Isolation and characterization of full-length genes encoding the anti-human CD45 antibody from the hybridoma cell line 16E8-F2

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ABSTRACT

Though the hybridoma technology has been widely applied in the production of monoclonal antibodies, it has existed some disadvantages including low yield and genetic instability. Therefore, an alternative approach should be taken into account. Recently, recombinant monoclonal antibody technology has emerged as the best choice to cure hybridoma related drawbacks. However, recombinant antibodies require known genes for their generation. The purpose of this study is to collect the full-length genes encoding the anti-human CD45 antibody derived from the hybridoma cell line 16E8-F2. In this research, we designed specific primer pairs to amplify the light and heavy chain genes of the antibody through the PCR method. Afterward, the genes were separately cloned into a cloning vector called pJET1.2/blunt. The generated recombinant pJET1.2 vectors will serve as the main material source to manufacture the recombinant monoclonal antibody recognizing human CD45 protein tomorrow.

1. Introduction

Monoclonal antibodies (mAbs) are monovalent antibodies derived from a single B cell clone and have an affinity with the same epitope (Liu, 2014; Wang, 2011). The first mAb was produced in mice in 1975 by hybridoma technology. This technique involves immunization of a certain species (for example mice) with a mixture of antigens and collection of B lymphocytes from the spleen of the animal (Liu, 2014; Steinitz, 2009; Wang, 2011). Then the B cells are fused (by a chemical- or virus-based method) with an immortal myeloma cell line deficient in the hypoxanthine-guanine-phosphoribosyltransferase (*HGPRT*) gene to transform into hybridoma cells. These cells are then grown in vitro in a selective medium including hypoxanthine - aminopterin - thymidine, where only the hybridomas can remain alive due to the inheritance of the immortality from the myeloma cells and the *hgprt* gene from the B cells (Liu, 2014). Meanwhile, the myeloma cells cannot grow because they lack the *HGPRT* enzyme necessary for nucleotide synthesis via the salvage pathway and are inhibited by the de novo biosynthesis of nucleotides by aminopterin (Hnasko & Stanker, 2015; Liu, 2014). The primary B lymphocytes possess the *HGPRT* enzyme and can use the salvage pathway for survival, but they will die in time when growing in cell culture owing to their mortality (Hnasko & Stanker, 2015).

At the initial stages of hybridoma cell culture, the cells secrete a variety of antibodies that bind to different antigens. The antibodies are derived from different B cell clones and are called polyvalent or polyclonal antibodies. Each clone can be separated by dilution into various 96-well plates. Afterward the conditioned media from hundreds of different wells are screened to find out

a clone that expresses the interested antibody (Liu, 2014). At this time, the antibody is monoclonal. The clone will then be proliferated in two ways to produce the mAb: (1) by injecting into the peritoneal chamber of another mouse (called the in vivo method) or (2) by culturing in vitro (called the in vitro method) (Groff, Brown, & Clippinger, 2015). However, the big drawbacks of hybridoma technology are low yield (Liu, 2014) and genetic instability (Costa, Rodrigues, Henriques, Azeredo, & Oliveira, 2010; Liu, 2014; Pasqualini & Arap, 2004). Meanwhile, the recombinant monoclonal antibody engineering is increasingly becoming popular and has received a great amount of attention from scientists around the world. The antibody built by this technique is called a recombinant antibody (rAb) or a genetically engineered antibody.

Recombinant antibody technology entails the collection of antibody coding genes from source cells, amplification and cloning of the genes into a proper vector, transfection of the recombinant vector into host cells, and accomplishment of expression of the desired antibody (Karu, Bell, & Chinet, 1995). Source cells are antibody-secreting cells, including B lymphocytes from the spleen or the peripheral blood of an immunized animal, hybridomas, or genes from a phage display model (Abcam, 2018; Babrak, McGarvey, Stanker, & Hnasko, 2017; Johnson & Bradbury, 2015; Karu et al., 1995). Therefore, this method will allow manufacturing antibodies in vitro without using animals (Echko & Dozier, 2010; Groff et al., 2015; Karu et al., 1995). In addition, recombinant antibody technology also has a lot of benefits compared to the hybridoma technique. Typically, recombinant antibody supplies are not in danger of dying out since the nucleotide sequence of the antibody is known. The batch-to-batch variability of rAb production is low. Its affinity and specificity can be improved by modifying the antibody's sequence (Groff et al., 2015). On those bases, we conducted to isolate the full-length genes encoding the anti-human CD45 antibody from the hybridoma cell line 16E8-F2 and used the genes as the main material source for the generation of the recombinant monoclonal anti-human CD45 antibody.

