A rapid and scalable method for selecting recombinant mouse monoclonal antibodies

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Abstract

Background: Monoclonal antibodies with high affinity and selectivity that work on wholemount fixed tissues are valuable reagents to the cell and developmental biologist, and yet isolating them remains a long and unpredictable process. Here we report a rapid and scalable method to select and express recombinant mouse monoclonal antibodies that are essentially equivalent to those secreted by parental IgG-isotype hybridomas.

Results: Increased throughput was achieved by immunizing mice with pools of antigens and cloning - from small numbers of hybridoma cells - the functionally rearranged light and heavy chains into a single expression plasmid. By immunizing with the ectodomains of zebrafish cell surface receptor proteins expressed in mammalian cells and screening for formalin-resistant epitopes, we selected antibodies that gave expected staining patterns on wholemount fixed zebrafish embryos.

Conclusions: This method can be used to quickly select several high quality monoclonal antibodies from a single immunized mouse and facilitates their distribution using plasmids.

Background

The ability of antibodies to bind, with high selectivity and affinity, to diverse chemical structures has made them an extremely important tool for biomedical research [1]. Their value is underlined by the numerous ongoing international efforts, both academic and commercial, to produce large antibody resources for the scientific community [2]. A great deal of effort has, therefore, been applied to both improve antibody selection and develop other types of affinity binders including the use of other protein scaffolds [3-5] or nonproteinaceous reagents [6].

Ideally, a binding reagent should have a high affinity and specificity for its target and be produced quickly with little resource. However, these two parameters are usually traded against each other. Monoclonal antibodies raised in vivo within the mammalian immune system are matured by somatic hypermutation and, therefore, usually produce high affinity antibodies, but they are relatively slow to isolate. Conversely, antibodies selected from in vitro libraries can be produced more rapidly but are typically of lower binding strength due to the lack of affinity maturation. Importantly, antibodies that have low affinities are often unsuitable for applications commonly used by cell and developmental biologists, such as wholemount immunohistochemistry, because of the extensive washing steps required by these protocols.

Although polyclonal antisera are an efficient and rapid way of raising high affinity antibodies, they are finite and can suffer from substantial batch-to-batch variation [7]. By providing essentially limitless amounts of a defined reagent, monoclonal antibodies selected from an immunized animal therefore remain the reagent of choice. They are, however, generally regarded as unsuitable for building large resources because of the requirement for long immunization schedules and a large tissue culture demand. Despite efforts to alleviate some aspects of this procedure, the large tissue culture burden of subsequent hybridoma cell cloning remains a significant barrier for large scale production [8,9]. Also, the use of chemically synthesized peptides as antigens is rapid and cost effective but, in many cases, these reagents do not adequately mimic the shape of a natively folded protein. Antibodies raised against peptides, therefore, often recognize only denatured proteins (for example on western blots) and do not stain native proteins found in wholemount fixed tissue, thus limiting their usefulness.
The zebrafish is an increasingly popular model organism used to understand early vertebrate developmental processes and model diseases [10-12]. The amenability to forward genetics and the ability to produce large numbers of translucent embryos that rapidly develop externally has enabled the genetic dissection of most vertebrate organ systems [13,14]. While significant advances have been made in genetic methods [15,16], the paucity of high quality antibodies is considered a significant limitation for zebrafish research [17]. The few antibody reagents available to zebrafish researchers have mostly come from ‘shotgun’ approaches where zebrafish tissue lysates were used as immunogens [9,18,19]. While this approach is valuable, the target antigen is not immediately known and the isolated antibodies frequently recognize highly immunogenic fish-specific glycans, which may not be protein specific [18,20].

In order to identify novel signalling pathways initiated by extracellular protein interactions between cell surface and secreted receptor-ligand pairs, we have recently compiled a library containing the ectodomain fragments of 249 zebrafish glycoproteins which mainly belong to the immunoglobulin (IgSF) and leucine-rich repeat (LRR) families [21,22]. This protein library represents a useful immunogenic fish-specific glycans, which may not be protein specific [18,20].

Here, we report the development and implementation of a convenient and scalable method for selecting mouse monoclonal antibodies against several zebrafish cell surface receptor proteins in parallel. We have derived a procedure for the rapid cloning of both the functionally rearranged heavy and light chains into a single expression plasmid from a small number of hybridoma cells. By screening for formalin-resistant epitopes we show that this procedure produces antibodies that display the expected staining on wholemount fixed tissues. The use of a single antibody expression plasmid facilitates the distribution of these reagents making it suitable for compiling an antibody resource.

