Published in final edited form as:

Methods Mol Biol. 2018 ; 1707: 39–49. doi:10.1007/978-1-4939-7474-0_3.

Expression of Exogenous Genes in Murine Primary B Cells and B Cell Lines Using Retroviral Vectors

Zhiyong Yang Christopher D.C. Allen

Abstract

B cells, after activation, can undergo class-switch recombination and somatic hypermutation of their immunoglobulin genes, and can differentiate into memory cells and plasma cells. Expressing genes in altered versions in primary B cells and B cell lines is an important approach to understanding how B cell receptor signaling leads to B cell activation and differentiation. Recombinant retrovirus-based transduction is the most efficient method to deliver exogenous genes for expression in B cells. In this chapter, we describe streamlined protocols for using recombinant retroviral vectors to transduce both murine primary B cells and B cell lines.

Keywords

Retroviral vector; Spinfection; Exogenous gene expression; Primary B cell; B cell lines; Transduction

1 Introduction

After encountering cognate antigens, naive B cells will become activated and then differentiate into short-lived extrafollicular plasma cells or go on to form microanatomical structures called germinal centers, where they undergo antibody affinity maturation and differentiation into memory B cells and long-lived plasma cells. Activated B cells can also undergo class-switch recombination, through which the B cell receptor (BCR) isotype switches from IgM/IgD to IgG, IgE, or IgA. How the binding of antigens to BCRs of different isotypes translates into B cell activation and differentiation is not fully understood.

The *in vivo* activation of B cells in a T cell-dependent or T cell-independent type 1 manner can be mimicked by culturing primary B cells with anti-CD40 or TLR ligands, respectively, together with certain cytokines. Expressing intact or engineered forms of components of BCR signaling pathways and transcription factors such as *Prdm1* (encoding Blimp-1) in cultured primary B cells remains as an important technique to study B cell activation and differentiation. Transfection by electroporation and retroviral transduction are the most commonly used approaches toward this aim [1]. Using a retroviral gene delivery system, others and we have demonstrated that the mouse IgE BCR exhibits elevated activity compared to the IgG1 BCR in the absence of cognate antigen [2, 3]. Using the same approach to express chimeric B cell receptors in primary B cells, we have characterized the contribution of different domains of the IgE BCR to this antigen-independent activity [2].

Compared to primary B cells, immortalized B cell lines offer some unique advantages to study BCR signaling. For example, unlike primary B cells, some B cell lines do not need the tonic signal activity of the BCR for survival. As a result, it is possible to reconstitute BCR signaling in B cell lines by adding or removing specific components in these cells. Additionally, B cell lines can also be maintained for extended periods and can be cultured in large quantities, making them suitable for biochemical studies. Exogenous genes are usually delivered to B cell lines by chemical transfection and electroporation. Various B cell lines, such as WEHI-231, BAL17, and M12 cells, can also be readily transduced by retroviruses [4]. We have studied the cell surface translocation of IgE, IgG1, and their chimeric receptors delivered to J558L cells by retroviral vectors [2].

In most cases, the recombinant retroviruses used to transduce primary B cells and B cell lines are replication incompetent. To generate such recombinant retroviruses, the gene of interest is cloned into a plasmid-based retroviral vector in place of the *gag*, *pol*, and *env* viral genes. The *gag* and *env* genes encode nucleocapsid (Gag) and envelope (Env) viral proteins respectively, while *pol* encodes protease, reverse transcriptase, and integrase. These viral proteins are necessary for retrovirus packaging and replication. To render the recombinant virus replication incompetent, the *gag*, *pol*, and *env* viral genes are either supplied in a separate plasmid vector and/or stably integrated in a packaging cell line [5]. Once a B cell is infected with recombinant retrovirus, the vector DNA with the exogenous gene will integrate into the genome of the B cell, resulting in the stable maintenance and expression of the exogenous gene in the B cell. Detailed protocols, from the construction of a retroviral vector, to the infection of murine primary B cells and B cell lines, are described below.

2 Materials

2.1 Construction of Retroviral Vector

1. Cloning vectors, such as pCR2.1 (Invitrogen).
2. Retroviral vectors, such as pQEF-T2A-Cerulean, pQCXIN (Clontech), MSCV-IRES-GFP (Addgene).
3. Packing plasmids: pCL-Eco (Addgene) or MSCV ecotopic gag-pol-env plasmid (G/P/E).
4. Molecular biology reagents, such as restriction enzymes, ligase, competent cells, plasmid DNA preparation kit.