2. Materials and methods

2.1. Hybridoma cell line

The hybridoma cell line 16E8-F2 with the ability to produce the anti-human CD45 antibody was generated by the Department of Medical Biotechnology at the Biotechnology Center of Ho Chi Minh City.

2.2. Total RNA extraction

Total RNA was extracted from the hybridoma cell line 16E8-F2 by a High pure RNA tissue kit (Roche) according to the manufacturer protocol.

2.3. cDNA synthesis

First-strand cDNA was synthesized from total RNA using oligo (dT)18 primer (Roche). For this purpose, 5µl of total RNA (approximately 1µg), 0.2µl of 50µM oligo (dT)18 primer, 4.8µl of DEPC treated water (Bioline) were mixed and incubated at 70^{o}C for 05 minutes, then lowered to room temperature for 05 minutes. Afterwards, this mixture was combined with 4µl of 5x RT buffer (Bioline), 1µl of 10mM dNTPs (Bioline), 1µl of 200 U/µl Reverse transcriptases (Bioline), and 4µl of DEPC treated water. The reaction was then incubated sequentially at 25^{o}C for 10 minutes, 45^{o}C for 60 minutes (to activate the reaction), and 85^{o}C for 05 minutes (to inactivate the enzyme). The reaction product was stored at -20^{o}C.

2.4. Primer design

The mouse Ig-Primer set (Merck) was used to amplify the heavy chain variable region (VH) and the light chain variable region (VL) by a PCR technique with the freshly synthesized

cDNA as a template. Setting up the PCR reaction accorded to the attached manual using Platinum® Taq DNA Polymerase High Fidelity (Thermo scientific). The PCR reactions for VH or VL had the same reverse primer but different, forward primers. Thanks to agarose gel electrophoresis and sequencing of PCR products, appropriate forward primers for the Heavy Chain gene (HC) and the Light Chain gene (LC) were selected from the forward primer mix of the Mouse Ig-Primer set.

On the other hand, reverse primers for HC and LC genes were designed in silico. Specifically, 20 nucleotide sequences of the mouse IgG2b constant region and 34 mouse kappa gene sequences were downloaded from IGMT (IMGT, n.d.), NCBI (NCBI, n.d.) and aligned by an online alignment tool, MUSCLE (MUSCLE, n.d.). The primers were picked up from the consensus sequences by the naked eye. The BLAST program served as a tool to check the specificity of primers.

2.5. Collection of HC and LC genes

Full-length HC and LC genes of the anti-human CD45 antibody were obtained by PCR using specific primer pairs previously designed. The PCR reaction of 20µl total volume contained 1x High Fidelity PCR buffer (Thermo scientific), 2mM $MgSO_4$ (Thermo scientific), 500nM of forwarding primer, 300nM of reverse primer, 200µM for each dNTP (Thermo scientific), 2µl of newly synthesized cDNA and 1 U of Platinum® Taq DNA Polymerase High Fidelity (Thermo scientific). Thermo cycling of the PCR reaction followed the manufacturer procedure with the annealing temperature of 59^{o}C for HC and 56^{o}C for LC.

2.6. Plasmid construction

HC and LC genes were individually inserted into the cloning vector pJET1.2/blunt through CloneJET PCR Cloning Kit (Thermo scientific) following the supplier procedure. The recombinant vector was then transformed into competent E. coli DH5α cells using the heat shock method as previously described (Froger & Hall, 2007) with LB medium instead of S.O.C.

2.7. Colony PCR

Only E. coli DH5α cells carrying the recombinant plasmid grew into colonies on the selection medium (LB agar plates contain 100 µg/ml ampicillin). The colony was checked if the gene of interest is present by a PCR technique called colony PCR.

The single bacterial colony for amplification was suspended in 5µl of DEPC treated water (Bioline). PCR was prepared in a 20µl volume reaction including 1x DreamTaq buffer (Thermo scientific), 300nM of each primer, 200µM for each dNTP (Thermo scientific), 2µl of the bacteria suspension, and 0.625 U of DreamTaq DNA Polymerase (Thermo scientific). The reaction conditions followed the manufacturer's instructions with the annealing temperature of 59^{o}C for HC and 56^{o}C for LC. The primers used herein were specific for HC and LC genes.

2.8. Gel electrophoresis and sequence analysis

10µl of a PCR product was electrophoresed on 1% agarose gels using GelRed (ATB) as a staining reagent.

The generated VH, VL, HC and LC sequences were visualized by a free software program called BioEdit. VH and VL sequences were analyzed by an online tool as IMGT/V-QUEST (www.imgt.org/IMGT_vquest).

