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Recombinant retroviral expression vectors based on pLXSN that encode EGFR, ERBB2, ERBB3, and ERBB4

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Abstract

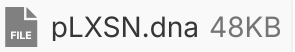
Recombinant retroviruses are commonly used to direct the ectopic expression of genes in infected cells. There are two significant advantages to this approach. Following infection of the target cell, the recombinant retroviral genome is reverse-transcribed and subsequently integrated into the host cell genome, enabling stable ectopic gene expression. Recombinant retroviral expression vectors typically contain a drug-resistance gene, allowing the selection and maintenance of stably infected cells.

A popular recombinant retroviral expression vector is pLXSN. This plasmid contains recombinant long-terminal repeat (LTR) sequences to permit the packaging of the RNA transcript into infectious recombinant retrovirus particles. The plasmid also includes a short poly-cloning region with unique EcoRI, HpaI, XhoI, and BamHI restriction enzyme sites. This region lies downstream of the 5' LTR and truncated retroviral gag sequence, thereby enabling the expression of a gene cloned into the poly-cloning region. An SV40 promoter lies downstream of the poly-cloning region; this promoter drives constitutive expression of a neomycin resistance gene (NeoR). These elements enable selection for stable infection of the recombinant retroviruses using the antibiotic G418. Finally, the plasmid contains a bacterial origin of replication and an ampicillin resistance gene to permit plasmid propagation in standard laboratory strains of *E. coli* (DH5alpha, DH10B, and related strains).

Here we describe the construction of pLXSN derivatives that contain the human *EGFR*, *ERBB2*, *ERBB3*, and *ERBB4* cDNAs. These cDNAs encode receptor tyrosine kinases (RTKs). The EGFR and ERBB2 RTKs are well-validated drivers of many types of human malignancies. However, the roles that the ERBB3 and ERBB4 RTKs play in human malignancies are more poorly defined. Thus, these recombinant retrovirus constructs permit us to study the function of these ERBB receptors, particularly ERBB3 and ERBB4, in various contexts.

Troubleshooting

Introduction

- 1 Recombinant retroviruses are commonly used to direct the ectopic expression of genes in infected cells. There are two significant advantages to this approach. Following infection of the target cell, the recombinant retroviral genome is reverse-transcribed and subsequently integrated into the host cell genome, enabling stable ectopic gene expression. Recombinant retroviral expression vectors typically contain a drug-resistance gene, allowing the selection and maintenance of stably infected cells.
- 2 A popular recombinant retroviral expression vector is pLXSN (**Figure 1**) [1]. This map is derived from the DNA sequence contained in  pLXSN.dna 48KB.

