

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	434
MMRRC Strain Name	STOCK <i>Pdx1^{tm2Cvw}</i> /Mmnc
Gene Name(s)	Pancreatic and duodenal homeobox 1 (Pdx1)
Breeding Protocol(s)	Backcross to B6D2
Protocol Date	11/11/13

MMRRC #434 PCR Reaction

	<u>1X</u>
ddH ₂ O	13
5X Buffer	5.0
25mM MgCl ₂	2
10mM dNTPs	0.5
10 μM Primer F	1
10 μM Primer R	1
Taq	0.5
DNA	2

Thermal Cycler:

Step 1: 94°C for 5 min
 Step 2: 94°C for 30 sec
 Step 3: 60°C for 30 sec
 Step 4: 72°C for 45 sec
 Step 5: 32x from step 2 to step 4
 Step 6: 72°C for 7 min

Taq: Apex and Chromataq 5X Buffer

Primer sequences 5' to 3': Primers are 10 μM with respect to each primer.

WT-F (434): GGTGCTTACACAGCGGAACCCGAGG

WT-R (434): GCGGTCGCAGTGGCGGATGCTGG

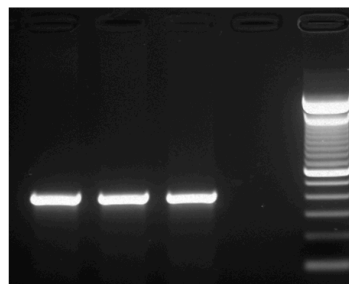
LacZ310R (434): CCTCTTCGCTATTACGCCAGCT

Bands expected: WT: WT-F (434) + WT -R (434), 430 bp
 MUTANT: WT-F (434) + LacZ310R (434), ~290 bp

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Run on 2% agarose gel in TAE.

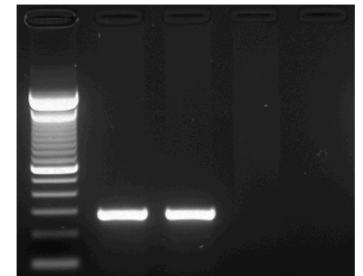
Het Het WT H₂O M



Primers: WT-F (434) + WT -R (434)
 M: 100 bp DNA ladder (Invitrogen)

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M Het Het WT H₂O



Primers: WT-F (434) + LacZ310R (434)
 M: 100 bp DNA ladder (Invitrogen)