

Design and characterization of a plasmid vector system capable of rapid generation of antibodies of multiple isotypes and specificities

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Abstract We present a detailed method for constructing a vector system composed of plasmids encoding immunoglobulin genes that have been constructed in such a fashion so as to allow for the generation and secretion of antibodies of multiple specificities and isotypes via a rapid and easy cloning-and-ligation scheme. Restriction sites within each plasmid allow for the removal of variable domains, constant domains, leader sequences, or the entire immunoglobulin gene. Degenerate primers are used to clone variable regions from hybridoma cDNAs, allowing for the creation of antibodies with varying binding specificities. Sequence-specific primers are used to clone antibody constant domains, allowing for the creation of antibodies of multiple isotypes from a variety of lineages. A high-efficiency leader sequence has been inserted into the start of each gene to improve secretion. Antibodies constructed through this system are efficiently secreted by transfected cells, and are fully functional in antigen-specific binding assays.

Keywords Antibody · Expression vectors · Hybridoma cDNA · Immunoglobulin · Plasmid · Vector

Introduction

Functional antibodies encoded by plasmids have a wide variety of uses in research settings. Such applications include *in vitro* uses such as ELISAs (Dechant et al. 2007; Berdoz et al. 1999) binding to cell surface proteins in culture (Dechant et al. 2007), stimulating cellular signaling (Dechant et al. 2007), blocking ligand binding to cellular receptors and antibody dependent cytotoxicity (ADCC) (Dechant et al. 2007). Applications for these types of plasmids may also have relevance *in vivo*, such as delivery of plasmids into muscle to create therapeutic monoclonal antibodies (Fang et al. 2007).

While the generation of an antibody from one or more plasmids may be useful in a variety of ways, a number of constructs created for such purposes have low plasticity in that the plasmid structure typically allows only for the generation and expression of a single antibody isotype and binding domain (Dechant et al. 2007; Berdoz et al. 1999; Koteswara et al. 2007). In this regard, these types of plasmids have limited potential in the scope of their application to experiments other than those for which they were expressly constructed. Moreover, while other plasmids have been created in order to combat this issue, such as pDR12 (Feng et al. 2006; Moroncini et al. 2004; Prabakaran et al. 2006), the description of these plasmids is generally restricted to their use in experiments, and not a description of how the

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plasmids themselves were constructed. Therefore, here we describe a vector expression system we have created consisting of antibody cassette plasmids encoding both heavy and light Ig chains which can be easily altered in both the antigen binding regions and constant domains via a rapid cloning-and-ligation method. Such a system allows for the rapid generation of multiple antibodies carrying various antigen binding domains as well as numerous constant domains and explains, in detail, how the plasmids were made.

To demonstrate the usefulness of the described plasmids, multiple variable and constant domains have been cloned, and co-expression of both antibody heavy and light chains, followed by heterodimerization and secretion is demonstrated after transfection *in vitro*. The functionality of antibodies formed by co-transfection is also shown, via the use of the recombinant antibody in a modified ELISA.

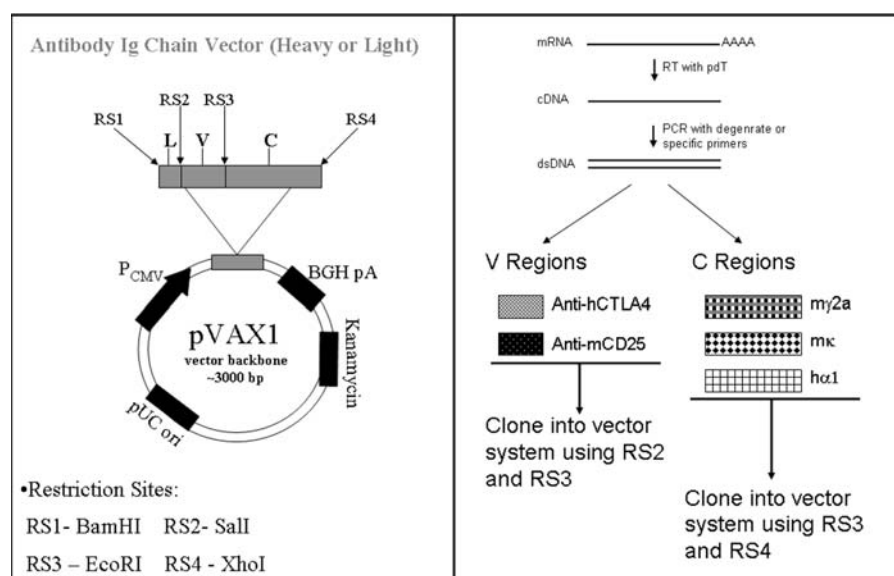
Materials and methods

Vector construction

Vectors encoding antibody heavy chain and antibody light chain were constructed in the pVAX1 backbone (Invitrogen). Antibody plasmids (both heavy and light chains) were constructed in three segments running from the start to the end of the

