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Integrated isotyping and CDR identification of mouse monoclonal antibodies using multiplex RT-PCR☆

Junmo Hwang [a], Eunbi Kim [a,b], Jina Kim [a], Sujin Shin [a,b], Hyun-Ho Lim [a,b,*]

[a] Neurovascular Unit Research Group, Korea Brain Research Institute (KBRI), Daegu 41062, South Korea
[b] Department of Brain Sciences, Daegu Gyeongbuk Institute of Science & Technology (DGIST), Daegu 42988, South Korea

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ABSTRACT

The complementarity-determining regions (CDRs) of monoclonal antibodies are essential for antigen recognition and antibody engineering. Accurate determination of CDR sequences typically requires cDNA synthesis from hybridoma-derived mRNA followed by sequencing of the variable regions. However, murine monoclonal antibodies are composed of diverse heavy and light chain isotypes, necessitating prior isotype determination to select appropriate primers for cDNA synthesis. Conventional workflows rely on immunoassays for isotype identification, which adds time and complexity. Here, we developed a streamlined, isotype-independent workflow for the molecular characterization of mouse monoclonal antibodies. A multiplex set of reverse transcription primers (Multiplex-RT) incorporating a universal adaptor sequence was designed to enable cDNA synthesis across major murine isotypes without prior isotype knowledge. Variable regions were subsequently amplified by isotype-specific PCR (Iso-PCR), allowing identification of antibody isotypes, IgG subclasses, and CDR sequences in a single workflow. We applied this method to characterize a murine antibody targeting the astrocytic membrane protein MLC1 and engineered a human-mouse chimeric antibody by grafting murine CDRs onto a human IgG1 backbone. The chimeric antibody retained antigen-binding activity, as demonstrated by immunoprecipitation and immunoblotting. This workflow provides a rapid and reliable strategy for sequencing and isotyping mouse monoclonal antibodies and facilitates downstream applications in antibody discovery, recombinant production, and engineering.

1. Introduction

Monoclonal antibodies (mAbs) have emerged as powerful therapeutic agents, and the global market for antibody-based drugs are expanding rapidly. In drug discovery, mAbs are preferred for their high specificity for target molecules and their origin as natural immune products [1–3]. Numerous mAbs have been approved or are under clinical evaluation for the treatment of cancers, autoimmune diseases, chronic inflammatory disorders, cardiovascular diseases, infectious diseases, osteoporosis, and neurodegenerative diseases as well as ophthalmologic and transplant-related indications [4]. Interestingly, antibodies targeting the same antigen can exhibit distinct mechanisms of action (MOAs), leading to diverse therapeutic applications. For example, CD20, a B lymphocyte surface antigen, is targeted by multiple mAbs that differ in isotype and MOA depending on the disease context. These examples highlight the importance of developing second-generation antibodies with improved pharmacokinetic properties, reduced immunogenicity, and broadened therapeutic potential [3,5,6].

Antibody engineering has enabled the development of various therapeutic antibody formats to meet specific clinical demands [3]. Several engineered antibody formats have obtained regulatory approval, including antibody fragments, bispecific antibodies, and conjugated antibodies linked to cytotoxic drugs, radioisotopes, or polyethylene glycol (PEG) [5]. One major challenge in therapeutic antibody development is the immunogenicity associated with Fc regions derived from non-human species. For example, murine-derived antibodies can elicit anti-mouse immune responses in humans, potentially triggering excessive cytokine release and cytotoxicity. To circumvent this risk, proteolytic enzymes such as papain or pepsin have been employed to remove the Fc region, thereby reducing immunogenicity. This approach has led to the development of genetically engineered antibody fragments, including monovalent formats (e.g., Fab and scFv)

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* Corresponding author at: Neurovascular Unit Research Group, Korea Brain Research Institute, 61 Cheomdan-ro, Dong-gu, Daegu, South Korea, 41062.
E-mail address: hhlim@kbri.re.kr (H.-H. Lim).

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and bivalent formats (e.g., F(ab')2, diabodies, and minibodies) [7,8]. However, in certain applications, full-length antibodies have been reconstituted to restore properties such as half-life, stability, and effector function [9–12].

The antigen-binding specificity and affinity of antibodies are primarily determined by the variable regions of the light and heavy chains. Within these regions, the CDRs mediate direct interactions with antigens and are thus essential for antibody function and engineering. To determine CDR sequences, conventional approaches rely on PCR amplification of the variable regions followed by Sanger sequencing [13,14]. While these methods are cost-effective and widely used, they require prior knowledge of the antibody isotype to select appropriate primers for amplification of isotype-specific CDRs. This limitation is particularly relevant for murine antibodies, which encompass a wide range of immunoglobulin isotypes: heavy chains include alpha (IgA), delta (IgD), epsilon (IgE), gamma (IgG; with subclasses 1, 2a, 2b, 2c, and 3), and mu (IgM), while light chains include kappa and lambda. Accurate isotype identification is therefore necessary before variable region amplification, adding time and complexity to the workflow.

In this study, we developed a streamlined workflow for the molecular characterization of antibodies produced from hybridoma cell cultures. To enable isotype-independent cDNA synthesis, we introduced a universal adaptor sequence at the 5′ end of the variable region and designed a multiplex set of reverse transcription primers (Multiplex-RT) covering all major murine isotypes. Using this approach, we successfully synthesized cDNA from hybridoma cells without prior isotype identification. Variable regions were subsequently amplified using an isotype-specific primer set (Iso-PCR) to determine the antibody isotype, IgG subclass, and CDR sequences (Fig. 1). We applied this method to characterize a murine monoclonal antibody against the astrocytic membrane protein MLC1. Based on the retrieved variable region sequences, we generated a chimeric antibody by grafting murine CDRs onto a human IgG1 backbone. The resulting chimeric antibody retained antigen-binding activity, as confirmed by immunoprecipitation and immunoblotting. This integrated approach provides a rapid and reliable strategy for isotyping and sequencing mouse monoclonal antibodies, enabling downstream applications in antibody engineering for both therapeutic and experimental purposes.

Figure 1. Overview of the Multiplex-RT/Iso-PCR workflow for molecular characterization of monoclonal antibodies