Methods
Protein production and purification
All zebrafish receptor ectodomain fragments were produced in HEK293E cells as rat Cd4 domains three- and four-tagged secreted proteins. The Cd4 tag was followed by either a six histidine-tag for purification or an enzymatic biotinylation site and cotransfected with the BirA enzyme, essentially as described [21]. Supernatants were harvested 6 days after transfection, filtered and stored at 4°C. Histidine-tagged proteins were purified with His-Trap HP columns (GE Healthcare, Buckinghamshire, UK) as previously described [21]. Proteins were over 90% pure as determined by SDS-PAGE and quantified by measuring absorbance at 280 nm. Antibodies were isotyped using the ISO-2 isotyping kit (Sigma, MO, USA).

Immunizations
Six-week-old male BALB/c mice were immunized subcutaneously with pools of five purified His-tagged proteins (5 μg each protein per immunization) in complete Freund’s adjuvant (once) and incomplete adjuvant (three times). Mice selected for hybridoma production were boosted intraperitoneally 3 days before dissecting the spleen.

Cell culture and hybridoma generation
The SP2/0 myeloma and SP2/mlL6 cell lines were grown in advanced DMEM/F12 medium (Invitrogen, CA, USA) supplemented with 20% fetal bovine serum, penicillin (100 U/mL), streptomycin (100 μg/mL) and L-glutamine (2 mM). SP2/mlL6 conditioned medium was harvested every 3 days. Following spleen dissection and dissociation, 10^8 splenocytes were fused to 10^7 SP2/0 myeloma in 50% PEG (PEG 1500, Roche, Hertfordshire, UK), using standard procedures. The resulting hybridomas were plated over 10 96-well plates and initially grown in advanced DMEM/F12 medium (Invitrogen) supplemented with 20% fetal bovine serum, 20% Sp2/mlL6 conditioned medium, penicillin (100 U/mL), streptomycin (100 μg/mL) and L-glutamine (2 mM) before addition of hypoxanthine aminopterin thymidine (HAT) selection medium 24 h after the fusion; after a regular exchange of selection medium for 2 weeks, hybridoma supernatants were harvested for screening. Positive clones were replated and grown for a further 5 days.

Antibody screening
Hybridoma supernatants were screened using an ELISA-based assay. Briefly, the biotinylated ectodomains of the injected proteins were bound to streptavidin-coated plates (NUNC, NY, USA) and incubated for 1 h with 50 μL hybridoma supernatant diluted 1:2 in phosphate buffered saline (PBS) and 0.2% bovine serum albumin (BSA). The plates were washed in PBS/0.1% Tween20 (PBST) before incubation with an anti-mouse immunoglobulin antibody coupled to alkaline phosphatase (Sigma) for 1 h at room temperature. After washes in PBST and PBS,
Table 1: Primers used for the generation of recombinant mouse monoclonal antibodies.

| Primers used for the generation of a leader sequence for the κ light chains |
|--------------------------------------------------------------------------|
| 1  GCCCACCAGCTGGAGTTTCAGGACCGACTTACTCAGTCCTCGTCTCGCAGTGCTGCTG          |
| 2  GGGCGCACAGACATCAGCACGGACAGACCGGGGACTATGACTTTGCTCGTGTGACGGAATGAAGTC  |

Degenerate forward primers used for the amplification of rearranged κ light chains

| Primer                          | Sequence                        |
|--------------------------------|--------------------------------|
| TCAATAGTTGACATAGGGGCCGCAAAAAT  | TTCGCTGCTCCAGGAGTTGGAATGGAAGTG  |
| TCAATAGTTGACATAGGGGCCGCAAAAAT  | TTCTGTCGTCTCGTCTCGTGTGACGGAATG  |

Reverse primer used for the amplification of rearranged κ light chains

| Primer                          | Sequence                        |
|--------------------------------|--------------------------------|
| TCAATAGTTGACATAGGGGCCGCAAAAAT  | TTCGCTGCTCCAGGAGTTGGAATGGAAGTG  |
| TCAATAGTTGACATAGGGGCCGCAAAAAT  | TTCTGTCGTCTCGTCTCGTGTGACGGAATG  |

