Short communication

A speedy method to detect inserted DNA fragment in cell lines transfected with retroviral vectors

Liang-Hao Ding¹, Emi Iimura¹, Kaoru Saijo¹, Hirofumi Hamada² & Tadao Ohno^{1*}

¹ RIKEN Cell Bank, RIKEN (The Institute of Physical and Chemical Research), Koyadai, Tsukuba Science City, Japan; ² Department of Molecular Medicine, Sapporo Medical University, S1 W17, Chuo-ku, Sapporo 060-8556, Japan

(* Author for correspondence; RIKEN Cell Bank, RIKEN (The Institute of Physical and Chemical Research), Koyadai 3-1-1, Tsukuba Science City, Ibaraki, Japan. Tel: +81-298-36-3611; Fax: +81-298-36-9130; E-mail: tad-ohno@rtc.riken.go.jp)

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Abstract

Cells transfected by retroviral vectors are brought in a gene of particular interest and are very useful in a variety of experiments. It is essential to testify that the DNA fragment was successfully introduced into the cells together with the retroviral vectors. Polymerase chain reaction is believed to be a fast and convenient method for this purpose when using primers flanking the cloning site of the inserted DNA. Unfortunately, a single PCR reaction often fails to amplify the targeted fragment because of the existence of endogenous virus DNA in cell genome. However, in this study we conducted a procedure for a single PCR, using vector-specific primers as well as a nested PCR, and successfully detected the DNA fragments cloned in MFG retroviral vectors in 22 transfected cell lines. We also proved that real time quantitative PCR in combination with MFG-specific primer is useful to determine copy number of the retroviral vector in murine producer cell lines.

Introduction

Retroviral vectors have been widely used in the delivery of genetic materials to eukaryotic cells because of their ability to integrate with the host genome and contribute to a stable and permanent transfection. Detecting the inserted DNA fragment is essential prior to further utilization of the transfected cells in basic research and applied science. It is also important for cell bankers to monitor the quality of the transfected cell lines before shipping.

In RIKEN Cell Bank, we have collected 22 retroviral vector-transfected cell lines, all from a parent murine cell line, Ψ CRIP-P131 (Danos et al., 1988), and a retroviral vector, MFG (Ohashi et al., 1992). The MFG vector is a simplified retroviral vector without a dominant selectable marker gene. The vec-

tor was derived from the Moloney murine leukemia virus (MMLV) in which the polymerase (*pol*) and envelope (*env*) gene sequence was deleted to render it replication-defective. Sequences in the *gag* gene up to base 1035 have been utilized to increase the packaging efficiency of the unspliced transcript. Viral donor and acceptor sites are used for splicing of the mRNA. Most frequently used cloning sites in MFG vector reside in the remaining *env* region, that is from Xba I to BamH I, or Nco I to BamH I. Theoretically, primers flanking these cloning sites could lead to PCR products amplified from the inserted DNA. However, this often fails because of a number of copies of endogenous viral DNA in cell lines. To overcome this disadvantage, we have searched for specific primers to the MFG vector and examined the results of nested PCR. Using these methods, we investigated the possibility of a

rapid and simple PCR procedure to detect the inserted DNA fragment in MFG retroviral vector transfected cell lines.

Recent study shows that real time quantitative PCR can be used as a powerful and reliable tool for determining retroviral DNA copies in human producer cells. However, the same method failed to measure retroviral copies from mouse cells because of the existence of endogenous viral DNA (Towers et al., 1999). Using a MFG-specific primer, we have conducted TaqMan[®] quantitative PCR and successfully measured copy number of the retroviral vectors without interference of endogenous viral DNA.

Materials and methods

Cell lines

22 MFG retroviral vector-transfected cell lines collected and stored at RIKEN Cell Bank and 1 GK vector-transfected cell line were used in the study (Table 1). The GK vector is a modified MFG vector in which *gag* transcription was suppressed by insertion of 2 stop codons and a 87 base pair-deletion from the 3' end of *env*. Retroviral vector packaging cell line Ψ CRIP-P131 was originally constructed from NIH3T3. These 2 cell lines were used as control.