gene (Fig. 1). Segment 1 is the IgE leader sequence. Segment 2 is reserved for the variable binding domain of the antibody that is being cloned and thus in the basic constructs is replaced with an 111 bp place holder. Segment 3 is the constant domain for the respective chain being made. In the case of the heavy chain, this domain was first selected as human IgA1 (GenBank P01876). Light chain antibodies were selected to have human Lambda constant domains (GenBank P01842). All genes are flanked by *Bam*HI cut sites on the 5'-end and *Xho*I cut sites on the 3'-end to allow for removal of the full gene from the vector. Two additional cut sites are found within the plasmids encoding the antibody chain. A *Sal*I cut site is found in between the leader sequence (Segment 1) and the antibody variable domain (Segment 2). An *Eco*RI cut site is found in between the antibody variable domain (Segment 2) and the constant domain of the antibody (Segment 3). All genes have an IgE leader sequence inserted at the start that is codon- and RNA-optimized (Andre et al. 1998; Fang et al. 2007; Muthumani et al. 2003; Schneider et al. 1997; Yang et al. 2001); have its GC content increased to prolong mRNA half life (Andre et al. 1998; Fang et al. 2007; Muthumani et al. 2003; Schneider et al. 1997; Yang et al. 2001) and had kozak sequences introduced to increase translational initiation. Gene optimization was conducted using GeneOptimizer by GeneArt (Regensburg, Germany).

Fig. 1 Vector maps for the plasmids encoding the antibody chains (both heavy and light). Restriction sites are located in 4 areas in and around the gene (denoted by "RS"). The cloning and ligation procedure that allows for the insertion of different variable or constant domains into the vector system is presented in the right panel



Cloning and ligation of antibody variable and constant domains

Variable domains of antibodies were cloned as follows: Hybridomas PC61 5.3 and A3.6B10, which encode antibodies with binding sites directed against mouse CD25 and human CTLA-4 respectively, were obtained from ATCC (Manassas, Va). Total RNA was isolated from hybridoma cell lines using TRIzol Reagent (Invitrogen) according to manufacturer protocols. Isolated RNA was used to synthesize mRNA-specific cDNA by employing oligo-p(dT) primers in the 1st Strand cDNA Synthesis Kit (Roche). Synthesized cDNA was subsequently used in PCR reactions employing a PCR Core Kit (Roche) according to manufacturer protocols. Primers used for PCR varied according to the chain being amplified. For amplification of light chain variable domains degenerate primers were used (Wang et al. 2000) and were as follows: 5'-GAYATTGTGMTSACMCARWCTMCA-3' as the forward primer where Y stands for T or C, M stands for A or C, R stands for A or G, and W stands for A or T. The reverse primer as follows: 5'-GGATACAGTTGGTGCAGCATC-3'. For amplification of heavy chain variable domains degenerate primers were also used (Wang et al. 2000) and were as follows: 5'-SARGTNMAGCTGSAGSAGTCWGG-3' for the forward primer where Y, M, R and W stand for the same nucleotides as above and S stands for G or C. The reverse primer was 5'-CTTGACGCATCCTAGAGTCA-3'. Restriction sites for *SalI* or *EcoRI* were inserted into primers as shown in Table 1 to allow for cutting and ligation of amplified binding domains into antibody heavy or light chain-encoding plasmids. For amplification of antibody constant domains, the same procedure as explained above was used, save for the use of sequence-specific primers. For mouse IgG2a, the forward primer was 5'-GCCAAAACAACAGCCCCATCGCTGTATCCA-3' and the reverse primer was 5'-CCCAGCAATGCCTGGGACCATGTAA-3'. For mouse Ig Kappa the forward primer was 5'-GCCCCGACTACGACGTGGTTGACATAGGTAG-3' and the reverse primer was 5'-CATTGTCAAGAGCTTCAACAGGAATGAGTGT-3'. Controls ensuring sequence-specific PCR amplification were established by including cDNA generated from irrelevant cell lines (HEK293T, ATCC). All PCR products were run out on a 1.3% (w/v) agarose gel and extracted using the GeneClean

