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RESEARCH
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A recombinant retroviral expression vector (pLXSN-HygR) based on pLXSN that confers resistance to hygromycin and pLXSN-HygR derivatives that encode EGFR, ERBB2, or ERBB3

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We use this protocol and it's working

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ABSTRACT

Here we describe the construction of a derivative of pLXSN, called pLXSN-HygR, that expresses a hygromycin resistance gene rather than a neomycin resistance gene. We also describe the construction of pLXSN-HygR derivatives that express the *EGFR*, *ERBB2, or ERBB3* genes. Thus, these constructs are ideal for studying the functional effects of ERBB4 heterodimerization with EGFR, ERBB2, or ERBB3.

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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 We have previously reported the construction of recombinant retroviral expression vectors based on pLXSN **[1]** that express the *EGFR*, *ERBB2*, *ERBB3*, or *ERBB4* receptor tyrosine kinase genes **[2, 3]**. These constructs are useful for studying the role of these genes in regulating cellular proliferation, particularly in tumor cells.

3 However, ERBB family receptor tyrosine kinases can undergo heterodimerization, thereby diversifying the effects of ERBB receptor signaling **[4, 5]**. Therefore, there is a need for constructs that enable the simultaneous ectopic expression of two ERBB receptor tyrosine kinase genes. Hence, here we describe the construction of a derivative of pLXSN, called pLXSN-HygR, that expresses a hygromycin resistance gene rather than a neomycin resistance gene. We also describe the construction of pLXSN-HygR derivatives that express the *EGFR*, *ERBB2, or ERBB3* genes. Thus, these constructs are ideal for studying the functional effects of ERBB4 heterodimerization with EGFR, ERBB2, or ERBB3.

Methods

4 **Construction of pLXSN-HygR**

We have previously briefly described the construction of pLXSN-HygR **[6]**. The plasmid pSV2-Hyg **[7]**, which encodes the hygromycin resistance gene **[8]**, is a generous gift from Daniel DiMaio.

5 The plasmid pSV2-Hyg was digested with BglII, after which the overhang was filled using the Klenow fragment of the *E. Coli* DNA polymerase I. This product was digested with HindIII, releasing a 1348 bp fragment that contains the hygromycin resistance gene; the hygromycin

resistance gene is 1026 bp in length (nt 2050 to 3075 of pLXSN-HygR) and encodes a 341 aa protein. Therefore, this fragment was used as the insert for pLXSN-HygR. The HindIII site of this fragment lies approximately 30 nt upstream of the start codon of the hygromycin resistance gene, whereas the former BglII site of this fragment lies approximately 290 nt downstream of the stop codon of the hygromycin resistance gene.

6 The plasmid pLXSN [1, 3] (pLXSN.dna – **Figure 1**) was digested with HindIII and NaeI. Note that NaeI produces blunt ends. The largest fragment (5071 bp) of this digestion is missing the neomycin resistance gene and was used as the vector for pLXSN-HygR.

