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

David J Riese
[1]

[1]Auburn University

David J Riese II
Auburn University

AUG 21, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.261gedd97v47/v1

Protocol Citation: David J Riese II 2023. Recombinant retroviral expression vectors based on pLXSN that encode EGFR, ERBB2, ERBB3, and ERBB4. **protocols.io** https://dx.doi.org/10.17504/protocols.io.261gedd97v47/v1

Protocol status: Working
We use this protocol and it's working

Created: Aug 21, 2023

DISCLAIMER

Last Modified: Aug 21, 2023

PROTOCOL integer ID: 86730

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.

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.

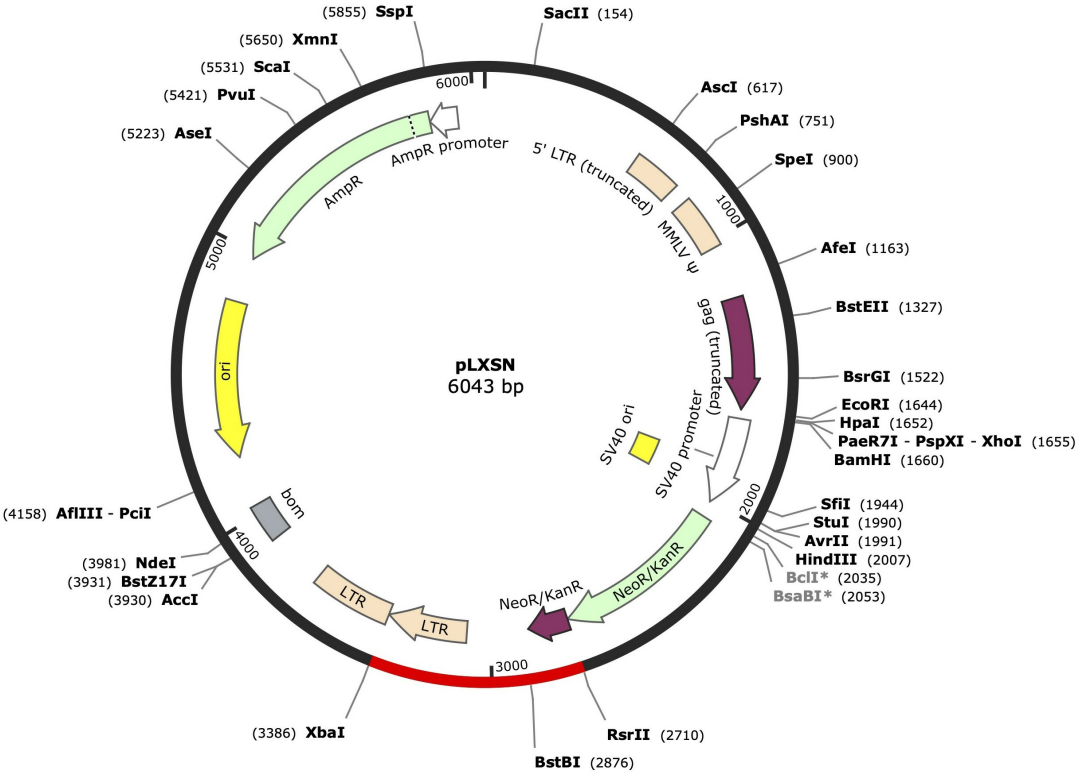

Figure 1. Map of the recombinant retroviral vector pLXSN. Noteworthy functional elements and unique restriction enzyme cleavage sites are depicted. Note that the 6043 bp sequence used to generate this map (**pLXSN.dna** - see link above) was generated by next-generation sequencing of our plasmid and 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) is shown in **Figure 2**. Note this strategy preserves the XhoI sites that flank the *EGFR* coding sequence.