3. Results and discussion

3.1. Primer design

The design of specific primers for genes encoding HC and LC genes was extremely necessary since this was a prerequisite for isolation of full-length HC and LC genes through a PCR method. In particular, forward primers were selected from the mouse Ig-Primer set (Merck) used to amplify variable regions of a mouse antibody. Meanwhile, reverse primers were built in silico.

In respect of forwarding primers, the PCR reaction using the commercial primer set with cDNA as a template was conducted. The cDNA was a product of RT-PCR with oligo (dT) primer and RNA template derived from the cell line hybridoma 16E8-F2. Because the oligo is specifically bound to the poly(A) tail at the 3' end of mRNA molecules, only mRNAs in the total RNA sample were converted into cDNA. PCR products were electrophoresed on 1% agarose gels. Amplification of the heavy chain variable region VH showed only one band of about 500 bp appeared on the gel when the forward primer IgVH5'-B was used (Figure 1a). Otherwise, up to three different products of some 500 bp corresponding to the forward primers IgVL5'-B, C and G were generated (Figure 1b). All these products were suspected to be variable regions of the antibody. To confirm if the variable regions were correctly amplified, therefore, all four products were sequenced and analyzed in silico.

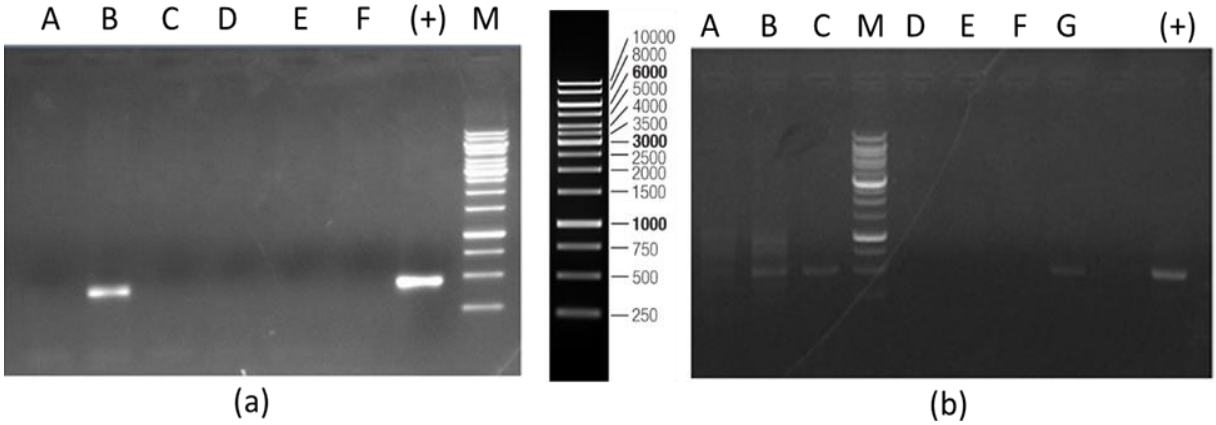

Figure 1. Agarose gel electrophoresis of the variable region of the heavy chain (a) and the light chain (b)

A, B, C, D, E, F, G: names of forward primers; M: DNA marker; (+): positive control using the forward primer B and the plasmid supplied in kit

Analysis of the PCR-generated sequences via IMGT/V-QUEST indicated the VH sequence amplified with the forward primer IgVH5'-B had a full structure containing CDR1, CDR2, CDR3, FR1, FR2, FR3 and FR4 regions (Figure 2). These components were also found in the VL sequence created by the forward primer IgVL5'-B (Figure 3). On the contrary, the remaining two VL products showed the same sequences but had unusual structures with presence of a premature stop codon (Figure 4). This phenomenon was possibly caused by the existence of aberrant kappa transcripts derived from the original myeloma cells (Carroll, Mendel, & Levy, 1988). Hence, the cell line hybridoma 16E8-F2 expressed not only normal kappa transcripts from B cells but also non-productive kappa mRNA molecules originating in myeloma cells.

As a result of these data, IgVH5'-B was used as a forward primer to amplify; the HC gene, the forward primer IgVL5'-G was chosen for the LC gene. However, IgVL5'-G is a mixture of different primers containing IgVL5'-G1, G2, G3 and G4. Basing on the sequencing result of the VL product, IgVL5'-G1 was selected and artificially synthesized.