Primers used for fusion polymerase chain reaction

| Primer                          | Sequence                        |
|--------------------------------|--------------------------------|
| TCAATAGTTGACATAGGGGCCGCAAAAAT  | TTCGCTGCTCCAGGAGTTGGAATGGAAGTG  |
| TCAATAGTTGACATAGGGGCCGCAAAAAT  | TTCTGTCGTCTCGTCTCGTGTGACGGAATG  |

Primers specific for the aberrant κ light chain from SP2/0 myeloma

| Primer                          | Sequence                        |
|--------------------------------|--------------------------------|
| TCAATAGTTGACATAGGGGCCGCAAAAAT  | TTCGCTGCTCCAGGAGTTGGAATGGAAGTG  |
| TCAATAGTTGACATAGGGGCCGCAAAAAT  | TTCTGTCGTCTCGTCTCGTGTGACGGAATG  |

Plasmid construction

The immunoglobulin expression vector was constructed from a derivative of pIT3 [23] that contains an expanded multiple cloning site. The leader sequence of the mouse variable κ light chain 7-33 was introduced by annealing of primers 1 and 2 and cloning between the multiple cloning site. The leader sequence for the κ light chain was expressed using the RNAqueous micro kit (Ambion, Texas, USA) and the reverse transcription performed with a polydT primer, using SuperscriptIII (Invitrogen). The functionally rearranged light variable region of a selected hybridoma light chain was amplified without its signal peptide sequence using a set of 17 degenerate forward primers containing a degenerate base code: M = A+C; R = A+G; W = A+T; S = C+G; Y = C+T; K = G+T; V = A+C+G; B = C+G+T.

RNA preparation and reverse transcriptase polymerase chain reaction (RT-PCR)

Total RNAs were prepared from selected hybridoma using the RNAqueous micro kit (Ambion, Texas, USA) and the reverse transcription performed with a polydT primer, using SuperscriptIII (Invitrogen). The functionally rearranged light variable region of a selected hybridoma light chain was amplified without its signal peptide sequence using a set of 17 degenerate forward primers containing a NotI site (primers 3 to 19) and one unique reverse primer corresponding to the constant region of the κ light chain.
and conjugate (Molecular Probes, Oregon, USA) diluted in 0.2% BSA and incubated with 1 μg/mL of an anti-mouse IgG-HRP conjugate. Membranes were blocked with 2% BSA in PBST and incubated with 1 μg/mL of an anti-mouse IgG-HRP conjugate (Molecular Probes, Oregon, USA) diluted in 0.2% BSA and detected with the Supersignal West pico chemiluminescent substrate (Pierce).

Biophysical analysis of antibody affinities
Antibody dissociation rates were determined using a BIACore T100 biosensor. Biotinylated Cd4d3 + 4-tagged recombinant proteins were immobilized onto streptavidin-coated SA sensor chip surfaces at low levels (Flrt3 ~750 RU, Jamc.2 ~400 RU, Lrrn1 ~1320 RU) and a molar equivalent of Cd4d3 + 4bio used as a reference. Purified Unc5bCd4d3 + 4-6H protein was covalently immobilized (~360 RU) to a CM5 sensor chip using the amine coupling kit (BIACore, Buckinghamshire, UK). Dissociation curves were exported and analysed in Microsoft Excel.

Wholemount in situ hybridization and immunohistochemistry
Zebrafish were maintained on a 14/10 h light/dark cycle at 28.5 °C according to the UK Home Office and local institutional regulations and staged according to Kimmel [24]. In situ hybridization was performed according to standard procedures [25] using 100 ng of either unc5b- or flrt3-RNA probes prepared as described [21]. Bright field images were captured on an Imager M1 microscope (Zeiss, Hertfordshire, UK). Purified parental or crude recombinant 5F11 antibody was diluted 1:500 or 1:2, respectively, in 10% goat serum, 2% BSA, 0.1% Triton X-100 in PBS and incubated with cryosections of 3-day-old formalin-fixed zebrafish embryos. For wholemount staining, zebrafish embryos were fixed overnight at 4°C in a 4% formalin solution and washed overnight in PBS/1% Triton X-100. Purified recombinant anti-Unc5b and anti-Flrt3 antibodies (0.5 mg/mL in PBS) were incubated with fixed embryos at 4°C overnight followed by three 15 min wash steps in PBST. For both cryosection and wholemount staining, an anti-mouse-Alexa568 secondary antibody was used (1:1000, Invitrogen) and tissues were counterstained with DAPI. Images of dissected and flat-mounted embryos were taken on a DM6000B confocal microscope (Leica, Wetzlar, Germany) and processed using Adobe Photoshop CS4.

