

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	36692
MMRRC Strain Name	C57BL/10J-Chr 14 ^{SWR/J} /NcaMmnc
Gene Name(s)	Chr 14 ^{SWR/J} /Nca chromosome substitution
Breeding Protocol(s)	Intra-strain mating
Protocol Date	8/5/13

MMRRC #36692 PCR Reactions

For the strain Chr14 (36692), there is a panel of 5 laboratory-developed microsatellite markers, which are D14Kar16, D14Kar19, D14Kar35, D14Kar24 and D14Kar40. This strain genotyping includes 5 reactions, which have same reagents mix recipe and thermal Cycler, but different primers and PCR product.

	<u>1X</u>
ddH ₂ O	13
5X Buffer	5
25mM MgCl ₂	2
10mM dNTPs	0.5
10 μM Primer Forward	1
10 μM Primer Reverse	1
Apex Taq	0.5
DNA	2

Thermal Cycler:

- Step 1: 94°C for 5 min
- Step 2: 94°C for 30 sec
- Step 3: 55°C for 30 sec
- Step 4: 72°C for 45 sec
- Step 5: 35x 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.

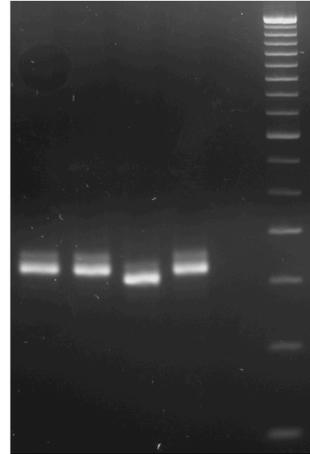
D14Kar16

16 F (36692): CCCTCTCTCCACACACTCGT
16 R (36692): TCCTGAAGGCTCTTGTGGC

Bands expected: WT ~150 bp
MUTANT ~170 bp

Run on 4% agarose gel in TAE.

36692 (D14Kar16)
1 2 3 4 5 M



Lane 1, 2, 4: Hom; Lane 3: WT; Lane 5: H₂O; M: 50 bp DNA ladder (Invitrogen)

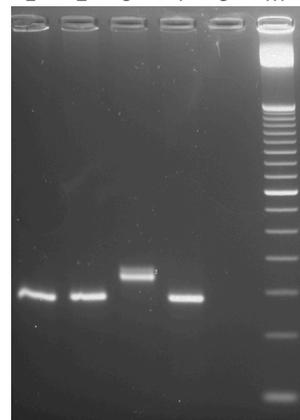
D14Kar19

19 F (36692): AGGACCATCCCAATCCTACC
19 R (36692): TGGCCCTTGGTACTTTTTGA

Bands expected: WT ~190 bp
MUTTANT ~145 bp

Run on 3 % agarose gel in TAE.

36692 (D14Kar19)
1 2 3 4 5 M



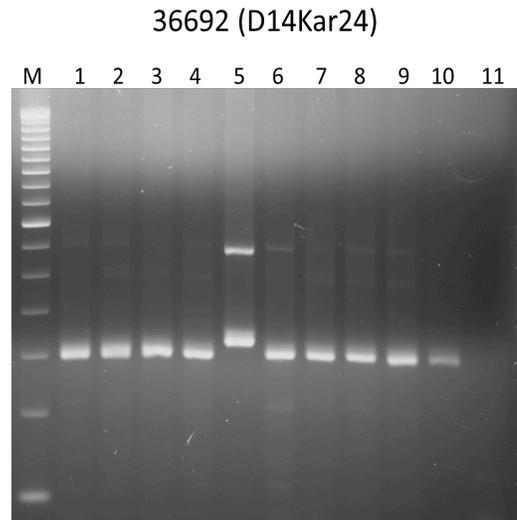
Lane 1, 2, 4: Hom; Lane 3: WT; Lane 5: H₂O; M: 50 bp DNA ladder (Invitrogen)

D14Kar24

24 F (36692): TCTGCTGCTTGCCTATCTCA
24 R (36692): AATCAGGAACCAGCAGCAAG

Bands expected: WT ~170 bp
MUTANT ~155 bp

Run on 4 % agarose gel in TAE.



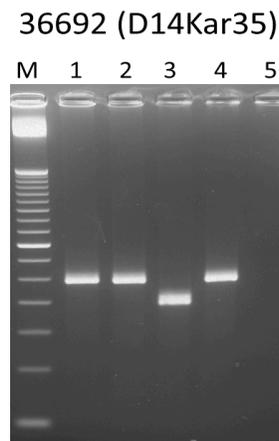
Lane 1-4, 6-10: Hom; Lane 5: WT; Lane 11: H₂O; M: 50 bp DNA ladder (Invitrogen)

D14Kar35

35 F (36692): CGCTGTTTCATAACCATCGTC
35 R (36692): TGGTGGGAAACCTTACTTCAA

Bands expected: WT ~200 bp
MUTANT ~250 bp

Run on 2% agarose gel in TAE.



Lane 1, 2, 4: Hom; Lane 3: WT; Lane 5: H₂O; M: 50 bp DNA ladder (Invitrogen)

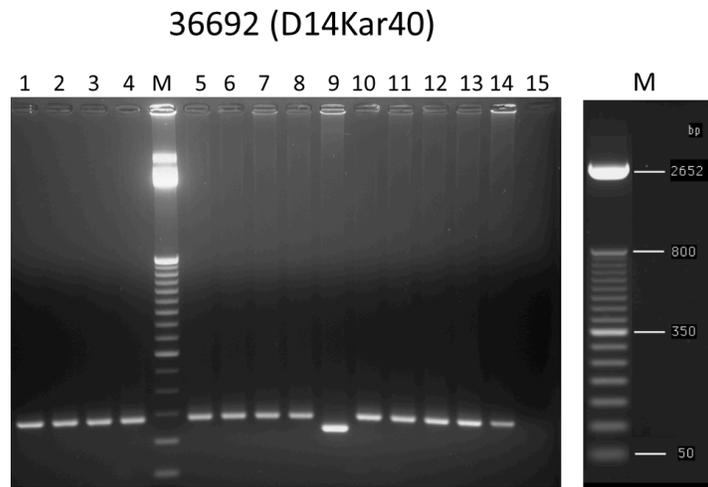
D14Kar40

40 F (36692): GATGTTCTGCAGCCAATTCA

40 R (36692): CCCAATGATGGGAAAGAAAG

Bands expected: WT 160 bp
MUT ~190 bp

Run on 3% agarose gel in TAE.



Lane 1-8, 10-14: Hom; Lane 9: WT; Lane 15: H₂O; M: 50 bp DNA ladder (Invitrogen)