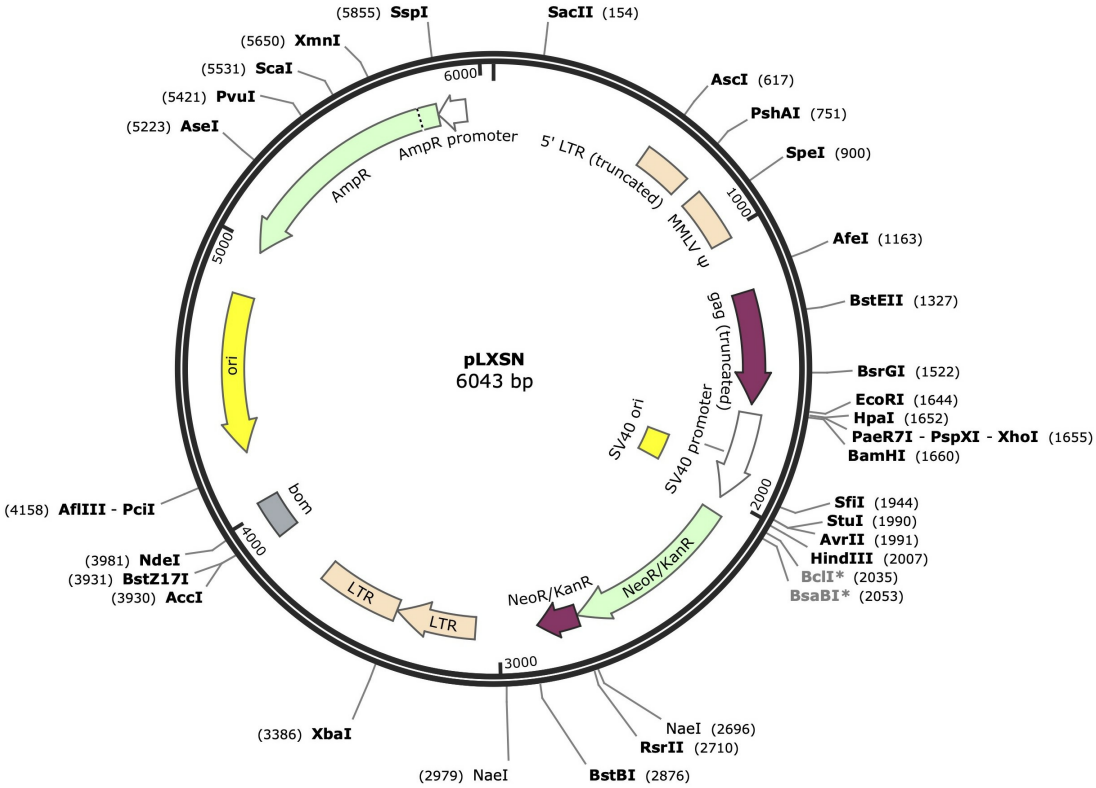

Figure 1. A map of pLXSN (**pLXSN.dna** – see link above) is shown. Note that the neomycin resistance gene is flanked at the 5' end by a unique HindIII site (nt 2007) and at the 3' end by a NaeI site (nt 2979). Thus, the 5071 bp NaeI-HindIII fragment of pLXSN serves as the vector for pLXSN-HygR.

7 The pLXSN-NaeI-HindIII fragment was ligated with the pSV2-Hyg-HindIII-Blunt fragment and the ligation reaction product was electro-transformed into DH10B *E. coli*. Ampicillin-resistant colonies of *E. coli* were expanded, and minipreps were screened for the presence and correct orientation of the hygromycin resistance gene insert. NGS has validated this construct, and the

map of the resulting sequence (pLXSN-HygR.dna) is shown in **Figure 2**.