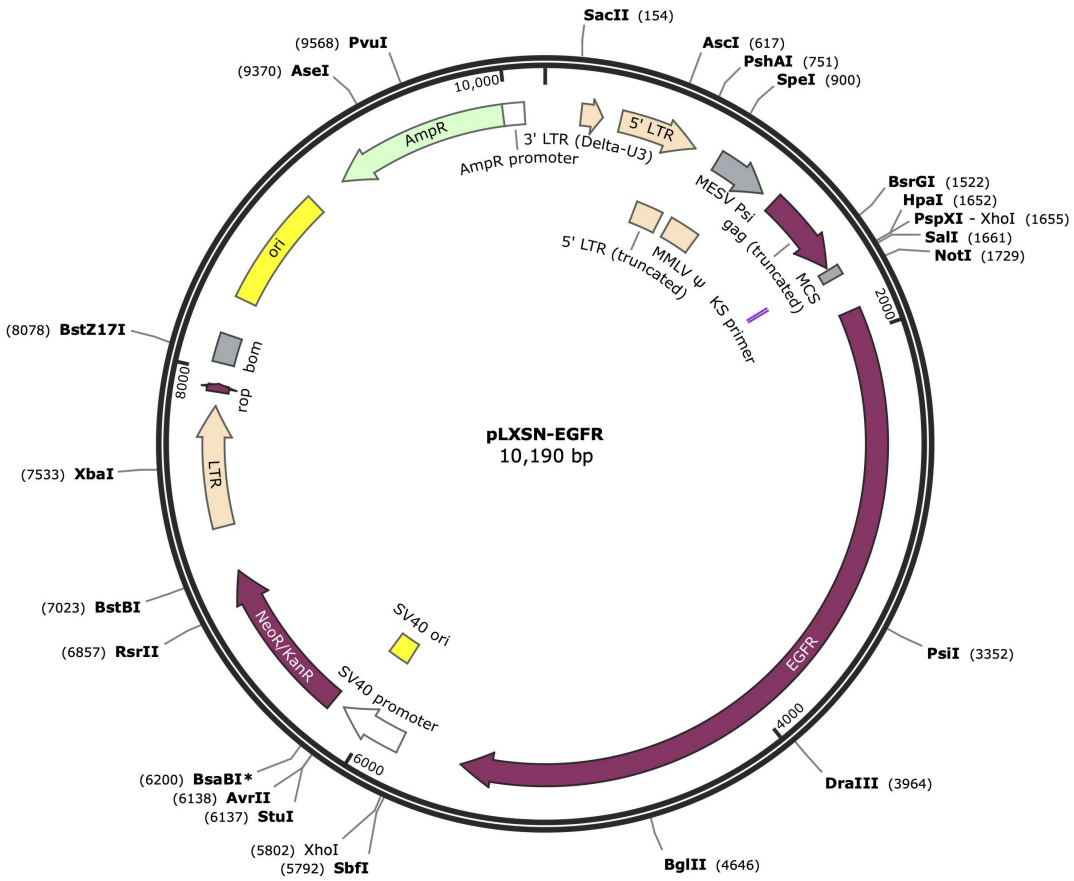

Figure 2. Map of the recombinant retroviral vector pLXSN-EGFR. This map is derived from the

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 NruI and DraI, 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 HpaI, which yields blunt ends. The ERBB2-blunt fragment was ligated to the pLXSN-HpaI 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) 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]**.

