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Pdx1-tTA - Mouse Strain RES220

Mouse Information

Common Name:	Pdx1-tTA
MGI Official Name:	STOCK Pdx1^{tm1Macd}/J
Description:	Mice homozygous for the targeted mutation fail to develop a pancreas. Heterozygous mice have normal pancreatic development, but have partially impaired glucose tolerance in adulthood. The substitution of the targeted Ipf1/Pdx1 gene with tTAoff inactivates the endogenous allele and places tTAoff expression under the control of the endogenous transcriptional regulatory sequences of the Pdx1 locus. Identical to the endogenous allele, mutant locus expression is detectable in the pancreas and adjacent duodenum but not in other visceral organs or salivary glands. This mutant may be useful to direct tetracycline-regulated expression of responder transgenes in studies of pancreatic endocrine/exocrine development and function and diabetes. This mutant can also be bred with other tetO/TRE strains for pancreas-specific applications. This mutant was originally designed to be mated with mice engineered with a heptameric tetracycline operator (tetO)-controlled bicistronic transgene coding for a normal PDX1 protein and with a beta-galactosidase or EGFP reporter (see BCBC mouse M561). The combined modifications allow normal pancreatic development and function until doxycycline-administration renders the mouse conditionally null of the Pdx1 gene. This configuration for conditional expression of Pdx1is most effective when the transgene locus is homozygous. This allows embryonic developmental arrest at desired stages or cessation of function in adult mice by tetracycline administration.
Categories:	Tet

Genetic Alterations

1) Targeted Mutagenesis	
Type of Allele	Gene Replacement
Targeted Gene	Pancreatic and duodenal homeobox 1 (Pdx1 - NCBI GeneID:18609)
Targeted Allele	targeted mutation 1 (Pdx1^{tmd1Macd} - MGI:2388676)
Description of Targeting Vector	The targeting vector contained a tetracycline-responsive tet-repressor/VP16 fusion transactivator (tTAoff) (Gossen & Bujard, PNAS 89:5547, 1992) and a neomycin-resistance gene flanked with 4.5-kb upstream and 1.3-kb downstream Pdx1 gene homology domains and was used to replace the entire coding region (both exons and the intron) of the endogenous Pdx1 locus by homologous recombination. Pdx1-tTA design Two additions helped ensure efficient production of the tTA protein and, in turn, effective levels of PDX1 from a tetO-Pdx1 transgene (see the schematic in attached Image 1): To help translation efficiency, an optimized Xenopus laevis beta-globin 5'UTR sequence (Falcone & Andrews, Mol Cell Biol 11:2656-64, 1991) was incorporated upstream of the tTA coding sequence, and the translation initiation codon and surrounding sequence was modified to conform to Kozak's rules. To help pre-mRNA processing, nuclear export and mRNA stability, a fragment of the rabbit beta-globin gene including the functional last intron and 3'UTR was added immediately downstream of the tTA stop codon. 36-nts separate the stop codon from the 5'