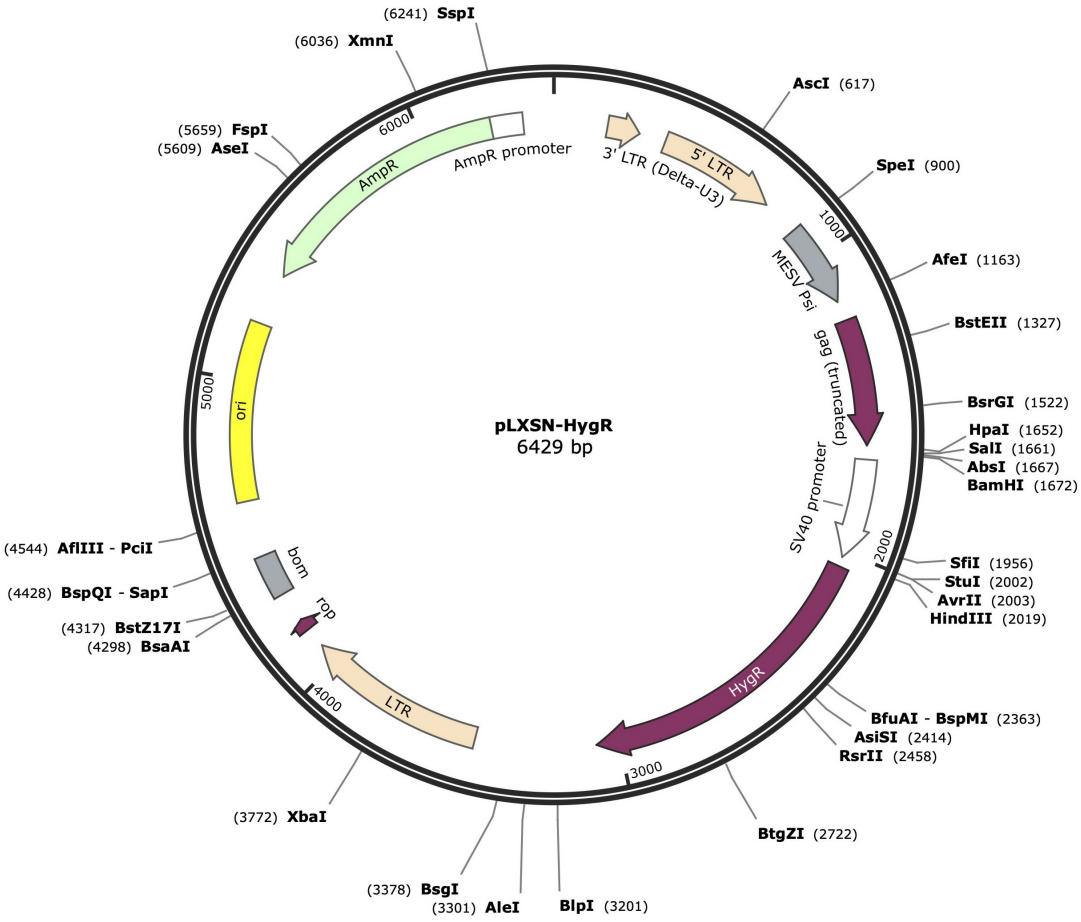

Figure 2. A map of pLXSN-HygR (**pLXSN-HygR.dna** – see above for link) is shown. Note the unique AscI, AvrII, XmnI, HindIII, AseI, and StuI sites used to generate pLXSN-HygR-EGFR, pLXSN-HygR-ERBB2 and pLXSN-HygR-ERBB3.

8 Construction of pLXSN-HygR-EGFR

To generate the pLXSN-HygR vector fragment, which contains the hygromycin resistance gene, we digested pLXSN-HygR (**pLXSN-HygR.dna** – see above for link) with AvrII and AscI (**Figure 2**) and isolated the 5043 bp fragment. To generate the *EGFR* cDNA fragment, we digested pLXSN-EGFR (pLXSN-EGFR.dna) [2, 3] with AscI and AvrII and isolated the 5521 bp fragment (**Figure 3**).

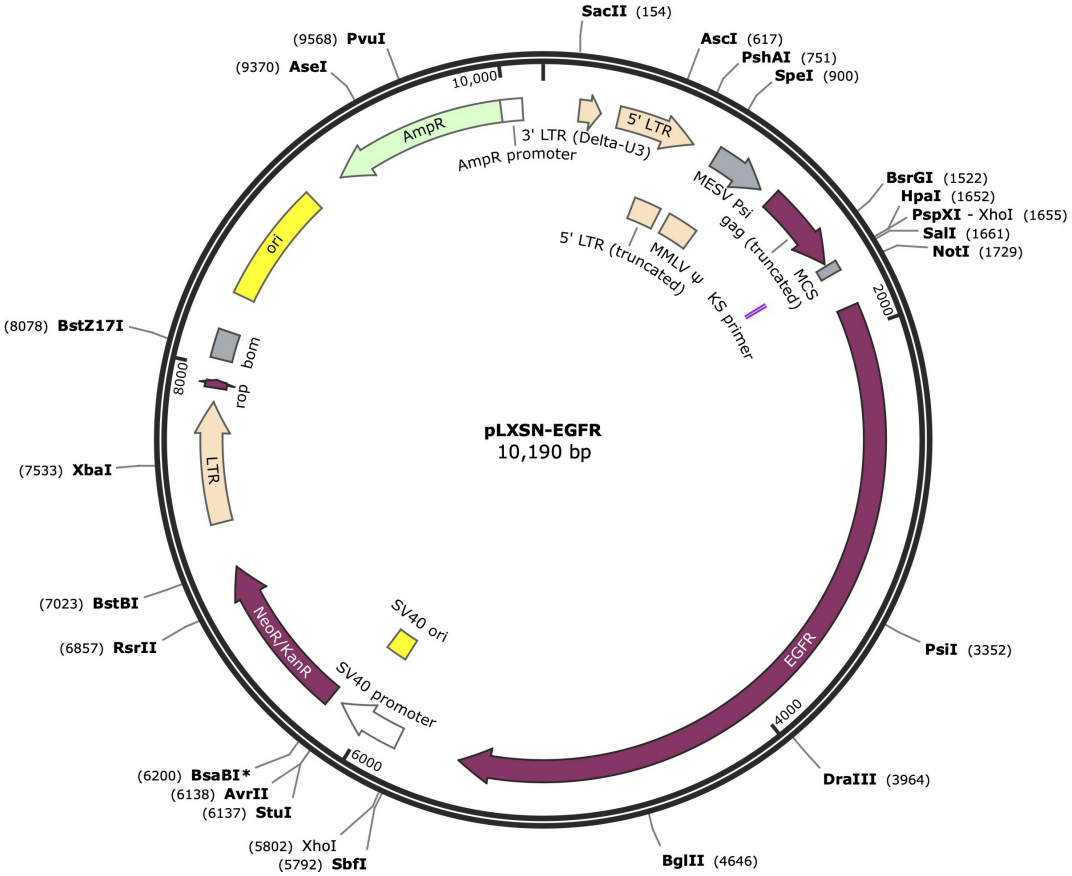

Figure 3. A map of pLXSN-EGFR (**pLXSN-EGFR.dna** – see above for link) is shown. Note the unique AscI site at nt 617 and the unique AvrII site at nt 6138. The 5521 bp AscI-AvrII fragment of pLXSN-EGFR encodes the *EGFR* cDNA and was used to construct pLXSN-HygR-EGFR.

