Gene Editing Rat Resource Center

Genotyping Your Model

Date:	**4/8/2020**	
Strain Symbol:	**WKY-Tph1^{em1MCWi}**	**RGD ID: 13207595**
Parental Strain:	**WKY/NCrl**	**Parental Strain RGD ID: 1358112**
Gene Targeted:	**Tph1**	**Gene RGD ID: 3895**
Mutation Description:	**Net 1 bp deletion in exon4 (1 bp substitution and 1 bp deletion)**	**Allele RGD ID: 13208230**
Deletion:	**AGCAGGGGAAAGATGTCATTCAGATGtTCTCGGTTGATGTCGCAGTCCAC (Changes in Red, lowercase is deletion, uppercase is a substitution)**	
Insertion		
Mutation Region:	**(RGSC 6.0/rn6): chr1:102,694,207-102,694,262**	
Target Site:	**AGATGTCATTCAGCTGTTCT CGG**	

Genotyping Primers:

Forward:	**ACAGAAGTTTCTTTTTATCCCCTTGTC**
Reverse:	**CTTTACAGCTAATTCTGACAAGTGTTG**
WT PCR Size:	**402 bp**
Mutant Size:	**401 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 net 1bp deletion in exon 4. Founder animals were genotyped by the Cel-1 assay

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and confirmed by sanger sequencing. The founders were then backcrossed to the parental strain and subsequent litters were genotyped by fluorescent genotyping.

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

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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