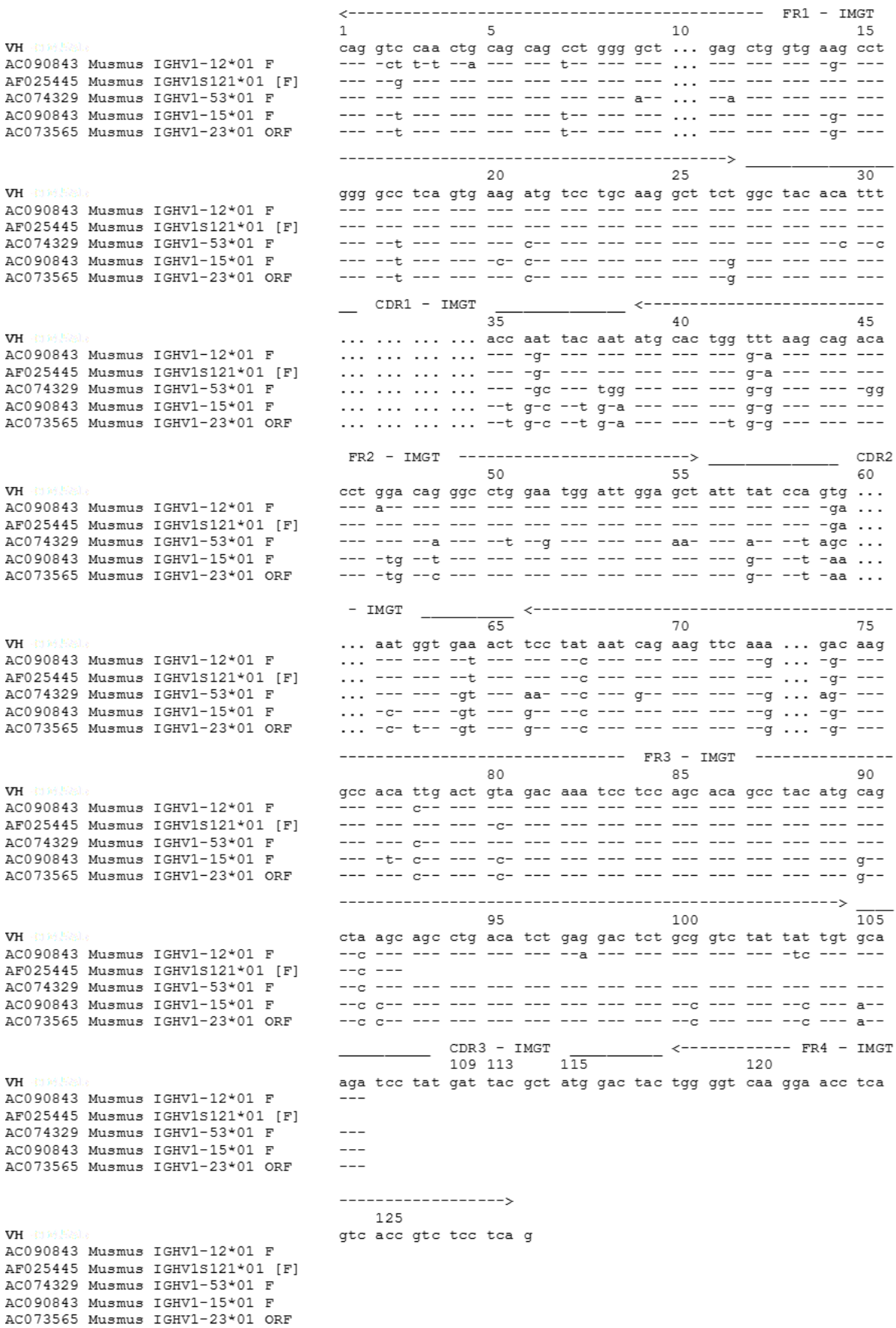

Figure 2. Analysis of VH sequence amplified by IgVH5'-B primer compared to different heavy chain variable regions of mouse antibodies