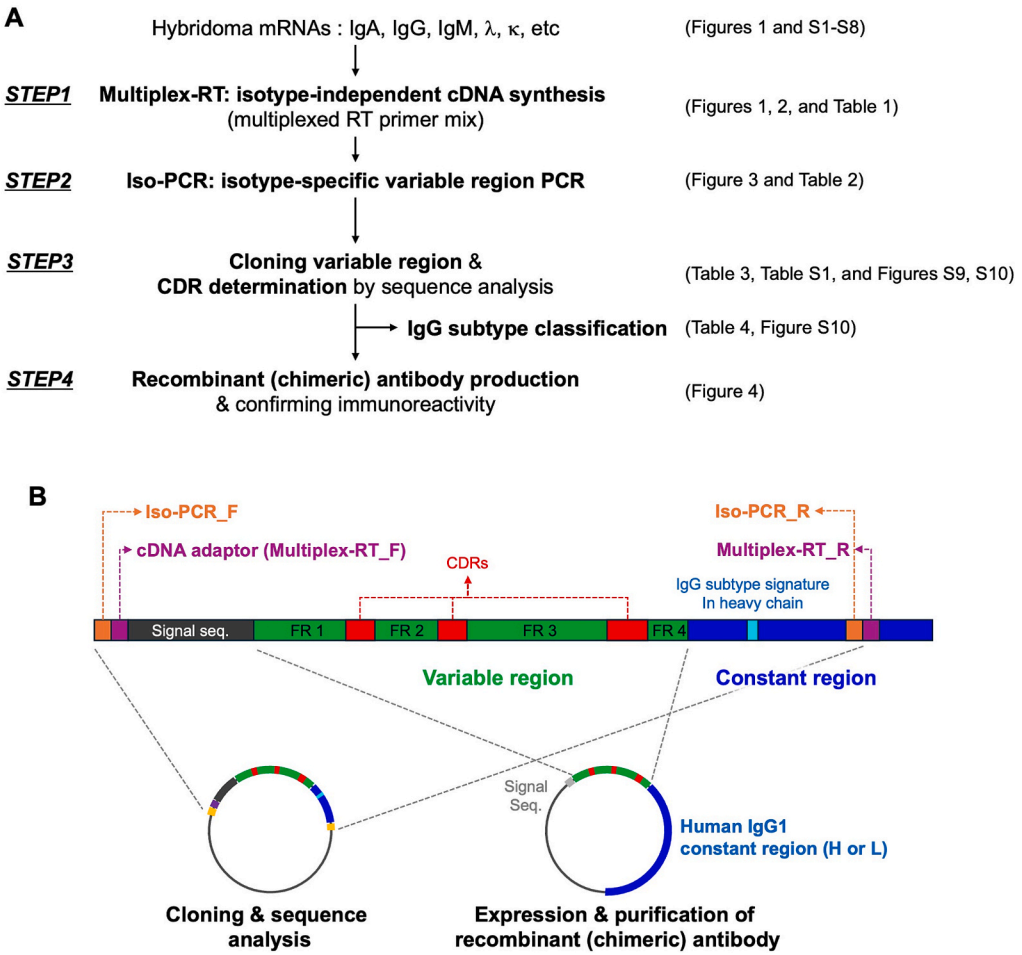

Fig. 1. Overview of the Multiplex-RT/Iso-PCR workflow for molecular characterization of monoclonal antibodies. (A) Overall experimental workflow with corresponding figures and tables referenced in this report. (B) Locations of Multiplex-RT_F (cDNA adaptor), Multiplex-RT_R and Iso-PCR_R primers in the immunoglobulin heavy and light chains. First-strand cDNA is synthesized by Multiplex-RT primer mix (*purple*) and subsequently amplified with Iso-PCR primer (*orange*). Isotypes are determined by presence of isotype-specific amplicons in the Iso-PCR products. FRs (*green*) and CDRs (*red*) are annotated using IgBLAST. IgG subclasses are classified based on the presence of a signature sequence in the constant region of heavy chain (*cyan*). Cloning scheme used for DNA sequencing and recombinant antibody production is shown below. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2. Results

2.1. Efficient isotype-independent cDNA synthesis enabled by Multiplex-RT primers

To develop a robust method for amplifying the variable regions of mouse immunoglobulin genes, we first collected available sequences of heavy and light chain isotypes from the IMGT database and performed multiple sequence alignments to identify conserved regions suitable for universal priming. However, the degree of sequence divergence among the different isotypes was too high to identify a single consensus region across all classes (Fig. S1). Consequently, it was not feasible to design a single universal primer for reverse transcription. Instead, we identified isotype-specific regions within the constant domains that allowed us to design a panel of subtype-specific reverse transcription primers (Multiplex-RT_R primers; Table 1). Alignments of constant region sequences from mouse heavy chain isotypes (IgA, IgD, IgE, IgM) and the kappa light chain revealed isotype-specific sequence stretches longer than 20 base pairs that could serve as priming sites (Fig. 1, Figs. S2–S4, S6, and S7). For IgG heavy chain and lambda light chain isotypes, where no single $>$ 20 bp consensus region was found, two distinct primers per isotype were designed to accommodate sequence variability (Figs. S5 and S8).

First-strand cDNA synthesis was performed from total RNA using the Multiplex-RT_R primer mixture (Table 1) and Moloney murine leukemia virus (M−MLV) reverse transcriptase, which is known to add a non-templated triple cytidine (CCC) overhang at the 3′ end of nascent cDNA strands [15–18]. To take advantage of this property, we adopted SMART (switching mechanism at 5′ end of RNA transcript) technology to design the universal cDNA adaptor (Multiplex-RT_F primer), which contains three guanosine ribonucleotides (rGrGrG) at its 3′ end. This adaptor design enables specific annealing to the triple cytidine overhang generated at the 5′ end of the variable region cDNA and allows first-strand cDNA synthesis to extend to the end of adaptor, thereby providing a universal forward priming site for subsequent amplification and cloning (Fig. 1 and Table 1). The Iso-PCR_F primer was designed to anneal to the universal adaptor sequence (Multiplex-RT_F) introduced during first-strand synthesis, rather than directly to immunoglobulin sequences. This adaptor strategy circumvents sequence variability at the 5′ end of immunoglobulin transcripts, ensures consistent amplification from FR1 through CDR3, and provides a defined priming site for downstream cloning. Non-target specificity of the Multiplex-RT_F primer was validated using Primer-BLAST [19,20].

Table 1
List of primers for multiplex RT reaction.

Name	Sequence (5′ to 3′)	Oligo length (mer)	% GC	Note
Multiplex-RT_F	5′-GCCGTAGAAC GATCGACAAT GAATTGArGrGrG-3′	30	50	Fig. 1
mIGH-A_Multiplex-RT_R	5′-CTGGCTGCTC ATGGTGTACC-3′	20	60	Fig. S2
mIGH-D_Multiplex-RT_R	5′-CAGTGACCTG GAGGACCATT G-3′	21	57	Fig. S3
mIGH-E_Multiplex-RT_R	5′-AGCTGGTCAC TTGGCTGGTG-3′	20	60	Fig. S4
mIGH-G_Multiplex-RT_R1	5′-AGCTGGGAAG GTGTGCACAC-3′	20	60	Fig. S5
mIGH-G_Multiplex-RT_R2	5′-AGATGAGACt GTGcGCACAC-3′	20	55	Fig. S5
mIGH-M_Multiplex-RT_R	5′-CCCTGGATGA CTTCAGTGTT G-3′	21	52	Fig. S6
mIGL-K_Multiplex-RT_R	5′-GCTGTAGGTG CTGTCTTTGC-3′	20	55	Fig. S7
mIGL-L_Multiplex-RT_R1	5′-GGGACAAACT CTTCTCCACA G-3′	21	52	Fig. S8
mIGL-L_Multiplex-RT_R2	5′-GAGACAGACT CTTCTCCACA G-3′	21	52	Fig. S8

2.2. Isotype-specific variable region amplification using Iso-PCR

To amplify the immunoglobulin variable regions from the first-strand cDNA, a PCR strategy was employed using isotype-specific primer sets. In the first round, isotype-specific reverse primers (Iso-PCR_R) targeting the 3′ constant region and a universal forward primer complementary to the adaptor sequence (Multiplex-RT_F primer) were used (Table 2). The Iso-PCR_R primers were designed for nested amplification to enhance specificity. Similar to Multiplex-RT primer design, two reverse primers were designed for IgG subclasses and three for the lambda light chain to accommodate sequence variability (Figs. S5 and S8). This approach enables the selective amplification of variable regions corresponding to IgA, IgD, IgE, IgG, and as IgM heavy chains as well as kappa and lambda light chains.

To optimize the conditions for variable region amplification, we tested different amounts of total RNA and varying concentrations of the Multiplex-RT_R primer mix during the cDNA synthesis step using the in-house generated hybridoma clone 37E5, which produces a monoclonal antibody targeting the astrocytic membrane protein MLC1. To evaluate the effect of total RNA input on multiplex reverse transcription, 0.1 ∼ 1 μg of total RNA was used for cDNA synthesis, followed by variable region amplification using Iso-PCR primer pairs. The resulting products were analyzed by agarose gel electrophoresis, and as little as 0.1 μg of total RNA was found to be sufficient for successful cDNA synthesis (Fig. 2A). Next, we examined the effect of Multiplex-RT_R primer concentration on cDNA synthesis efficiency. Evaluation of the multiplex primer mix in the isotype-specific PCR amplification step showed that a concentration of at least 3 μM each primer was required to achieve efficient cDNA synthesis (Fig. 2B). Finally, to determine the appropriate amount of cDNA template for the Iso-PCR reaction, 0.5 ∼ 4.0 μL of cDNA derived from the 37E5 hybridoma was used as input. When 4.0 μL of cDNA was used, weak non-specific bands were observed in lanes corresponding to other isotypes. In contrast, using 0.5–2.0 μL of cDNA as the template consistently yielded a clear, isotype-specific amplicon without detectable non-specific amplification (Fig. 2C and Table S1). We further evaluated the effect of total RNA input on the multiplex-RT reaction using another in-house generated hybridoma, T3A11C2. In addition, we tested different annealing temperatures in both the multiplex-RT and Iso-PCR reactions. Because all primers designed in this study had melting temperature ranging from 59 to 61 °C, we examined reaction performance at 50, 55, 60, and 65 °C using the T3A11C2 clone. While non-specific amplification was observed at 50 °C, reactions performed at 55–65 °C produced specific and robust amplificons (Fig. S9). These results demonstrate that our Multiplex-RT/Iso-PCR protocol is both robust and sensitive, enabling reliable amplification of immunoglobulin variable regions from as little as 0.1 μg of total RNA across multiple Ig isotypes.