Genomic DNA isolation

Genomic DNA of the cell lines were isolated using DNAzol Genomic Isolation Reagent (Molecular Research Center, Inc., Cincinnati, USA). Briefly, cells were washed twice with PBS, and then were centrifuged. The cell pellet containing $1-3 \times 10^6$ cells was lysed by adding 1 ml DNAzol and pipetting repeatedly. The homogenate was centrifuged for 10 minutes at 10000 g at 4 °C. Supernatant was transferred into a fresh tube. A half ml of 100% ethanol was added to precipitate DNA. The DNA was spooled onto a pipette tip and was transferred into tubes containing 1.0 ml of 95% ethanol. The DNA was washed twice with 1.0 ml of 95% ethanol and was dissolve in 50 μ l of distilled water. DNA concentration was determined by measuring ultraviolet light absorbency at 260 nm.

Primers

Primers were designed on the basis of the sequence of MFG vector (Figure 1). For nested PCR, 2 primer pairs were designed with the 2nd primers corresponding to

the sequence of the 1st primer pair PCR product. The primers were synthesized by ESPEC Oligo Service Inc., Tsukuba, Japan and were dissolved in distilled water to make a final concentration of 10 μ M.

Primer 011: designed 414 base pairs upstream from Xba I site, 5'-CTTGAACCTCCTCGTTCGAC-3', primer 010: designed 30 base pair downstream from BamH I site, 5'-AAAAGTACAGCCTGGACCACTG-3'. Primer 007: designed 91 base pairs upstream from Xba I site, 5'-AAAGGACCTTACACAGTCCTGCT-3', primer 008: designed 17 base pairs downstream from BamH I site, 5'-TGGACCACTGATATCCTGTCTTT-3'.

A pair of MFG vector specific primers was also designed. The left primer codes for the newly formed *gag-pol* junction upon the deletion of wild-type MMLV. Primer 012: designed 359 base pairs upstream from Xba I site, 5'-CTTCTCTAGGCGCCCATATG-3', primer 013: designed 23 base pairs downstream from BamH I site, 5'-GCCTGGACCACTGATATCCT-3'.

Polymerase chain reaction

TaKaRa Ex Taq PCR system (TaKaRa, Ohtsu, Japan) and GeneAmp 9600 thermal cycler (PE Applied Biosystems, USA) were used for PCR amplification. The amplification was carried out in 50 μ l of each reaction mixture containing 5 μ l of 10 $\times$ Ex Taq buffer, 4 μ l of dNTP mixture, 1 μ l of each primer for nested PCR or 2 μ l for MFG specific primers, 0.1 μ g of genomic DNA and 0.25 μ l of Ex Taq polymerase. For the second reaction of the nested PCR, 0.5 μ l of the 1st PCR product was applied as template. The two amplifying reactions of nested PCR were carried out using an identical protocol. Briefly, the reaction mixtures heated to 95 °C for 3 minutes, and then cycled 25 times; each cycle consisted of denaturation at 95 °C for 30 seconds, annealing at 67 °C for 30 seconds, and elongation at 74 °C for 30 seconds per 1,000 bps. PCR using MFG specific primers was conducted as follows, heated to 95 °C for 3 minutes, and then cycled 25 times; each cycle consisted of denaturation at 95 °C for 30 seconds, annealing at 58 °C for 30 seconds, and elongation at 74 °C for 30 seconds per 1,000bps. PCR products were subjected to electrophoresis in 2% agarose gel. DNA bands were stained with ethidium bromide afterwards.

Table 1. Retroviral vector transfected cell lines used in this study

Name of Cell Line	cDNA inserted	Cloning Site	cDNA Length (bps)
ΨCRIP-MFGmIL-1 α	Mouse interleukin 1 alpha	Xba I – BamH I	–
ΨCRIP-MFGmIL-2	Mouse interleukin 2	Xba I – BamH I	533
ΨCRIP-MFGmIL-3	Mouse interleukin 3	Nco I – BamH I	520
ΨCRIP-MFGmIL-6	Mouse interleukin 6	Xba I – BamH I	661
ΨCRIP-MFGmIL-7	Mouse interleukin 7	Xba I – BamH I	500
ΨCRIP-MFGmIL-12p40	Mouse interleukin 12p40	Nco I – BamH I	1,000
ΨCRIP-MFGmIL-12p35	Mouse interleukin 12p35	Xba I – BamH I	650
ΨCRIP-MFGmTNF- α	Mouse tumor necrosis factor alpha	Xba I – BamH I	733
ΨCRIP-MFGmTNF- α	Human tumor necrosis factor alpha	Xba I – BamH I	–
ΨCRIP-MFGmLT	Mouse lymphotoxin	Xba I – BamH I	632
ΨCRIP-MFGmG-CSF	Mouse granulocyte colony-stimulating factor	Nco I – BamH I	645
ΨCRIP-MFGmM-CSF	Human macrophage colony-stimulating factor	Xba I – BamH I	1,600
ΨCRIP-MFGmINF- γ	Mouse interferon gamma	Xba I – BamH I	492
ΨCRIP-MFGmMIF	Human macrophage migration inhibitory factor	Xba I – BamH I	360
ΨCRIP-MFGmLIF	Mouse leukemia inhibitory factor	Xba I – BamH I	633
ΨCRIP-MFGmKitLigand	Mouse stem cell factor	Xba I – BamH I	640
ΨCRIP-MFGmOncost-M	Human oncostatin-M	Nco I – BamH I	780
ΨCRIP-MFGmB7	Mouse B7	Nco I – BamH I	–
ΨCRIP-MFGmB7-2	Mouse B7-2	Nco I – BamH I	–
ΨCRIP-MFGm γ INFR	Truncated form interferon- γ receptor	–	–
ΨCRIP-MFGmIL1RA	Rabbit interleukin 1 receptor antagonist	Xba I – BamH I	550
ΨCRIP-MFGmGM-CSF	Rat granulocyte-macrophage colony-stimulating factor	Xba I – BamH I	–

