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
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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
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.