Using the optimized conditions, we applied the Multiplex-RT/Iso-PCR method to amplify mouse immunoglobulin genes of various isotypes from one commercial hybridoma clone obtained from ATCC (anti-HLA type II, HB-145) [21] and four in-house generated hybridoma clones: anti-human LCN2 (3F1), anti-MLC1 (37E5), anti-human TREM2 (T3A11C2), and anti-mouse SHLP2 (5E3). Successful amplification was achieved for all samples, producing variable region amplicons corresponding to heavy chains of IgA, IgG, and IgM isotypes as well as kappa light chains (Fig. 3A). To validate the isotyping results obtained by

Table 2
List of primers for Iso-PCR.

Name	Sequence (5′ to 3′)	Priming seq. length (mer)	%GC *	Note
	5′-TTAAA-(AflII-HindIII-KpnI)-priming seq-3′			
Iso-PCR_F	5′-TTAAA-(CTTAAGCTTGGTACC)-GCCGTAGAAC GATCGACAAT G-3′	21	52	Fig. 1
	5′-C-(XbaI-XhoI-NotI)-priming seq-3′			
mIGH-A_Iso-PCR_R	5′-C-(TCTAGACTCGAGCGGCCGC)-CCACTCTTTC CCCAGGTCAC-3′	20	60	Fig. S2
mIGH-D_Iso-PCR_R	5′-C-(TCTAGACTCGAGCGGCCGC)-CCCAGCTGAT TTTCAGTGGC-3′	20	55	Fig. S3
mIGH-E_Iso-PCR_R	5′-C-(TCTAGACTCGAGCGGCCGC)-GAATACCAGG TCACAGTCAC AGG-3′	23	52	Fig. S4
mIGH-G_Iso-PCR_R1	5′-C-(TCTAGACTCGAGCGGCCGC)-GGACAGGGAT CCAGAGTTCC-3′	20	60	Fig. S5
mIGH-G_Iso-PCR_R2	5′-C-(TCTAGACTCGAGCGGCCGC)-GGACAGGGCT CCATAGTTCC-3′	20	60	Fig. S5
mIGH-M_Iso-PCR_R	5′-C-(TCTAGACTCGAGCGGCCGC)-GGCCACCAGA TTCTTATCAG AC-3′	22	50	Fig. S6
mIGL-K_Iso-PCR_R	5′-C-(TCTAGACTCGAGCGGCCGC)-GTTCACTGCC ATCAATCTTC CAC-3′	23	48	Fig. S7
mIGL-L_Iso-PCR_R1	5′-C-(TCTAGACTCGAGCGGCCGC)-GGGGTACCAT CTACCTTCCA G-3′	21	57	Fig. S8
mIGL-L_Iso-PCR_R2	5′-C-(TCTAGACTCGAGCGGCCGC)-GGGGTACCAT CTGCCTTCCA G-3′	21	62	Fig. S8
mIGL-L_Iso-PCR_R3	5′-C-(TCTAGACTCGAGCGGCCGC)-GGTGTACCAT TTGCCTTCCA G-3′	21	52	Fig. S8

* GC content (%) of the priming sequence.

Figure 2. Optimization of input RNA, primer concentration, and cDNA template volume for Iso-RT/Iso-PCR amplification.

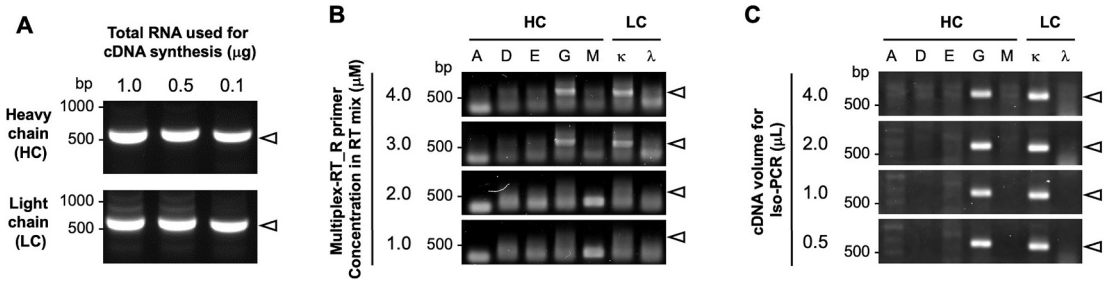

Fig. 2. Optimization of input RNA, primer concentration, and cDNA template volume for Multiplex-RT/Iso-PCR amplification. (A) Effect of total RNA input (μg) on the variable region amplification products. Varying amounts of total RNA isolated from 37E5 hybridoma cells were used for cDNA synthesis, and Iso-PCR products were analyzed by agarose gel electrophoresis. (B) Effect of Multiplex-RT_R primer concentration (μM) in the multiplex primer mix. All the reactions were carried out with 0.5 μg total RNA and 2 μL cDNA. (C) Effect of cDNA volume (μL) used as template in the Iso-PCR reaction. Arrowheads indicate the expected PCR products (approximately 600–700 bp). DNA size markers (bp) are shown to the left of each gel. HC and LC indicate heavy chain and light chain, respectively.

Multiplex-RT/Iso-PCR, we cross-checked the isotypes of each antibody using two commercially available immunological subtyping methods. Because the concentration of secreted antibodies can vary among hybridoma cultures, we first purified each antibody using protein L resin, which binds kappa light chain-containing antibodies regardless of heavy chain isotype (Fig. S10). Both the lateral flow-based isotyping strip and ELISA-based subtyping assays confirmed that 3F1, HB-145, 37E5, T3A11C2, and 5E3 antibodies contained IgA, IgG1, IgG2a, IgG2b, and IgM heavy chains, respectively, and that all antibodies possessed kappa light chains (Figs. 3B and 3C). Together, these results confirm that the Multiplex-RT/Iso-PCR method provides robust and accurate isotype determination across multiple murine Ig classes without prior isotype information (Fig. 3).

2.3. Sequence analysis and determination of CDRs, framework regions (FRs), and IgG subtype

The Iso-PCR primers were designed with extended 5′ sequences complementary to the multiple cloning site (MCS) of the pcDNA3.1 vector, enabling efficient subcloning either by conventional restriction enzyme digestion and ligation or by Gibson assembly without the need for enzymatic digestion (Table 2). Using this strategy, isotype-specific amplicons from anti-MLC1 (37E5) and anti-human TREM2 (T3A11C2) immunoglobulin heavy and light chain genes were cloned into the pcDNA3.1 vector by Gibson assembly. The sequences of each variable region were subsequently analyzed using IgBLAST against mouse germline gene database [22]. Light chain variable regions were aligned to the corresponding V and J gene segments, whereas heavy chain sequences aligned to the V, D, and J segments. IgBLAST analysis further enabled the annotation of CDRs and FRs for each chain (Table 3; Table S2; Figs. S11 and S12). To compare our sequence determinations with known mouse V-genes, we analyzed the variable region sequences of the 37E5, HB-145, and T3A11C2 clones using IgBLAST and quantified their sequence identities to the closest germline V genes. Overall, the heavy and light chains from the tested clones exhibited 96.6–99 % identity to their respective top germline V-gene hits (Table S3).

