pNEBR-X1Hygro

Sequence file available at www.neb.com
See page 165 for ordering information.

There are no restriction sites for the following enzymes: AarI(x), Acc65I, AfeI, AflII, AgeI, AleI, AvrII, BaeI, BbsI, BbvCI, BclI, BglII, BlpI, BmgBI, Bpu10I, BseRI, BsiWI, BspDI, BstEII, BstXI, BstZ17I, Bsu36I, ClaI, CspCI, EcoNI, FspAI(x), I-CeuI, I-SceI, KpnI, MscI, NruI, NsiI, PI-PspI, PI-SceI, PmeI, PmlI, SbfI, SexAI, SfiI, SgrAI, SmaI, SnaBI, SpeI, SrfI(x), SwaI, TspMI, XmaI, XmnI

(x) = enzyme not available from NEB

pNEBR-X1Hygro is a plasmid cloning vector capable both of replication in *E. coli* and stable transfection of mammalian cells. It is designed for inducible expression of recombinant proteins in mammalian cells using the RheoSwitch Mammalian Inducible Expression System (NEB #E3000).

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 *hpt* (Hyg^R) marker under control of the thymidine kinase promoter; thus, following transfection into mammalian cells, it can be used to form stable cell lines by selection with hygromycin.

The multiple cloning site (MCS) is positioned downstream of a promoter apparatus consisting of 5 tandem copies of the yeast *GAL4* response element (5XRE) followed by a minimal TATA box and a short leader sequence, and upstream of the SV40 polyadenylation (polyA) sequence. When co-transfected in mammalian cells with pNEBR-R1 (which encodes the RheoReceptor-1 protein), expression from the *GAL4*-derived promoter can be induced by the synthetic RSL1 ligand and controlled in a RSL1 concentration-dependent manner. A transcription terminator upstream of the 5XRE (not shown) prevents read-through transcription from other sources.

pNEBR-X1 is identical to pNEBR-X1Hygro except that it does not contain the *hpt* (Hyg^R) marker, so it is intended to be used for transient transfection. pNEBR-X1GLuc is a control plasmid with the reporter gene GLuc (the humanized coding sequence for the secreted *Gaussia princeps* luciferase) (1) cloned into the HindIII-NotI sites of pNEBR-X1.

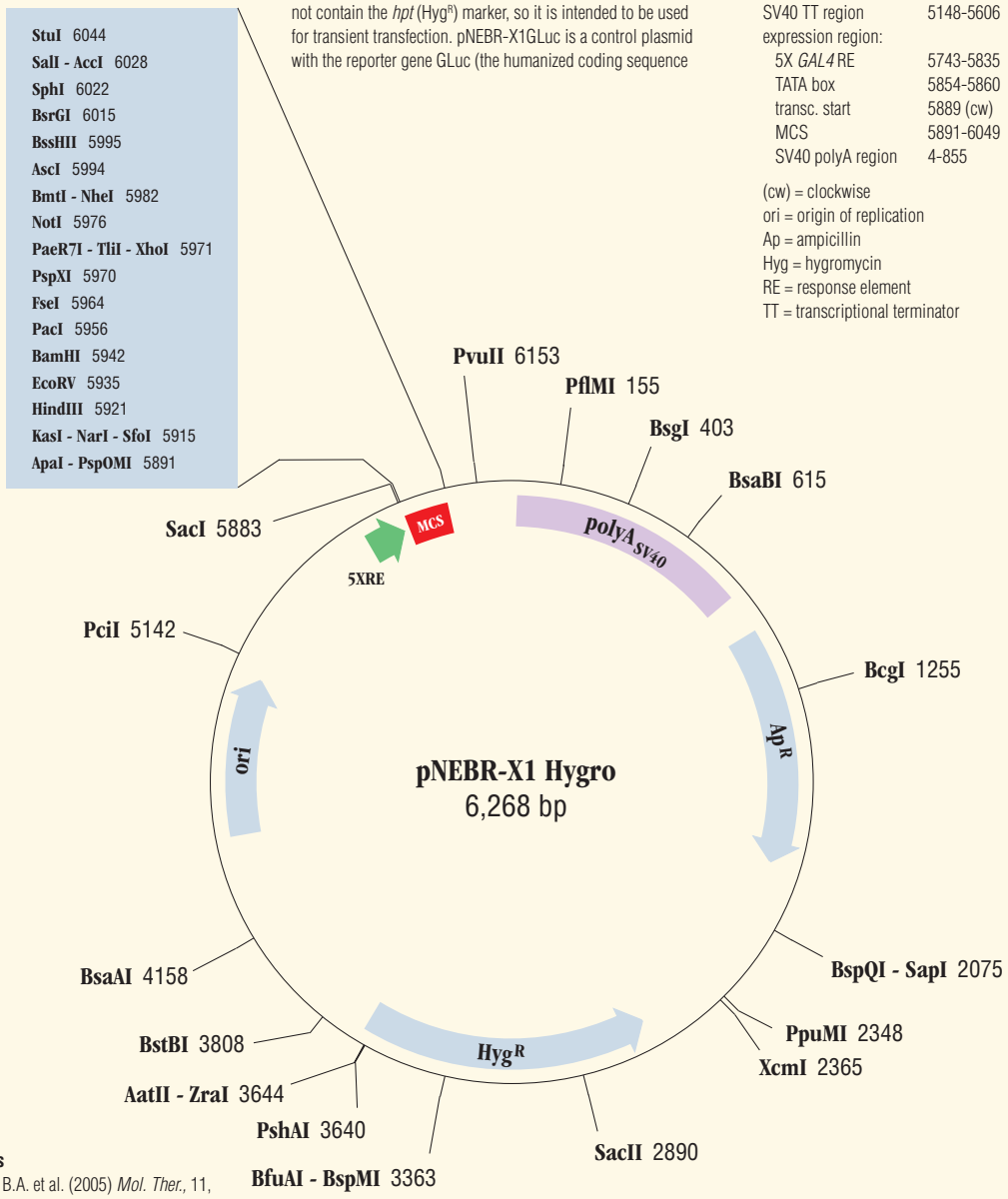
Enzymes with unique restriction sites are shown in **bold** type. Location of sites of all NEB restriction enzymes can be found on the NEB web site (choose Technical Reference > DNA Sequences and Maps). Restriction site coordinates refer to the position of the 5'-most base on the top strand in each recognition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

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

Feature	Coordinates	Source
bla (Ap^R)	995-1855	*Tn3*
aph-IV (hpt; Hyg^R)	3672-2635	*S. hygroscopicus*
origin	4498-5086	pMB1
SV40 TT region	5148-5606	SV40
expression region:		
5X *GAL4* RE	5743-5835	*S. cerevisiae*
TATA box	5854-5860	–
transc. start	5889 (cw)	–
MCS	5891-6049	–
SV40 polyA region	4-855	SV40

(cw) = clockwise
ori = origin of replication
Ap = ampicillin
Hyg = hygromycin
RE = response element
TT = transcriptional terminator



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