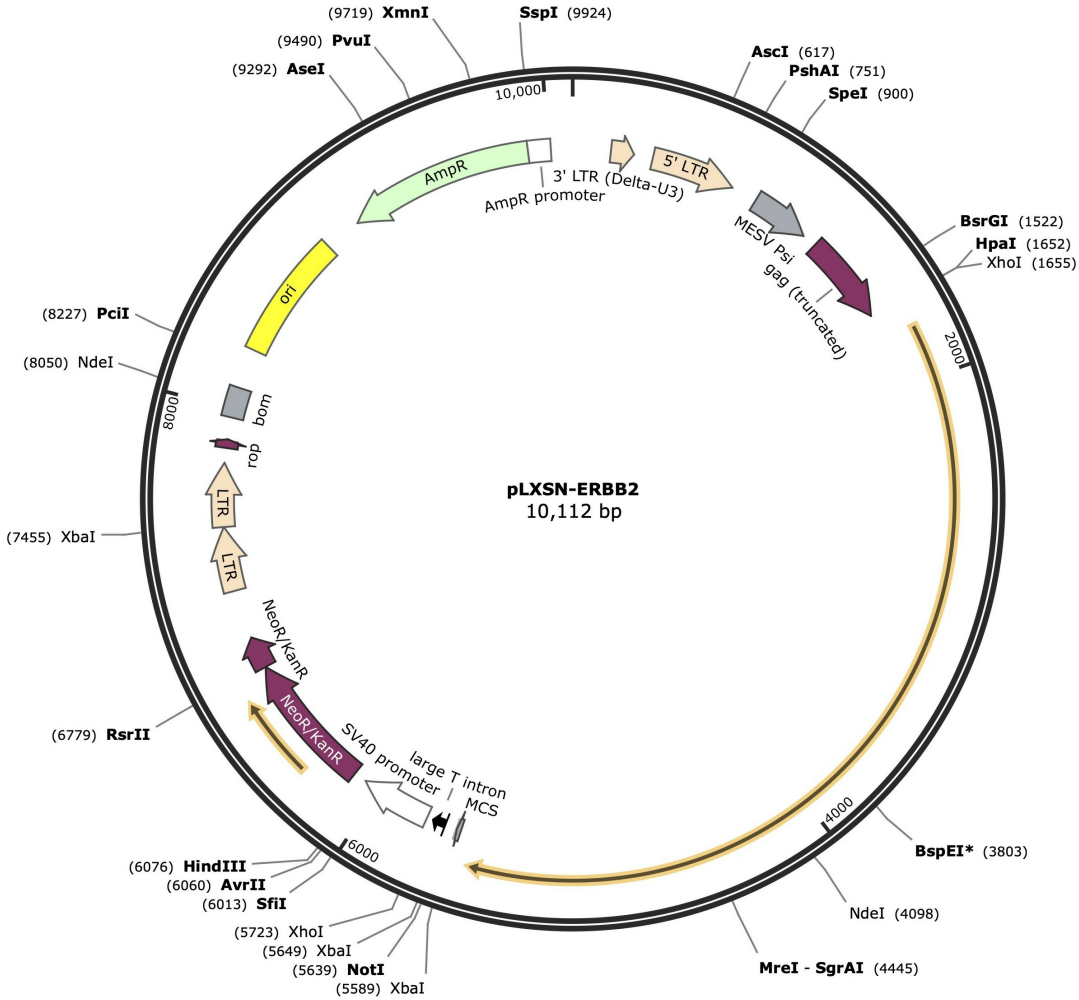

Figure 3. Map of the recombinant retroviral vector pLXSN-ERBB2. This map is derived from the NGS sequence of pLXSN-ERBB2 (**pLXSN-ERBB2.dna** - see link above). The sequence of this plasmid's region derived from pLXSN matches our sequence of pLXSN (**pLXSN.dna** - see link above). The sequence of the 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) 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.

