SCREENING MFGE8/Tmbgeo mice

New protocol set up 05/2009
(the protocol described in Silvestre et al, Nature Med 2005, 5 : 499 required to set up 2 separate reactions for the WT and mutant allele. This new protocol works in a single reaction with the 3 primers included)

Digestion mix ears + tails

Tris 1M pH8	10 µL	
NaCl 2M	10 µL	
Proteinase K 10mg/ml	2 µL for tails	(1 µL for ears)
TWEEN 20	0.5 µL	
H2O to final 100 µL :	77.5 µL	78.5 µL for ears

Incubation 2h30 (for ears) to 4h (for tails) at 56°C
vortex at mid-time
Stop digestion by incubating 20 min at 95° C

PCR MIX (*kit Gotaq flexi DNA polymerase 500 units, Promega*)

Buffer	5X	Final : 1X	5 µL	
MgCl2	25 mM	Final : 1.5 mM	1.5 µL	
dNTP	25 mM	Final : 0.2 mM	0.2 µL	
primer	100 µM	Final : 0.25 µM	0.06 µL	gF1
primer	100 µM	Final : 0.5 µM	0.12 µL	gR1D
primer	100 µM	Final : 0.125 µM	0.03 µL	gR2
Gotaq	5U/µl	Final : 0.025 U/µl	0.125 µL	
DNA from ear or tail			2 µL	
H2O to final 25 µL			15.965 µL	

PCR REACTION

95°C	**5 min**	
94°C	**45 sec**	
60°C	**45 sec**	**35 cycles**
72°C	**1 min**	
72°C	**10 min**	

GEL

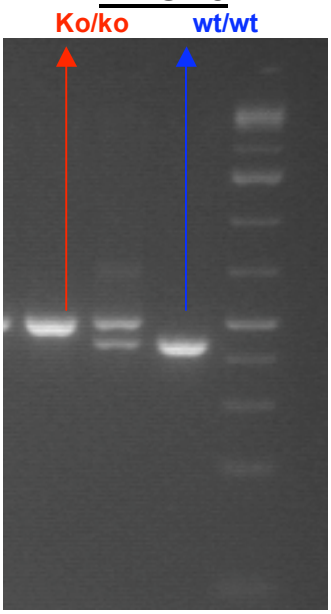
Agarose 2 %
10 µL of PCR product

PRIMERS

- **MFGE8**

- gF1A	gggggactagaaagggacag	(forward primer for both alleles)
- gR1D	ctatgtagagcgatcctatctcaaaa	(reverse primer for WT allele)
- gR2	ggaattcctccgcaaactcctatttctg	(reverse primer for mutant allele)

- **MFGE8**



band for wt : 450 bp

band for mutant (ko): 500 pb