2.2 Preparation of Recombinant Retrovirus, In Vitro Culture of Primary B Cells, and Spinfection of the Cultured Primary B Cells with Retrovirus

1. Complete DMEM medium (cDMEM): DMEM high glucose medium with 10% fetal bovine serum (FBS), 10 mM HEPES, 1× penicillin/streptomycin/L-glutamine.
2. Opti-MEM reduced serum medium (Invitrogen).
3. TransIT-LT1 Transfection Reagent (Mirus Bio).
4. ViralBoost (Alstem).

5. Retrovirus packaging cell line, such as Phoenix-Eco [6].
6. 5–3/4" Pasteur pipettes.
7. DNase I (10 mg/ml, Sigma-Aldrich).
8. Anti-CD43 (clone S7) biotin (0.5 mg/ml, BD Pharmingen).
9. Anti-CD11c (clone N418) biotin (0.5 mg/ml, Biolegend).
10. ACK Lysis Buffer (Quality Biological).
11. Streptavidin MyOne T1 DynaBeads (Invitrogen).
12. Magnetic stand (Invitrogen).
13. Complete RPMI growth medium (cRPMI): RPMI 1640 medium with 10% FBS, 10 mM HEPES, 1× penicillin/streptomycin/L-glutamine, 50 μ M β -mercaptoethanol.
14. Recombinant murine interleukin-4 (IL-4, R&D Systems or Peprotech).
15. Anti-CD40 (FGK-45, 2 mg/ml, Miltenyi Biotec).
16. Swinging bucket centrifuge that can accommodate 15/50 ml tubes and plates.
17. Tissue culture incubator.
18. Flow cytometer.

3 Methods

3.1 Construction of Retroviral-Expression Vectors

Most retroviral vectors use the inherent promoters in their long terminal repeats (LTRs) to drive the expression of exogenous genes. However, we have found that the strength of the LTR promoter can be affected dramatically by the differentiation status of B cells (*see Note 1*). To address the issue, we have engineered a new type of retroviral vector. The new vector, pQEF-Ceru-T2A, has a unique combination of following features (Fig. 1). First, it is derived from the pQCXIN vector, which has the U3 enhancer region deleted in the 3'LTR. In the resultant "self-inactivating" virus, the LTR is thus transcriptionally inactive, such that gene expression requires an inserted promoter sequence [7]. Second, for the pQEF-Ceru-T2A vector, the immediate early CMV promoter in pQCXIN, which is only weakly active in B cells, was replaced with a human EF-1 α promoter [8], which allows uniform expression of exogenous genes in primary B cells irrespective of their differentiation status. Third, the pQEF-Ceru-T2A vector uses Cerulean [9], a variant of cyan fluorescent protein, as a reporter

¹-Retroviral plasmid vectors that use the intrinsic promoters residing in the LTRs of Moloney murine leukemia virus (MoMLV) or murine stem cell virus (MSCV) have been widely used for generating recombinant retroviruses for B cell infection. However, we have found that the expression of exogenous genes under the LTR promoter varies widely, with nearly an order of magnitude higher expression in plasma cells (PCs) compared with non-PCs (Fig. 3). In some cases, the low expression of exogenous genes in non-PCs could potentially make it difficult to clearly separate the transduced cells from non-transduced cells. To address these issues, we replaced the CMV promoter in the pQCXIN vector with EF-1 α promoter. Additionally, we cloned a Cerulean-T2A expression cassette immediately downstream of the EF-1 α promoter so that the fluorescent protein Cerulean can be used as a reporter for retroviral infection and exogenous gene expression. The resultant new retroviral vector, pQEF-Ceru-T2A (Fig. 1), allows relatively uniform expression of exogenous genes in both PCs and non-PCs, and all the transduced cells can be unequivocally separated from the untransduced cells (Fig. 3).

for viral infection and foreign gene expression. Finally, one or more genes of interest can be joined to the Cerulean gene by coding sequence of 2A peptides [10]. The multicistronic expression cassette will be transcribed in one transcript but the protein products of different genes will be “spliced” into individual proteins at the site of the 2A peptide during translation.

Using standard molecular biology techniques, genes of interest can be cloned into the pQEF-Ceru-T2A vector following the procedures described below and outlined in Fig. 1. A similar strategy could be used to clone genes of interest into other retroviral vectors, such as MSCV-IRES-GFP.

