

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	34767
MMRRC Strain Name	C3.B6- <i>Opa1</i> ^{lilr3} /Mmnc
Gene Name(s)	optic atrophy 1 homolog (human); lilr3 (<i>Opa1lilr3</i>)
Breeding Protocol(s)	Backcross to C3H/HeJ (Homozygous lethal)
Protocol Date	10/2/12

MMRRC #34767 PCR Reaction

2,2 Primer Reaction
(CommonF + WTR) for wild-type and
(CommonF + MutR) for mutant reaction

Thermal Cycler:

94°C for 30 sec
60°C for 30 sec
72°C for 30 sec
32x

	<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

Taq: **Apex and Chromataq 5X Buffer**

Bands: MUTANT: 253bp
WT: 253bp

Primer sequences 5' to 3':

CommonF: 5'-CTCATGCCTCGGTTATCTTCTAATAAGCC-3',
MutR: 5'-GCTGCAGGATTTCCCTGAGTC-3',
WTR: 5'-GCTGCAGGATTTCCCTGAGTT-3'

Run on 2.0% agarose gel in TAE.