Although it was challenging to identify suitable priming sites for the specific amplification of IgG subclasses 1, 2a, 2b, 2c, and 3 during the Iso-PCR step, we identified an 11-nucleotide sequence patch located about 50 bp downstream of FR 4 that is distinct among these subclasses (Fig. 1B, S5, S11A, and S12A; Table 4). This short sequence enables IgG subclass discrimination through DNA sequencing of the Iso-PCR products. To validate this approach, we analyzed the variable region sequences obtained from three hybridoma clones, 37E5, T3A11C2, and HB-145, which are known to produce IgG2a, IgG2b, and IgG1 antibodies, respectively (Fig. 3). In all cases, the subclass-specific sequence signatures were clearly identifiable, confirming the utility of this method for accurate subclass determination without the need for subclass-specific primers or immunoassays (Figs. S11 ~ S13).

2.4. 37E5 chimeric antibody construction and functional validation

To generate a recombinant antibody for downstream validation and potential large-scale production, the variable region sequences of the

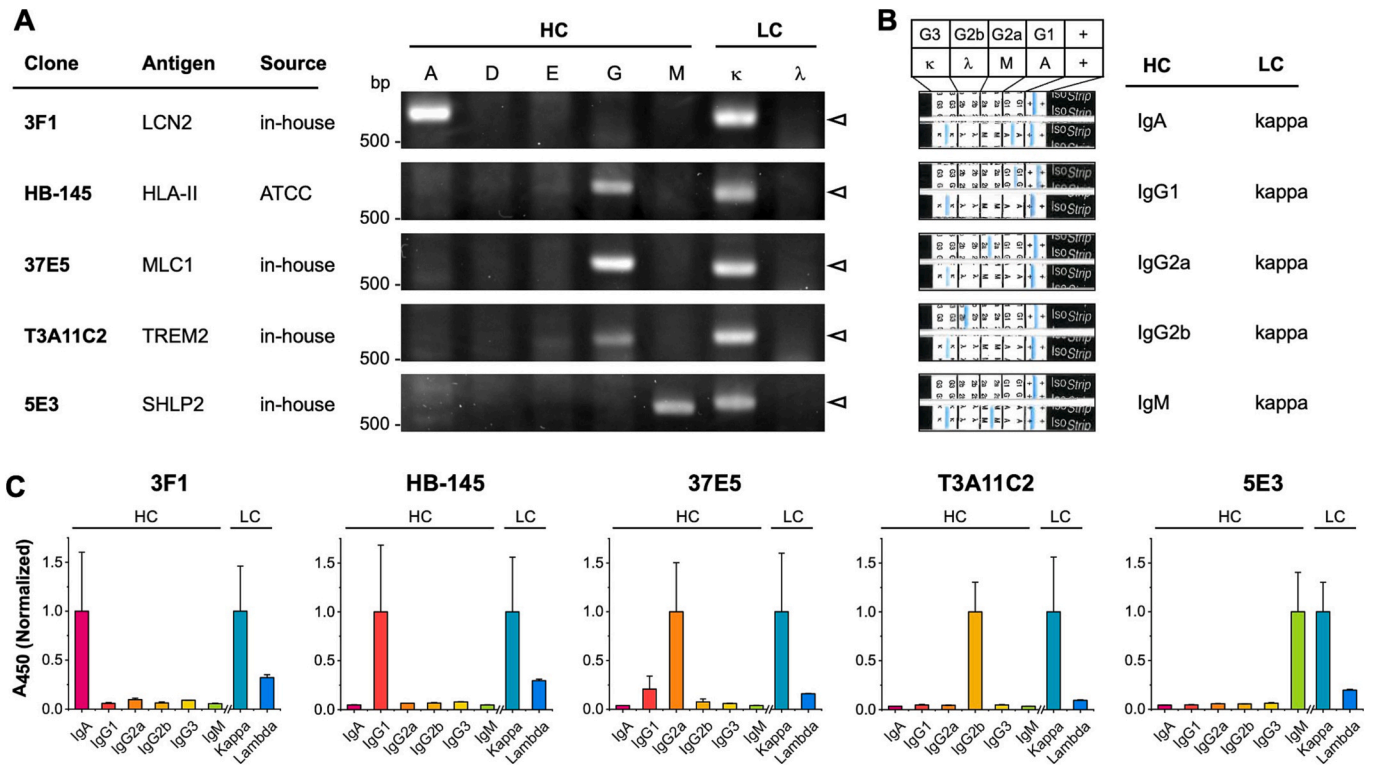

Fig. 3. Amplification and isotype validation of immunoglobulin genes from diverse hybridoma-derived antibodies. (A) Agarose gel electrophoresis of Multiplex-RT/Iso-PCR products from one commercial hybridoma clone (HB-145) and four in-house hybridoma clones (3F1, 37E5, T3A11C2, and 5E3). (B) Isotype determination of purified antibodies using an isotyping strip. (C) ELISA-based isotype and light chain subtyping of purified antibodies. Duplicate measurements were normalized to the maximum heavy or light chain signal, and error bars represent the range of the values.

Table 3
Amino acid sequences of CDRs and FRs of 37E5 and T3A11C2 monoclonal antibodies.

Chain	Region	Deduced amino acid sequence	
		37E5 antibody	T3A11C2 antibody
Light chain	FR 1	DIVMSQSPSS	DVLMTQTPLS
		LAVSAGEKVT MSCRSS	LPVSLGDQAS ISCRSS
	CDR 1	QSLLNSRTRK NY	QSLLHSNGNT Y
	FR 2	LAWYQQKPGQ SPKLLIY	LEWYLQKAGQ SPKLLIY
	CDR2	WAS	KVS
	FR 3	TRESGVPDRF	NRFSGVPDRF
		TGSGSGTDFT LTISSVQAED	SGSGSGTDFT LKISRVEAED
		LAVYYC	LGVYYC
	CDR 3	KQSYDLPYT	FQGSQVPFT
	FR 4	FGGGTKLEIK	FGSGTKLEIK
Heavy chain	FR 1	QIQLVQSGPD	QVQLKESGPG LVAPSQSLSI
		LKKPGETVKI SCKAS	TCTVS
	CDR 1	GYTFTNYG	GFSLTSYG
	FR 2	MSWVKQAPGK	VHWVRQPPGK GLEWLGV
		VLKWMGW	
	CDR2	INTYTGEP	IWTGGST
	FR 3	TYADDFKGRF	NYNSALMSRL
		AFSLETSANT	SISKDNSKSQ
		AYLQINNLKN EDTATYFC	AFLKMNSLQT DDTAMYYC
	CDR 3	AREGATSGFP Y	AKVGRLYATD Y
	FR 4	WGQGTLVTVS A	WGQGTSVIVS S

37E5 heavy and light chains were cloned into EEV expression vectors containing the constant regions of human IgG1 and human kappa, respectively (Fig. 4A). The resulting chimeric antibody constructs were transiently transfected into HEK293 GnTI⁻ cells, and secreted antibodies were collected from the culture supernatant. Recombinant chimeric antibodies (chi37E5) were purified using Protein G affinity chromatography followed by size-exclusion chromatography (SEC). The SEC elution profile and SDS-PAGE analysis under both reducing and non-reducing conditions confirmed proper expression and correct assembly of the recombinant chi37E5 antibody, comparable to the mouse antibody purified from the original 37E5 hybridoma culture supernatant (Figs. 4B and 4C).

Table 4
IgG subtype-specific signature sequence.