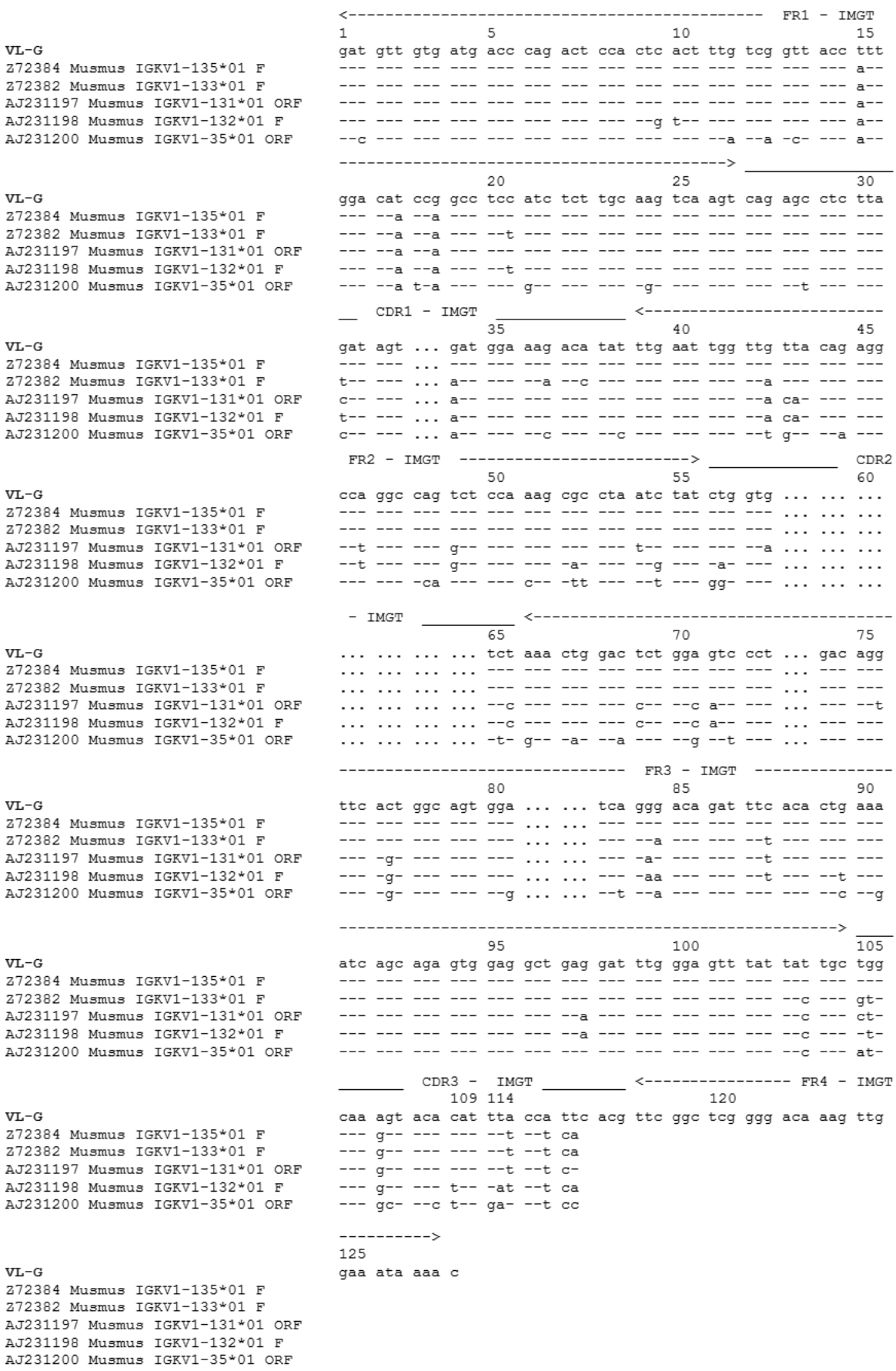

Figure 3. Analysis of VL sequence amplified by IgVL5'-G primer compared to different light chain variable regions of mouse antibodies

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>VL-B/C
gacattgtgctgacacagtctcctgcttccttagctgtatctctggggcagagggccacc
atctcatacagggccagcaaaagtgtcagtacatctggctatagttatatgcactggaac
caacagaaaccaggacagccacccagactcctcatctatcttgtatccaacctagaatct
ggggtccctgccaggttcagtggcagtgggtctgggacagacttcaccctcaacatccat
cctgtggaggaggaggatgctgcaacctattactgtcagcacattagggagcttacacgt
tcggagggggggaccaagctggaaataaaac
                    (Stop codon)
```

Figure 4. Analysis of VL sequences amplified by IgVL5'-B, C primers compared to different light chain variable regions of mouse antibodies

With regard to reverse primers, 34 mouse kappa nucleotide sequences and 20 gene sequences of the mouse IgG2b constant region were downloaded from IGMT, NCBI and aligned by an online alignment tool called MUSCLE. This is because the anti-human CD45 antibody from the hybridoma cells 16E8-F2 belongs to the IgG2b isotype and has the kappa light chain type (Ta, Nguyen, & Nguyen, 2020). Alignment results revealed a very great similarity between the mouse kappa sequences (Figure 5) as well as the mouse IgG2b constant sequences (Figure 6). On this basis, reverse primers were designed to specifically amplify HC and LC genes. Information on primers were listed in Table 1.