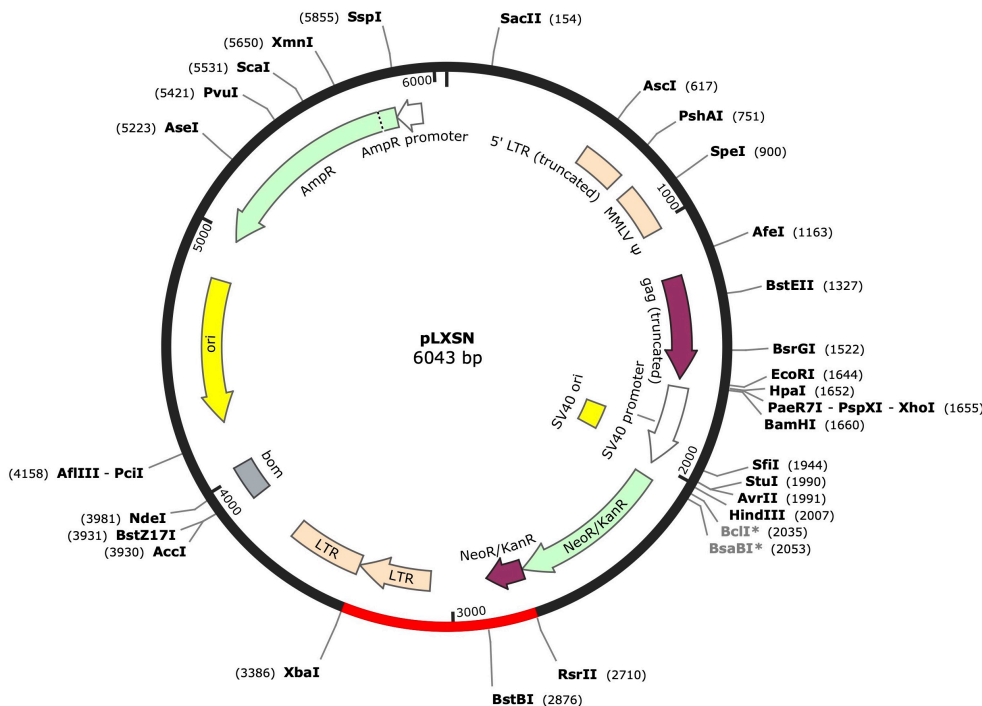


Figure 1. Map of the recombinant retroviral vector pLXSN. Noteworthy functional elements and unique restriction enzyme cleavage sites are depicted. Note that the 6043 bp sequence used to generate this map (**pLXSN.dna** - see link above) was generated by next-generation sequencing of our plasmid and does not match the 5874 bp published sequence of pLXSN [10, 11]. However, we have identified the apparent differences between our sequence and the published sequence and confirmed the differences in our plasmid by next-generation sequencing of a PCR product generated from the divergent region.


This plasmid contains recombinant long-terminal repeat (LTR) sequences to permit the packaging of the RNA transcript into infectious recombinant retrovirus particles. The plasmid also includes a short poly-cloning region with unique EcoRI, HpaI, XhoI, and BamHI restriction enzyme sites. This region lies downstream of the 5' LTR and truncated retroviral gag sequence, thereby enabling the expression of a gene cloned into the poly-

cloning region. An SV40 promoter lies downstream of the poly-cloning region; this promoter drives constitutive expression of a neomycin resistance gene (NeoR). These elements enable selection for stable infection of the recombinant retroviruses using the antibiotic G418. Finally, the plasmid contains a bacterial origin of replication and an ampicillin resistance gene to permit plasmid propagation in standard laboratory strains of *E. coli* (*DH5alpha*, *DH10B*, and related strains).

- 3 Here we describe the construction of pLXSN derivatives that contain the human *EGFR* [2], *ERBB2* [3], *ERBB3* [4], and *ERBB4* [5] cDNAs. These cDNAs encode receptor tyrosine kinases (RTKs). The EGFR and ERBB2 RTKs are well-validated drivers of many types of human malignancies. However, the roles that the ERBB3 and ERBB4 RTKs play in human malignancies are more poorly defined. Thus, these recombinant retrovirus constructs permit us to study the function of these ERBB receptors, particularly ERBB3 and ERBB4, in various contexts.

Methods

4 Construction of pLXSN-EGFR

We have previously briefly described the construction of pLXSN-EGFR [6]. The plasmid pSKEGFR [6], which encodes the human EGFR cDNA [2], was generated (unpublished data) from pCO12EGFR [7] and is a generous gift from David F. Stern. This plasmid (pSKEGFR) contains XhoI sites that flank the EGFR coding region of the plasmid. Thus, this plasmid was digested with XhoI to yield the complete *EGFR* cDNA fragment. The recombinant retroviral vector pLXSN was linearized at the polycloning region by digestion with XhoI. The EGFR-XhoI fragment was ligated to the pLXSN-XhoI fragment, and the ligation product was electro-transformed into DH10B *E. coli*. Ampicillin-resistant colonies of *E. coli* were screened for the *EGFR* cDNA insert by colony hybridization using a radioactive probe. Positive colonies were expanded, and minipreps were screened for the correct orientation of the *EGFR* cDNA insert. NGS has validated this construct, and the map of the resulting sequence ( pLXSN-EGFR.dna 69KB) is shown in **Figure 2**. Note this strategy preserves the XhoI sites that flank the *EGFR* coding sequence.