–: Data not known.

Figure 1. Map for PCR primers. 011/010 and 007/008 were used for nested PCR. 012/013 was used for MFG specific PCR. Primer 012 codes for the unique gag-pol junction formed by deletion of the wild-type MMLV.

Figure 2. Results of the nested PCR using primers 011/010 and 007/008 show the amplification of the targeted DNA inserts. Arrows indicate a band of about 2,000 base pairs that was detected in the packaging cell line Ψ CRIP-P131 and some other cell lines. This band codes for the amphotropic 4070A virus that was one of the constituents for the construction the packaging cell line. The lanes represent the cell samples as follows: 1, no sample; 2, NIH3T3; 3, Ψ CRIP-P131; 4, Ψ CRIP-MFG Δ IL1RA; 5, Ψ CRIP-MFG Δ MIF; 6, Ψ CRIP-MFG Δ LIF; 7, Ψ CRIP-MFG Δ IL-6; 8, Ψ CRIP-MFG Δ IL-2; 9, Ψ CRIP-MFG Δ KitLigand; 10, Ψ CRIP-MFG Δ GM-CSF; 11, no sample; 12, Ψ CRIP-MFG Δ IL-3; 13, Ψ CRIP-MFG Δ IL-12p40; 14, Ψ CRIP-MFG Δ IL-12p40; 15, Ψ CRIP-MFG Δ GM-CSF.

Figure 3. Results of PCR using primers 012/013 that code for a unique sequence in MFG vector. The expected DNA fragments were specifically amplified. No DNA was amplified in NIH3T3 or the packaging cell line Ψ CRIP-P131. The lanes represent the cell samples as follows: 1, no sample; 2, NIH3T3; 3, Ψ CRIP-P131; 4, Ψ CRIP-MFG Δ Oncost-M; 5, Ψ CRIP-MFG Δ IL-1 α ; 6, Ψ CRIP-MFG Δ IL-1 α ; 7, Ψ CRIP-MFG Δ IL-1 α ; 8, Ψ CRIP-MFG Δ IL-1 α ; 9, Ψ CRIP-MFG Δ IL-1 α ; 10, Ψ CRIP-MFG Δ IL-1 α ; 11, Ψ CRIP-MFG Δ IL-1 α ; 12, Ψ CRIP-MFG Δ IL-1 α ; 13, Ψ CRIP-MFG Δ IL-1 α ; 14, Ψ CRIP-MFG Δ IL-1 α ; 15, Ψ CRIP-MFG Δ IL-1 α ; 16, Ψ CRIP-MFG Δ IL-1 α ; 17, Ψ CRIP-MFG Δ IL-1 α ; 18, Ψ CRIP-MFG Δ IL-1 α ; 19, Ψ CRIP-MFG Δ IL-1 α ; 20, Ψ CRIP-MFG Δ IL-1 α ; 21, Ψ CRIP-MFG Δ IL-1 α ; 22, Ψ CRIP-MFG Δ IL-1 α ; 23, Ψ CRIP-MFG Δ IL-1 α ; 24, Ψ CRIP-MFG Δ IL-1 α ; 25, Ψ CRIP-MFG Δ IL-1 α .