Kit I (GeneClean). PCR products for antibody variable domains were digested using *SalI* and *EcoRI* restriction enzymes (Roche), while PCR products for constant domains were cut with *EcoRI* and *XhoI* (NEB). Ligations of PCR products into parent antibody chain vectors occurred using T4 DNA ligase (NEB) at room temperature for 15 min. Ligation products were transformed into Max Efficiency DH5 α (Invitrogen) and new plasmids were extracted using plasmid maxi kits (Qiagen). Control for confirmation of the presence of RNA after extraction from cell lines was accomplished via RT-PCR for fragments of mouse or human (Dakhama et al. 1996) beta-actin messages, which amplified fragments of ~250 and ~150 bp, respectively.

Generation and isolation of recombinant antibodies

Antibodies were generated via transfection of the desired antibody constructs into HEK293T cells (ATCC). Briefly, HEK293T cells were seeded into 6-well plates and were grown to 80% confluence. At this time point, transfection reactions consisting of desired plasmids (antibody light and/or heavy chains) mixed with Lipofectamine 2000 (Invitrogen) at a ratio of 1:4 were added drop-wise to cultures. Cells were grown at 37°C for 48–72 h after which culture cell lysates were taken and analyzed by Western Blot. Separately, contents of cell culture supernatants were collected and centrifuged at 1,800g for 10 min to remove floating cells and cell debris for analysis. Antibody complexes were pulled out of culture supernatants via use of Jacalin beads (Pierce) via a batch-purification method. Briefly, 1.3 ml of antibody culture supernatant was incubated with 60 μ l Jacalin beads in a 1.5 ml Eppendorf tube at 4°C overnight. Beads were then removed by centrifuging and the culture supernatant removed, followed by 3 washes with DPBS (Dulbecco's Phosphate Buffered Saline).

Western blotting for antibody components

Western blotting for antibody light and heavy chains from cell lysate was performed as follows. Cell lysis occurred 48–72 h post transfection in a lysis buffer consisting of 0.1% Triton X-100, 0.05% SDS, 5 mM CHAPS, 20 mM Tris/HCl (pH 7.5), and 50 mM

Table 1 List of primers used in PCR reactions

Primer sequence	Region amplified/primer name
5'-GGGTCGACGACATTGTGATCACCCAGACTCCA-3'	Light chain forward-V domain
5'-GGTGAATTCGGATACAGTTGGTGCAGCATC-3'	Light chain reverse-V domain
5'-CCGCCGGTCGTTTCAGCTGCAGGAGTCAGG-3'	Heavy chain forward-V domain
5'-GAAGAATTCCTTGACCAGGCATCCTAGAGTCA-3'	Heavy chain reverse-V domain
5'-GAATTCGCCAAAAACAACAGCCCCCATCGCTGTATCCA-3'	Mouse IgG2a forward
5'-CTCGAGCCCAGCAATGCCTGGGACCATGTAA-3'	Mouse IgG2a reverse
5'-GAATTCGCCCGACTACGACGTGGTTGACATAGGTAG-3'	Mouse kappa forward
5'-CTCGAGCATTGTCAAGAGCTTCAACAGGAATGAGTGT-3'	Mouse kappa reverse

Degenerate primers were used for variable domain amplification. Sequence specific primers were used for constant domain amplification. Restriction sites are included within primer sequences to allow for cutting and ligation of products into antibody constructs

Restriction enzyme cut sites are underlined

Variable domain primers have been described elsewhere (Moroncini et al. 2004)

NaCl. BioRad protein assay was performed on lysates to ensure equal loading onto 10–20% Tris/HCl gels (BioRad) and this loading was subsequently confirmed via Western blot for β -actin. Gels were run at 100 V for 90 min and transferred to PVDF membranes (BioRad) overnight at 40 V at 4°C. The membrane was subsequently blocked in 10% (v/v) milk, and washed with TBS with 0.1% Tween20. Primary chicken polyclonal antibodies to human IgA (Abcam) were added to 5% (v/v) milk at a 1:2,000 dilution and allowed to blot the membrane overnight. After washing again with TBS/Tween, HRP (horse-radish peroxidase)-conjugated anti-chicken antibodies (Abcam) were added to 3% (v/v) milk at 1:4,000 dilution and the membrane was blotted for 1 h at room temperature. After washing again with TBS/Tween, the membrane was developed using the ECL reagent from GE/Amersham Biosciences. Blots for β -actin were accomplished in the same fashion using primary monoclonal antibodies to actin at a 1:1,000 dilution and both proteins were subsequently blotted for using secondary anti-mouse HRP conjugated antibodies at 1:1,000. Western blotting for antibody complexes isolated using Jacalin beads occurred as follows: beads were resuspended in 30 μ l Laemmli buffer with β -mercaptoethanol (BioRad) after being washed with 1 $\times$ DPBS as above. Bead samples were loaded onto 10–20% Tris/HCl gels and run and blotted for IgA antibody chains using the same antibodies in the same fashion as described for cell lysates.