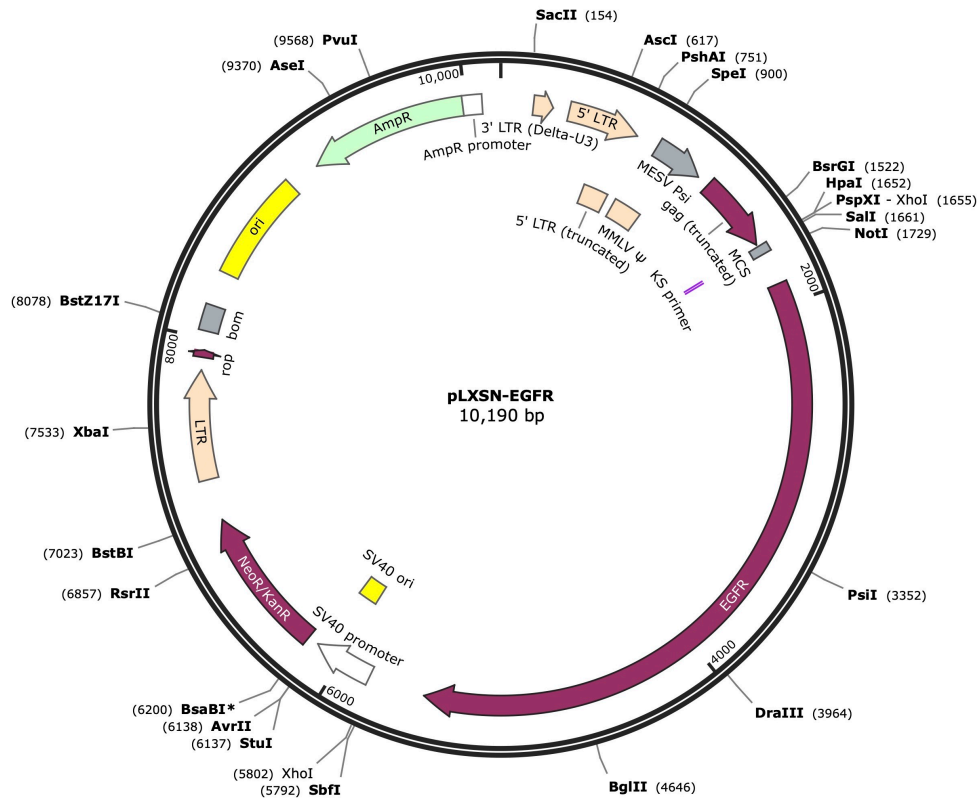


Figure 2. Map of the recombinant retroviral vector pLXSN-EGFR. This map is derived from the NGS sequence of pLXSN-EGFR (**pLXSN-EGFR.dna** - see link above). The sequence of this plasmid's region derived from pLXSN matches our sequence of pLXSN (**pLXSN.dna** - see link above). The sequence of this plasmid's region derived from the *EGFR* cDNA encodes the entire *EGFR* coding sequence (nt 1882-5514; 1210 aa) and perfectly matches the corresponding sequence of the *EGFR* cDNA [2].

5 Construction of pLXSN-ERBB2

We have previously briefly described the construction of pLXSN-ERBB2 [6]. The plasmid pCDNEU [8], which encodes the complete human *ERBB2* cDNA, was a generous gift from Gregory D. Plowman. This plasmid was digested with *Nru*I and *Dra*I, which cut the plasmid at sites that flank the coding region of the *ERBB2* cDNA and generate blunt ends. The recombinant retroviral vector pLXSN was linearized at the poly-cloning region by digestion with *Hpa*I, which yields blunt ends. The ERBB2-blunt fragment was ligated to the pLXSN-*Hpa*I fragment, and the ligation product was electrotransformed into DH10B *E. coli*. Minipreps were generated from ampicillin-resistant colonies and were screened by restriction mapping for the correct orientation of the *ERBB2* cDNA insert. This yielded the plasmid pLXSN-Long-ERBB2.

Unfortunately, pLXSN-Long-ERBB2 did not direct adequate ectopic expression of ERBB2 protein in eukaryotic cells that were transduced with this construct [6]. We postulate

that the inadequate expression was due to excessive DNA (>800 bp) upstream of the *ERBB2* transcriptional start site. Thus, we subcloned the XhoI fragment of pLXSN-Long-ERBB2 into the XhoI site of pLXSN, yielding the plasmid pLXSN-ERBB2. NGS has validated this construct, and the map of the resulting sequence (



pLXSN-ERBB2.dna 83KB) is shown in **Figure 3**. Note this strategy preserves the XhoI sites that flank the *ERBB2* coding sequence. This plasmid directs abundant ectopic expression of ERBB2 protein in eukaryotic cells that were transduced with this construct [6].

