

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	127
MMRRC Strain Name	B6.Cg-Tg(Ttr-Cdkn1a)36Tvd/Mmnc
Gene Name(s)	Transthyretin (Ttr), Cyclin-dependent kinase inhibitor 1A (Cdkn1a), transgene insertion 36 (Tg(Ttr-Cdkn1a)36Tvd)
Breeding Protocol(s)	Backcross to B6/J (Mutant males x B6/J females)
Protocol Date	9/7/13

MMRRC #127 PCR Reaction

	<u>1X</u>
ddH ₂ O	13
5X Buffer	5.0
25 mM MgCl ₂	2
10 mM dents	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: 56°C for 30 sec
 Step 4: 72°C for 1 min
 Step 5: 34x 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

F (127): AAAGTCCTGGATGCTGTCCGAG

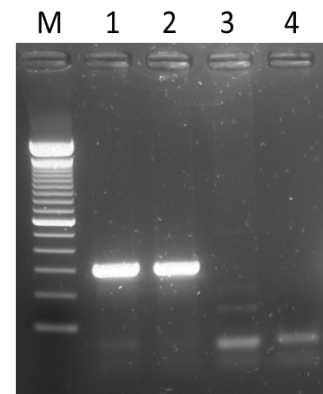
R (127): CAGACATGATAAGATACATTGATG

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Bands expected: Tg +: ~300bp

WT: no band

Run on 1% agarose gel in TAE.



Lane 1, 2: Tg+; Lane 3: WT; Lane 4: H₂O

M: 100 bp DNA ladder (Invitrogen)