ELISA testing for recombinant antibody function

In order to determine whether vector-encoded recombinant antibodies were functional, a modified ELISA was carried out. For these purposes, recombinant antibodies to human CTLA-4 were generated from transfection of relevant plasmids into HEK293T cells (variable domain cloned from hybridoma A3.6B10, human IgA1 constant domain). These antibodies in particular (with variable domains directed against human CTLA-4) were selected for functional testing due to the fact that there are more commercial reagents available in respect to antibody function against human CTLA-4 in an ELISA than there are for mouse CD25. Therefore, in order to show the fidelity of our recombinant antibody function in comparison to those in commercial use, synthesizing antibodies directed against human CTLA-4 from our system was the logical choice.

The human sCTLA-4 ELISA Kit (Bender Med-Systems), which has the ability to measure soluble CLTA-4 (sCTLA-4) in a variety of sample types, was modified for use in the following protocol: microwell strips coated with monoclonal antibody to human sCTLA-4 were washed according to manufacturer instructions, and recombinant sCTLA-4 was added at 2.5 ng/ml for 2 h at room temperature. Wells were then washed and cell culture supernatant from HEK293T cells that had been transfected with relevant plasmids was added to wells that were to be tested for antibody function. Plates were incubated

for 2 h at room temperature and washed. Wells serving as negative controls (no detector antibody) or positive controls (detector antibody supplied by kit) had RPMI added in equal volumes. Wells serving as negative or positive controls for kit antibodies then received biotin-conjugated secondary antibodies as provided by the kit. Wells serving as negative or positive controls for plasmid-encoded antibodies received biotin-conjugated anti-human α chain secondary antibodies (Jackson ImmunoResearch). Plates were incubated for 2 h at room temperature and were washed as above. All wells then received streptavidin-HRP as supplied by the kit, and were incubated for 1 h at room temperature before washing. TMB (tetramethyl benzidine) substrate was then added to all wells and color development was monitored and read at 450 nm after addition of Stop Solution.

Results

Plasmid generation and characterization

To allow for the generation of numerous antibodies of multiple isotypes and binding specificities, we constructed a set of expression vectors containing several

cut sites that permits users to clone in and out variable or constant domains of interest via a simple procedure (Fig. 1). The vectors are all constructed in the pVAX1 backbone, using the *Bam*HI and *Xho*I cut sites in the multiple cloning region to insert the full length gene. In plasmids encoding antibody chains, *Sal*I and *Eco*RI cut sites are also present to allow for manipulation of different segments of the antibody chain as desired (Fig. 1). To ensure high levels of secretion, the IgE immunoglobulin leader sequence was added to the start of the gene (Andre et al. 1998; Fang et al. 2007; Muthumani et al. 2003; Schneider et al. 1997; Yang et al. 2001).

To demonstrate the utility of having multiple cut sites within the antibody chains, the basic constructs were cut using different combinations of enzymes to remove various parts of the insert. The restriction sites used in these constructs were chosen to be compatible for double-digests, thus reducing the time required for this type of manipulation when compared to sequential, single-digest reactions. The ability to remove specific sections using double-digests allows for manipulation of the gene in various ways to quickly and easily generate different antibodies of interest. For example, digestion of the Ig-encoding constructs using *Bam*HI and *Eco*RI resulted in