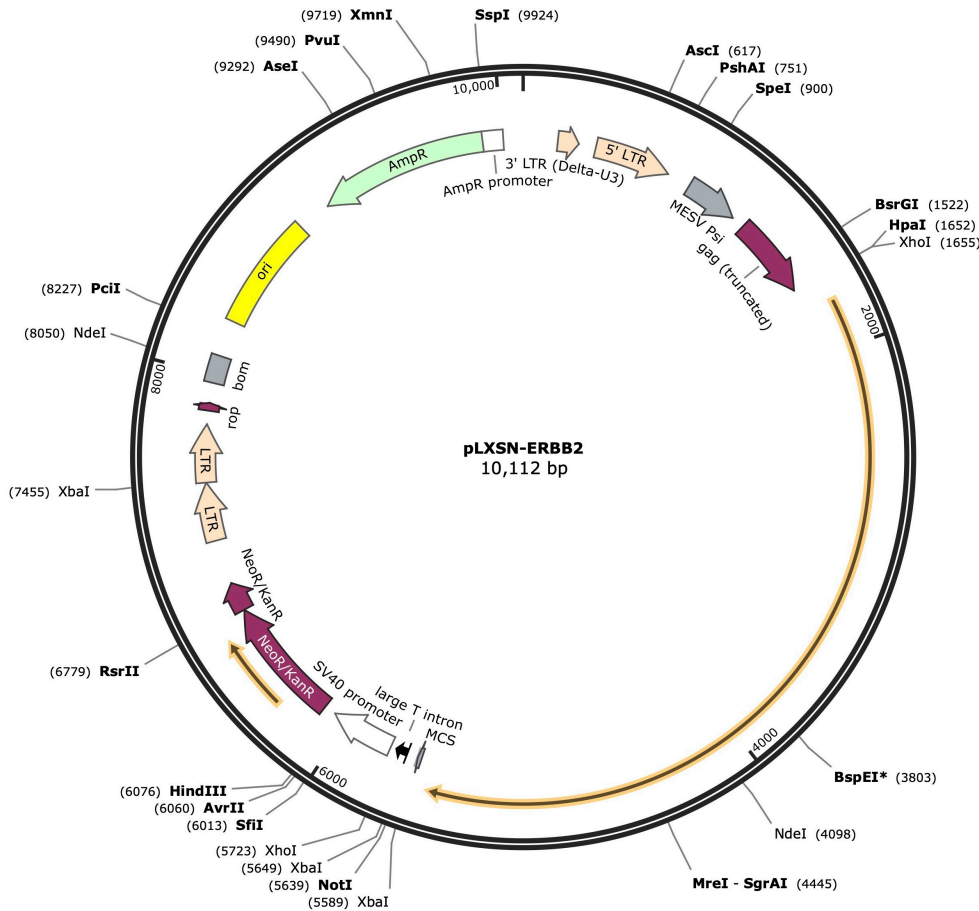



Figure 3. Map of the recombinant retroviral vector pLXSN-ERBB2. This map is derived from the NGS sequence of pLXSN-ERBB2 (pLXSN-ERBB2.dna - see link above). The sequence of this plasmid's region derived from pLXSN matches our sequence of pLXSN (pLXSN.dna - see link above). The sequence of the region of this plasmid that is derived from the *ERBB2* cDNA encodes the entire *ERBB2* coding sequence (nt 1759-5526; 1255 aa) and perfectly matches the corresponding sequence of the *ERBB2* cDNA [3].

6 Construction of pLXSN-ERBB3

We have previously briefly described the construction of pLXSN-ERBB3 [6]. The plasmid pBSHER3X [6], which encodes the human *ERBB3* cDNA [9], was a generous gift from Gregory D. Plowman. This plasmid was digested with BssHII, which cuts the plasmid at sites that flank the coding region of the *ERBB3* cDNA. The sticky ends were filled using the Klenow fragment of *E. coli* DNA polymerase I, yielding a fragment with blunt ends. The recombinant retroviral vector pLXSN was linearized at the poly-cloning region by digestion with HpaI, which generates blunt ends. The *ERBB3*-blunt fragment was ligated to the pLXSN-HpaI fragment, and the ligation product was electro-transformed into DH10B *E. coli*. Ampicillin-resistant colonies of *E. coli* were screened for the *ERBB3* cDNA insert by colony hybridization using a radioactive probe. Positive colonies were expanded, and minipreps were screened for the correct orientation of the *ERBB3* cDNA insert. NGS has validated this construct, and the map of the resulting sequence ( pLXSN-ERBB3.dna 69KB) is shown in **Figure 4**. Note that this subcloning strategy destroys the BssHII restriction enzyme sites of the *ERBB3* cDNA fragment and the HpaI restriction enzyme site of pLXSN.