1. Clone desired expression cassette into a common cloning vector, such as pCR2.1. Verify the expression cassette by sequencing.
2. Clone the sequence-verified expression cassette from the cloning vector into the retroviral vector. For cloning into the pQEF-Ceru-T2A retroviral vector, the expression cassette is released from the cloning vector by digestion with restriction enzymes SnaBI and XhoI.
3. Gel purify the expression cassette, and ligate with gel-purified pQEF-Ceru-T2A that has been digested with the same restriction enzymes.
4. Transform competent *Escherichia coli* cells with the ligation sample.
5. Prepare plasmid DNA of the putative recombinant retroviral vector. Verify the identity of the clones by restriction enzyme digestion.

3.2 Preparation of Recombinant Retrovirus, In Vitro Culture of Primary B Cells, and Spinfection of the Cultured Primary B Cells with Retrovirus

We always use freshly generated retroviruses to infect mouse B cells. The whole experiment, including preparing recombinant retroviruses, setting up the B cell culture, infecting cultured B cells with the retroviruses, and analyzing the transduced B cells, takes about 6 days. Some of these parts of the experiment are overlapping. For better planning and executing the experiment, we find it is helpful to summarize workflow of the experiment in a timeline flowchart (Fig. 2). The detailed protocol is described below, using a 12-well plate to culture the packaging Phoenix-Eco cells, and a U-bottom 96-well plate to culture B cells as examples (see Note 2).

3.2.1 Preparation of Recombinant Retroviruses

1. (Day 0) Seed 0.35 million Phoenix-Eco cells in 1 ml complete DMEM medium per well, so that the cell density will be at about 50%–70% confluency on the next day.

²Several categories of reagents, such as calcium phosphate, and liposome-based reagents, have been successfully used for transfecting the Phoenix-Eco packing cells. Among these reagents, we have found that *TransIT-LT1* Transfection Reagent has several advantages. First, it has low toxicity to cells and does not need to be removed several hours after transfection. Second, it works well even in a small volume of the transfection mixture, and is thus suitable for preparing tens of different transfection mixtures in parallel.

2. (Day 1) Transfect the Phoenix-Eco cells. Add 0.7 µg retroviral plasmid DNA, 0.3 µg G/P/E plasmid DNA to 100 µl Opti-MEM reduced serum medium in a 1.5 ml Eppendorf tube, then add 3 µl *TransIT*-LT1 Transfection Reagent (*see* Note 3). Mix and incubate at room temperature for 15–30 min. Then add the *TransIT*-LT1:DNA complex mixture dropwise to the cell culture.
3. (Day 2) Replace the medium of the transfected Phoenix-Eco cells with fresh medium in the morning, and again replace the medium with new medium with 1× ViralBoost in the evening (*see* Note 4).

3.2.2 Purification of Mouse Primary B Cells (See Note 5)

1. (Day 2) Euthanize a mouse of the desired genotype, harvest the spleen into a 15 ml conical tube with 5 ml cDMEM medium on ice. Mash the spleen on a 40 µm cell strainer in a 10 cm Petri dish using the bulb of a 3 cc syringe. Pipette the cell suspension three times through a 5–¾" Pasteur pipette to break tissue particles. Then pass the cell suspension through the cell strainer again to remove unbroken cell aggregates. Centrifuge the cell suspension at $400 \times g$ at 4 °C for 5 min.
2. Remove the supernatant and add 10 µl DNase I (10 mg/ml) to the cell pellet, then resuspend the cell pellet in 1 ml cDMEM by pipetting slowly and carefully using a P1000 pipette.
3. Count the cells.
4. Add biotin conjugated anti-CD43 and anti-CD11c antibodies to the splenocyte sample. Use 10 µl of each antibody per 1×10^8 cells. Tap the tube to mix, and incubate 25–60 min on ice with gentle mixing every 10–15 min.
5. Lyse red blood cells by adding 9 ml ACK Lysis Buffer at room temperature to the tube. Invert tubes to mix. Spin at $400 \times g$ at 4 °C for 7 min.
6. Meanwhile, prepare Streptavidin MyOne T1 DynaBeads:
 - a. Resuspend beads by pipetting up and down and gentle vortexing.
 - b. Transfer 250 µl beads per 1×10^8 cells into a 1.5 ml Eppendorf tube.
 - c. Add 1 ml cDMEM media per tube to wash.
 - d. Put the tube on a magnet stand, wait 2 min. The beads will bind to the sides of the tube. Aspirate media.

