG is light sensitive and may decay during prolonged storage. Use stocks aliquoted and stored in brown 1.5ml tubes at –20°C for not longer than 2 months.

17. A maximum of functional scFv fragments was achieved at 25°C. Incubation times longer than 3 hours lead to a slight increase in the amount of secreted protein, but a significantly higher contaminant concentration as well, possibly due to increased cell death. However, depending on the hybridoma antibody sequence, differences were found in solubility and the ability to be secreted. The expression of some antibodies even lead to a strong growth inhibition during induction.

18. For detection of scFv produced from pOPE101, the monoclonal antibody Myc1-9E10 recognizing the c-myc tag or an antibody to the his tag is recommended. HRP-conjugated antibodies to mouse immunoglobulins should be applied before TMB

stabilized substrate for HRP (Progen, Heidelberg, Germany) can be used for the detection of bound enzymatic activity.

19. Soluble antibody fragments can be isolated from induced *E. coli* cultures by osmotic shock. For immunodetection in ELISA, the same antibodies as in Note 18 are suitable. Protocols for both methods can be found in Schmiedl *et al.* (25).

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Table 1.

Oligonucleotides for the amplification of mouse immunoglobulin variable region DNA (28)

Heavy chain:

heavy chain constant domain:

MHC.F 5'- GGCCAGTGGATAGTCAGATGGGGGTGTCGTTTTGGC - 3'

heavy chain variable domain

MHV.B1 5'- GATGTGAAGCTTCAGGAGTC - 3'

MHV.B2 5'- CAGGTGCAGCTGAAGGAGTC - 3'

MHV.B3 5'- CAGGTGCAGCTGAAGCAGTC - 3'

MHV.B4 5'- CAGGTTACTCTGAAAGAGTC - 3'

MHV.B5 5'- GAGGTCCAGCTGCAACAATCT - 3'

MHV.B6 5'- GAGGTCCAGCTGCAGCAGTC - 3'

MHV.B7 5'- CAGGTCCAAGTGCAGCAGCCT - 3'

MHV.B8 5'- GAGGTGAAGCTGGTGGAGTC - 3'

MHV.B9 5'- GAGGTGAAGCTGGTGGAAATC - 3'

MHV.B10 5'- GATGTGAACTTGGAAAGTGTC - 3'

MHV.B12 5'- GAGGTGCAGCTGGAGGAGTC - 3'

Light chain:

Kappa chain constant domain

MKC.F 5'- GGATACAGTTGGTGCAGCATC - 3'

Kappa chain variable domain

MKV.B1 5'- GATGTTTTGATGACCCAACT - 3'

MKV.B2 5'- GATATTGTGATGACGCAGGCT - 3'

MKV.B3 5'- GATATTGTGATAACCCAG - 3'

MKV.B4 5'- GACATTGTGCTGACCCAATCT - 3'

MKV.B5 5'- GACATTGTGATGACCCAGTCT - 3'

MKV.B6 5'- GATATTGTGCTAACTCAGTCT - 3'

MKV.B7 5'- GATATCCAGATGACACAGACT - 3'

MKV.B8 5'- GACATCCAGCTGACTCAGTCT - 3'

MKV.B9 5'- CAAATTGTTCTCACCCAGTCT - 3'

MKV.B10 5'- GACATTCTGATGACCCAGTCT - 3'

Lambda chain constant domain

MLC.F 5'- GGTGAGTGTGGGAGTGGACTTGGGCTG - 3'

Lambda chain variable domain

MLV.B 5'- CAGGCTGTTGTGACTCAGGAA - 3'

Table 2.

Oligonucleotides for the reamplification of mouse immunoglobulin variable region DNA and introduction of appropriate restriction sites

Heavy chain

heavy chain constant domain:

MHC.F.Hind 5'- GGCCAGTGGATAAAGCTTTGGGGGTGTCGTTTTGGC - 3'

heavy chain variable domain

MHV.B1.Nco 5'- GAATAGGCCATGGCGGATGTGAAGCTGCAGGAGTC - 3'
 MHV.B2.Nco 5'- GAATAGGCCATGGCGCAGGTGCAGCTGAAGGAGTC - 3'
 MHV.B3.Nco 5'- GAATAGGCCATGGCGCAGGTGCAGCTGAAGCAGTC - 3'
 MHV.B4.Nco 5'- GAATAGGCCATGGCGCAGGTTACTCTGAAAGAGTC - 3'
 MHV.B5.Nco 5'- GAATAGGCCATGGCGGAGGTCCAGCTGCAACAATCT - 3'
 MHV.B6.Nco 5'- GAATAGGCCATGGCGGAGGTCCAGCTGCAGCAGTC - 3'
 MHV.B7.Nco 5'- GAATAGCCATGGCGCAGGTCCAAGCTGCAGCAGCCT - 3'
 MHV.B8.Nco 5'- GAATAGGCCATGGCGGAGGTGAAGCTGGTGGAGTC - 3'
 MHV.B9.Nco 5'- GAATAGGCCATGGCGGAGGTGAAGCTGGTGGGAATC - 3'
 MHV.B10.Nco 5'- GAATAGGCCATGGCGGATGTGAACTTGGAAGTGTC - 3'
 MHV.B12.Nco 5'- GAATAGGCCATGGCGGAGGTGCAGCTGGAGGAGTC - 3'

Light chain:

Kappa chain constant domain

MKC.F.Not 5'- TGACAAGCTTGCGGCCGCGGATACAGTTGGTGCAGCATC - 3'

Kappa chain variable domain

MKV.B1.Mlu 5'- TACAGGATCCACGCGTAGATGTTTTGATGACCCAAACT - 3'

MKV.B2.Mlu 5'- TACAGGATCCACGCGTAGATATTGTGATGACGCAGGCT - 3'

MKV.B3.Mlu 5'- TACAGGATCCACGCGTAGATATTGTGATAACCCAG - 3'

MKV.B4.Mlu 5'- TACAGGATCCACGCGTAGACATTGTGCTGACCCAATCT - 3'

MKV.B5.Mlu 5'- TACAGGATCCACGCGTAGACATTGTGATGACCCAGTCT - 3'

MKV.B6.Mlu 5'- TACAGGATCCACGCGTAGATATTGTGCTAACTCAGTCT - 3'

MKV.B7.Mlu 5'- TACAGGATCCACGCGTAGATATCCAGATGACACAGACT - 3'

MKV.B8.Mlu 5'- TACAGGATCCACGCGTAGACATCCAGCTGACTCAGTCT - 3'

MKV.B9.Mlu 5'- TACAGGATCCACGCGTACAAATTGTTCTCAGCCAGTCT - 3'

MKV.B10.Mlu 5'- TACAGGATCCACGCGTAGACATTCTGATGACCCAGTCT - 3'

Lambda chain constant domain

MLC.F.Not 5'- TGACAAGCTTGCGGCCGGGTGAGTGTGGGAGTGGACTTG
GGCTG - 3'

Lambda chain variable domain

MLV.B.Mlu 5'- TACAGGATCCACGCGTACAGGCTGTTGTGACTCAGGAA - 3'