Results
A method for the rapid selection of recombinant mouse monoclonal antibodies
Our aim was to rapidly select monoclonal antibodies of high affinity and specificity that could be used in wholemount immunohistochemistry protocols and could be easily distributed. The successful procedure is outlined in Figure 1 and is based around standard animal immunization and hybridoma technology, which enabled the selection of antibodies of a given specificity and ensured that they were affinity matured. Throughput was increased by immunizing mice with pools of antigens from our library.
of zebrafish cell surface and secreted proteins, so that antibodies to several proteins could be selected simultaneously from a single animal. In order to circumvent the burdensome tissue culture cloning procedures usually associated with the isolation of hybridomas, the productively rearranged heavy and light chain loci were amplified from small numbers of selected hybridoma cells by RT-PCR. The amplified light and heavy chains were then joined into a contiguous PCR product and cloned into a mammalian expression plasmid so that large amounts of antibody could be quickly produced by transiently transfected mammalian cells. Recombinant antibodies were then validated by staining fixed wholemount zebrafish embryos and the patterns checked for congruence with the known in situ expression profiles.

**Proof-of-principle: cloning of the 5F11 monoclonal antibody**

In order to clone productively rearranged antibody loci from hybridomas, we expanded a set of primers to mouse immunoglobulin V-regions originally published by Krebber [26] by using more recent genomic sequence information (Table 1). This new set of primers can recognize 97 and 98% of functional heavy and kappa (κ) light chain V-regions, respectively. Lambda light chains only constitute 5% of those used in mouse immunoglobulins [27] and primers were, therefore, not designed to this light chain. Hybridomas were generated using the SP2/0 myeloma cell line, which lacks transcription from the heavy chain locus due to a genomic inversion [28], but is known to transcribe an aberrantly rearranged κ light chain [29]. We anticipated that this aberrant light chain as well as the possible transcription of non-productively rearranged heavy or light chain loci present in the original B-cell could be amplified and would have to be identified and excluded.

As a proof of principle, we used a hybridoma (5F11) which secretes a mouse IgG1 recognizing an epitope restricted to zebrafish basement membranes [18]. Using our set of primers, we successfully amplified PCR products of expected sizes corresponding to the productively rearranged variable heavy and light chain regions (Figure 2a). These two products were then assembled by fusion PCR using a joining fragment that contained (5’ to 3’) a κ light chain constant region and its polyadenylation signal, a CMV promoter and a signal peptide for the heavy chain (Figure 2b). The primers used for the fusion PCR contained the rare-cutting restriction enzymes NotI and Ascl to facilitate cloning into a single mammalian expression plasmid, which included a signal peptide for the light chain and the constant region of the mouse IgG1 antibody isotype. The final construct contained both light and heavy chains, each flanked by a CMV promoter and polyadenylation sequence (Figure 2c). Although some clones contained the aberrant SP2/0-derived light chain, we selected a clone containing both productively rearranged light and heavy chains.

Western blotting of supernatants from transiently transfected HEK293E cells under reducing and non-reducing conditions showed that the heavy and light chains were stoichiometrically balanced in their expression level and capable of association (Figure 2d). The unpurified tissue culture supernatant was used in place of the primary antibody in an immunohistochemistry protocol using cryosections of 3-day old zebrafish larvae and strong staining of the basement membranes was
observed with the recombinant antibody (Figure 2e) which was indistinguishable from that obtained with antibodies secreted by the original hybridoma (Figure 2f). These data validate the overall strategy for producing recombinant antibodies from a single expression plasmid.

