

Taz Floxed allele GT protocol

Mouse tail digestion:

- 1) To each sample, add 300 ul of 50 mM NaOH
- 2) Incubation in 95-degree heat block for 30 mins.
- 3) Add 50ul of 1M Tris-HCl, pH 8.0.
- 4) Vortex to mix.

PCR reaction:

Primers: TL-F + TL-R

PCR product size:

Wild-type allele: 135bp

Flox allele: 169bp

or

Primers: TR-F + TR-R

PCR product size:

Wild-type allele: 121bp

Flox allele: 155bp

Dream Taq (Thermo Fisher Scientific, # K1081) PCR Protocol:

2x green Dream Taq Mix: 10ul

Forward Primer: 0.5ul

Reverse Primer: 0.5ul

ddH₂O: 6ul

DNA: 3ul

20ul total reaction volume

Temp settings for PCR

95°C 5min

94 °C 30s

58 °C 30s } 33 cycles

72 °C 1min }

72 °C 5min

Load PCR product in 2.5% agarose Gels.

Taz KO allele GT protocol

Mouse tail digestion:

- 1) To each sample, add 300 ul of 50 mM NaOH
- 2) Incubation in 95-degree heat block for 30 mins.
- 3) Add 50ul of 1M Tris-HCl, pH 8.0.
- 4) Vortex to mix.

Mice carrying floxed allele can be genotyped with the following primers:

Reaction 1:

Primers (for KO allele): TG-L + TG-R

PCR product size:

Wild-type allele: no band

Flox allele: 303bp

Reaction 2:

Primers (for WT allele): TL-F + TL-R

PCR product size:

Wild-type allele: 135BP

KO allele: no band

Dream Taq (Thermo Fisher Scientific, # K1081) PCR Protocol:

2x green Dream Taq Mix:	10ul
Forward Primer:	0.5ul
Reverse Primer:	0.5ul
ddH ₂ O:	6ul
DNA:	3ul

20ul total reaction volume

Temp settings for PCR

95°C	5min	} 33 cycles
94 °C	30s	
58 °C	30s	
72 °C	1min	
72 °C	5min	

Load PCR product in 2.5% agarose Gels.