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Resource Tags

mouse, mouse strain, Pdx1-tTA

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Resource History & Actions

Approved on Dec 31, 2007
Last modified on Sep 24, 2009

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	(donor) splice junction of the beta-globin intron, well within the 50-nts maximum (Marquat et al., Curr Biol 12:R196-7, 2005) to avoid nonsense mediated destruction of the chimeric mRNA. Pdx-tTA vector construction A 4.3kb 5' flanking sequence from a Pdx1 genomic clone (a gift of C. V. E. Wright) was subcloned into XbaI/SacI sites of a modified pBS polylinker following ablation of a single XhoI site at position 2570 of the 4.3-Kb sequence by silent mutation. A fragment containing an additional (approx) 200-bp of flanking sequence and 20-bp of Pdx1 exon1 was generated by PCR from a genomic Pdx1 clone. This was fused to the 5' flanking sequence to generate the p(Pdx-pro) construct. A modified tTA gene was engineered by fusing a 50-bp double stranded oligonucleotide encoding the Xenopus beta-globin 5'UTR sequence to the tTA gene from pUHG15-1 (Gossen & Bujard, PNAS 89:5547-51, 1992) and optimizing the initiator codon by PCR mutagenesis to conform to Kozak's rules, ablating an NdeI site in the process. A 1041-bp BamHI/NruI rabbit beta-globin gene fragment was attached to the XbaI site immediately downstream of the tTA stop codon. The fragment contained, in order: a 22-bp linker, 14-bp of the 3' end of the b-globin exon 2, 573-bp of intron 2, the entire 364-bp exon 3 (including the stop codon and 98 bp of 3'UTR containing the poly A site), and 39-bp of gene flanking DNA. The 2 Kb modified XltTAbglob gene was excised via HindIII and NdeI restriction and subcloned into pMCS5 (Mobitec, Gottingen Germany) to facilitate later cloning. An approximately 1.4-Kb BglII/EcoRI fragment of 3' Pdx1 flanking sequence was subcloned from a genomic Pdx1 clone (C. V. E Wright) into BamHI/EcoRI restricted pKO V902 vector (Lexicon genetics). A Neomycin resistance positive selection cassette from pKO SelectNeo (Lexicon genetics) was then subcloned into an AscI site followed by insertion of the XltTA gene into the pKO V902 vector at the HindIII/XhoI sites. The 5' Pdx1 flanking sequence from p(Pdx-pro) was subcloned into the HpaI/HindIII sites of the pKO vector before a thymidine kinase selection cassette (pKO SelectTK - Lexicon Genetics) was inserted into the RsrII site of the final construct to generate the plasmid p(Pdx-tTA) bearing the targeting fragment.
Targeting Vector Genbank File	*Not provided*
Citations	**PubMedID** **Citation** 16126192 The homeodomain protein PDX1 is required at mid-pancreatic development for the formation of the exocrine pancreas. (2005) *Dev Biol* **286**: 225-37 (Added 2011-03-30 12:40:55.542748) 12221286 Experimental control of pancreatic development and maintenance. (2002) *Proc Natl Acad Sci U S A* **99**: 12236-41 (Added 2011-03-30 12:41:54.858482)

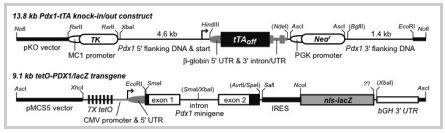
Strain Information

Strain Type:	Mixed
Chimera/Founder Genetic Background:	Not provided
Current Genetic Background:	Not provided (date recorded: Not provided)
Strain Description:	A targeting vector containing a tetracycline-responsive tet-repressor/VP16 fusion transactivator (tTAoff) and the neomycin-resistance gene flanked with 4.5-kb upstream and 1.3-kb downstream homology domains was used to replace the entire coding region (both exons and the intron) of the endogenous locus. The construct was electroporated into the (129X1/SvJ x 129S1/Sv)F1-derived R1 embryonic stem (ES) cells. Correctly targeted ES cells were injected into C57BL/6

blastocysts. The resulting chimeric males were bred with C57BL/6 females to generate heterozygotes. At some point, mutant mice were bred to B6SJL hybrid transgenic mice.

Associated Images

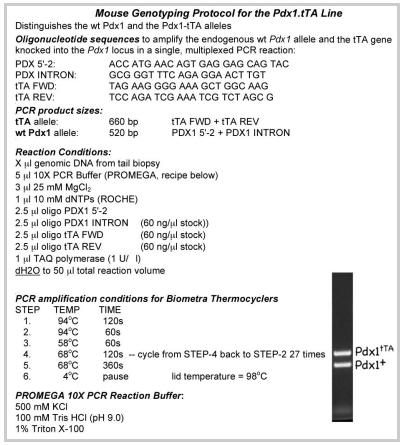
Image 1



Description:
The Pdx1-tTA targeting vector and its companion tTA-regulated transgene (tetO-Pdx1-lacZ). When combined they regulate Pdx1 and the reporter lacZ expression in mice in response to tetracycline/doxycycline administration.

Reference:
Not provided

Image 2



Description:
Protocol for PCR-genotyping that distinguishes the tTA and wild type alleles.

Reference:
Not provided

Repositories

The Jackson Laboratory

No URL supplied for repository

Stock #: STOCK 5701
Availability Notes: *Not provided*

MacDonald Lab

Request this resource

Stock #: Pdx1-tTA
Availability Notes: *Not provided*

Contact Information

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Associated Publications

No publications associated

Comments

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