**Multiple different antibodies per fusion using pooled antigen immunization**

To increase the throughput of monoclonal antibody generation and show that antibodies could be cloned from hybridomas at an early stage after selection, we immunized mice with two pools of five different proteins selected from our library, and performed the fusions using standard procedures (Table 2). Hybridoma supernatants were screened by ELISA using the appropriate biotinylated recombinant proteins immobilized on streptavidin-coated 96-well plates. Hybridomas secreting antibodies of interest were expanded for 5 days before removing ~10^6 cells for total RNA extraction; the supernatant was retained for antibody isotyping and future comparisons with recombinant antibodies. For each hybridoma, cDNA was synthesized and used to independently amplify both light and heavy chains before joining them in a single expression construct. All six hybridomas for which a heavy chain was cloned contained a productively rearranged V_H region. Only one also had an aberrant heavy chain transcript, which presumably originated from an unproductive rearrangement of the other heavy chain allele within the fusing B-cell. The only aberrant κ light chain identified originated from SP2/0 and was present in 26% to 70% of plasmids depending on the hybridoma studied; these were quickly identified and eliminated by colony PCR using a primer specific to this sequence. In general, cloning of the productively rearranged antibody chains was readily achieved (Table 2). In two cases (clones 1.2.3 and 1.4.2) cloning was halted due to the successful completion of a different antibody for that antigen. In the three other cases where antibody cloning was not successful, two were due to very small hybridoma colonies which stopped dividing (clones 1.1.1 and 1.3.1) or because the antibody secretion was lost (clone 2.4.1).

Single plasmids containing productively rearranged light and heavy chains were transfected into HEK293E cells and the supernatant was tested by ELISA to ensure that the recombinant antibodies retained specificity for their antigens. After purification from bulk cultures, yields ranged from 10 μg/mL (SI4-Jamc.2) to 40 μg/mL (SI3-Flrt3). Of the nine different immunized proteins, 10 positive hybridoma supernatants were identified for seven antigens and five recombinant antibodies that retained binding specificity were isolated.

Fixation of biological samples, usually necessary to maintain tissue architecture for subsequent analysis, can modify the chemical structure of the protein resulting in the destruction of antibody epitopes. This is a particular problem for monoclonal antibodies which recognize a single epitope. We therefore screened each antibody against recombinant proteins that had been formalin-treated for different lengths of time. Of the two antibodies that recognize the Unc5b receptor, one (SI2-Unc5b) was insensitive and the other (SI1-Unc5b) sensitive to formalin-fixation (Table 2). We found, however, that most epitopes were insensitive to formalin-fixation (Table 2).

**Recombinant and parental hybridoma IgG antibodies have comparable affinities**

Antibodies cloned and produced recombinantly will differ in several respects from those secreted by hybridomas. First, all antibodies contain two amino acid changes (KT to RP) at the junction of the VH and CH1 domains due to the incorporation of the AccI cloning site. The degene-
erate primers used to amplify the VL and VH are also likely
to introduce amino acid changes at the N-terminus of
each chain. The antibody isotype, if not originally an
IgG1, will be altered, and finally the antibodies produced
in HEK293E cells will contain different glycans. In order
to determine the consequences of these changes on the
antibody affinity, we compared each recombinant anti-
bodv to the original hybridoma supernatant using a BIA-
core instrument. Each antigen and a control were
immobilized at approximate molar equivalence on a sen-
sor chip; either the hyridoma or the recombinant anti-
bodv was injected until saturation had been achieved
data not shown) and the rate of dissociation of the anti-
bodv was then followed for several hours. Of the four
recombinant antibodies tested, three (clones SI2-Unc5b,
SI3-Flrt3, and SI4-Jamc.2) were essentially indistinguish-
able from the original hybridoma demonstrating that the
changes due to the *AscI* site at the V<sub>H</sub>-CH<sub>1</sub> junction, and
those due to the V<sub>L</sub> and V<sub>H</sub>-region degenerate primers, do
not affect antibody affinity, at least for these antibodies
(Figure 3b-d). In contrast, the affinity of the recombinant
SI5-Lrrn1 antibody was reduced >200-fold (Figure 3e).
Isotyping revealed that the original hybridoma antibody
was an IgM which would have more avid binding than the
dimeric IgG of the recombinant form. Eliminating IgM
isotype antibodies by screening with isotype-specific sec-
tary antibodies could avoid this problem.

**Recombinant antibodies have expected staining patterns
on wholemount fixed tissue**

In order to test our recombinant antibodies on whole-
mount fixed tissue, we determined the embryonic expres-
sion patterns of the *flrt3*, *unc5b* and *jamc.2* genes and
compared them to the antibody staining. At 24 hpf, *flrt3*
was expressed in a broad stripe at the mid-hindbrain
boundary (Figure 4a) and the recombinant anti-Flrt3
antibody (SI3-Flrt3) also showed cell surface localization.
of the Flr3 receptor in the same territory (Figure 4b, c). Similarly, unc5b was expressed in the dorsal part of the retina (Figure 4d) and this staining was recapitulated using the recombinant anti-Unc5b (SI2-Unc5b) antibody which showed cell surface staining in the dorsal retina (Figure 4e, f). Neither the jamc.2 gene nor SI4-Jamc.2 antibody showed staining on zebrafish embryos at different stages up to 48 hpf demonstrating that this gene was not expressed during early embryogenesis.

