Mills Lab Protocol
10.1028

B6.Cg-Tg(Cr2-cre)3Cgn/J Genotyping

1. Prepare DNA by standard isopropanol/ethanol extraction.
2. Bring final volume of each oligo to 100 pmol/ μl (each) using ultrapure H_2O.

wt	KMO280	CTAGGCCACAGAATTGAAAGATCT
	KMO281	GTAGGTGGAAATTCTAGCATCATCC
mut	KMO278	GCGGTCTGGCAGTAAAAACTATC
	KMO279	GTGAAACAGCATTGCTGTCACTT

3. Set up a master mix (on ice) for each primer as follows:

12.5 uL Promega GoTaq Mastermix
.25 uL Primer Mix (=10pmol each primer)
19.8 uL H20

} X No. of samples

4. Add 1μl template DNA to corresponding well in PCR plate.
5. Add 24μl master mix to each well in PCR plate
5. Seal using Microseal film.
6. Keep reactions on ice and load onto PCR block pre-heated to 94º.

PCR Program

94° 3 min.
94° :30 sec
51.7° 1 min
72° 1 min
} Repeat steps 2-4 for 35 cycles
72° 2 min
10° forever

Expected band sizes:

WT: approx. 324bp
Tg: approx. 100bp