

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	34788
MMRRC Strain Name	STOCK <i>Clic4</i> ^{tm1Jce} /Mmnc
Gene Name(s)	chloride intracellular channel 4 (mitochondrial) (<i>Clic4</i>)
Breeding Protocol(s)	Intra-strain Mating Outcross to CD-1 (Charles River)
Protocol Date	6/27/13

#34788 PCR

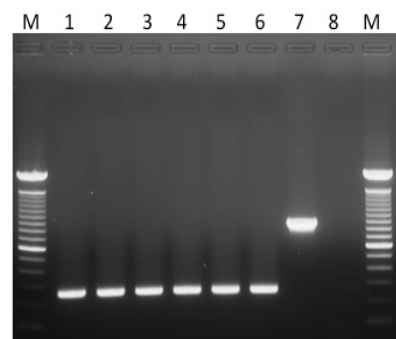
	<u>1X</u>
ddH ₂ O	13
5X Buffer	5.0
25mM MgCl ₂	2
10mM dNTPs	0.5
10 uM Primer F	1
10 uM Primer R	1
Taq	0.5
DNA	2

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 30 sec
 Step 5: Step 2 to 4; Cycles: 35
 Step 6: 72 °C for 7 min

Taq: **Apex and chromataq Buffer**

34788 Picture 1



Mutant-F (34788) + C4mR (34788). Lane 1- 6: Hom; Lane 7: WT; Lane 8: ddH₂O; M: 100 bp DNA ladder (Invitrogen)

Primers are 10uM with respect to each primer

Primer sequences 5' to 3':

WT-F (34788): 5'- GCCGGAAGTGATGGTCAAAGCATT-3'

Mutant-F (34788): 5'- TGACCACGGCAACTCCTAGAAGGAC-3'

C4mR (34788): Agg ACT Cgg ggT gAC ACT gTA AAT CgA C

Bands: WT-F (34788) + C4mR (34788): WT 288 bp

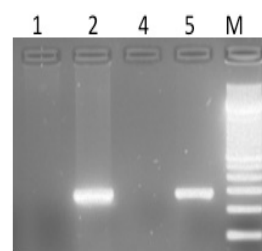
Mutant: No band

Mutant-F (34788) + C4mR (34788): Mutant 240 bp

WT: 864 bp

NOTE: For “Mutant-F + C4mR “ reaction, the KO markedly out-amplifies the longer WT gene in the hets particularly if the DNA is poor quality, so it can be easy to mistake hets for KOs. Diluting the DNA can help, but it is recommended to do the “WT-F + C4mR” reaction as well to distinguish het from KO.

34788 Picture 2



WT-F (34788) + C4mR (34788): Lane 1: Hom; Lane 2 and 5: WT; Lane 4: ddH₂O; M: 100 bp DNA ladder (Invitrogen)

Run on 1 % agarose gel in TAE.