Discussion

High quality, validated monoclonal antibodies are valuable biomedical reagents but their isolation can be time-consuming, unpredictable and expensive. By immunizing with pools of antigens and quickly cloning the productively rearranged variable antibody regions, we have greatly reduced the time, number of animals, and resources needed to isolate monoclonal antibodies that work on fixed wholemount tissues. Importantly, this procedure is accessible to smaller laboratories since it does not require expensive automated infrastructures or large clone libraries.

Antibodies that work in immunohistochemistry protocols are of most use for developmental and cell biologists but the protocols used often have long wash steps and therefore require high affinity antibodies. Here we have shown that the recombinant antibodies produced can have half-lives of many hours and are suitable for whole-mount immunohistochemistry protocols. We have also shown that amino acid changes introduced within the variable regions by using degenerate primers to amplify the rearranged heavy and light fragments do not significantly affect the specificity or affinity of the recombinant antibody. In contrast, we observed a large decrease in binding avidity when the antibody isotype was changed from an avid decameric IgM to a bivalent IgG1. In order to avoid this problem, positive antibodies of the IgM isotype should be identified and eliminated at an early stage of the screening process using isotype-specific secondary antibodies.

Previous attempts to raise mouse monoclonal antibodies to zebrafish cell surface blood cell differentiation markers using fluorescence-activated cell sorting (FACS)-sorted zebrafish cells met with little success, most likely...
due to the presence of highly immunogenic glycans that were not protein-specific [20]. Zebrafish proteins expressed in a human cell line contain glycans that are not immunogenic in mice, making our recombinant protein library an ideal source of antigens for generating a zebrafish antibody resource. Importantly, although we have used this procedure to raise antibodies against cell surface zebrafish proteins, it could, in principle, be used to raise antibodies against any antigen that develops an immune response in mice, including intracellular proteins.

Recent technical advances could allow further refinements of the method: specificity screening on protein microarrays should permit to increase the number of pooled antigens (possibly 50 or more) used for the immunizations. Quicker immunization procedures such as the Repetitive Immunization at Multiple Sites (RIMMS) [30] in which antigens are administered over just an 11-day period could also further reduce the time taken to select antibodies. Methods for selecting monoclonal antibodies from single antibody-secreting cells have been recently developed but, crucially, do not enable collection of sufficient reagent for specificity screening before single cell RT-PCR amplification [31,32]. However, we have recently established that cell culture time can be reduced further by bypassing the 5-day expansion step before RT-PCR.

Cloning the productively rearranged light and heavy chains of selected monoclonal antibodies into a single mammalian expression vector facilitates their distribution and is an ideal format to build large reagent resources. It is also a very flexible system since additional properties such as purification tags (FLAG, poly-His) and reporter proteins (alkaline phosphatase, horseradish peroxidase or fluorescent proteins) can be easily added at the C-terminus of the heavy chain. Recent improvements in mammalian expression systems can now rapidly yield over 1 g/L of recombinant antibody from a transient transfection [33], raising new hopes for the rapid and large scale production of these valuable reagents.

Conclusions
We have reported here the development of a procedure for cell and developmental biologists which streamlines the production of monoclonal antibodies recognizing antigens in wholemount fixed tissue. In this paper, we have used the method to raise antibodies against zebrafish proteins but it could also be broadly applied to any antigen that can raise a humoral immune response in mice. As only minimal equipment is required, this technique can also be used by smaller laboratories. Cloning of the rearranged light and heavy chains of selected monoclonal antibodies into a single mammalian expression vector facilitates their distribution, making scope for the creation of valuable antibody resources.

Abbreviations
BSA: bovine serum albumin; CMV: cytomegalovirus; IgG: immunoglobulin G; PBS: phosphate buffered saline; PEST: PBS Tween; PCR: polymerase chain reaction; RT-PCR: reverse transcription PCR.

Authors’ contributions
CC designed the study and performed all the experiments except the whole-mount immunohistochemistry, which was performed by NS, and the BiAcore studies which were done by GW. GW conceived the study and wrote the manuscript which was edited by CC and NS. All authors read and approved the final manuscript.

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