

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	11679
MMRRC Strain Name	B6;129S5- <i>Vcp</i> ^{Gt(OST196720)Lex} /Mmnc
Gene Name(s)	valosin containing protein (Vcp)
Breeding Protocol(s)	Backcross to C57BL/6J
Protocol Date	4/1/14

MMRRC #11679 PCR Reaction

2, 2 primer reactions: (3' + 5') and (3' + LTR2)

Thermal Cycler:

94°C for 3 min
 94°C for 15 sec
 65°C for 30 sec, decrease 1°C/cycle
 72°C for 40 sec
 Steps 2-4, 10x
 94°C for 15 sec
 55°C for 30 sec
 72°C for 40 sec
 Steps 5-7, 30x
 72°C for 5 min

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

Taq: Apex Taq and Chromataq 5X Buffer

Bands: MUTANT: 134bp WT: 197bp

Primer sequences 5' to 3':

NIH-1805-3': TTGCAGTGTTTTCCCTTCTCAGGC
 NIH-1805-5': TCGGAGCCTGGACAAGATGGTGC
 NIH-1805-LTR2: AAATGGCGT TACTTAAGCTAGCTTGC

Run on 2% agarose gel.

- Lanes
1. Heterozygous Sample
 2. Heterozygous Sample
 3. Wild-type Sample
 4. Wild-type Sample
 5. Wild-type Sample
 6. Wild-type Control
 7. Negative Control
 8. 100bp Marker

