R26(SYNbglA)R Mouse

Catalogue number: 151653
Sub-type: Mouse
Images:

Contributor

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Images:

Tool details

***FOR RESEARCH USE ONLY**

Name: R26(SYNbglA)R Mouse

Alternate name:

Class:
Conjugate:
Description: Scientific value: The reporter gene has been characterised and compared with LacZ driven beta-galactosidase (b-gal). B-gal and b-glu can both be detected using colourimetric assays and standard IHC, and display similar levels of protein stability. b-glu was resistant to epigenetic silencing in stably transfected cell lines (LacZ occasionally shows epigenetic silencing- although has not been directly compared in this system). In contrast to beta-gal (LacZ driven), beta-glu is thermostable, allowing for preperation of paraffin embedded versus frozen sections, and an increased in image/IHC quality. The distribution of high endogenous activity of beta-glu is also different to beta-gal, which may provide an advantage in certain tissues. Endogenous b-glu can be inactivated due to the thermostability of the enzyme. When crossed with Cre-expressing mice (for example AhCre), the efficiency of Cre-recombination is also more consistent in R(SYNbglA)26R mice compared to the R26R (LacZ-ROSA26) reporter mouse. In the AhCre-R(SYNbglA)26R cross this manifests in a reduced gradient of b-glu expression from proximal to distal in the gut epithelium compared to b-gal in the AhCre-R26R mice. This is likely to be a result of the mammalian codon usage optimization which has been performed for SYNbglA, but not for LacZ. The specific product, the R(SYNbglA)26R mouse, allows for monitoring of Cre-recombination efficiency, and can be used to in combination with temporal/tissue specific Cre expression to stain tissues and/or cells. This has been validated using the AhCre model. Commerical value: b-glu clearly offers a number of advantages over b-gal as a reporter for use in vivo and in vitro. In particular, the R(SYNbglA)26R mouse may be sold on single use license to academic institutes or through a resale licensing deal to mouse model companies through CRTs reagents business (DaRT). No patent protection is possible as the sequence of SYNbglA has been published on Genbank. SYNbglA is a synthetic gene generated from bglA, which was cloned from

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Caldocellum saccharolyticum. SYNbglA has been optimised for mammalian codon usage and resistance to epigenetic silencing. The SYNbgl1 gene product is a thermostable (up to 85oC) beta-glucosidase (b-glu).A ROSA26-SYNbglA reporter mouse (R(SYNbglA)26R) has been generated by targeting the ubiquitously expressed murine ROSA26 locus with SYNbglA containing a floxP-flanked STOP cassette preventing expression of the downstream SYNbglA gene. When crossed with a Cre transgenic strain, the STOP sequence is removed and SYNbglA is expressed in cells/tissues where Cre is expressed. These mutant mice may be used as a Cre-reporter strain; to test the tissue/cellular expression pattern of Cre transgenic mice, or to produce tissue specific expression of b-glu for the purposes of cell labelling.

Purpose:

Parental cell:

Organism:

Tissue:

Model:

Gender:

Isotype:

Reactivity:

Selectivity:

Host:

Immunogen:

Immunogen UNIPROT ID:

Sequence:

Growth properties:

Production details: The bglA sequence was initially PCR amplified from pNZ1065 (Pacific Enzymes Limted) and subcloned into pCR3 (Invitrogen) using primers (5â?? ttccatggggatcctaagtttcccaaaaggatttttgtgg 3â?? and â??ttagatctgtcgacttacgaattttcctttatatactg 3â??) designed to introduce a consensus translation start sequence and flanking restriction sites for subsequent cloning. This bglA fragment was subcloned into pEF1alpha to generate an expression construct (pEFbglA) containing the human type 1 elongation factor promoter and SV40 polyadenylation sequence. For comparative purposes pEFlacZ was also generated by conventional subcloning. To generate the final gene targeting construct conventional cloning was performed to make a cassette comprising loxP-PGKneo.pA-loxP SYNbglA.pA which was subcloned into pROS-MCS-13 containing the two arms of ROSA26 locus homology. Additional cloning details are available upon request. Gene targeting of a construct containing the loxP-PGKneo-loxP SYNbglA cassette (pROSA-MCS13-puro) was performed at the ROSA26 locus to generate R26(SYNbglA)R.ES cell manipulations procedures were performed by the Gene Targeting Service, Babraham Institute (Babraham, Cambridge, UK). E14 129Ola ES clones were selected with G418 and colonies initially screened by PCR using primers anchored 5â?? to the shorter targeting arm (R26TOPV: 5â?? ggtagtggggtcgactagatgaaggagagcc 3â??) and at the introduced splice acceptor (R26SAmut: gtcctcaaccgcgagctgtg) which amplified a unique 4kb band. Two clones were selected following further screening by Southern blotting using a probe located 5â?? to the targeting vector as described previously (Kim DW et al (1990). Gene, 91: 217-223). These clones, C8 and C10, were microinjected into blastocysts and the resultant chimeras used to establish R26(SYNbglA)R mice. Both clones were evaluated for expression of reporter following induction of Cre in double transgenic Ahcre/ R26(SYNbglA)R mice and were found to behave identically.

Formulation:

Recommended controls:

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Bacterial resistance:
Selectable markers:
Additional notes:

Target details

Target: SYNbglA/beta-glucosidase

Target alternate names:

Target background:

Molecular weight:

Ic50:

Applications

Application:
Application notes:

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Handling

Format:
Concentration:
Passage number:
Growth medium:
Temperature:
Atmosphere:
Volume:
Storage medium:
Storage buffer:
Storage conditions:
Shipping conditions: Embryo/Spermatoza- Dry Ice

Related tools

Related tools:

References

References:

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