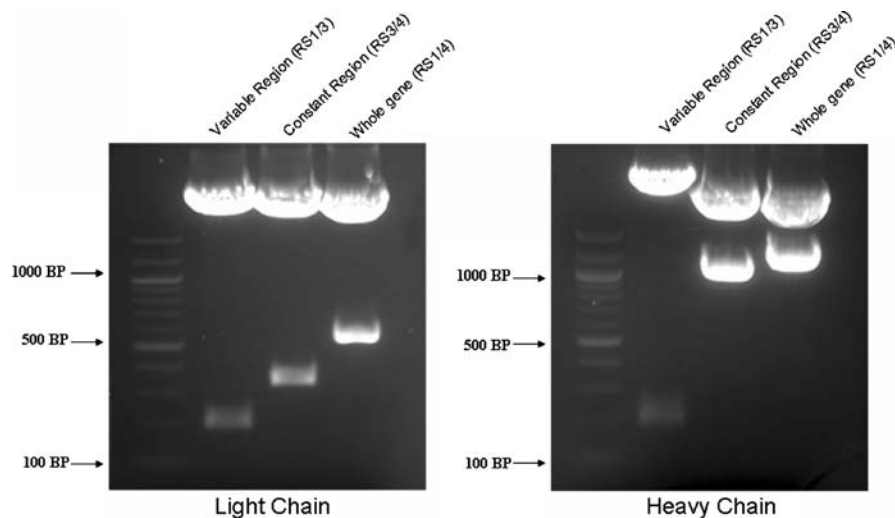


Fig. 2 Efficient restriction of plasmids encoding antibody light chains (left) or antibody heavy chains (right). Double digests show the ability to manipulate specific sections of each chain. Plasmids were subjected to double digestion followed by electrophoresis on 1.3% (w/v) agarose gels. Variable regions (in this case, 111 bp spacers including IgE leader sequences) were removed using *Bam*HI and *Eco*RI. The constant domain

of the antibody light chain (human lambda) was removed using *Eco*RI and *Xho*I double digestion. The constant domain of the heavy chain (human IgA1) was removed from the plasmid in the same fashion. The entire gene was able to be removed from the plasmid using *Bam*HI and *Xho*I double digestion for both constructs

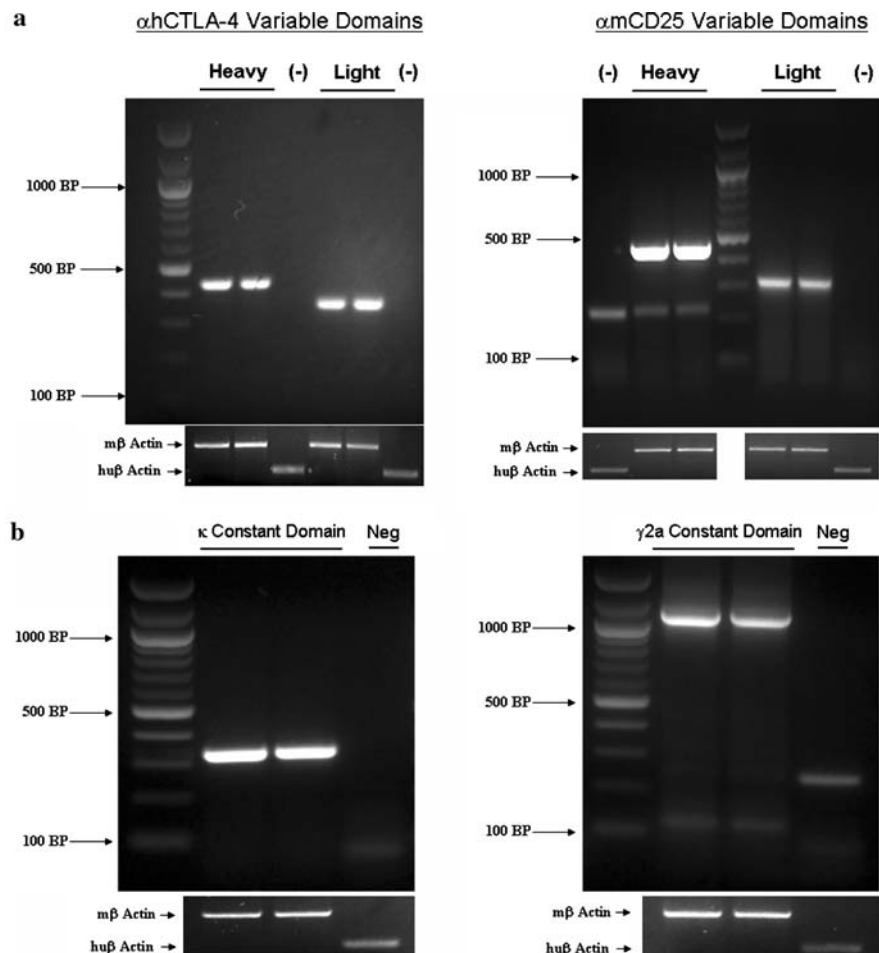
removal of the leader sequence and variable binding domain of the antibody chains (Fig. 2). Use of *EcoRI* and *XhoI* allowed for the removal of the constant domains of the Ig genes, which in the current plasmids are human IgA1 for the heavy chain and human Lambda for the light chain (Fig. 2). Employing *BamHI* and *XhoI* in digestion removed the entire gene from the plasmid (Fig. 2), which allow for its subsequent ligation into other expression vectors if preferred. Thus, all constructs can be manipulated simply as desired using different combinations of restriction enzymes.

