

Rag1 Genotyping

1. Prepare DNA by standard isopropanol/ethanol extraction.
2. Bring final volume of each oligo to 100 pmol/ μ l (each) using ultrapure H₂O.

KMO269 GAGGTTCCGCTACGACTCTG	wild type forward
KMO270 CCGGACAAGTTTTTCATCGT	common
KMO271 TGGATGTGGAATGTGTGCGAG	mutant forward

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

12.5 μ L Promega GoTaq Mastermix
.2 μ L Primer Mix (=10pmol each primer)
11.1 μ L H₂O



X No. of samples

4. Add 1 μ l template DNA to corresponding well in PCR plate.
5. Add 24 μ l 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 94°.

PCR Program

94° 2 min.	} Steps 2-4 for 35 cycles
94° :30 sec	
58° :45 sec	
72° 45 sec	
72° 2 min	
10° forever	

Expected band sizes:

Notes an artifact band of 640bp may be amplified in heterozygous mutant mice.

Mut: 530bp
Het: 474bp and 530bp
Wt: 474bp