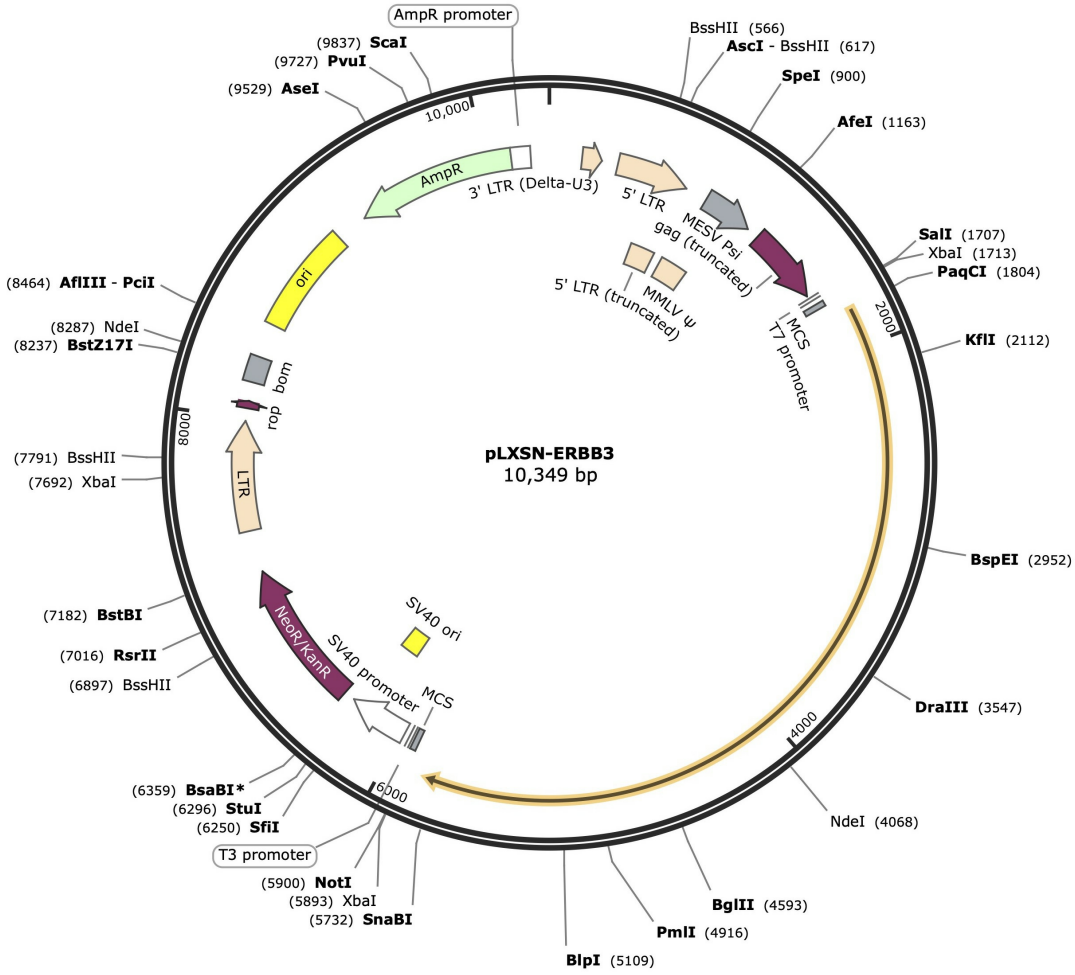

Figure 4. Map of the recombinant retroviral vector pLXSN-ERBB3. This map is derived from the NGS sequence of pLXSN-ERBB3 (**pLXSN-ERBB3.dna** - see link above). The sequence of this plasmid's region derived from pLXSN matches our sequence of pLXSN (**pLXSN.dna** - see link above). The sequence of the 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 SnaBI and SmaI, each cutting the plasmid at a single site and yielding the complete *ERBB4* cDNA with blunt ends. SalI linkers were added to this fragment. The recombinant retroviral vector pLXSN was linearized at the poly-cloning region by digestion with XhoI. The *ERBB4*-SalI 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 *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) is shown in **Figure 5**. Note that a SalI site is retained at the 5' end of the *ERBB4* cDNA insert, and multiple SalI sites are retained at the 3' end of the *ERBB4* cDNA insert.