Cloning of variable and constant domains for ligation

In order to demonstrate the ease of introducing new variable binding domains into the Ig-chain encoding

constructs of this system, variable domains from anti human CTLA-4 and anti mouse CD25 antibodies were cloned. RT-PCR was performed using cDNA generated from hybridomas expressing antibodies against human CTLA-4 (Kuchroo and Greenfield 2001) or mouse CD25 (Trowbridge et al. 1982; Zubler et al. 1984). PCR amplification of cDNAs generated from mouse hybridoma mRNAs was accomplished using sets of degenerate primers previously described (Wang et al. 2000; see Table 1). The use of degenerate primers for this purpose allows for the amplification of a wide variety of antibody variable domains from numerous hybridomas while eliminating the need for new primer construction, and thus variable domains that can be cloned using these primers are not restricted to the ones demonstrated here. PCR products for the variable domain of the anti-mouse CD25 antibody (Fig. 3a) and the variable

Fig. 3 RT-PCR of the variable domains of both the heavy and light chains from described hybridomas carried out via the use of degenerate primers. Use of these primers allowed for the targeted amplification of variable domains from both antibody chains (heavy and light) from two distinct antibodies—one directed against human CTLA4 and one directed against mouse CD25 (a). In contrast, the constant domains of the selected antibody chains could be easily amplified using sequence-specific primers designed to match mouse IgG2a and mouse Ig kappa. Specificity of these primers was verified by the fact that products generated using these primers migrate at the correct molecular weight on agarose gels (b). Beta-actin controls for the presence human (“hu β actin” ~150 bp) and mouse (“m β actin” ~250 bp) RNA are shown



domain of the anti-human CTLA-4 antibody (Fig. 3a) were run on a 1.3% (w/v) agarose gel, visualized and removed from the gel. Products from the PCR amplifying the variable domain of the anti-human CTLA-4 antibody were cut using *SaII* and *EcoRI* restriction enzymes and ligated back into the plasmids encoding heavy or light Ig chains for later use in antibody function studies.

The constructs described here not only permit the changing of antibody variable binding domains, but also the changing of antibody constant regions. To demonstrate the ease of this process, cloning of immunoglobulin heavy and light chain constant domains for use in these vectors was also carried out via PCR amplification after cDNA generation from the same hybridomas as used above. Primers designed for this purpose, however, were sequence specific (Table 1), as opposed to degenerate. The constant domains of the mouse kappa-light chain as well as the constant domain of the mouse IgG2a heavy chain were able to be amplified using this system (Fig. 3b). Primers specific for both light and heavy constant domains include restriction sites that allow them to be cut and ligated into the parent heavy or light Ig chain plasmid (Table 1). Taken together, the use of both degenerate and sequence-specific primers containing different restriction sites allows for the amplification of numerous target sequences and subsequent manipulation of encoded antibody chains in a wide variety of ways. The ability to “mix and match” different variable and constant domains in this fashion allows for the creation of numerous types of recombinant antibodies from these plasmids.

Characterization of antibody heavy and light chains after transfection

To verify proper construct expression, the plasmids were transfected into HEK293T cells using the Lipofectamine 2000 reagent. After 48 h, cells were lysed and lysates were run on 10–20% Tris/HCl gels to resolve antibody heavy and antibody light chains. Transfection of the antibody light chain construct into cells resulted in high expression of the Ig light chain protein, as evidenced by Western blots showing a specific band migrating at the appropriate molecular weight (Fig. 4). Transfection of the heavy chain construct alone also showed high expression, with an antibody specific band running at correct weight.