```
AB048528.1|Mus  CGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGTTAA
BC091750.1|Mus  CGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCACAAGACATCAACTTCACCCATCGTCAAGAGCTTCAACAGGAATGAGTGTTAG
BC092090.1|Mus  CGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCACAAGACATCAACTTCACCCATCGTCAAGAGCTTCAACAGGAATGAGTGTTAG
BC092064.1|Mus  CGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGTTAG
L35138.1|Mus    CGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGTTAA
U29147.1|Mus    CGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGT---
BC080787.1|Mus  CGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGTTAG
U04353.1|Mus    CGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCACAAGACATCAACTTCACCCATCGTCAAGAGCTTCAACAGGAATGAGTGTTAA
AJ131289.1|Mus  CGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGT---
AF290569.1|Mus  CGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACCGAAATGAGTGTTAA
AB048522.1|Mus  CGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGTTAA
D14630.1|Mus    CGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCACAAGACATCAACTTCACCCATCGTCAAGAGCTTCAACAGGAATGAGTGTTAG
BC106161.1|Mus  CGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGTTAG
M63550.1|Mus    CGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGTTAG
BC092097.1|Mus  CGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCACAAGACATCAACTTCACCCATCGTCAAGAGCTTCAACAGGAATGAGTGTTAG
AB048526.1|Mus  CGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGTTAA
AB048524.1|Mus  CGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGTTAA
U00941.1|Mus    CGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCACAAGACATCAACTTCACCCATCGTCAAGAGCTTCAACAGGAATGAGTGTTAA
BC094049.1|Mus  CGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGTTAG
L27438.1|Mus    CGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGTTAG
M12177.1|Mus    CGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCACAAGACATCAACTTCACCCATCGTCAAGAGCTTCAACAGGAATGAGTGTTAG
J00560.1|Mus    CGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGTTAG
AY607096.1|Mus  CGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCACAAGACATCAACTTCACCCATCGTCAAGAGCTTCAACAGGAATGAGTGT---
AY607098.1|Mus  CGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCACAAGACATCAACTTCACCCATCGTCAAGAGCTTCAACAGGAATGAGTGT---
AY005823.1|Mus  CGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCACAAGACATCAACTTCACCCATCGTCAAGAGCTTCAACAGGAATGAGTGTTAG
BC091754.1|Mus  CGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGTTAG
E00398.1|Mus    CGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGTTAG
AF178456.1|Mus  CGAGTATGGACGACATAACAGCTATACCTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGT---
BC092080.1|Mus  CGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGTTAG
AY672134.1|Mus  CGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGT---
BC094013.1|Mus  CGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGTTAG
365921.1|Mus    CGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGTTAG
U28969.1|Mus    CGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGT---
AF043114.1|Mus  CGAGTATGAACGAGATAACAGCTATACCTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGT---
AF043116.1|Mus  CGAGTATGAACGAGATAACAGCTATACCTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGT---
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Figure 5. Alignment of the mouse kappa sequences