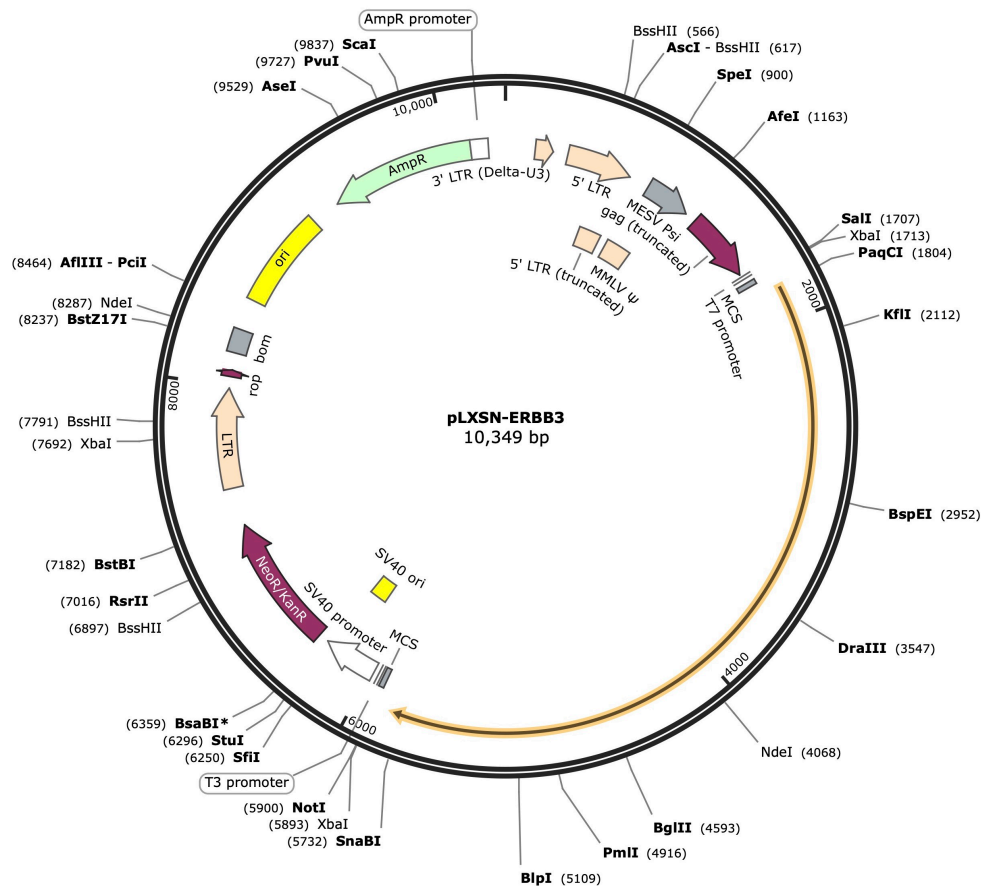



Figure 4. Map of the recombinant retroviral vector pLXSN-ERBB3. This map is derived from the NGS sequence of pLXSN-ERBB3 (**pLXSN-ERBB3.dna** - see link above). The sequence of this plasmid's region derived from pLXSN matches our sequence of pLXSN (**pLXSN.dna** - see link above). The sequence of the region of this plasmid that is derived from the *ERBB3* cDNA encodes the entire *ERBB3* coding sequence (nt 1793-5821; 1342 aa) and perfectly matches the corresponding sequence of the *ERBB3* cDNA [4].

7 Construction of pLXSN-ERBB4

We have previously briefly described the construction of pLXSN-ERBB4 [6]. The plasmid cH4M2 [6], which encodes the human *ERBB4* cDNA [8], was a generous gift from Gregory D. Plowman. This plasmid was digested with *Sna*BI and *Sma*I, each cutting the plasmid at a single site and yielding the complete *ERBB4* cDNA with blunt ends. *Sall* linkers were added to this fragment. The recombinant retroviral vector pLXSN was linearized at the poly-cloning region by digestion with *Xho*I. The *ERBB4*-*Sall* fragment was ligated to the pLXSN-*Xho*I fragment, and the ligation product was electro-transformed into DH10B *E. coli*. Ampicillin-resistant colonies of *E. coli* were screened for the *ERBB4* cDNA insert by colony hybridization using a radioactive probe. Positive colonies were expanded, and minipreps were screened for the correct orientation of the *ERBB4* cDNA insert. NGS has validated this construct, and the map of the resulting

sequence ( pLXSN-ERBB4.dna 85KB) is shown in **Figure 5**. Note that a *Sall* site is retained at the 5' end of the *ERBB4* cDNA insert, and multiple *Sall* sites are retained at the 3' end of the *ERBB4* cDNA insert.