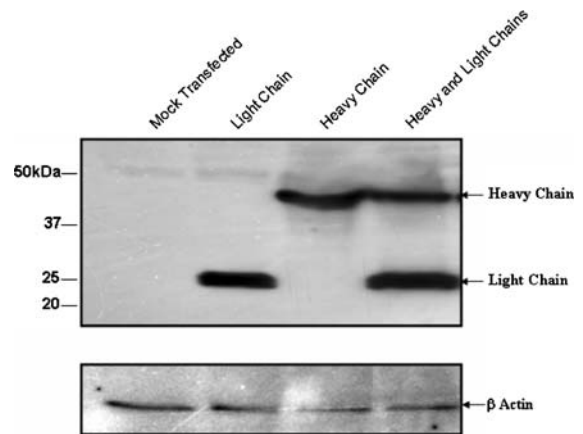


Fig. 4 Expression of light and heavy chain Ig proteins after transfection. HEK293T cells were mock transfected, transfected with a single Ig-encoding construct, or co-transfected with both constructs and allowed to culture for 48 h. Lysates were taken from the transfected cells and blotted for human IgA1 and human lambda chains. Western blot analysis shows bands migrating at appropriate molecular weights in both single transfections and in co-transfection, suggesting that both Ig-encoding constructs express well individually and can be expressed together

Confirmation of both heavy and light chain protein expression via western blot suggests that the insertion of restriction sites into the antibody genes (Fig. 1) did not adversely affect their synthesis. Co-transfection of the heavy and light antibody chain constructs together resulted in expression of both chains, suggesting the possibility that co-expression of these constructs in vitro could result in the formation of a complete antibody complex consisting of heavy and light chains heterodimerized together. In order to determine if co-expression of Ig chains was leading to heterodimerization, we next ran these same cell lysates under non-reducing conditions to attempt to detect the full antibody complex. Western blotting confirmed the expression of these constructs in homodimerized forms and in a heterodimer complex that migrated at a higher weight (data not shown), suggesting the formation of an antibody complex composed of light and heavy Ig chains.

Confirmation of antibody multimerization and secretion

Upon detecting a high molecular weight band under non-reducing conditions that may correspond to an Ig

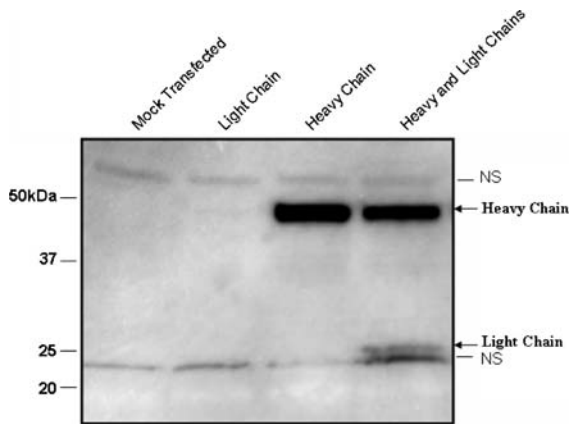


Fig. 5 Confirmation of antibody multimerization and secretion after transfection. The use of human IgA-specific Jacalin bead incubation with supernatant from single and co-transfected cultures followed by Western blotting shows no lambda light chain from single transfection but that both Ig chains can be visualized from supernatants taken from co-transfected cultures, suggesting the presence of antibody complexes consisting of both heavy and light chains

light chain/Ig heavy chain heterodimer, we decided to attempt to confirm the formation of this complex and its subsequent secretion post-transfection. As all Ig antibody chain constructs contain a high efficiency IgE leader sequence (Fig. 1) that would target them for secretion from cells into culture media, we reasoned that culture supernatants could be used to confirm release of Ig chains from transfected cells, and moreover, could be used to confirm whether or not the heavy and light Ig chains were heterodimerizing. In order to accomplish this, we employed the use of Jacalin coated beads, which are specific for human IgA1. We reasoned that if the heavy Ig chains that were seen intracellularly (Fig. 4) were being actively released from the cells, they should be able to be pulled out of culture supernatant using Jacalin coated beads. Indeed, western blotting using these beads after incubation with supernatants confirm the presence of the heavy Ig chain in cultures transfected with this construct (Fig. 5). Conversely, beads incubated with cultures transfected with only the construct encoding the light Ig chain which contained the Lambda constant domain, for which the beads have no affinity, showed no bands (Fig. 5). Most importantly, beads incubated with supernatants from cultures cotransfected with both antibody chain constructs showed the presence of both heavy and

light chains after western blotting (Fig. 5). The presence of the light Ig chain band in addition to the heavy Ig chain from beads incubated with the supernatants taken from cotransfected cultures but not in cultures transfected with light chain alone suggests that this band is being pulled down via an interaction with the heavy IgA1 chain. Therefore, both chains are expressed together, heterodimerize into an antibody complex, and can be isolated from culture supernatants as a complete antibody. Additionally, we were able to estimate the amount of antibody released from transfected cells into culture supernatant via ELISA (data not shown).