Real time quantitative PCR

TaqMan[®] real time quantitative PCR was performed using an ABI PRISM 7700 sequence detector (Perkin-Elmer). Platinum quantitative PCR super mix-UDG (GIBCO BRL, MD, USA) was used for PCR reaction. TaqMan[®] quantitative PCR kit were purchased from GIBCO. TaqMan[®] primers and probes were designed by *PrimerExpress* 3 software (Perkin-Elmer). The forward primer for MFG vectors codes for the unique *gag-pol* junction. The sequence of the primers and probes are as follows:

MFG vector:

Forward primer CCTTCTCTAGGCGCCCATATG

Reverse primer TTAGTAACTCTTGTTCATGTCAGG-GTCA

Probe FAM-CACCCCCGCCCCCTTGTAAGTCC-TAMRA

Mouse β -actin:

Forward primer GGCCAGAGCAAGAGAGGT

Reverse primer CGTCCCAGTTGGTAACAATGC

Probe FAM-TCCTGACCCTGAAGTACCCCATTT

GAACAT-TAMRA

PCRs were held at 50 °C for 3 min, 95 °C for 2 min, and then cycled 45 times at 95 °C 15 sec, 62 °C 1 min. The fluorescent emission was measured at each annealing/extension phase. Data were analyzed by Sequence Detector Software (Perkin-Elmer).

Plasmid pMFG Δ IL-2 was provided by DNA Bank, RIKEN, Japan. The plasmid was serially diluted for obtaining a standard curve of the copy number of MFG vector. A PCR product was amplified using mouse β -actin primers, separated from agarose gel to exclude the genomic template, and was used for the standard of β -actin copy number of the cell lines. The same genomic DNA sample for each cell line was used to obtain the copy number of MFG vector as well as β -actin gene that is exactly correspond to the cell number. Number of MFG copies per cell was calculated for each cell line tested.

Retrovirus infection and titration

Retrovirus producer cell line Ψ CRIP-GKLacZ and

Table 2. Quantitation of MFG vectors

Sample Name	β -actin Copies	MFG Copies	MFG Copies/Cell
Ψ CRIP-MFGmIL-12p40	5.80E+06	1.20E+07	2.07E+00
Ψ CRIP-MFGmIL-3	1.40E+06	1.90E+06	1.36E+00
Ψ CRIP-MFGmGratGM-CSF	3.70E+06	2.90E+06	7.84E-01
Ψ CRIP-MFGmM-CSF	1.80E+06	1.80E+06	1.00E+00
Ψ CRIP-MFGmINF- γ	7.80E+05	7.30E+05	9.36E-01
Ψ CRIP-MFGmIL-12p35	5.10E+06	5.60E+06	1.10E+00
Ψ CRIP-MFGmKit-Lig.	1.80E+06	1.40E+06	7.78E-01
Ψ CRIP-MFGmTNF- α	8.00E+06	1.20E+07	1.50E+00
Ψ CRIP-MFGmIL-6	5.70E+05	3.50E+05	6.14E-01
Ψ CRIP-MFGmIL-2	1.30E+06	2.20E+06	1.69E+00
Ψ CRIP-MFGmMIF	2.70E+06	9.90E+05	3.67E-01
Ψ CRIP-MFGmTry-INFR	1.20E+06	1.30E+06	1.08E+00
Ψ CRIP-MFGmIL-7	8.30E+06	6.00E+06	7.23E-01
Ψ CRIP-MFGmTNF- α	9.40E+05	1.40E+06	1.49E+00
Ψ CRIP-MFGmIL-1RA	6.90E+05	6.90E+05	1.00E+00
Ψ CRIP-MFGmB7-2	2.30E+06	3.00E+06	1.30E+00
Ψ CRIP-MFGmLIF	5.10E+06	2.60E+06	5.10E-01
Ψ CRIP-MFGmOncosta-M	7.50E+05	5.90E+05	7.87E-01
Ψ CRIP-MFGmG-CSF	7.60E+06	1.10E+07	1.45E+00
Ψ CRIP-MFGmB7	1.70E+06	1.10E+07	6.47E+00
Ψ CRIP-MFGmLT	7.90E+06	3.40E+06	4.30E-01
NIH3T3	2.70E+05	0.00E+00	0.00E+00

target cell line NIH3T3 were used for retrovirus titration. 24hrs before infection, 5×10^5 NIH3T3 cells were seeded into 100 mm dishes. The cell number at the time of infection was counted. 350 μ l and 70 μ l of the producer cell supernatant was added into NIH3T3 medium respectively. The NIH3T3 cells were then incubated for 2 days. Half of the cells were collected for DNA extraction and were subjected to quantitative PCR. The remaining cells were stained for β -galactosidase expression. The percentage of the positive cells was calculated.

