



Gene Editing Rat Resource Center

Genotyping Your Model



Date:	4/8/2020	
Strain Symbol:	WKY-Sik2 ^{em5MCWi}	RGD ID: 12790944
Parental Strain:	WKY/NCrl	Parental Strain RGD ID: 1358112
Gene Targeted:	Sik2	Gene RGD ID: 1559698
Mutation Description:	Net 5 bp del in exon 4	Allele RGD ID: 12790945
Deletion:	CTTCCG gctgtg GCAATAATCAACAG CAGA (Deletion in red)	
Insertion	CTTCCG t GCAATAATCAACAGCAGA CAGAA (Insertion in red)	
Mutation Region:	(RGSC 5.0/rn5) chr8:53,942,155	
Target Site:	GCACTACCTTCCGGCTG TGG	

Genotyping Primers:

Forward:	CTCAGACCCTCCAAAGTACAGC
Reverse:	TTTTGTGTGTTTGTTCATT
WT PCR Size:	422 bp
Mutant Size:	417 bp

Derivation Information:

This strain was produced by injecting Crispr targeting the sequence GCACTACCTTCCGGCTG TGG into WKY rat embryos. The resulting mutation is a net 5-bp deletion in exon4.

Suggested Genotyping:

Questions? Send an email to: mcwcustomrats@mcw.edu

HpaII RFLP assay. PCR using above primers and set up a digest with 10ul of PCR product and HpaII. If sample is WT it will be cleaved, if it is a KO it destroys restriction site.

PCR

5.0 ul of template (approximately 200ng)

12.5 µl of Accuprime Supermix II (Invitrogen, Carlsbad, CA)

1.0 µl of primer set at 10 µM

8.5 µl of water

25 uL / rxn

PCR cycling parameters:

95°C- 5 min

35 cycles of:

95°C- 30 sec

60°C- 30 sec

68°C- 45 sec

68°C- 5 min

4°C- forever