pFB and pFB-Neo Retroviral Vectors

Instruction Manual

Catalog #217563 (pFB Retroviral Vector) and #217561 (pFB-Neo Retroviral Vector)

Revision C.0

For Research Use Only. Not for use in diagnostic procedures.

217561-12

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pFB and pFB-Neo Retroviral Vectors

Contents

pFB and pFB-Neo Retroviral Vectors

MATERIALS PROVIDED

Material provided	Quantity	
	Catalog #217561	Catalog #217563
pFB-Neo retroviral vector	10 µg	—
pFB retroviral vector	—	10 µg
pFB-Luciferase expression control vector	10 µg	10 µg

STORAGE CONDITIONS

All components:–20°C

NOTICE TO PURCHASER

FOR LABORATORY USE ONLY
A license (from Promega for research reagent products and from The Regents of the University of California for all other fields) is needed for any commercial sale of nucleic acid contained within or derived from this product.

BIOSAFETY CONSIDERATIONS

The host range of a retrovirus is determined not by the vector DNA but by the specific *env* gene used to construct the packaging cell line. Viruses produced from amphotropic or polytropic packaging lines are capable of infecting human cells. The National Institutes of Health has designated retroviral vectors, such as MMLV, as Biosafety Level 2. Appropriate caution should be used in the production and use of recombinant retrovirus. For more information see *Biosafety in Microbiological and Biomedical Laboratories* at *www.nih.gov/od/ors/ds/pubs/ bmbl/contents.htm*.

Revision C.0

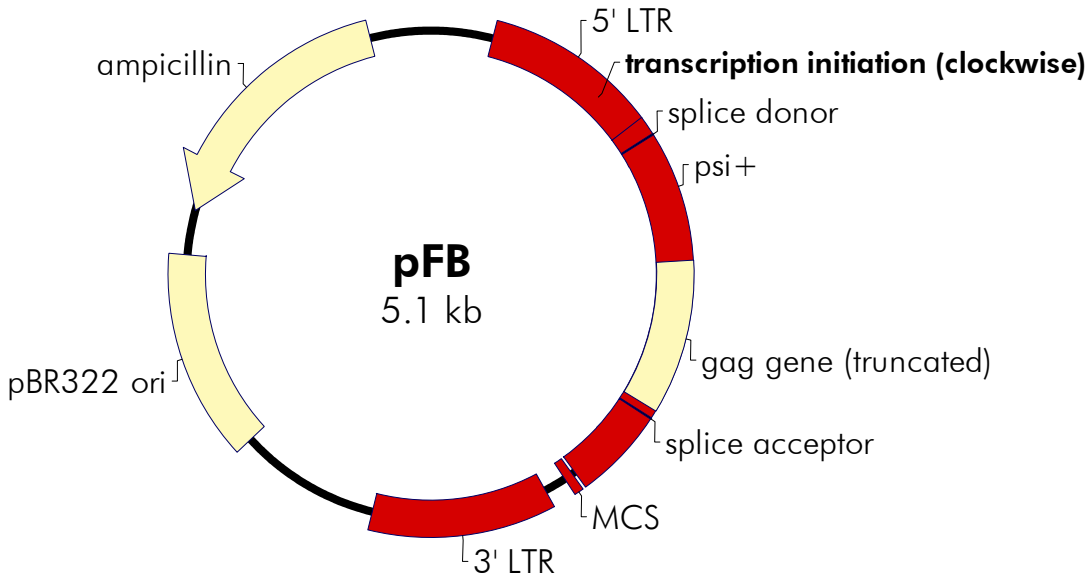
INTRODUCTION

The Agilent pFB and pFB-Neo plasmid vectors for retroviral gene delivery and expression are derived from the Moloney murine leukemia virus (MMLV) and can be used to produce high titer viral stocks with MMLV-based packaging cell lines.[1,2] Both vectors contain the bacterial origin of replication and ampicillin-resistance gene from pBR322, an extended MMLV packaging signal (ψ+), and a multiple cloning site (MCS) located between the MMLV 5´ and 3´ long terminal repeat sequences (LTRs), see Figures 1 and 2.

The pFB vector is a minimal MMLV-based vector that can accommodate larger inserts than pFB-Neo. The pFB vector does not contain any exogenous sequence other than the sequence of the MCS.

The pFB-Neo vector differs from the pFB vector only by the presence of a cassette consisting of the internal ribosome entry site (IRES) from the encephalomyocarditis virus (EMCV) and the neomycin-resistance gene (neo^r). The open reading frame (ORF) for the neo^r gene is positioned downstream of the MCS and follows the IRES. A dicistronic message encoding the gene of interest and the neo^r gene is expressed from the viral promoter within the 5´ LTR.