Results

Nested PCR

As expected, a single pair of primers flanking the cloning site, i. e., primers 011/010 or 007/008, failed to amplify the targeted DNA fragment. Nested PCR, first with the primers 011/010 and then with the primers 007/008, under high annealing temperature and low primer and template concentrations, resulted in a major band corresponding to the inserted DNA frag-

ments. A band of about 2 kb was amplified in the packaging cell line Ψ CRIP-P131 (Figure 2, lane 3). The same band was also detected in Ψ CRIP-MFGmMIF, Ψ CRIP-MFGmLIF, and Ψ CRIP-MFGmIL-6 (Figure 2, lanes 5, 6, and 7, respectively) together with the DNA inserts, although the band from Ψ CRIP-MFGmLIF is very weak. No DNA was amplified in the host cell line, NIH3T3.

MFG specific PCR

Using the single pair of primers 012/013, the expected DNA inserts of all the 22 cell lines were specifically amplified (Figure 3). The sequences of PCR products from some of the cell samples were analyzed and confirmed to be the expected cDNA inserts. In sample Ψ CRIP-MFGmIL12p35, a second band was detected simultaneously corresponding to the vectors without inserted DNA (Figure 3, lane 12). Weak amplification was found in Ψ CRIP-MFGmMIF, Ψ CRIP-MFGmLIF, and Ψ CRIP-MFGmIL-6 (Figure 3, lanes 20, 21, and 22, respectively). These samples were detected possessing a 2 kb DNA fragment by the above mentioned

A **B**

Figure 4. (A) Primers and probe positions for TaqMan[®] quantitative PCR. The forward primer is positioned at the unique Δgag - Δpol junction. (B) Plot of TaqMan[®] quantitative PCR result against PCR cycle number. NIH3T3 remains zero after 45 cycles of amplification.

nested PCR. No amplification occurred in Ψ CRIP-P131 or NIH 3T3.

Real time quantitative PCR

The forward primer of MFG vectors for quantitative PCR was designed at the specific $\Delta gag/\Delta RT$ junction (Figure 4A). MFG specific sequence was detected in

all Ψ CRIP-MFG cell lines tested. ΔRn plotted against PCR cycles for some of the representative cell lines was shown in Figure 4B. No fluorescent signals were measured in NIH3T3 cells after 45 cycles of amplification, indicating that endogenous viral DNA was not detected by this primer/probe set. Plasmid pMFGmIL-2 was serially diluted and subjected to quantitative PCR (Figure 5A). A plot of the standard plasmid cop-

Figure 5. (A) ΔR_n plotted against PCR cycle number for a representative pMFGmIL-2 standard. The threshold at which Ct value is determined is shown. (B) Standard curve of Ct vs pMFGmIL-2 copy number.

ies against Ct (threshold cycle) was obtained (Figure 5B). Purified mouse β -actin PCR product amplified from mouse genome was also serially diluted serially, subjected to quantitative PCR. The standard curve of β -actin copies against Ct was plotted (Figure 6A, B).

Copy numbers of MFG vector as well as β -actin gene in Ψ CRIP-MFG producer cell lines and NIH3T3 were quantified. MFG copies per β -actin gene, i.e., MFG copies/cell were calculated (Table 2). Many cell lines contain more than one copy of the viral vector. Some of the cell lines, such as Ψ CRIP-MFGhMIF, Ψ CRIP-MFGmLIF, and Ψ CRIP-MFGmIL-6, which

were poorly amplified by MFG-specific PCR (Figure 3), were detected to have low MFG/cell ratios by TaqMan[®] quantitative PCR. An exception was the Ψ CRIP-MFGmLT cell, for which a higher concentration of genomic DNA template was used in primer 012/013-primed PCR. MFG sequence was not detected in NIH3T3 cells.

To further confirm the feasibility of TaqMan[®] quantitative PCR on detection of MFG vector copies, NIH3T3 cells were infected with GKLacZ retroviral vector. The infectious titer obtained from quantitative PCR is slightly higher than those determined by β -

Table 3. Comparison of infectious titer on NIH3T3 by X-gal staining and by real time quantitative PCR

The amount of virus used for infection	Copy number measured by TaqMan [®] PCR		Percentage of infected cells	
	β -actin	MFG	X-gal staining	TaqMan [®] PCR
70 μ l	1.40E+06	4.40E+03	0.30%	0.31%
350 μ l	5.90E+05	4.00E+03	0.63%	0.69%

Figure 6. ΔRn plotted against cycle number for a standard mouse β -actin PCR product. The threshold at which Ct value is determined is shown. (B) Standard curve of Ct vs β -actin PCR product copy number.

galactosidase staining, indicating that MFG/cell ratio is more than 1 in some of the cells (Table 3).