9 The pLXSN-HygR-AvrII-AscI fragment was ligated with the pLXSN-EGFR-AscI-AvrII fragment and the ligation reaction product was electro-transformed into DH10B *E. coli*. Ampicillin-resistant colonies of *E. coli* were expanded, and minipreps were screened for the correct recombinant plasmid. NGS has validated this construct, and the map of the resulting sequence (pLXSN-HygR-EGFR.dna) is shown in **Figure 4**.

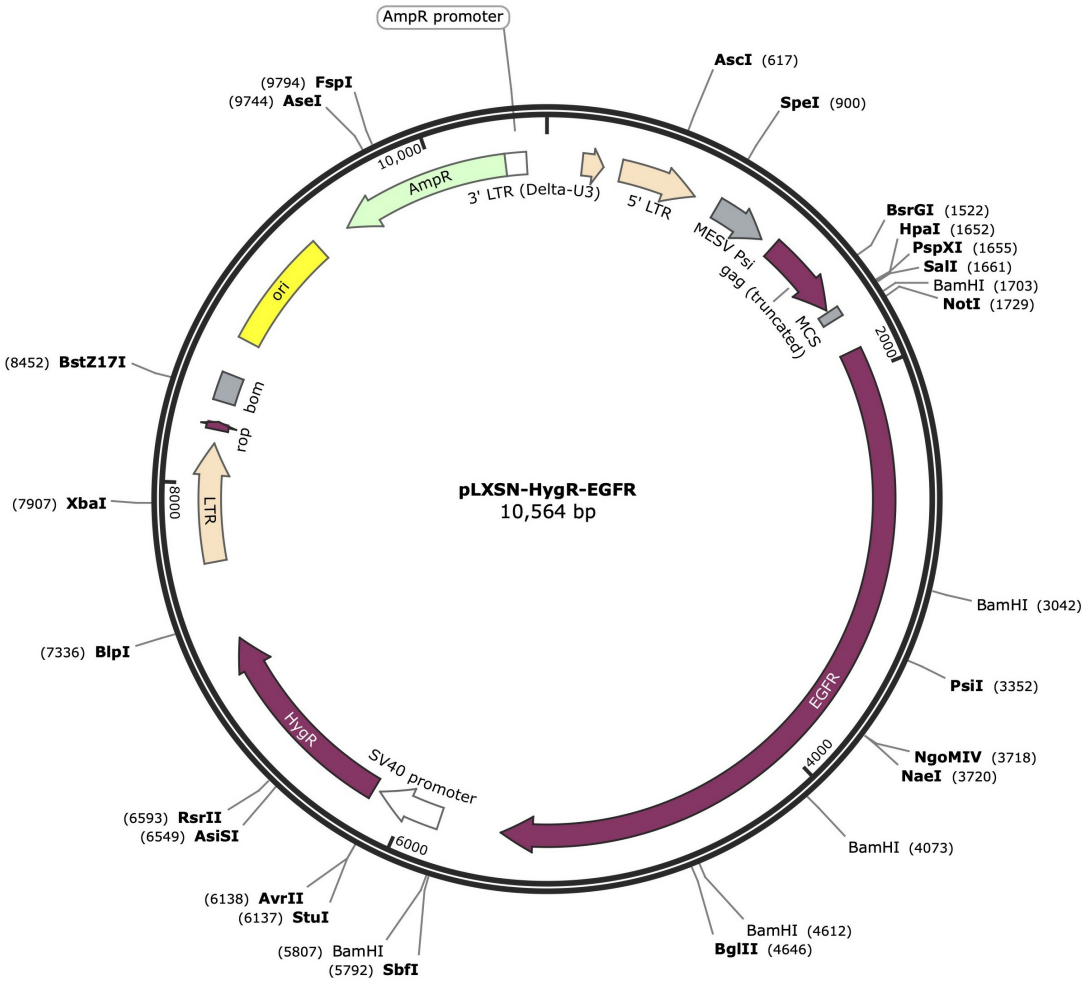

Figure 4. A map of pLXSN-HygR-EGFR (**pLXSN-HygR-EGFR.dna** – see above for link) is shown.