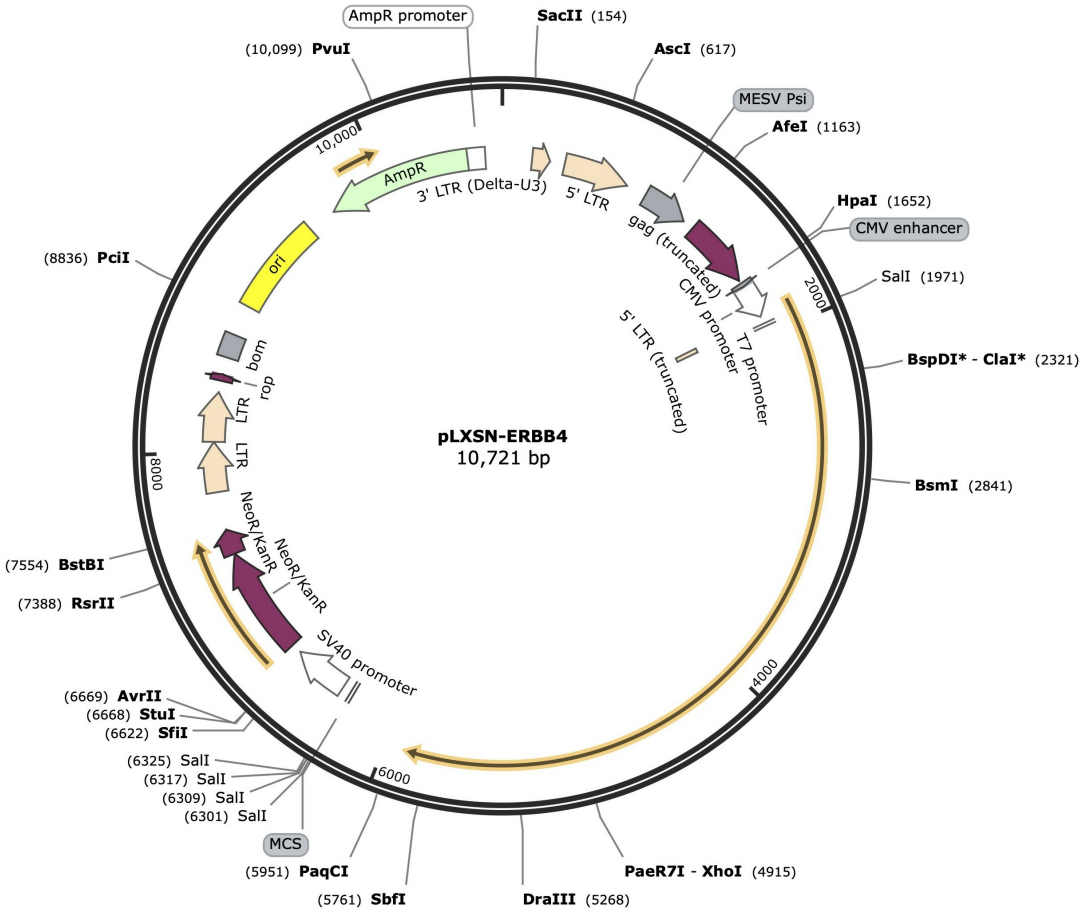

Figure 5. Map of the recombinant retroviral vector pLXSN-ERBB4. This map is derived from the NGS sequence of pLXSN-ERBB4 (**pLXSN-ERBB4.dna** - see link above). The sequence of this plasmid's region derived from pLXSN matches our sequence of pLXSN (**pLXSN.dna** - see link above). The sequence of the 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 paper.

References

9

1. Miller, A.D. and G.J. Rosman, *Improved retroviral vectors for gene transfer and expression.* Biotechniques, 1989. **7**(9): p. 980-2, 984-6, 989-90.

2. *Human EGFR cDNA - NM_005228.5*. [Accessed August 18, 2023]; Available from: https://www.ncbi.nlm.nih.gov/nuccore/NM_005228.

3. *Human ERBB2 cDNA - NM_004448.4*. [Accessed August 18, 2023]; Available from: https://www.ncbi.nlm.nih.gov/nuccore/NM_004448.4.

4. *Human ERBB3 cDNA - NM_001982.4*. [Accessed August 18, 2023]; Available from: https://www.ncbi.nlm.nih.gov/nuccore/NM_001982.

5. *Human ERBB4 cDNA - NM_005235.5*. [Accessed August 18, 2023]; Available from: https://www.ncbi.nlm.nih.gov/nuccore/NM_005235.3.

6. Riese, D.J., 2nd, et al., *The cellular response to neuregulins is governed by complex interactions of the erbB receptor family.* Mol Cell Biol, 1995. **15**(10): p. 5770-6.

7. Velu, T.J., et al., *Epidermal-growth-factor-dependent transformation by a human EGF receptor proto-oncogene.* Science, 1987. **238**(4832): p. 1408-10.

8. Plowman, G.D., et al., *Ligand-specific activation of HER4/p180erbB4, a fourth member of the epidermal growth factor receptor family.* Proc Natl Acad Sci U S A, 1993. **90**(5): p. 1746-50.

9. Plowman, G.D., et al., *Molecular cloning and expression of an additional epidermal growth factor receptor-related gene.* Proc Natl Acad Sci U S A, 1990. **87**(13): p. 4905-9.

10. *pLXSN - Addgene*. [Accessed August 18, 2023]; Available from: https://www.addgene.org/vector-database/3492/.

11. *pLXSN - Genbank M28248.1*. [Accessed August 18, 2023]; Available from: https://www.ncbi.nlm.nih.gov/nuccore/M28248.1.