Vanderbilt Genome Editing Resource

GENOTYPING PROTOCOL: *Rr$^{Chr12Etv1\Delta801Mgn}$/Vu*

Investigator: Mark Magnuson
Genome edit: ΔChr12:38,928,700-38,929,500 (mm10)
Common allele name: *Rr$^{Chr12Etv1\Delta801Mgn}$/Vu*

PCR Primers:
Etv1Fwd1: GCCTGCAACCATTGTGTTCC
Etv1Rev1: AACGAAGGAACTAACAGGCAAGG

Predicted PCR product sizes:
Homozygous = *Rr$^{Chr12Etv1\Delta801Mgn}$/Vu* = 362 bp
Heterozygous = *Rr$^{Chr12Etv1\Delta801Mgn}$/Vu* = 362 bp and 1157 bp
WT = 1157 bp

Component	25 ul reaction	Final concentration	PCR program
5X Phusion Reaction Buffer (NEB #M0530)	5.0 µL	1X	98ºC, 30 seconds
10 mM dNTPs	0.5 µL	200 µM	98ºC, 10 seconds
10 µM Etv1_Fwd1	1.25 µL	0.5 µM	65ºC, 10 seconds
10 µM Etv1_Rev1	1.25 µL	0.25 µM	72ºC, 30 seconds
Phusion DNA Polymerase (NEB #M0530)	0.25 µL	0.02 U/µl	Go to 2, 35 X
Nuclease-free water	16.25 µL		72ºC, 2 minutes
Genomic DNA	0.5 µL	Less than 1 µg	4ºC, ∞

PCR products can be digested with XhoI to confirm presence of the designed deletion, but this line has been sequence validated. No other type of *Etv1* intergenic deletion is present:

Example Etv1 intergenic deletion genotyping gel: N1-24 and N1-27 contain the desired XhoI positive deletion while N1-25 and N1-26 do not. This genotyping assay under the PCR conditions described here shows a faint band between the two PCR products that results from a minor fraction of products having non-specific binding between the full length and truncated PCR products. F0-03 was the original founder from which the line was established.