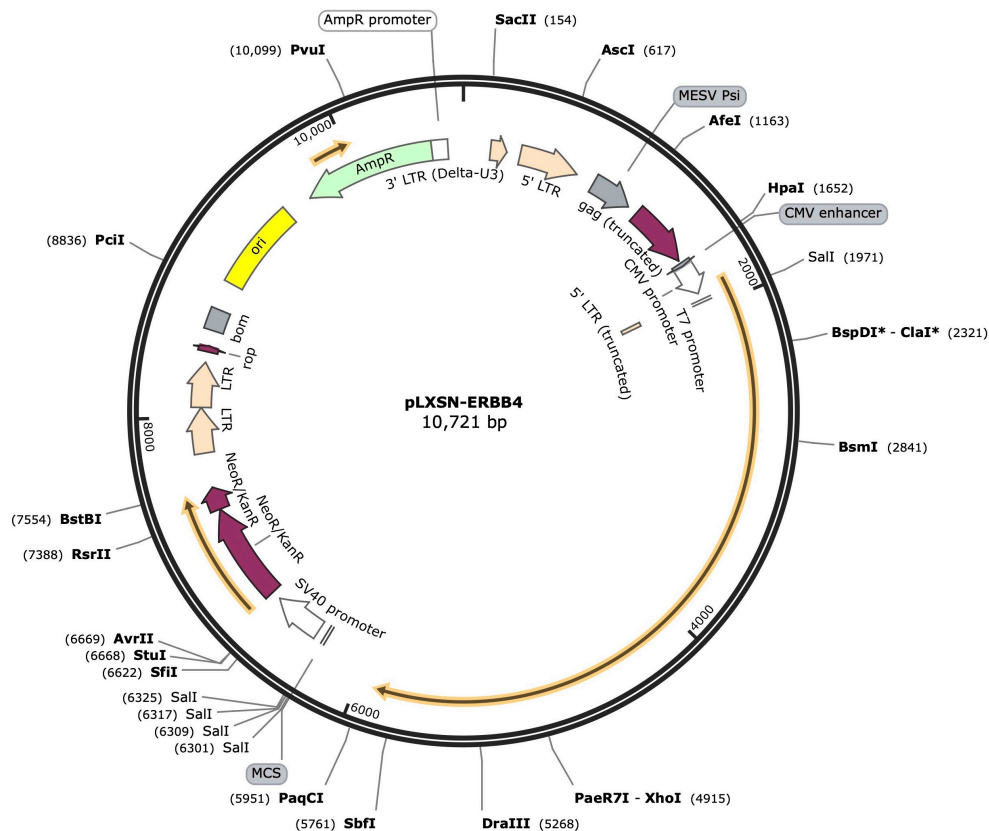


Figure 5. Map of the recombinant retroviral vector pLXSN-ERBB4. This map is derived from the NGS sequence of pLXSN-ERBB4 (**pLXSN-ERBB4.dna** - see link above). The sequence of this plasmid's region derived from pLXSN matches our sequence of pLXSN (**pLXSN.dna** - see link above). The sequence of the region of this plasmid that is derived from the *ERBB4* cDNA encodes the entire *ERBB4* coding sequence (nt 1983-5909; 1308 aa) and perfectly matches the corresponding sequence of the *ERBB4* cDNA [5].

Conclusion

- 8 Here we describe the construction of recombinant retroviral constructs based on pLXSN and carrying the human *EGFR*, *ERBB2*, *ERBB3*, or *ERBB4* genes. We have used these constructs to ectopically express these genes in various cell types, with the first example being the mouse BaF3 pro-B lymphocyte cell line [6]. In this example, we stably transduced the BaF3 cells with the recombinant retroviral constructs by electroporation. In subsequent studies, we packaged the recombinant retroviral constructs into high-titer amphotropic retrovirus particles suitable for infection of both

murine and human cell lines. We will describe the procedure for packaging and titering these recombinant retroviruses in a subsequent [protocols.io](https://doi.org/10.17504/protocols.io.261gedd97v47/v1) paper.

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