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tetO-Pdx1-lacZ - Mouse Strain RES221**Mouse Information**

Common Name:	tetO-Pdx1-lacZ
MGI Official Name:	STOCK Tg(tetO-Pdx1,lacZ)958.1Macd
Description:	Mice hemizygous and homozygous for the transgenic insert are viable, fertile, of normal size, and do not display any behavioral abnormalities. Expression of the bicistronic transgene is directed by a heptameric tetO repeat fused to a minimal promoter (collectively the tetracycline-response element, or TRE). Transgenic mice do not express lacZ until a tetracycline-transactivator (tTA) protein is introduced; thereafter Pdx1 and lacZ from the transgene are expressed. All cells expressing transgenic Pdx1 coexpress the reporter. Further, mRNA levels of the transgene and endogenous Pdx1 fluctuate in concert during development. This mouse was originally designed to be mated to an apacnetic targeted mutant with the coding sequence of the Pdx1 locus replaced with that for tTAoff (see BCBC #M541). The combined mutations allow normal pancreatic development and function until doxycycline treatments render the mouse conditionally null of the endogenous gene. For this configuration, rescue of pancreatic development is most effective when the transgene locus is homozygous. This allows embryonic developmental arrest at desired stages or cessation of function in adult mice by administration of tetracycline/doxycycline. This transgenic strain may be useful in studies of pancreatic development and endocrine/exocrine function and diabetes. This strain can also be bred with other tTA strains for regulating Pdx1 expression in other contexts.
Categories:	LacZ Tet


Genetic Alterations

1) BAC or Transgene Insertion	
Type of Vector	Plasmid
Promoter	tetracycline response element with CMV minimal promoter (TRE)
Expressed Gene	bicistronic Pancreatic and duodenal homeobox 1 -- beta-galactosidase (bicistronic Pdx1-lacZ)
Description of Transgene	A bicistronic transgenic vector was generated containing an Pdx1 minigene (both exons linked with a shortened intron and preceded by a truncated 5' untranslated region) and an internal ribosome entry site-linked beta-galactosidase gene (lacZ) all under transcriptional control of seven direct repeats of the tetracycline operator (tetO) sequence fused to the human cytomegalovirus (hCMV) immediate-early promoter. See the schematic in the attached Image 1. The heptameric tetracycline operator and CMV minimal promoter (the TRE) were subcloned as a unit from the pUHG10-3 vector (Gossen & Bujard, PNAS 89:5547-51, 1992) into the XhoI/EcoRI sites of the cloning vector pMCS5 to generate pMCS5 (O7). The PDX1 mini-gene was created by sequentially subcloning a Smal-Smal fragment containing exon 1 (and part of intron 1) into a filled in Apal site, and an XbaI-AvrII fragment containing exon 2 (including partial 3' end of intron 1 and partial 3'UTR) from Pdx1 genomic clones (C. V. E. Wright) into the SspI site of pMCS5(O7) to generate pMCS5(O7-PDX). The nlacZ gene was obtained from the pLacZ.t vector (Dymecki, Gene 171:197-201, 1996) and subcloned into pMCS5. A bGH 3'UTR with polyA addition signal and addition site was incorporated downstream of nlacZ. To fuse the

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Resource Tags

mouse, mouse strain, tetO-Pdx1-lacZ

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Approved on Dec 31, 2007
Last modified on Dec 31, 2007

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nIacZ gene to an IRES sequence, an NcoI site was generated at the initiation codon of the nIacZ gene and also at AUG #11 (Jang et al., *Enzyme* 44:292-309, 1990) of the IRES sequence in pNTRLacZpGKNeoLox (R. Behringer via R. Hammer). The Sall-NcoI IRES fragment was then fused to nIacZ via the Sall-NcoI sites to create pMCS5(ILacZbGH). The pMCS5(ILacZbGH) construct was then cut with Sall, filled with Klenow, cut with Ascl and cloned into the EcoRV/Ascl sites of the pMCS5(O7-PDX) construct to generate the final p(O7-PDXILacZ) construct.

Vector Genbank File

Not provided

Citations

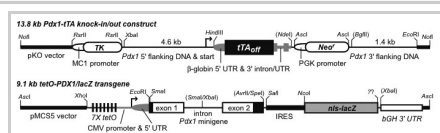
PubMedID	Citation
16126192	The homeodomain protein PDX1 is required at mid-pancreatic development for the exocrine pancreas. (2005) <i>Dev Biol</i> 286: 225-37 (Added 2011-03-30 12:40:55.542748)

Strain Information

Strain Type:	Mixed
Chimera/Founder Genetic Background:	C57Bl6/SJL
Current Genetic Background:	C57Bl6/SJL (date recorded: 04/23/2015)
Strain Description:	Transgenic mice were generated by pronuclear injection of the construct into fertilized eggs from a female C57BL/6 mated with a SJL male. Two-cell stage eggs were implanted into pseudopregnant foster mothers. Founder line 958.1 was obtained and maintained by transgenic positive sibling intercross. At some point, transgenic mice were bred to B6;129 mice with a targeted mutation. The targeted mutation was selected against in subsequent breedings and this transgenic mouse is now maintained as hemizygotes in a C57BL/6: SJL hybrid background.

Associated Images

Image 1



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

Reference:
Not provided

Image 2

Mouse Genotyping Protocol for the tetO-Pdx1 Transgenic Lines

Oligonucleotides to amplify the tetO-Pdx1 lacZ OR the tetO-Pdx1 EGFP transgene:
 PDX1 5'-2: ACC ATG AAC AGT GAG GAG CAG TAC
 PDX1 3'-2: TAG GTT AAG TTC CCT TAT CCA GCT G

PCR product size
transgene Pdx1: 1436 bp (PDX1 5'-2 + PDX1 3'-2); the corresponding region from the wt and Pdx1 locus has a much longer intron and is too long to amplify under these conditions.

Reaction Conditions:
 X µl genomic DNA from tail biopsy
 5 µl 10X PCR Buffer (PROMEGA, recipe below)
 3 µl 25 mM MgCl₂
 1 µl 10 mM dNTPs (ROCHE)
 2.5 µl oligo PDX1 5'-2
 2.5 µl oligo PDX1 3'-2 (60 ng/µl stock)
 2.5 µl oligo tTA FWD (60 ng/µl stock)
 2.5 µl oligo tTA REV (60 ng/µl stock)
 1 µl TAQ polymerase (1 U/ l)
 dH₂O to 50 µl total reaction volume

PCR amplification conditions:

STEP	TEMP	TIME
1.	94°C	120s
2.	94°C	60s
3.	58°C	60s
4.	68°C	120s
5.	68°C	360s
6.	4°C	pause

lid temperature = 98°C

PROMEGA 10X PCR Reaction Buffer:
 500 mM KCl
 100 mM Tris HCl (pH 9.0)
 1% Triton X-100

Description:
The PCR-genotyping protocol distinguishes the tetO-Pdx1-lacZ transgene from the wt and tTA alleles of Pdx1.

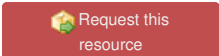
Reference:
Not provided

Repositories

The Jackson Laboratory

No URL supplied for repository **Stock #:** 5728
Availability Notes: Not provided

MacDonald Lab

 **Stock #:** tetO-Pdx1-lacZ
Availability Notes: Not provided

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Associated Publications

No publications associated

Comments

There are no comments for this entry.

