



Gene Editing Rat Resource Center

Genotyping Your Model



Date:	4/8/2020	
Strain Symbol:	WKY-Tph1 ^{em3MCWi}	RGD ID: 13208231
Parental Strain:	WKY/NCrl	Parental Strain RGD ID: 1358112
Gene Targeted:	Tph1	Gene RGD ID: 3895
Mutation Description:	1 bp insertion in exon4	Allele RGD ID: 13208232
Deletion:	N/A	
Insertion	GGGAAAGATGTCATTCAGCTGTT C TCGGTTGATGTCGCAGTC (insertion in red)	
Mutation Region:	(RGSC 6.0/rn6): chr1:102,694,238-102,694,239	
Target Site:	AGATGTCATTCAGCTGTTCT CGG	

Genotyping Primers:

Forward:	ACAGAAGTTTCTTTTTATCCCCTTGTC
Reverse:	CTTTACAGCTAATTCTGACAAGTGTTG
WT PCR Size:	402 bp
Mutant Size:	403 bp

Derivation Information:

This strain was produced by injecting a CRISPR targeting the sequence AGATGTCATTCAGCTGTTCT CGG into WKY rat embryos. The resulting mutation is a 1bp insertion in exon 4. Founder animals were genotyped by the Cel-1 assay and confirmed by sanger sequencing. The founders were then backcrossed to the parental strain and subsequent litters were genotyped by fluorescent genotyping.

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

Suggested Genotyping:

Suggested genotyping is fluorescent genotyping or direct sequencing. If these do not work a potential assay of mutation specific primer and WT specific primer can be used to determine zygosity.

If mutation and deletion specific primers are preferred method we can help design these if needed.

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