pGLuc-Basic

Sequence file available at www.neb.com.

There are no restriction sites for the following enzymes: AarI(x), AfeI, AflII, AgeI, AleI, ApaI, AscI, AsiSI, BaeI, BbvCI, BlpI, BmgBI, BmtI, Bpu10I, BsiWI, BsmBI, BspDI, BspEI, BsrGI, BstEII, BstXI, Bsu36I, ClaI, CspCI, EcoNI, EcoO109I, FseI, FspAI(x), HpaI, MfeI, MluI, NdeI, NheI, PacI, PflMI, PmeI, PmlI, PpuMI, PshAI, PspOMI, SanDI(x), SbfI, SfiI, SgrAI, SnaBI, SpeI, SrfI(x), SwaI, XcmI

(x) = enzyme not available from NEB

pGLuc-Basic is a plasmid cloning vector capable both of replication in *E. coli* and stable transfection of mammalian cells. It is designed for the cloning of promoter sequences and measurement of their transcription activity using the *Gaussia* Luciferase Assay Kit (NEB #E3300).

In *E. coli*, it replicates using the pMB1 origin of replication from pBR322 (although the *rop* gene is missing) and carries the *bla* (Ap^R) marker for selection with ampicillin. It also carries the *nptII* (Nm^R) marker under control of an SV40 promoter; thus, following transfection into mammalian cells, it can be used to form stable cell lines by selection with geneticin (G418).

The multiple cloning site (MCS) is positioned immediately upstream of a promoterless reporter gene, GLuc (the humanized coding sequence for the secreted *Gaussia princeps* luciferase) (1), which is followed by a synthetic polyadenylation (polyA) sequence (not shown). Thus, in mammalian cells the transcriptional activity of promoter sequences cloned into the MCS can be assessed by measuring GLuc activity in the culture medium.

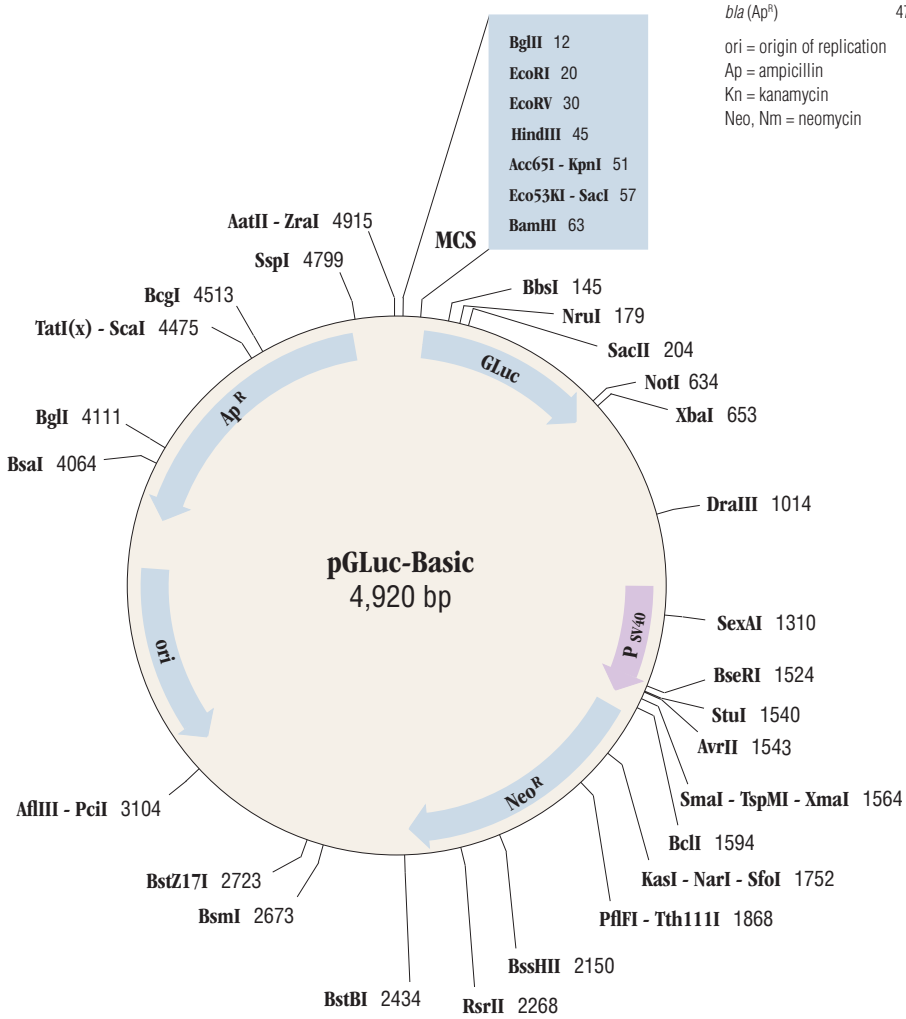
Enzymes with unique restriction sites are shown in **bold** type. Location of sites of all NEB restriction enzymes can be found on the NEB web site (choose Technical Reference > DNA Sequences and Maps). Restriction site coordinates refer to the position of the 5'-most base on the top strand in each recognition sequence.

Open reading frame (ORF) coordinates are in the form "translational start – translational stop"; numbers refer to positions on the top (clockwise) strand, regardless of the direction of transcription and include the start and stop codons.

pMB1 origin of replication coordinates include the region from the –35 promoter sequence of the RNAII transcript to the RNA/DNA switch point. SV40 promoter coordinates represent the cloned region and not necessarily the precise functional elements.

Feature	Coordinates	Source
GLuc	76-633	*G. princeps*
SV40 promoter region	1238-1573	SV40
aph(3´)-II (*nptII*; Nm^R; Kn^R)	1625-2419	*Tn5*
origin	3753-3165	pMB1
bla (Ap^R)	4784-3924	*Tn3*

ori = origin of replication
Ap = ampicillin
Kn = kanamycin
Neo, Nm = neomycin

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              BglII   EcoRI     EcoRV          HindIII
 1   GACGGATCGGGAGATCTTGGAATTCTGCAGATATCCTCGAGCCCAAGCTT     50

     KpnI  SacI  BamHI
51   GGTACCGAGCTCGGATCCAGCCACCATGGGAGTCAAAGTTCTGTTTGCCC    100
                          M  G  V  K  V  L  F  A...
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GLuc

Reference

(1) Tannous, B.A. et al. (2005) *Mol. Ther.*, 11, 435-443.