The pFB Vector



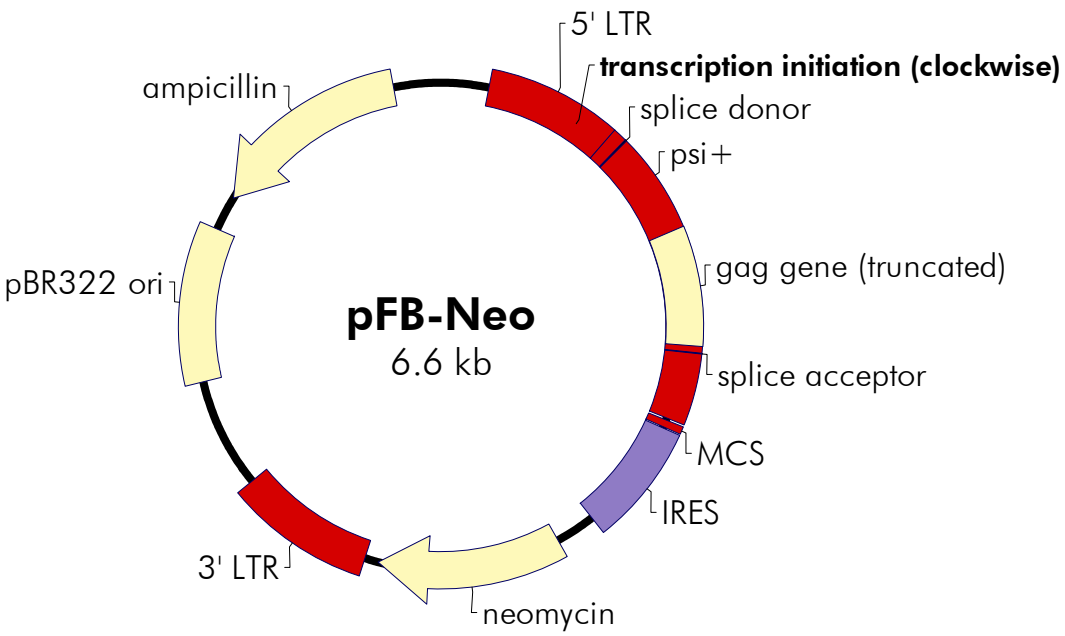
pFB Multiple Cloning Site Region
(sequence shown 2057–2086)

```
Sal I     EcoR I    BamH I  Xho I    Not I
  |         |         |       |        |
GTCGACGAATTCGGATCCTCGAGCGGCCGC
```

Feature	Nucleotide position
5′-long terminal repeat (LTR)	209–760
transcription initiation (clockwise)	616
splice donor	818–822
ψ+ extended viral packaging signal	760–2046
gag gene (truncated)	1236–1723
splice acceptor	1751–1753
5′ retro primer binding site	2008–2028
multiple cloning site	2057–2086
3′ pFB primer binding site	2121–2101
3′-long terminal repeat (LTR)	2163–2756
pBR322 origin of replication	3237–3904
ampicillin resistance (*bla*) ORF	4055–4912

FIGURE 1 Circular map and features for the pFB retroviral vector. The complete nucleotide sequence and list of restriction sites can be found at *www.genomics.agilent.com*.

The pFB-Neo Vector



pFB-Neo Multiple Cloning Site Region
(sequence shown 2057–2086)

Sal I EcoR I BamH I Xho I Not I

GTCGACGAATTCGGATCCTCGAGCGGCCGC

Feature	Nucleotide Position
5′-long terminal repeat (LTR)	209–760
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5′ retro primer binding site	2008–2028
multiple cloning site	2057–2086
3′ pFB primer binding site	2147–2127
internal ribosome entry site (IRES)	2093–2586
neomycin resistance ORF	2763–3557
3′-long terminal repeat (LTR)	3617–4210
pBR322 origin of replication	4691–5358
ampicillin resistance (*bla*) ORF	5509–6366

FIGURE 2 Circular map and features for the pFB-Neo retroviral vector. The complete nucleotide sequence and list of restriction sites can be found at *www.genomics.agilent.com*.

pFB-LUCIFERASE CONTROL VECTOR

The pFB-Luc plasmid vector (Figure 3), which contains the luciferase gene, is included with the vectors as an expression control. A retroviral vector expressing a reporter gene is useful for optimizing transfection efficiency, confirming viral production, as well as ascertaining whether or not a target cell line can be infected by a given viral stock.