*: indicates all the sequences have the same nucleotide

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LT727354.1      AACAGATTCCTTCTCATGCAACGTGAGACACGAGGGTCTGAAAAATTACTACCTGAAGAAGACCATCTCCCGGTCTCCGGGTAAATGA
LT727357.1      AACAGATTCCTTCTCATGCAACGTGAGACACGAGGGTCTGAAAAATTACTACCTGAAGAAGACCATCTCCCGGTCTCCGGGTAAATGA
LT727624.1      AACAGATTCCTTCTCATGCAACGTGAGACACGAGGGTCTGAAAAATTACTACCTGAAGAAGACCATCTCCCGGTCTCCGGGTAAATGA
LT727633.1      AACAGATTCCTTCTCATGCAACGTGAGACACGAGGGTCTGAAAAATTACTACCTGAAGAAGACCATCTCCCGGTCTCCGGGTAAATGA
LT727584.1      AACAGATTCCTTCTCATGCAACGTGAGACACGAGGGTCTGAAAAATTACTACCTGAAGAAGACCATCTCCCGGTCTCCGGGTAAATGA
LT727621.1      AACAGATTCCTTCTCATGCAACGTGAGACACGAGGGTCTGAAAAATTACTACCTGAAGAAGACCATCTCCCGGTCTCCGGGTAAATGA
X13188.1|Mus    AACAGATTCCTTCTCATGCAACGTGAGACACGAGGGTCTGAAAAATTACTACCTGAAGAAGACCATCTCCCGGTCTCCGGGTAAATGA
AK134155.1      AACAGATTCCTTCTCATGCAACGTGAGACACGAGGGTCTGAAAAATTACTACCTGAAGAAGACCATCTCCCGGTCTCCGGGTAAATGA
BC092269.1|Mus  AACAGATTCCTTCTCATGCAACGTGAGACACGAGGGTCTGAAAAATTACTACCTGAAGAAGACCATCTCCCGGTCTCCGGGTAAATGA
BC092049.1|Mus  AACAGATTCCTTCTCATGCAACGTGAGACACGAGGGTCTGAAAAATTACTACCTGAAGAAGACCATCTCCCGGTCTCCGGGTAAATGA
LC050825.1      AACAGATTCCTTCTCATGCAACGTGAGACACGAGGGTCTGAAAAATTACTACCTGAAGAAGACCATCTCCCGGTCTCCGGGTAAATGA
FJ232994.1      AACAGATTCCTTCTCATGCAACGTGAGACACGAGGGTCTGAAAAATTACTACCTGAAGAAGACCATCTCCCGGTCTCCGGGTAAATGA
V00799.1|Mus    AACAGATTCCTTCTCATGCAACGTGAGACACGAGGGTCTGAAAAATTACTACCTGAAGAAGACCATCTCCCGGTCTCCGGGTAAATGA
AH002571.2      AACAGATTCCTTCTCATGCAACGTGAGACACGAGGGTCTGAAAAATTACTACCTGAAGAAGACCATCTCCCGGTCTCCGGGTAAATGA
EF392841.1|Mus  AACAGATTCCTTCTCATGCAACGTGAGACACGAGGGTCTGAAAAATTACTACCTGAAGAAGACCATCTCCCGGTCTCCGGGTAAATGA
GQ984290.1|Mus  AACAGATTCCTTCTCATGCAACGTGAGACACGAGGGTCTGAAAAATTACTACCTGAAGAAGACCATCTCCCGGTCTCCGGGTAAATGA
EF392837.1|Mus  AACAGATTCCTTCTCATGCAACGTGAGACACGAGGGTCTGAAAAATTACTACCTGAAGAAGACCATCTCCCGGTCTCCGGGTAAATGA
X67210.1        AACAGATTCCTTCTCATGCAACGTGAGACACGAGGGTCTGAAAAATTACTACCTGAAGAAGACCATCTCCCGGTCTCCGGGTAAATGA
LT160968.1      AACAGATTCCTTCTCATGCAACGTGAGACACGAGGGTCTGAAAAATTACTACCTGAAGAAGACCATCTCCCGGTCTCCGGGTAAATGA
FJ232992.1      AACAGATTCCTTCTCATGCAACGTGAGACACGAGGGTCTGAAAAATTACTACCTGAAGAAGACCATCTCCCGGTCTCCGGGTAAATGA
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Figure 6. Alignment of the mouse IgG2b sequences

*: indicates all the sequences have the same nucleotide

Table 1

Primers for amplification of HC and LC genes

Sequence	Primer	Target
5'- GGGAATTCATGRAATGSASCTGGGTYWTYCTCTT-3'	IgV_H5'-B	HC
5'-TCATTTACCCGGAGACCGGGAGATG-3'	Rv-IgG2b	
5'- ACTAGTCGACATGAAGTTGCCTGTTAGGCTGTTGGTGCT-3'	IgV_L5'-G1	LC
5'-TCAACACTCATTCCTGTTGAAGCTCTTG-3'	Rv-LC	

3.2. Isolation of HC and LC genes

Clone 16E8-F2 secreting the antibody of interest was used as source cells to obtain HC and LC genes. With the constructed primers (Table 1), PCR reactions amplifying HC and LC genes were carried out with the cDNA template. After electrophoresis of PCR products, the expected DNA bands appeared in the agarose gel (Figure 7), namely a length of around 1,500bp for the HC gene and approximately 750bp for the LC gene.