Subtype	Signature sequence (5′ to 3′)	Oligo length (mer)	Note
IgG1	CCCAAACTAAC	11	Fig. S5
IgG2a	ATACAACTGGC	11	Fig. S5 and S10
IgG2b	ATACAACTGGT	11	Fig. S5 and S11
IgG2c	GTACAACTGGC	11	Fig. S5
IgG3	ACACATCTGGA	11	Fig. S5

To assess whether the chimeric antibody retained the antigen-binding specificity of the original hybridoma-derived monoclonal antibody 37E5, we performed immunoprecipitation and immunoblotting of MLC1 protein transiently expressed in HEK293 GnTI⁻ cells. The results showed that both the original and chimeric antibodies were capable of immunoprecipitating natively folded MLC1 from cell lysates and detecting SDS-denatured MLC1 in immunoblot assays (Fig. 4D). The robust immunoreactivity of the chimeric antibody comparable to that of the parental mouse antibody, indicating that the epitope-paratope interaction was preserved in the humanized recombinant format (Fig. 4D). These findings demonstrate that the CDR and framework region (FR) sequences identified through our workflow are sufficient to faithfully recapitulate the original antigen specificity. Collectively, this workflow offers a platform for molecular characterization and recombinant production of monoclonal antibodies.

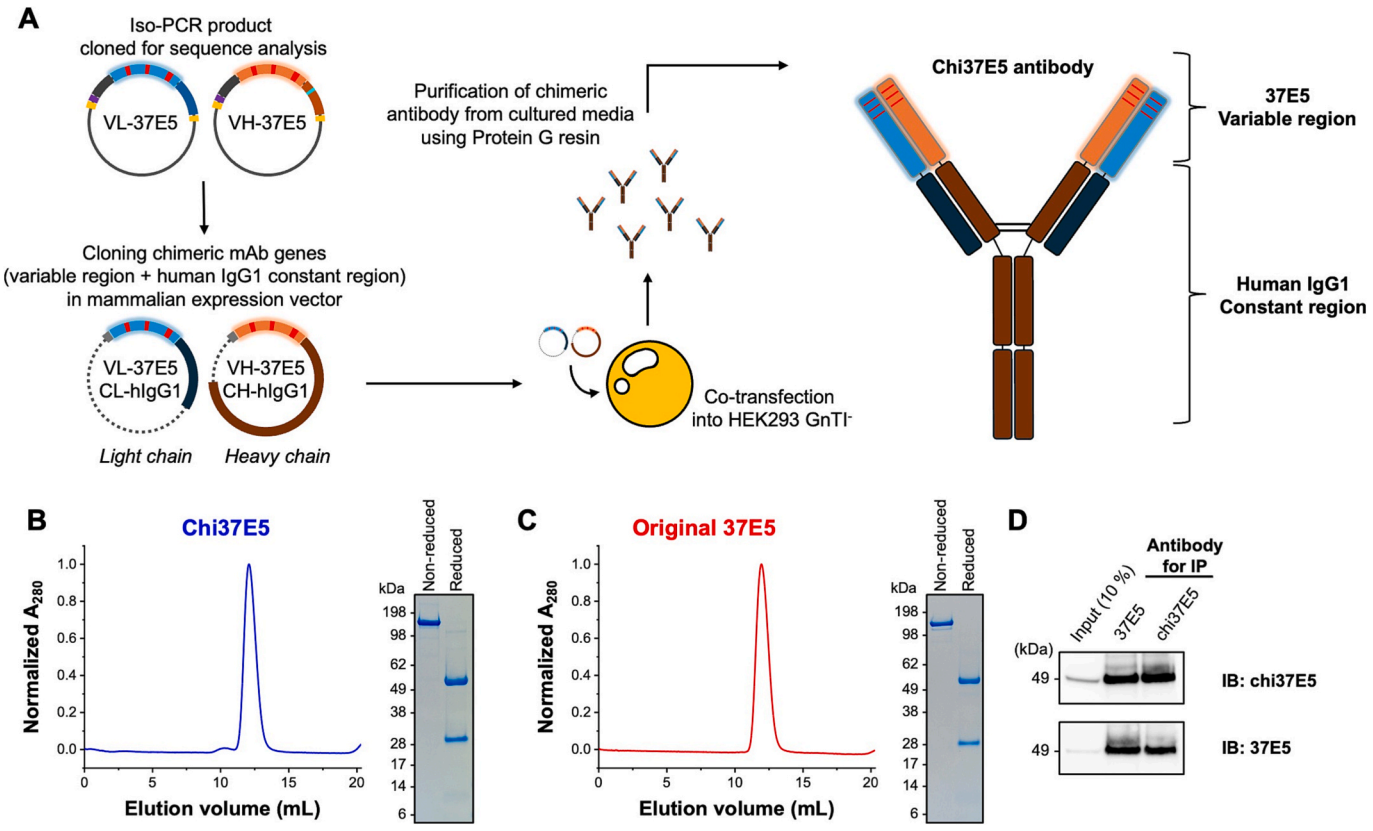

Fig. 4. Recombinant production and functional validation of the chimeric antibody chi37E5. (A) Schematic representation of cloning the variable regions of 37E5 heavy and light chains into expression vectors carrying human IgG1 and human kappa constant regions. SEC profile and SDS-PAGE analysis under reducing and non-reducing conditions of purified chi37E5 (B) and mouse 37E5 antibody (C). (D) Immunoprecipitation and immunoblotting of MLC1 protein transiently expressed in HEK293 GnTI⁻ cells using chi37E5 and mouse 37E5 antibody. Protein molecular weight markers (kDa) are indicated to the left of gels and blots.

3. Discussions

Previous PCR-based strategies for sequencing antibody variable regions have faced difficulties in designing universal primers capable of amplifying variable regions. Alternative approaches employing degenerate primers targeting 5′ region achieved only 80–90 % success, primarily due to non-specific priming or amplification failure [23–26]. Methods relying on 5′ RACE also involve multiple steps, including mRNA tailing and cDNA purification, making them labor-intensive and susceptible to RNA degradation [27,28]. It is also noteworthy that hybridomas can occasionally express multiple functional variable regions, which complicates accurate sequence identification and may compromise downstream antibody production [29]. Therefore, it is critical to use sequence-validated antibodies for reliable experimental and therapeutic applications [30].

Compared to next-generation sequencing (NGS)-based approaches, our workflow offers a cost-effective and rapid alternative for labs seeking to characterize or re-engineer monoclonal antibodies without requiring transcriptome-wide coverage or bioinformatic expertise [31–36]. A recently described monoclonal antibody sequencing method employed SMART (switching mechanism at 5′ end of RNA transcript) technology [15,16], in combination with IgG heavy chain, kappa/lambda light chain-specific reverse transcription primers and nested PCR primers, to sequence the variable regions of five mouse IgG1 antibodies [13]. Although this method achieved 100 % success in recovering functional variable region sequences from the IgG1 antibodies tested, its performance with other isotypes (e.g., IgA, IgM, IgD, or IgE) and across all IgG subclasses was not evaluated. In fact, the reverse transcription primer (mIGHG RT) for mouse IgG heavy chains does not match the IgG3 constant region, and the PCR primer (mIGHG PCR) shows mismatches with both IgG2a and IgG3 subclasses (Figure S5). Similarly, mismatches were observed in the light chain–specific primers (Figs. S7 and S8).

In this study, we present a streamlined workflow for the characterization of mouse monoclonal antibodies that bypasses the need for prior isotype determination. By combining a multiplex reverse transcription primer mix (Multiplex-RT) with an isotype-specific PCR strategy (Iso-PCR), our approach enables the synthesis and amplification of immunoglobulin variable regions from hybridoma-derived total RNA in a highly efficient and scalable manner. This framework simplifies the process of CDR and framework region identification and facilitates downstream cloning, sequencing, and antibody reconstitution. A key strength of our method is its isotype-independence at the cDNA synthesis step. The use of M−MLV reverse transcriptase and adaptor-based template switching enables the generation of full-length variable region transcripts with a universal 5′ handle, allowing for standardized forward priming in subsequent amplification. While amplification still requires isotype-specific reverse primers, it serves the dual purpose of selectively enriching variable regions and simultaneously allowing for accurate isotype identification including subclass discrimination within the IgG family via sequence-based analysis. Indeed, we identified an 11-nucleotide IgG subclass-specific sequence motif located upstream of the constant region, which enabled robust subclass annotation without the need for subclass-specific primers or immunoassays.