³The protein products of retroviral genes *gag*, *pol*, and *env* are necessary for the replication and packaging of recombinant retroviruses. These genes have been stably integrated into the Phoenix-Eco packaging cells. However, the Phoenix-Eco cells tend to gradually lose these genes with extended passaging. So it is advisable to use Phoenix-Eco cells with fewest passages possible. Additionally, we have found that co-transfection of a plasmid containing the MSCV ecotropic gag-pol-env DNA together with the retroviral vector DNA improves the yield of recombinant viruses. We co-transfect the MSCV ecotropic gag-pol-env plasmid DNA and the retroviral DNA at a mass ratio of 3:7.

⁴Addition of ViralBoost is optional. We have observed an approximately two-fold increase in the expression of retrovirally-delivered exogenous genes with 1× ViralBoost.

⁵We have found that unpurified splenocytes and white blood cells can be used in place of purified B cells for most applications [11]. In the presence of anti-CD40 and IL-4, B cells are the primary cell type to undergo activation and proliferation whereas most other cells die out. Purified B cells should be used in applications in which the contribution of non-B cells needs to be excluded or for analyses of total cell lysates, such as measurements of mRNA or protein abundance.

- e. Remove the tube from the magnet, resuspend beads in the original volume of cDMEM.
7. Take the 15 ml tube with cells out of centrifuge, aspirate the supernatant, and add 10 μ l DNase I to the cell pellet. Slowly, carefully resuspend cells in 1 ml media using a P1000 pipette.
8. Transfer the cells to the Eppendorf tube with the washed beads. Invert the tube to mix, incubate on a tube rotator at 4 °C for 10 min.
9. Put the Eppendorf tube on a magnet stand at room temp for 2 min. The beads will bind to the sides of the tube.
10. Carefully remove negative fraction (without beads) using a short Pasteur pipette and transfer to a new 1.5 ml Eppendorf tube.
11. Centrifuge at $400 \times g$ at 4 °C for 2 min. Remove the supernatant, resuspend the cell pellet in 0.2 ml cRPMI.
12. The purity of the cell sample can be assessed by flow cytometry after staining with fluorescently conjugated anti-CD19, anti-CD11c, and anti-CD3e antibodies. The purity of B cells using this protocol is usually above 95%.

3.2.3 Setting Up Splenocyte/B Cell Culture (See Notes 6 and 7)

1. (Day 2) Add 100 μ l cRPMI with $2\times$ final concentration of IL-4 and anti-CD40 to each well of a U-bottom 96-well plate.
2. Then add 100 μ l cRPMI with 100,000 splenocytes or purified B cells to the same wells of **step 1** (see Note 8).
3. Place the culture in a humidified tissue culture incubator at 37 °C with 5% CO₂.

3.2.4 Spinfection (See Note 9)

1. (Day 3) Transfer the medium of the transfected Phoenix-Eco cells into new tubes, centrifuge $1000 \times g$ at room temperature for 2 min. Transfer the supernatant containing retroviral particles into new tubes, add 1/100 volume of 1 M HEPES, and polybrene to a final concentration of 5 μ g/ml (see Note 10).

⁶We have observed that the source of FBS makes a substantial qualitative difference in the success of in vitro B cell cultures. Thus, it is advisable to screen different lots of FBS, possibly from different commercial sources, to identify the FBS that allows the most robust B cell culture. Then the FBS from the same lot can typically be acquired in affordable quantities and stored in a -80 °C freezer for long-term use.

⁷We have found that the potency of anti-CD40 antibody from different sources varies dramatically (even with supposedly the same clone). In addition, the concentration of anti-CD40 antibody can have a significant impact on B cell differentiation [2]. We usually use 125 ng/ml of anti-CD40 (clone FGK-45, Miltenyi Biotec) for culturing B cells.

⁸We suggest setting up B cell culture plates using “two component” system, one component with a $2\times$ concentration of activation reagents and the second component with cells in media. This approach is convenient, especially under conditions where multiple treatments or multiple sources of cells are desired.

