Genotyping protocol

General information:

Strain name	Gfm1R671C

Primers:

Name	Sequence	Primer type
Gfm1 KI Forward	CTGTCTCATGTAGAGCTGGAGAG	gene-specific
Gfm1 KI Reverse	AGATAAGACAGGTACAGATCAGGG	gene-specific
		please select one
		please select one

In case more than two primers are introduced, please indicate how they should be combined:

	Forward primer	Reverse primer
e.g. wt		
e.g. mut		

Reaction mix:

rTaq (Takara) (5 Units/µL)	0,12	µl
10X PCR Buffer (Mg2+ plus)	2,50	µl
dNTPs Mixture (2.5 mM each)	2,00	µl
Forward primer (2 µM)	6,25	µl
Reverse primer (2 µM)	6,25	µl
Water	5,87	µl
DNA	1,00	µl
		µl
Final volume	25,00	µl

PCR program:

95	°C	5	min	
95	°C	30	sec	x30
60	°C	30	sec	
72	°C	60	sec	
72	°C	10	min	

Expected fragment size:

wt	613	bp
mutant	613	bp

Comments/Additonal information:

It is necessary to perform an enzyme digestion of the PCR product to discrimenate between WT and mutant alleles. The digestion conditions are the following:

Reaction mix:

DNA PCR product	5,00	µl
NcoI (20 000 units/ml)	1,00	µl
CutSmart® Buffer 10X	1,00	µl
Water	3,00	µl
Final volume	25,00	µl

Enzyme activity conditions:

37	°C	60	min

Expected fragment size:

wt	613	bp
mutant	432	bp
	181	bp

General information:

Strain name	Gfm1 KO

Primers:

Name	Sequence	Primer type
Gfm1 Forward	TGACCTTTCCAGCACCAGG	gene-specific
Gfm1 WT Reverse	CACCCGACCAGTTTTCATCC	gene-specific
Gfm1 KO Reverse	CCTGACAGACACATCTGCAGAC	gene-specific
		please select one

In case more than two primers are introduced, please indicate how they should be combined:

	Forward primer	Reverse primer
e.g. wt	Gfm1 Forward	Gfm1 WT Reverse
e.g. mut	Gfm1 Forward	Gfm1 KO Reverse

Reaction mix:

rTaq (Takara) (5 Units/µL)	0,10	µl
Uracil N-glycosylase (2 Units/µL)	0,20	µl
10X PCR Buffer (Mg2+ plus)	2,00	µl
dNTPs Mixture (2.5 mM each)	1,60	µl
Forward primer (2 µM)	4,00	µl
Reverse primer WT (2 µM)	2,00	µl
Reverse primer KO (2 µM)	2,00	µl
Water	7,10	µl
DNA	1,00	µl
Final volume	20,00	µl

PCR program:

25	°C	10	min	
95	ºC	2	min	
95	°C	30	sec	x30
60	°C	30	sec	
72	°C	60	sec	
72	°C	10	min	

Expected fragment size:

wt	548	bp
mutant	318	bp

Comments/Additonal information:

This PCR strategy is also useful to discriminate between KO and KI alleles. KI allele amplification product size is 548 using « Gfm1 Forward » and « Gfm1 WT Reverse » primers.