For the design of Multiplex-RT primers, Iso-PCR primers, and the IgG subclass-specific signature sequences, we curated constant region sequences from the IMGT database, including 8 IgA, 2 IgD, 4 IgE, 12 IgG (comprising 2 IgG1, 2 IgG2a, 3 IgG2b, 3 IgG2c, and 2 IgG3), 4 IgM, 3 kappa, and 4 lambda entries. Although this collection may not fully capture the diversity within each isotype or subclass, it provided

sufficient coverage for effective primer design and classification. Importantly, our method was validated using diverse hybridoma clones producing IgA, IgG1, IgG2a, IgG2b, and IgM antibodies. Although we were unable to evaluate hybridoma cells producing rarer isotypes such as IgD, IgE, and lambda light chain, future validation using a broader range of isotype sequences will aid in refining primer design and improving the generalizability of our workflow. Continued expansion of publicly available immunoglobulin databases, such as IMGT, will further support consensus-based optimization of isotype-specific primers.

Furthermore, we demonstrated that the variable region sequences obtained by our method are functionally relevant by generating a recombinant chimeric antibody in which the murine 37E5 CDRs and framework regions were grafted onto a human IgG1 backbone. The chimeric antibody retained antigen-binding activity equivalent to the parental hybridoma-derived antibody, validating the functional fidelity of the identified sequences. The compatibility of Iso-PCR products with mammalian expression vectors further facilitates direct translation of sequence data into functional recombinant antibodies.

In conclusion, this method enables rapid, accurate, and scalable molecular profiling of mouse monoclonal antibodies and provides a versatile platform for antibody engineering, functional validation, and therapeutic development. Future improvements may include integration of expanded sequence datasets and applications for use with other species-specific immunoglobulins.

4. Materials and methods

4.1. Cell culture and transfection

Home-made hybridoma cell lines were produced by fusing splenocytes and SP2/0 myeloma cell line (ATCC #CRL-1581) as described previously [37]. Splenocytes were isolated from mice immunized with either synthetic peptide or recombinant protein: synthetic peptides of mouse MLC1 (37E5 clone) and mouse SHLP2 (5E3 clone); recombinant proteins of human TREM2 (T3A11C2 clone) and human Lcn2 (3F1 clone). The IVA12 hybridoma (HB-145) was purchased from ATCC. Hybridoma cells were maintained in Hybridoma-SFM medium (Gibco #12045078) supplemented with 4 % (v/v) FBS, ultra-low IgG (Gibco #16250–078).

HEK293 GnTI$^-$ cells were maintained in FreeStyle 293 Expression medium (ThermoFisher #12338–026) supplemented with 2 % (v/v) FBS (Gibco #16000–044) and 0.025 % (v/v) Poloxamer 188 (Merck #P5556). Cells were cultured in humidified CO_2 (5 %) incubators at 37 °C. For suspension culture, an orbital shaker was installed in the incubator and operated at 150 rpm. For transfection, plasmid DNA (10 μg/mL, heavy: light chain ratio = 1: 1.2) and PEI MAX (60 μg/mL; Polyscience #24765) were prepared in Opti-MEM (ThermoFisher #31985–062). The transfection mixture (50 μL) was added to a 35 mm culture dish containing 1 mL of culture medium.

4.2. Total RNA extraction and cDNA synthesis

Total RNA was extracted from hybridoma cells using the NucleoSpin RNA Mini kit (MACHEREY-NAGEL, #740955.50) followed manufacturer's instruction. cDNA synthesis was performed using SMARTScribe Reverse Transcriptase (Takara, #639537). Briefly, 1 μL of total RNA (100–500 ng/μL), 2 μL of the Multiplex-RT reverse primer mix (3 μM each), and 6 μL of RNase-free water were combined for each reaction. The RNA secondary structure was melted by incubation at 72 °C for 3 min, followed by immediate chilling on ice. Next, 2 μL of 10 mM dNTPs, 2 μL of 20 mM DTT, 2 μL of 30 μM Multiplex-RT_F primer, and 1 μL of reverse transcriptase (100 U/μL) were added to bring the final reaction volume to 20 μL. cDNA synthesis was carried out at 42 °C for 60 min. The reaction was terminated by heating at 70 °C for 15 min and the cDNA was stored on ice for subsequent isotyping PCR.

4.3. Isotyping PCR

Isotyping PCR was performed using PfuUltra II fusion HS DNA polymerase (Agilent #600672). Each 50 μL reaction contained 1 mM dNTPs, 200 nM Iso-PCR_F primer, 200 nM isotype-specific Iso-PCR_R primer, 2 μL cDNA, and 0.5 μL DNA polymerase in the supplied reaction buffer. Thermal cycling was initiated with a denaturation step at 95 °C for 1 min, followed by 37 cycles of 95 °C for 20 sec (denaturation), 60 °C for 20 sec (annealing), and 72 °C for 30 sec (extension). A final extension was carried out at 72 °C for 3 min. PCR products (~3 μL) were analyzed by agarose gel electrophoresis. Isotype-specific amplicons of approximately 600–700 bp were detected.

4.4. Hybridoma culture, antibody purification, and SDS-PAGE

Monoclonal hybridomas were cultured in hybridoma serum-free media (Hybridoma-SFM, Gibco #12045076) supplemented with 4 % (v/v) FBS (Gibco #16000–044). Cultured supernatants were harvested and filtered through a 0.22 μm polyethersulfone (PES) membrane filter. Antibodies were purified by affinity chromatography using Protein G or L resin (GenScript #L00209 or #L00239, respectively). After washing with PBS (pH 7.4), bound antibodies were eluted with 0.1 M glycine (pH 2.2) and immediately neutralized by adding an equal volume of 1.0 M Tris-HCl (pH 8.0).

Purified antibodies were analyzed by SDS-PAGE under both reducing and non-reducing conditions. Samples (~10 μg) were mixed with 4X LDS sample buffer (ThermoFisher, #NP0007) containing reducing reagent (reducing condition) or without reducing reagent (non-reducing condition). Samples were heated at 95 °C for 5 min (reducing condition) or left at room temperature (non-reducing condition). Proteins were resolved in 4 ~ 12 % Bis-Tris gradient gels (ThermoFisher, #NP0322BOX) using MES running buffer and visualized by Coomassie Brilliant Blue staining.

4.5. Isotype determination using immunological assays

For isotype confirmation using the Mouse Monoclonal Antibody Isotyping Kit (IsoStrip, Roche, #11493027001), purified antibodies were diluted in PBS (pH 7.4) containing 1 % (w/v) BSA to a final concentration of 350 ng/mL. All subsequent steps were performed according to the manufacturer's instructions. Briefly, diluted antibodies were mixed with latex beads in the development tube, and isotyping strips were inserted into the mixtures. Isotypes were determined based on the position of blue bands on the strips.

A second confirmation was performed using the Rapid ELISA Mouse mAb Isotyping Kit (Pierce, #37503). Antibodies were diluted in tris-buffered saline (TBS) to a final concentration of 250 ng/mL, and 50 μL of the diluted samples was added to wells precoated with antibodies specific for mouse immunoglobulin isotypes (IgA, IgG1, IgG2a, IgG2b, IgG3, IgM, and kappa or lambda light chains). After incubation with 50 μL goat anti-mouse IgG + IgA + IgM-HRP conjugate for 1 h at 23 °C, the wells were washed three times with the provided wash buffer, and 75 μL substrate solution was added for signal development. The reaction was stopped by adding 75 μL stop solution, and absorbance was measured at 450 nm using a spectrophotometer. Color development was performed using the TMB substrate solution supplied with the kit, and the reaction was terminated with the manufacturer-provided stop solution, generally consisting of 0.18 M H_2SO_4 in standard TMB-based ELISA reactions. Duplicate measurements were normalized to the maximum heavy or light chain signal and plotted using Origin 2018b software (OriginLab).