⁹A previous protocol concentrated retrovirus by centrifugation of viral-containing supernatant and then used the concentrated retrovirus to transduce B cells [12]. The transduction efficiency for primary B cells using that protocol was reported to be 20%–50%. With the protocol described in this chapter, and without the steps to concentrate retroviruses, we can routinely achieve 30%–50% transduction efficiency for primary B cells. Under ideal conditions, the transduction efficiency for primary B cells can be as high as 90%.

¹⁰Before being added to B cell pellets to be infected, individual retroviral supernatants can be placed in the wells of a 96-deep well plate. This allows transfer up to 12 samples simultaneously with a multichannel pipette.

2. Centrifuge the plate with cultured B cells at $750 \times g$ at room temperature for 5 min. Carefully transfer the growth media to wells in a new plate. Save the plate in the same tissue culture incubator.
3. Immediately add 195 μ l/well viral supernatant to the wells with B cell pellet. Centrifuge at $1100 \times g$ at room temperature for 90 min.
4. Aspirate the supernatant after spinfection, and immediately add the growth media saved in **step 2** back to the cell pellet.
5. Return the plate(s) back to 37 °C incubator for further culture until the time to be analyzed.

3.2.5 Spinfect B Cell Lines with Recombinant Retroviruses (See Note 11)—

The protocol for infecting B cell lines with retroviruses is almost identical to the one for infecting primary B cells. The major difference is that 10,000 cells in logarithmic growth phase can be directly added to each well of a U-bottom 96-well plate for spinfection.

Acknowledgments

These protocols were developed for research projects supported by the UCSF Sandler Asthma Basic Research Center, the UCSF Cardiovascular Research Institute, the Weston Havens Foundation, and grant DP2HL117752 from the National Institutes of Health. C.D.C.A. is a Pew Scholar in the Biomedical Sciences, supported by The Pew Charitable Trusts. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or of the Pew Charitable Trusts.

References

1. Moghimi B, Zolotukhin I, Sack BK, Herzog RW, Cao O (2011) High efficiency ex vivo gene transfer to primary murine B cells using plasmid or viral vectors. *J Genet Syndr Gene Ther* 2(103): 10.4172/2157-7412.1000103
2. Yang Z, Robinson MJ, Chen X, Smith GA, Taunton J, Liu W, Allen CD (2016) Regulation of B cell fate by chronic activity of the IgE B cell receptor. *eLife* 5:e21238 10.7554/eLife.21238 [PubMed: 27935477]
3. Haniuda K, Fukao S, Kodama T, Hasegawa H, Kitamura D (2016) Autonomous membrane IgE signaling prevents IgE-memory formation. *Nat Immunol* 17(9):1109–1117. 10.1038/ni.3508 [PubMed: 27428827]
4. Krebs DL, Yang Y, Dang M, Haussmann J, Gold MR (1999) Rapid and efficient retrovirus-mediated gene transfer into B cell lines. *Methods Cell Sci* 21(1):57–68 [PubMed: 10733259]
5. Chang T, Yee JK (2012) General principles of retrovirus vector design. *Methods Enzymol* 507:1–14. 10.1016/B9780-12-386509-0.00001-6 [PubMed: 22365766]
6. Pear WS, Scott ML, Nolan GP (1997) Generation of high-titer, helper-free retroviruses by transient transfection. *Methods Mol Med* 7:41–57. 10.1385/0-89603-484-4:41 [PubMed: 24493417]
7. Yu SF, Vonruden T, Kantoff PW, Garber C, Seiberg M, Ruther U, Anderson WF, Wagner EF, Gilboa E (1986) Self-inactivating retroviral vectors designed for transfer of whole genes into mammalian-cells. *Proc Natl Acad Sci U S A* 83(10):3194–3198. 10.1073/pnas.83.10.3194 [PubMed: 3458176]

¹¹B cell lines are invaluable to study B cell biology. These cells are usually either transfected by electroporation [13] or by retroviral vectors [14]. Compared to transient transfection by electroporation, transduction by retroviral infection has at least two advantages. First, the retroviral method does not require an electroporator. Second, the infected cells are stably “transfected,” by definition, due to retroviral integration. However, we observed that the amenability of B cell lines to retroviral infection varies dramatically, from close to 0% of A20 cells to nearly 100% of J558L cells (Fig. 4). Therefore, it is advisable to test the infectivity of B cell lines by retroviruses if the retroviral transduction approach is desired for future experiments.