Discussion

Several hybridization methods, such as dot blot, Southern blot, etc, could also be considered to detect

the inserted DNA in cells transfected by recombinant retroviruses. Among these methods, PCR is the favorite for cell bankers in sense of fast, less laborious and radiation-free.

Retrovirus is characterized by the insertion of proviral DNA into the genome of host cells. When the insertions take place in germline cells of an animal, the

provirus is transmitted to the offspring and to succeeding generations. Such viruses are called endogenous viruses and may be genetically silent. These endogenous viral copies of DNA integrated in the cell genome interfered with the priming of primers flanking the cloning sites and resulted in the consequent failure of detection of the inserted DNA. Primers coding for regions within the inserted DNA are not favorable because various optimal primers and PCR reaction conditions have to be set in accordance with a variety of DNA inserts. Cell bankers usually receive recombinant cell lines with different DNA inserts transfected by one kind of viral vector. It is practical to design primers and standardize the PCR conditions based on viral vector sequences.

Although strict reaction conditions were applied for nested PCR, such as high annealing temperature, low template and primer concentrations, some of the extra bands were unavoidable. Nevertheless, nested PCR is still a useful tool because it successfully amplified the targeted DNA inserts as the major bands (Figure 2). In contrast, PCR reaction using the single primer set, 012/013, was proven to be very specific to MFG retroviral vector. Recombinant DNA inserts in all the 22 cell lines tested were amplified as a unique band (Figure 3). Since most commonly used cloning sites in MFG reside in *env*, this primer pair can cover most cases of the detection regarding to MFG vector mediated cell transfection. As dual bands were amplified in some cell lines by the nested PCR, the 2 kb band is considered to be a fragment in the packaging cell line Ψ CRIP-P131. It codes for the *env* of 4070A virus that was constructed into Ψ CRIP-P131 for packaging of amphotropic recombinant viruses (Chattopadhyay et al., 1981). This fragment should not exist in recipient cell lines other than the Ψ CRIP series. It is not quite clear why other cell lines derived from Ψ CRIP-P131 were not detected to have the 4070A fragment. One assumption is that the 4070A fragment was amplified in cells that have lesser number of copies of the recombinant MFG vectors. This was supported by the MFG specific PCR that the bands of recombinant DNA inserts were weaker in those cells (Figure 3, lane 20, 21, and 22) that had dual bands for nested PCR (Figure 2, lane 5, 6, and 7). In contrast, PCR reaction using the single primer set, 012/013, was proven to be very specific to MFG retroviral vector. Recombinant DNA inserts in all the 22 cell lines tested were amplified as a unique band (Figure 3).

TaqMan[®] quantitative PCR has been used to determine the infectious titer by detecting the number

of integrated retroviral vectors in a population of infected cells (Gerard CJ et al., 1996; Towers et al., 1999; Yang S et al., 1999). The technique has been successfully applied in human cells transfected with retroviral vectors but failed to detect in murine cell lines due to the large number of endogenous retroviral sequences as described by Towers et al. We positioned TaqMan[®] forward primer at the unique site of MFG vector thereby overcome the interference of endogenous viral copies. This was clued by that 1). PCR kinetics was identical for pMFGmIL-2 template as well as the Ψ CRIP-MFG cell genome template; 2). No fluorescent signals were detected in NIH3T3 cells after 45-cycle PCR. Due to the restricted position, the forward primer has relatively high tendency of duplex formation, and the sensitivity for pMFGmIL-2 standard quantification was not as high as described by Towers et al. However, the primer/probe set is still useful in high titer retroviral producers.

Current study confirms that, while the nested PCR is practical, the single set of primers and PCR conditions provided in this paper specifically detect the recombinant DNA inserts in cells transfected by MFG retroviral vectors, thus, these primers and PCR conditions are useful in MFG-transfected recombinant cell lines. In combination with the MFG specific primers, real time quantitative PCR assay is reliable to quantify the retroviral copies in murine cell lines. It is predictable that the recombinant DNA inserts cloned in other retroviral vectors may also be successfully detected using other vector-specific primers.

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