10 Construction of pLXSN-HygR-ERBB2

We have previously briefly described the construction of pLXSN-HygR-ERBB2 [9]. To generate the pLXSN-HygR vector fragment, which contains the hygromycin resistance gene, we digested pLXSN-HygR (**pLXSN-HygR.dna** – see above for link) with HindIII and XmnI (**Figure 2**) and isolated the 4017 bp fragment. To generate the *ERBB2* cDNA fragment, we digested pLXSN-ERBB2 (pLXSN-ERBB2.dna) [2, 3] with XmnI and HindIII and isolated the 6469 bp fragment (**Figure 5**).

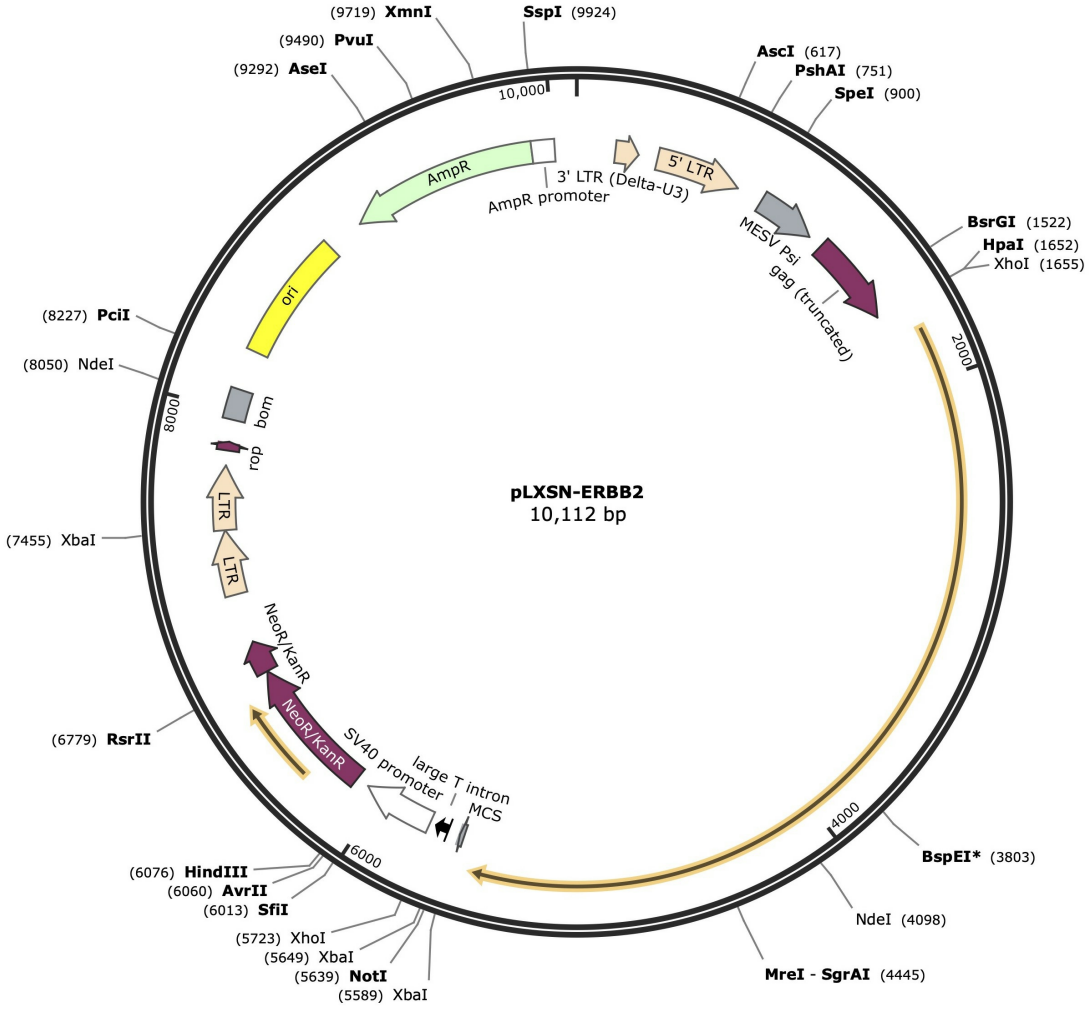

Figure 5. A map of pLXSN-ERBB2 (**pLXSN-ERBB2.dna** – see above for link) is shown. Note the unique HindIII site at nt 6076 and the unique XmnI site at nt 9719. The 6469 bp XmnI-HindIII fragment of pLXSN-ERBB2 encodes the *ERBB2* cDNA and was used to construct pLXSN-HygR-ERBB2.

