EMMA ID: *01705*
Gene: *Tfap2a*
Common name: *Doarad*
Allele: *Tfap2a* ^{Mhdador}

Genotyping Information

Genotyping by end-point PCR based on gel is composed of a genespecific short range PCR using primers on wild type allele and a mutant allele-specific short range PCR. The combined results show the genotype of the mice. For example: mutant positive, wild type positive = Heterozygous.

PCR primer pairs and expected size bands

Assay	Forward Primer	Reverse Primer	Expected Size Band (bp)
Wildtype	Doarad_F	Doarad_R	378
Mutant	same as wt	same as wt	278 / 317

Primer sequences

Primer Name	Sequence 5' --> 3'
Doarad_F	CCGACTTCCAGCCTCCACACTTC
Doarad_R	CGATGGCGTGAGGTAAGGAGTG

PCR setup (Qiagen, Hot Start Plus)

Component	Volume (µl) 1x	Final conc.
DNA (~ 50-100 ng)	2	
Q-Solution (5x)	2,5	0,5
PCR-Buffer (10x)	2,5	1
DNTP mix (10 mM)	0,5	0,2
$MgCl_2$ (25 mM)	1,5	1,5
Primer 1 (10 pmol/µl)	1	0,4
Primer 2 (10 pmol/µl)	1	0,4
Taq Polymerase (5 U/µl)	0,3	0,06
H_2O*	13,7	
Final volume	25	

*** The amount of H_2O is adjusted with the number of primer.**

Amplification conditions

PCR Settings	Temperature (°C)	Time	# of cyles
1 Denaturation (Melting)	95°C	5 min	1
2 Amplification (Melting, Annealing, Polym.)	94°C 62°C 72°C	30 sec 45 sec 45 sec	39
3 Polymerisation	72°C	10 min	1
4 Cooling	4°C	hold	1

These PCR conditions have been optimized for our methods and preparation kits. Adaptions may be required.

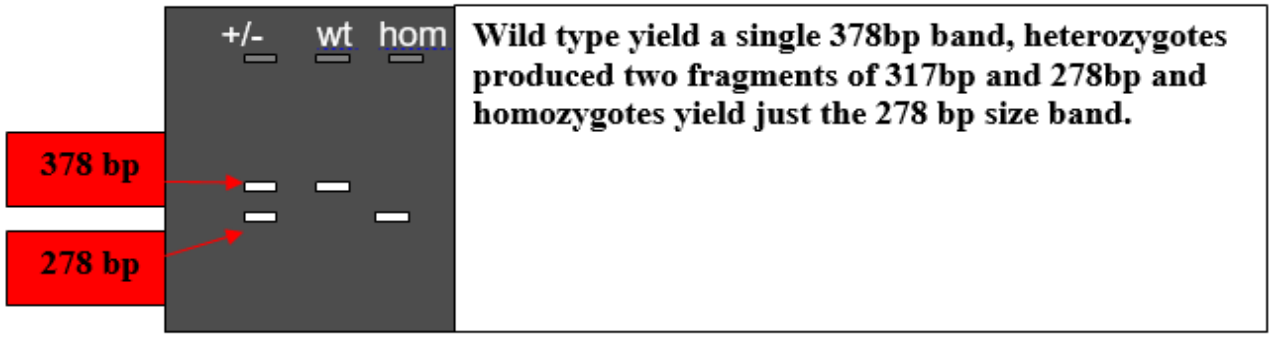
Restriction

Restriction enzyme: BsaXI (ordered from Biolabs Company, catalog number #RO6O9L)
Buffer for the restriction enzyme: NEBuffer4 (ordered from Biolabs company, catalog number B70045)

Incubation at 37 C° for 8 hours (Overnight preferred)

10µl	PCR product
2µl	NEBuffer4
1 µl	BSAXI
7µl	H20
20µl	**TOTAL**

Gel Image



Separated by gel electrophoresis on a 3% agarose gel.