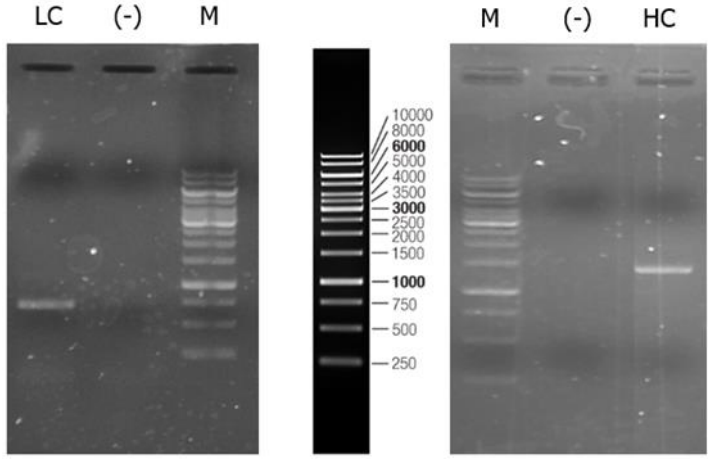

Figure 7. Agarose gel electrophoresis showing PCR products of HC and LC genes

(-): Negative control (water instead of cDNA); M: DNA marker

3.3. Cloning HC and LC genes into pJET1.2 plasmid

The PCR product of each HC and LC genes was directly ligated into the pJET1.2 plasmid. The presence of the HC or LC gene on the pJET1.2 vector was checked by colony PCR. Four colonies of E. coli DH5α cells growing on LB agar plates were randomly opted for the colony PCR followed by electrophoresis. In case of the cells transformed with the pJET1.2-HC vector in which the HC gene was inserted, colonies numbered 1, 2 and 3 gave the expected bands of about 1,500bp in the agarose gel (Figure 8). Likewise, as for the pJET1.2-LC vector, PCR products of colonies marked 1, 2 and 3 showed the intended size of around 750bp (Figure 8).

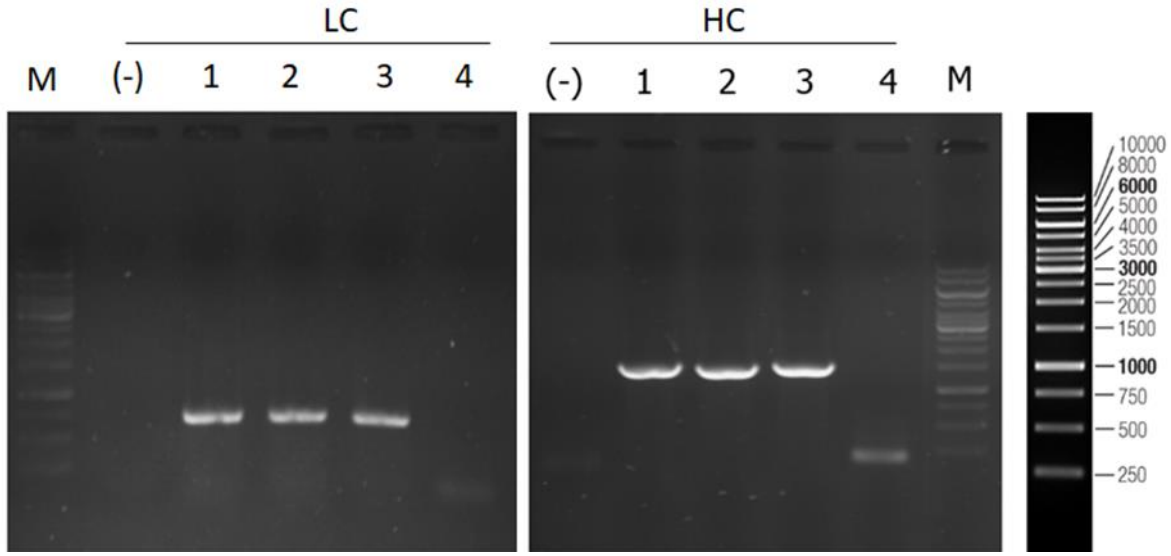

Figure 8. Colony PCR products of HC and LC genes cloned into pJET1.2 vector

HC: pJET1.2-HC; LC: pJET1.2-LC; 1-4: colonies of E. coli DH5α cells; (-): Negative control (water instead of the bacterial colony); M: DNA marker

Because the nucleotide sequences of HC and LC genes were unknown and it was to avoid mutations of these two genes during the proliferation of E. coli cells, the pJET1.2-HC and pJET1.2-LC vectors each were collected from two different colonies (colonies labeled 1, 2 for each) and sequencing afterward. Sequencing results of the HC gene from both colonies showed the same sequences with 1,427bp in length. Similarly, the LC gene of 721bp isolated from colony 1 was identical to that from colony 2. Therefore, the likelihood of mutations during the plasmid propagation was excluded. In addition, analyses of HC and LC nucleotide sequences in silico demonstrated the genes contain fully functional domains agreeing with a typical antibody structure. Variable regions were composed of the necessary domains mentioned before. As a result of BLAST, the constant region of the HC gene was the same as mouse IgG2b sequences. In like manner, the constant region of the LC gene was identical to mouse kappa sequences.

4. Conclusion

The results of this research showed that HC and LC genes encoding the anti-human CD45 antibody originating in the hybridoma cell line 16E8-F2 were successfully isolated by PCR method. The two genes were then cloned into the pJET1.2 cloning vector and served as primary source materials for the generation of a recombinant antibody in the future.

ACKNOWLEDGMENTS

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