11 The pLXSN-HygR-HindIII-XmnI fragment was ligated with the pLXSN-ERBB2-XmnI-HindIII fragment and the ligation reaction product was electro-transformed into DH10B *E. coli*. Ampicillin-resistant colonies of *E. coli* were expanded, and minipreps were screened for the correct recombinant plasmid. NGS has validated this construct, and the map of the resulting sequence (pLXSN-HygR-ERBB2.dna) is shown in **Figure 6**.

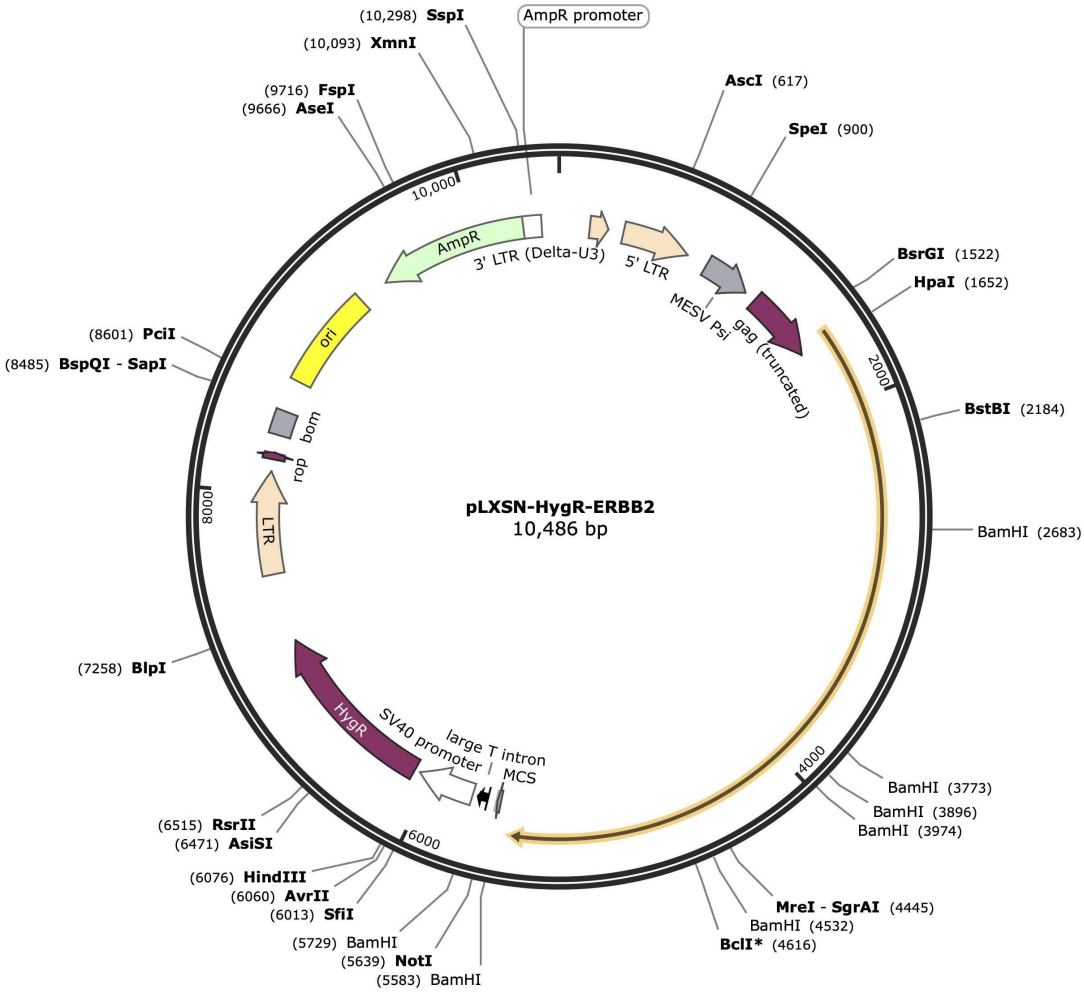

Figure 6. A map of pLXSN-HygR-ERBB2 (**pLXSN-HygR-ERBB2.dna** – see above for link) is shown.