8. Wakabayashi-Ito N, Nagata S (1994) Characterization of the regulatory elements in the promoter of the human elongation factor-1 alpha gene. *J Biol Chem* 269 (47):29831–29837 [PubMed: 7961976]
9. Rizzo MA, Springer GH, Granada B, Piston DW (2004) An improved cyan fluorescent protein variant useful for FRET. *Nat Biotechnol* 22(4): 445–449. 10.1038/nbt945 [PubMed: 14990965]
10. Szymczak-Workman AL, Vignali KM, Vignali DA (2012) Design and construction of 2A peptide-linked multicistronic vectors. *Cold Spring Harb Protoc* 2012(2):199–204. 10.1101/pdb.ip067876 [PubMed: 22301656]
11. Yang Z, Sullivan BM, Allen CD (2012) Fluorescent in vivo detection reveals that IgE(+) B cells are restrained by an intrinsic cell fate pre-disposition. *Immunity* 36(5):857–872. 10.1016/j.immuni.2012.02.009 [PubMed: 22406270]
12. Lin KI, Calame K (2004) Introduction of genes into primary murine splenic B cells using retrovirus vectors. *Methods Mol Biol* 271:139–148. 10.1385/1-59259-796-3:139 [PubMed: 15146118]
13. Liu W, Chen E, Zhao XW, Wan ZP, Gao YR, Davey A, Huang E, Zhang L, Crocetti J, Sandoval G, Joyce MG, Miceli C, Lukszo J, Aravind L, Swat W, Brzostowski J, Pierce SK (2012) The scaffolding protein synapse-associated protein 97 is required for enhanced signaling through isotype-switched IgG memory B cell receptors. *Sci Signal* 5(235):ra54 10.1126/scisignal.2002820 [PubMed: 22855505]
14. Lee AH, Iwakoshi NN, Anderson KC, Glimcher LH (2003) Proteasome inhibitors disrupt the unfolded protein response in myeloma cells. *Proc Natl Acad Sci U S A* 100 (17):9946–9951. 10.1073/pnas.1334037100 [PubMed: 12902539]

Fig. 1.

Diagram of the cloning vector and the retroviral vector and the strategy for cloning exogenous genes into the retroviral vector. In *step (1)* the exogenous gene is cloned into the cloning vector pCR2.1 and verified by sequencing. In *step (2)* the exogenous gene is released from the cloning vector by restriction digestion. In *step (3)* the purified exogenous gene is cloned into the pQEF-Ceru-T2A retroviral vector. *GOI* gene of interest, *LTR* long terminal repeat, *3'-LTR*3'* self-inactivating LTR, Ψ^+ extended packaging signal. EF-1 α : human EF-1 α promoter. Amp^R, ampicillin resistance gene cassette

Day 0	Day 1	Day 2	Day 3	Day 4-6
Seed Phoenix cells	Transfect Phoenix cells	Change media.	Prepare retroviral suspension	
		Set up splenocyte or B cell culture	Spinfect the cultured splenocytes or B cells	Analysis by flow cytometry etc.

Fig. 2.

Workflow for preparing recombinant retroviruses and transducing splenocytes or B cells by spinfection. Tasks are grouped into distinct days. Tasks involving handling of Phoenix-Eco cells are listed above the line, whereas those involving handling of splenocytes/B cells are listed beneath the line

Fig. 3.

Comparison of expression of an exogenous gene driven by different promoters. The bicistronic expression cassette Cerulean-T2A-mIgE was cloned into a MSCV-based vector under the endogenous promoter in the LTR (pMSCV-Ceru-T2A-mIgE) or in pQEF-Ceru-T2A retroviral vector under the EF-1a promoter (pQEF-Ceru-T2A-mIgE). Splenocytes from Blimp1-gfp knock-in reporter mouse were spininfected with the resultant recombinant retroviruses after 2 days of in vitro culture with IL-4 and anti-CD40 and then analyzed by flow cytometry after 2 more days of in vitro culture. Cerulean was used as a reporter for viral transduction and exogenous gene expression. GFP was used as a reporter for Blimp-1 expression and plasma cell differentiation

Fig. 4.

Examples of expression of exogenous genes delivered by retroviral vectors in B cell lines. Histogram of the Cerulean reporter expressed in J558L cells infected with or without viruses (**a**) and A20, Bal17 and M12 cells infected with viruses (**b**). The retroviruses used in the experiments were generated from pQEF-Ceru-T2A-mIgG1 vector that expresses membrane IgG1