4.6. Cloning and sequence analysis of variable regions

Gibson assembly was performed using the Gibson Assembly Master Mix (NEB, #E2611L). The pcDNA3.1 vector was digested with *KpnI* and *NotI*, and gel-purified along with the amplicons generated by Iso-PCR

using the NucleoSpin Gel and PCR Clean-up Mini Kit (MACHEREY-NAGEL, #740609.50). Approximately 30–40 ng of vector was mixed with a 4-molar excess of amplicons. An equal volume of 2X Gibson Assembly Master Mix was added, and the mixture was incubated at 50 ℃ for 30 min.

The assembled products were transformed into DH5α competent cells (RBC, #RH617) and plated on LB agar containing 100 μg/mL ampicillin. Candidate colonies were screened by colony PCR using the vector-specific T7 forward and BGH reverse primers. Colonies yielding the expected PCR product (~1 kb) were selected for plasmid purification. The cloned immunoglobulin DNA sequences were subsequently determined and analyzed. The sequences of each clone were analyzed using IgBLAST [22]. Productivity, chain type, and clonotype were confirmed from the IgBLAST results, and the final FRs and CDRs were determined.

4.7. Purification of chi37E5 recombinant antibody

To produce a recombinant chimeric antibody, the variable regions of the 37E5 clone was fused to the human IgG1 heavy and kappa light chain constant region. Chimeric heavy and light chain sequence was inserted into CAGs-MCS EEV Vector (System Biosciences # EEV600A-1).

HEK293 GnTI$^-$ cells were transfected with vectors expressing the heavy and light chains of chi37E5 and cultured for 2 days. The same volume of fresh culture medium was then added to boost up the expression, and cells were incubated for an additional 4 days. After incubation, cells were removed by centrifugation, and the culture supernatant was collected. Hybridoma cells secreting parental mouse native 37E5 were cultured for 3 weeks to obtain a parallel source of antibody for purification. Culture supernatants containing chi37E5 or native 37E5 antibodies were loaded onto a HiTrap Protein G HP column (Cytiva, #29048581) using an ÄKTA start system (Cytiva). The column was washed with 1X phosphate-buffered saline (PBS, pH 7.4), and antibodies were eluted with 0.1 M glycine (pH 2.2). The eluates were immediately neutralized by mixing with an equal volume of 1 M Tris-HCl (pH 8.0). The buffer was exchanged with 1X PBS through multiple rounds of concentration using Amicon® Ultra-4 Centrifugal Filter Units (Merck, #UFC805024). For SEC analysis, ~100 μg of purified antibody was injected onto a Superdex 200 Increase 10/300 GL column (Cytiva) pre-equilibrated with 1X PBS (pH 7.4). Chromatography was performed using an ÄKTA pure system (Cytiva) at a flow rate of 15 mL/min, and the elution profile was monitored at 280 nm.

4.8. Immunoprecipitation and immunoblotting of MLC1 with chimeric antibody

HEK293 GnTI$^-$ cells were transfected with a human MLC1 (NM_015166.3) construct containing a C-terminal BRIL-TwinStrep tag. Cells were washed with 1 × PBS and resuspended in lysis buffer containing 1 % Triton X-100, 1X PBS, and 1X cOmplete EDTA-free protease inhibitor cocktail (Roche, #05056489001). After incubation for 1 h at 4 °C, the lysates were clarified by centrifugation, and the supernatants were incubated with either original 37E5 or chi37E5 antibodies pre-bound to Protein G resin. Following 16 h incubation at 4 °C, unbound proteins were removed by washing, and bound proteins were eluted with 2X LDS sample buffer. Immunoblotting was performed using either the 37E5 or chi37E5 antibody (1 μg/mL) as the primary antibody and HRP-conjugated anti-mouse or anti-human secondary antibodies (0.2 μg/mL), respectively.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used ChatGPT5 in order to check for the grammatical erros. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the published article.

CRediT authorship contribution statement

Junmo Hwang: Writing – original draft, Conceptualization, Investigation, Data curation, Validation, Formal analysis. **Eunbi Kim:** Writing – review & editing, Investigation, Data curation, Validation. **Jina Kim:** Writing – review & editing, Investigation, Data curation, Validation. **Sujin Shin:** Writing – review & editing, Investigation, Data curation, Validation. **Hyun-Ho Lim:** Writing – original draft, Writing – review & editing, Conceptualization, Resources, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Hyun-Ho Lim has patent #KR-10–2156165 issued to DGIST, ASAN foundation, and Ulsan University. Hyun-Ho Lim has patent #KR-10–2023-0029962 pending to DGIST. Junmo Hwang has patent #KR-10–2023-0029962 pending to DGIST. Hyun-Ho Lim has patent #KR-10–2025-0070978 pending to DGIST. Junmo Hwang has patent #KR-10–2025-0070978 pending to DGIST. Given his/her/their role as, had no involvement in the peer review of this article and had no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to another journal editor. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ymeth.2025.09.008.

Data availability

No data was used for the research described in the article.

References

[1] E.C. Lee, Q. Liang, H. Ali, L. Bayliss, A. Beasley, T. Bloomfield-Gerdes, L. Bonoli, R. Brown, J. Campbell, A. Carpenter, S. Chalk, A. Davis, N. England, A. Fane-Dremucheva, B. Franz, V. Germaschewski, H. Holmes, S. Holmes, I. Kirby, M. Kosmac, A. Legent, H. Lui, A. Manin, S. O'Leary, J. Paterson, R. Sciarrillo, A. Speak, D. Spensberger, L. Tuffery, N. Waddell, W. Wang, S. Wells, V. Wong, A. Wood, M.J. Owen, G.A. Friedrich, A. Bradley, Complete humanization of the mouse immunoglobulin loci enables efficient therapeutic antibody discovery, Nat. Biotechnol. 32 (4) (2014) 356–363.

[2] K. Basu, E.M. Green, Y. Cheng, C.S. Craik, Why recombinant antibodies - benefits and applications, Curr. Opin. Biotechnol. 60 (2019) 153–158.

[3] A. Beck, T. Wurch, C. Bailly, N. Corvaia, Strategies and challenges for the next generation of therapeutic antibodies, Nat. Rev. Immunol. 10 (5) (2010) 345–352.

[4] R.M. Lu, Y.C. Hwang, I.J. Liu, C.C. Lee, H.Z. Tsai, H.J. Li, H.C. Wu, Development of therapeutic antibodies for the treatment of diseases, J. Biomed. Sci. 27 (1) (2020) 1.

[5] P.J. Carter, G.A. Lazar, Next generation antibody drugs: pursuit of the 'high-hanging fruit', Nat. Rev. Drug Discov. 17 (3) (2018) 197–223.

[6] J. Cummings, A.M.L. Osse, D. Cammann, J. Powell, J. Chen, Anti-Amyloid Monoclonal Antibodies for the Treatment of Alzheimer's Disease, BioDrugs 38 (1) (2024) 5–22.