12 Construction of pLXSN-HygR-ERBB3

We have previously briefly described the construction of pLXSN-HygR-ERBB3 [9]. To generate the pLXSN-HygR vector fragment, which contains the hygromycin resistance gene, we digested pLXSN-HygR (**pLXSN-HygR.dna** – see above for link) with StuI and AseI (**Figure 2**) and isolated the 3607 bp fragment. To generate the *ERBB3* cDNA fragment, we digested pLXSN-ERBB3 (pLXSN-ERBB3.dna) [2, 3] with AseI and StuI and isolated the 7116 bp fragment (**Figure 7**).

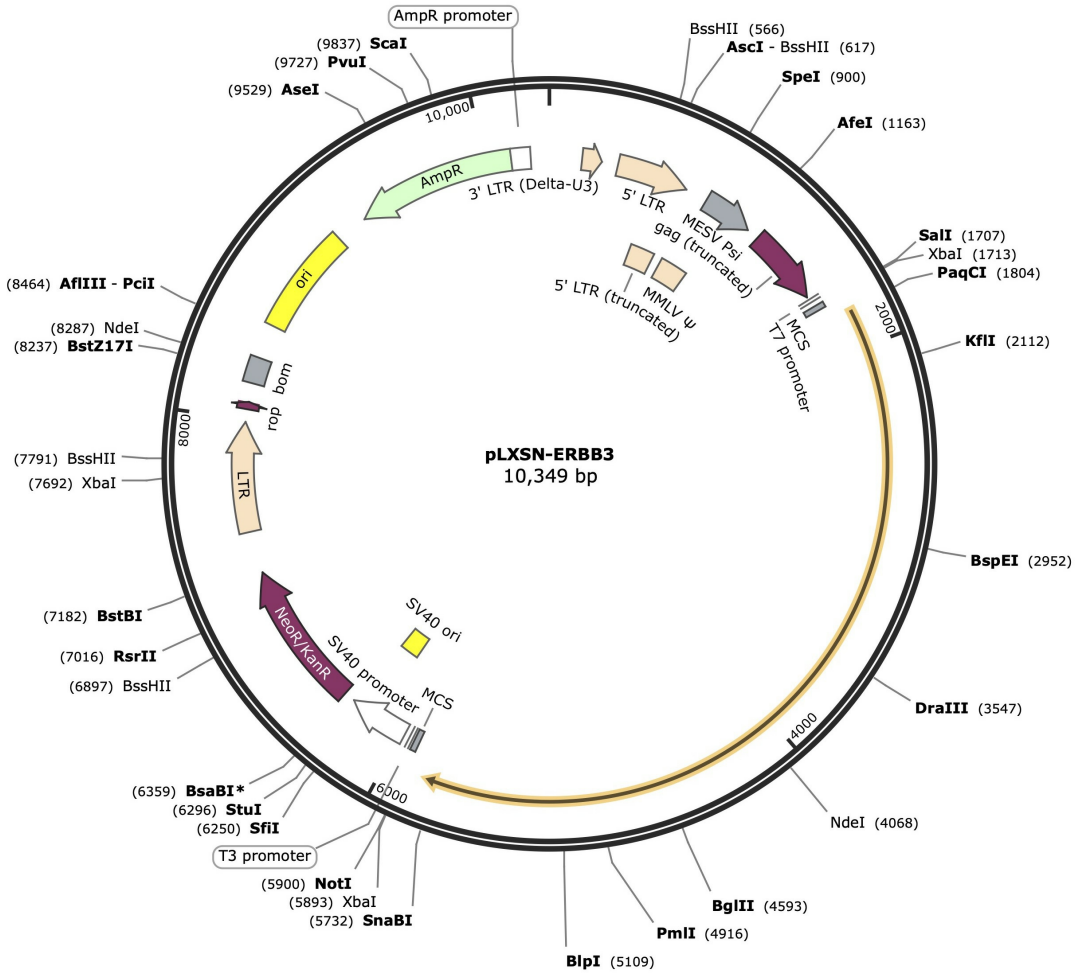

Figure 7. A map of pLXSN-ERBB3 (**pLXSN-ERBB3.dna** – see above for link) is shown. Note the unique StuI site at nt 6296 and the unique AseI site at nt 9529. The 7116 bp AseI-StuI fragment of pLXSN-ERBB3 encodes the *ERBB3* cDNA and was used to construct pLXSN-HygR-ERBB3.

