

Genotyping Protocol: **MMRRC 32650**

Assay Type: PCR- can distinguish heterozygous animals from homozygous animals

DNA Extraction: DNA from tail snips was extracted using Qiagen's DNeasy Blood and Tissue kit (Cat# 69506). Kit directions for animal tissues were performed with a few minor modifications as follows: repeat AW1 and AW2 wash steps one time, elute in 200µl of AE buffer once.

Strain Description: This strain carries a targeted deletion in the runt-related transcription factor 2 isoform type II gene (*Runx2-II*). The P1 promoter and exon 1 of *Runx2-II* are replaced by a neomycin cassette. Details can be found in Xiao, Z.S. et al (2004) J. Biol. Chem. 279, 20307– 20313.

Primer Information:

- | | |
|-----------------------|---|
| 1) Name: M32650 WT F | Sequence: 5'- GCG TCA AAC AGC CTC TTC AGC GCA GTG ACA -3' |
| 2) Name: M32650 MUT F | Sequence: 5'- ATC GCC TTC TAT CGC CTT CTT GAC GAG TTC -3' |
| 3) Name: M32650 R | Sequence: 5'- AGC ACT GGA GAT CTT TAC AGA ACA GCT CTG -3' |

Primer location: M32650 WT F is located in exon 1 of *Runx2-II*. M32650 MUT F is located in the neomycin cassette. M32650 R is located in intron 1 of *Runx2-II*.

Assay name: Runx2-II deletion PCR**PCR Master Mix Components:**

component	manufacturer	concentration	µl/rxn
Buffer with MgCl ₂ (green cap)	Roche	10X	2
dNTP	Promega (Cat# U1515)	1.25mM	3.2
M32650 WT F	Sigma	25µM	0.3
M32650 MUT F	Sigma	25µM	0.3
M32650 R	Sigma	25µM	0.3
FastStart <i>Taq</i>	Roche (Cat# 12032953001)	5 U/µl	0.2
sterile water			12.7

PCR Setup:

Final Reaction: 19µl master mix & 1µl DNA template (10-20ng/µl)

All reactions were performed in 200µl thin walled PCR tubes and were run in Perkin Elmer 2400 thermocycler or Applied Biosystems 2700 thermocycler.

Cycle Parameters:

- | | | |
|----|--|--------------------------------|
| 1) | 95°C | 3 minutes |
| 2) | 94°C | 30 seconds |
| 3) | 70°C | 30 seconds |
| 4) | 72°C | 1 minute |
| 5) | Repeat steps 2-4 34 times for a total of 35 cycles | |
| 6) | 72°C | 10 minutes |
| 7) | 4°C | hold until refrigerate product |

Product Analysis:

All products were analyzed on the Qiaxcel (instrument and all supplies from Qiagen) with the Qiaxcel DNA Screening Kit (Cat# 929004).

Alignment Marker: QX Alignment Marker 15bp/3Kb (Cat# 929522)

Size Marker: QX DNA Size Marker 100-3Kb (Cat# 929553)

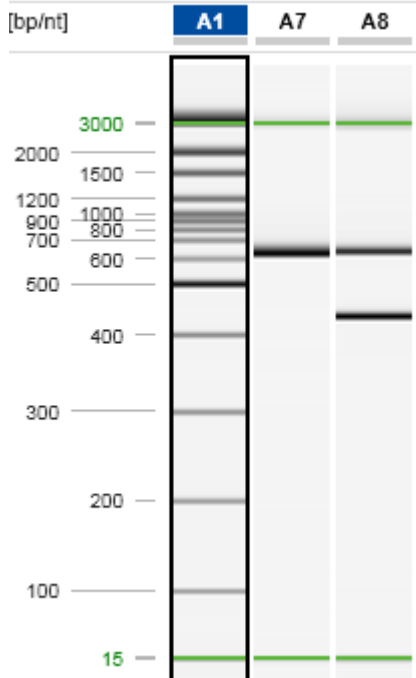
Method: AM320 Injection: 10s at 5KV
 Separation: 320s at 6KV

Expected products:

Wild-type allele: 720bp

Mutant allele: 435bp

Example gel:



Lane A1 displays a 15bp-3kb size marker.
Lane A7 displays a wild-type sample (720bp product).
Lane A8 displays a heterozygous sample (720bp and 435bp products).

Please note: the 15bp and 3kb bands are reference markers specific to the Qiaxcel method and do not represent expected products.