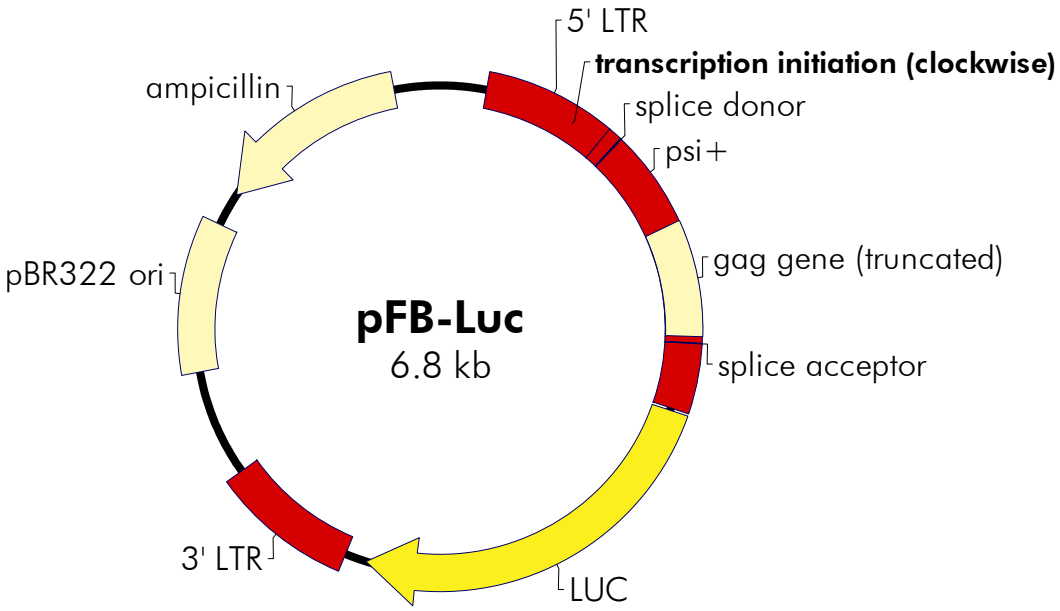

Figure 3 Circular map for the pFB-Luc control vector. The complete nucleotide sequence and list of restriction sites can be found at *www.genomics.agilent.com*.

REPLICATION-DEFECTIVE RETROVIRAL GENE TRANSFER SYSTEMS

Replication-defective retroviral vectors contain all of the *cis* elements required for transcription of a gene of interest and packaging of the transcripts into infectious viral particles.[2] The retroviral vectors typically consist of an *E. coli* plasmid backbone containing a pair of viral LTRs between which the gene of interest is inserted. In order to generate infectious virus particles that carry the gene of interest, specialized packaging cell lines have been generated that contain chromosomally integrated expression cassettes for viral proteins Gag, Pol, and Env, all of which are required in *trans* for production of virus.

After the packaging cell line is transfected with the vector DNA, either the supernatant is collected for transiently produced viral stocks, or stable viral producer cell lines are selected (provided the vector has an appropriate selectable marker). The supernatant is used to infect dividing target cells. Upon infection, the viral RNA molecule is reverse transcribed by reverse transcriptase (which is present in the virion particle), and the gene of interest, flanked by the LTRs, is integrated into the host DNA. Because the vector itself does not express viral proteins, once a target cell is infected, the LTR expression cassette is incapable of another round of virus production.

BIOSAFETY CONSIDERATIONS

The host range of a retrovirus is determined not by the vector DNA but by the specific *env* gene used to construct the packaging cell line. Viruses produced from amphotropic or polytropic packaging lines are capable of infecting human cells. The National Institutes of Health has designated retroviral vectors, such as MMLV, as Biosafety Level 2. Appropriate caution should be used in the production and use of recombinant retrovirus. For more information see *Biosafety in Microbiological and Biomedical Laboratories* at *www.nih.gov/od/ors/ds/pubs/ bmbl/contents.htm*.

LIGATION OF VECTOR AND INSERT

Ligation Guidelines

Because the size of retroviral RNA that can be efficiently packaged is limited to ~11 kb (including the 5´ and 3´ LTRs),[1] the size of the insert should be <8.4 kb for the pFB vector and <7.0 kb for the pFB-Neo vector. Inserts should include both initiation and stop codons. Design the insert to contain a Kozak sequence. A complete Kozak sequence includes CCACC**ATG**G, although CC**ATG**G, or the core **ATG**, is sufficient.

Dephosphorylate the digested plasmid DNA with alkaline phosphatase prior to ligation with the insert DNA. If more than one restriction enzyme is used, the background can be reduced further by gel purification of the digested plasmid DNA. After gel purification, resuspend the DNA in TE buffer (see *Preparation of Media and Reagents*) or water.