13 The pLXSN-HygR-StuI-AseI fragment was ligated with the pLXSN-ERBB3-AseI-StuI fragment and the ligation reaction product was electro-transformed into DH10B *E. coli*. Ampicillin-resistant colonies of *E. coli* were expanded, and minipreps were screened for the correct recombinant plasmid. NGS has validated this construct, and the map of the resulting sequence (pLXSN-HygR-ERBB3.dna) is shown in **Figure 8**.

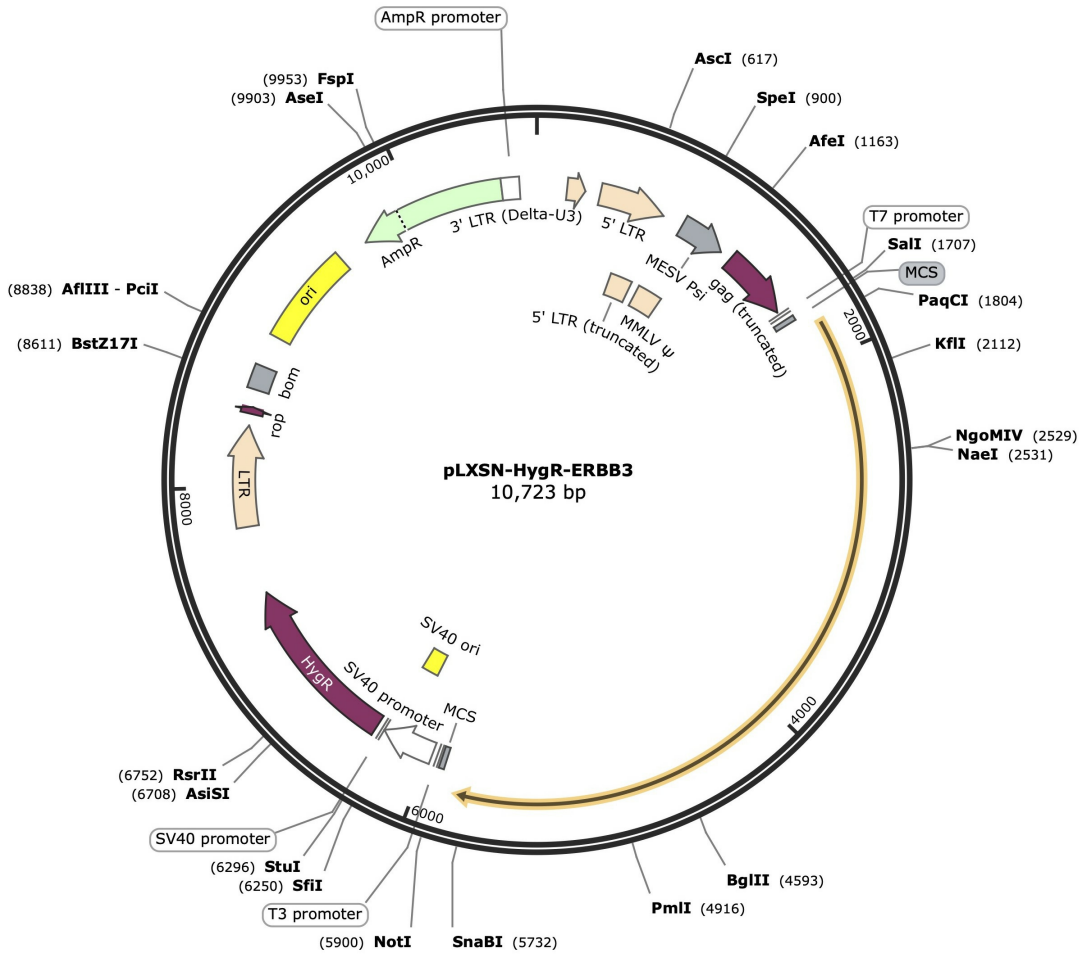

Figure 8. A map of pLXSN-HygR-ERBB3 (**pLXSN-HygR-ERBB3.dna** – see above for link) is shown.

Conclusion

14 Here we describe the construction of a recombinant retroviral vector (pLXSN-HygR) that carries the hygromycin resistance gene instead of the neomycin resistance gene, as well as derivatives that also carry the *EGFR*, *ERBB2*, or *ERBB3* cDNAs (pLXSN-HygR-EGFR, pLXSN-HygR-ERBB2, and pLXSN-HygR-ERBB3, respectively). These constructs, together with the recombinant retroviral vector that carries the neomycin resistance gene and the *ERBB4* cDNA (pLXSN-ERBB4) **[2, 3]**, will enable us to generate bi-recombinant cell lines to study the effects of ERBB4 receptor heterodimerization with the other ERBB family receptors.

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