[7] P. Holliger, P.J. Hudson, Engineered antibody fragments and the rise of single domains, Nat. Biotechnol. 23 (9) (2005) 1126–1136.
[8] M. Jain, N. Kamal, S.K. Batra, Engineering antibodies for clinical applications, Trends Biotechnol. 25 (7) (2007) 307–316.
[9] L. Persic, A. Roberts, J. Wilton, A. Cattaneo, A. Bradbury, H.R. Hoogenboom, An integrated vector system for the eukaryotic expression of antibodies or their fragments after selection from phage display libraries, Gene 187 (1) (1997) 9–18.
[10] D.B. Powers, P. Amersdorfer, M. Poul, U.B. Nielsen, M.R. Shalaby, G.P. Adams, L. M. Weiner, J.D. Marks, Expression of single-chain Fv-Fc fusions in Pichia pastoris, J. Immunol. Methods 251 (1–2) (2001) 123–135.
[11] C. De Lorenzo, A. Tedesco, G. Terrazzano, R. Cozzolino, P. Laccetti, R. Piccoli, G. D'Alessio, A human, compact, fully functional anti-ErbB2 antibody as a novel antitumour agent, Br. J. Cancer 91 (6) (2004) 1200–1204.
[12] C. De Lorenzo, R. Cozzolino, A. Carpentieri, P. Pucci, P. Laccetti, G. D'Alessio, Biological properties of a human compact anti-ErbB2 antibody, Carcinogenesis 26 (11) (2005) 1890–1895.
[13] L. Meyer, T. Lopez, R. Espinosa, C.F. Arias, C. Vollmers, R.M. DuBois, A simplified workflow for monoclonal antibody sequencing, PLoS One 14 (6) (2019) e0218717.
[14] H.C. Foreman, A. Frank, T.T. Stedman, Determination of variable region sequences from hybridoma immunoglobulins that target Mycobacterium tuberculosis virulence factors, PLoS One 16 (8) (2021) e0256079.
[15] M.G. Wulf, S. Maguire, P. Humbert, N. Dai, Y. Bei, N.M. Nichols, I.R. Correa Jr., S. Guan, Non-templated addition and template switching by Moloney murine leukemia virus (MMLV)-based reverse transcriptases co-occur and compete with each other, J. Biol. Chem. 294 (48) (2019) 18220–18231.
[16] Y.Y. Zhu, E.M. Machleder, A. Chenchik, R. Li, P.D. Siebert, Reverse transcriptase template switching: a SMART approach for full-length cDNA library construction, Biotechniques 30 (4) (2001) 892–897.
[17] R. Kraivong, P. Luangaram, N. Phaenthaisong, P. Malasit, W. Kasinrerk, C. Puttikhunt, A simple approach to identify functional antibody variable genes in murine hybridoma cells that coexpress aberrant kappa light transcripts by restriction enzyme digestion, Asian Pac. J. Allergy Immunol. 39 (4) (2021) 287–295.
[18] D. Dermic, S. Ljubic, M. Matulic, A. Procino, M.C. Feliciello, D. Ugarkovic, I. Feliciello, Reverse transcription-quantitative PCR (RT-qPCR) without the need for prior removal of DNA, Sci. Rep. 13 (1) (2023) 11470.
[19] V. Giudicelli, D. Chaume, M.P. Lefranc, Imgt, gene-db,, a comprehensive database for human and mouse immunoglobulin and T cell receptor genes, Nucleic Acids Res. 33 (Database issue) (2005) D256–D261.
[20] J. Ye, G. Coulouris, I. Zaretskaya, I. Cutcutache, S. Rozen, T.L. Madden, Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction, BMC Bioinf. 13 (2012) 134.
[21] S. Shaw, A. Ziegler, R. DeMars, Specificity of monoclonal antibodies directed against human and murine class II histocompatibility antigens as analyzed by binding to HLA-deletion mutant cell lines, Hum. Immunol. 12 (4) (1985) 191–211.
[22] J. Ye, N. Ma, T.L. Madden, J.M. Ostell, IgBLAST: an immunoglobulin variable domain sequence analysis tool, Nucleic Acids Res 41(Web Server issue) (2013) W34-40.
[23] Z. Wang, M. Raifu, M. Howard, L. Smith, D. Hansen, R. Goldsby, D. Ratner, Universal PCR amplification of mouse immunoglobulin gene variable regions: the design of degenerate primers and an assessment of the effect of DNA polymerase 3' to 5' exonuclease activity, J. Immunol. Methods 233 (1–2) (2000) 167–177.
[24] A. Krebber, S. Bornhauser, J. Burmester, A. Honegger, J. Willuda, H.R. Bosshard, A. Pluckthun, Reliable cloning of functional antibody variable domains from hybridomas and spleen cell repertoires employing a reengineered phage display system, J. Immunol. Methods 201 (1) (1997) 35–55.
[25] O. Ihle, K.J. Beckstrom, T.E. Michaelsen, Cloning, sequencing and expression of immunoglobulin variable regions of murine monoclonal antibodies specific for the P1.7 and P1.16 PorA protein loops of Neisseria meningitidis, Scand. J. Immunol. 57 (5) (2003) 453–462.
[26] S. Koren, M. Kosmac, A. Colja Venturini, S. Montanic, V., Curin Serbec, Antibody variable-region sequencing as a method for hybridoma cell-line authentication, Appl. Microbiol. Biotechnol. 78 (6) (2008) 1071–1078.
[27] F. Ruberti, A. Cattaneo, A. Bradbury, The use of the RACE method to clone hybridoma cDNA when V region primers fail, J. Immunol. Methods 173 (1) (1994) 33–39.
[28] A. Doenecke, E.L. Winnacker, M. Hallek, Rapid amplification of cDNA ends (RACE) improves the PCR-based isolation of immunoglobulin variable region genes from murine and human lymphoma cells and cell lines, Leukemia 11 (10) (1997) 1787–1792.
[29] A.R.M. Bradbury, N.D. Trinklein, H. Thie, I.C. Wilkinson, A.K. Tandon, S. Anderson, C.L. Bladen, B. Jones, S.F. Aldred, M. Bestagno, O. Burrone, J. Maynard, F. Ferrara, J.S. Trimmer, J. Gornemann, J. Glanville, P. Wolf, A. Frenzel, J. Wong, X.Y. Koh, H.Y. Eng, D. Lane, M.P. Lefranc, M. Clark, S. Dubel, When monoclonal antibodies are not monospecific: Hybridomas frequently express additional functional variable regions, MAbs 10 (4) (2018) 539–546.
[30] R.A. Kahn, H. Virk, C. Laflamme, D.W. Houston, N.K. Polinski, R. Meijers, A. I. Levey, C.B. Saper, T.M. Errington, R.E. Turn, A. Bandrowski, J.S. Trimmer, M. Rego, L.P. Freedman, F. Ferrara, A.R.M. Bradbury, H. Cable, S. Longworth, Antibody characterization is critical to enhance reproducibility in biomedical research, Elife 13 (2024).
[31] M.F. Erasmus, F. Ferrara, S. D'Angelo, L. Spector, C. Leal-Lopes, A.A. Teixeira, J. Sorensen, S. Nagpal, K. Perea-Schmittle, A. Choudhary, W. Honnen, D. Calianese, L. Antonio Rodriguez Carnero, S. Cocklin, V. Greiff, A. Pinter, A.R.M. Bradbury, Insights into next generation sequencing guided antibody selection strategies, Sci. Rep. 13 (1) (2023) 18370.
[32] M. Schanz, T. Liechti, O. Zagordi, E. Miho, S.T. Reddy, H.F. Gunthard, A. Trkola, M. Huber, High-throughput sequencing of human immunoglobulin variable regions with subtype identification, PLoS One 9 (11) (2014) e111726.
[33] R. Rouet, K.J.L. Jackson, D.B. Langley, D. Christ, Next-Generation Sequencing of Antibody Display Repertoires, Front. Immunol. 9 (2018) 118.
[34] K. Mitchell, S. Hunter, L. Froenicke, K. Murray, M. Settles, J.S. Trimmer, clonevdjseq: a workflow and bioinformatics management system for sequencing, archiving, and analysis of VDJ sequences from clonal libraries, BMC Bioinf. 26 (1) (2025) 186.
[35] H.P. Subas Satish, K. Zeglinski, R.T. Uren, S.L. Nutt, M.E. Ritchie, Q. Gouil, R. M. Kluck, NAb-seq: an accurate, rapid, and cost-effective method for antibody long-read sequencing in hybridoma cell lines and single B cells, MAbs 14 (1) (2022) 2106621.
[36] N. Pascual, T. Belecciu, S. Schmidt, A. Nakisa, X. Huang, D. Woldring, Single-Cell B-Cell Sequencing to Generate Natively Paired scFab yeast Surface Display Libraries, Methods Mol. Biol. 2681 (2023) 175–212.
[37] H.H. Lim, Y. Fang, C. Williams, High-efficiency screening of monoclonal antibodies for membrane protein crystallography, PLoS One 6 (9) (2011) e24653.