For ligation, the ideal ratio of insert-to-vector DNA is variable; however, a reasonable starting point is 2:1 (insert:vector), measured in available picomole ends. This ratio is calculated as follows:

$$\text{picomole ends/microgram of DNA} = \frac{2\times10^6}{\text{number of base pairs}\times 660}$$

Ligation Protocol

1. Prepare the following two experimental and three control ligation reactions by adding the following components in order to separate 0.5-ml microcentrifuge tubes:

Component	Insert control[a]	Digest control[b]	Phosphatase control[c]	Test I 2:1	Test II X:1
Prepared vector (10 ng/µl)	—	1 µl	1 µl	1 µl	1 µl
Prepared insert (10 ng/µl)	X µl	—	—	X µl	X µl
10 mM rATP (pH 7.0)	0.5 µl	0.5 µl	0.5 µl	0.5 µl	0.5 µl
10× ligase buffer[d]	0.5 µl	0.5 µl	0.5 µl	0.5 µl	0.5 µl
T4 DNA ligase (4 U/µl)	0.5 µl	—	0.5 µl	0.5 µl	0.5 µl
Double-distilled water to a final volume of 5 µl	X µl	3.0 µl	2.5 µl	X µl	X µl

[a] Lacks the prepared vector and controls for contamination of the insert by vector DNA.

[b] Lacks the prepared insert and T4 DNA ligase and controls for residual undigested vector.

[c] Lacks the prepared insert and controls for the effectiveness of the alkaline phosphatase treatment; should result in significantly fewer colonies than the test plates.

[d] See *Preparation of Media and Reagents*.

2. Mix the ligation reactions gently.

3. Incubate the ligation reactions overnight at 16°C.

4. Transform the appropriate competent bacteria with 1–2 µl of ligation reaction (see *Recombination and Host Strains for Subcloning and Propagation*). Plate transformation reactions on LB–ampicillin agar plates (see *Preparation of Media and Reagents*).

RECOMBINATION AND HOST STRAINS FOR SUBCLONING AND PROPAGATION

Retroviral vectors are more likely to experience recombination events than other plasmid vectors because of repeat sequences in the LTR regions. We offer RecA$^-$ *E. coli* strains such as XL10-Gold ultracompetent cells or XL1-Blue supercompetent cells which address this issue.

PRIMERS

The nucleotide sequence and vector coordinates for primers suitable for PCR amplification and sequencing of inserts in the pFB and pFB-Neo vectors are as follows:

Primer	Coordinates (bp)	Sequence
5′ Retro	2008–2028	5′-GGCTGCCGACCCCGGGGGTGG-3′
3′ pFB	2121–2101	5′-CGAACCCCAGAGTCCCGCTCA-3′
3′ pFB-Neo	2147–2127	5′-GCCAGGTTTCCGGGCCCTCAC-3′

PREPARATION OF MEDIA AND REAGENTS

LB Agar (per Liter)	LB–Ampicillin Agar (per Liter)
10 g of NaCl 10 g of tryptone 5 g of yeast extract 20 g of agar Add deionized H_2O to a final volume of 1 liter Adjust pH to 7.0 with 5 N NaOH Autoclave Pour into petri dishes (~25 ml/100-mm plate)	1 liter of LB agar, autoclaved Cool to 55°C Add 10 ml of 10-mg/ml filter-sterilized ampicillin Pour into petri dishes (~25 ml/100-mm plate)
TE Buffer 10 mM Tris-HCl (pH 7.5) 1 mM EDTA	**10× Ligase Buffer** 500 mM Tris-HCl (pH 7.5) 70 mM $MgCl_2$ 10 mM DTT ***Note*** *rATP is added separately in the ligation reaction.*

REFERENCES

1. Miller, A. D. (1997). Development and Applications of Retroviral Vectors. In *Retroviruses*, J. M. Coffin, S. H. Hughes and H. E. Varmus (Eds.), pp. 437-473. Cold Spring Harbor Laboratory Press, Plainview, NY.
2. Felts, K., Bauer, J. C. and Vaillancourt, P. (1999) *Strategies* 12(2):74-77.

SUPPLEMENTAL REFERENCES

1. Miller, A. D. (1997) Development and Applications of Retroviral Vectors. In *Retroviruses* (eds., Coffin, J. M., Hughes, S. H., and Varmus, H. E.) pp. 437–473. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
2. Cepko, C., and Pear, W. (1996) In *Current Protocols in Molecular Biology*, pp. 9.9.1–9.9.16. John Wiley & Sons Inc., New York.

MSDS INFORMATION

Material Safety Data Sheets (MSDSs) are provided online at *http://www.genomics.agilent.com*. MSDS documents are not included with product shipments.