Testing antibody function

To determine whether or not the antibodies produced using our recombinant vector system were functional and able to bind their target antigen, we employed them in a modified ELISA for their ability to bind human CTLA-4. By altering the protocol of a commercial ELISA kit, we were able to utilize our plasmid-encoded antibody as a secondary antibody in a sandwich ELISA for soluble human CTLA-4. This was accomplished simply by adding culture media from transfected cells to ELISA wells which contained recombinant antibody at 0.05 mg/ml. Unfortunately, the antibodies supplied by the kit are proprietary and thus we were unable to retrieve a concentration for them. As our antibody is unconjugated, we added an additional step consisting of applying a streptavidin-conjugated antibody against human IgA, which matched the isotype of our antibody heavy chain. When OD readings were taken of ELISA samples, the antibodies generated through our system gave comparable results to the secondary antibodies supplied by the kit itself (Fig. 6), suggesting that antibodies made using our vector system were indeed functional in their ability to bind target antigen. These results also suggest that the constant domain of the antibody (human IgA1) is correct in that the Streptavidin conjugated antibodies we employed were able to detect them. Moreover, the fact that antibodies generated in our system were able to bind antigen in an ELISA suggests that they have retained the ability to bind the protein in its native conformation, a characteristic that may prove important in some applications of this system.

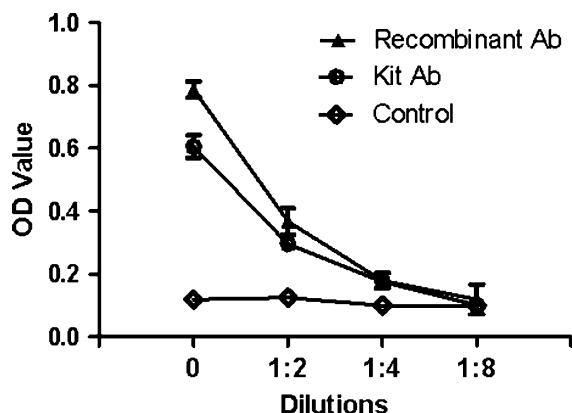


Fig. 6 Testing of recombinant antibody function. Antibodies generated in vitro via transfection of HEK 293T cells were tested for their ability to bind human CTLA-4 in an ELISA. Comparison of binding activity of the recombinant antibody produced by in vitro plasmid transfection versus the anti CTLA-4 antibody supplied by the kit shows equivalent or better binding by the recombinant antibody. Control for the ELISA was addition of secondary antibody without prior incubation with recombinant or kit-provided antibodies directed against CTLA-4

Discussion

Here we describe a set of expression vectors that allow for the generation of recombinant antibodies via transfection. The plasticity of this vector system allows for the generation of a wide variety of antibodies with varying binding specificities and isotypes. Cut sites are included within the vector genes for antibody heavy and light chains that allow the end user to swap in and out the variable and constant domains of their choice. Co-transfection of the vectors into cell culture allows for the generation and subsequent secretion of antibodies in vitro. We have shown that these antibodies can be isolated from culture supernatants if desired and that antibodies produced in this way have functional antigen binding sites and correct isotype specificities.

Variable domains of the vector system are cloned via the use of degenerate PCR primers. Employing degenerate primers for this purpose means that numerous antibodies of varying binding specificities can be made without the need for designing new primer sets for each antigen binding site the user desires to clone. Moreover, use of these types of primers also allows for the amplification of variable domains of which the specific nucleotide sequence is unknown. Therefore, any antibody binding site from

antibodies made by mouse hybridomas can theoretically be cloned into this vector system. The use of sequence specific primers allows for the cloning of antibody chain constant domains, which may also be inserted into the vector system.

This vector system forms complete antibody complexes, as evidenced by their isolation from cell culture supernatants and subsequent analysis for both antibody chains via western blot. Employing these antibodies in functional assays such as ELISAs shows that variable domains cloned into the vector system retain their antigen binding specificities and the constant domains encoded by the system are correct. Therefore, the employment of degenerate primers for cloning binding domains and subsequent verification of antibody function suggests that this vector system can be used to create a vast number of correctly formed, functional antibodies for use in a wide array of experimental settings. Further study of this system is currently in progress.

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