PCR protocol

Sheet ID PS_07097
Release date : 12/18/2018
Print date : 12/18/2018

RBRC 06307 **Strain name** ICR;129(B6)-Mafk<tm1.1Jeng>

Primer	primer_name	primer_seq	length		Reference
1	mafK Ex2.1	CCTACCGTTTCTGTCTTTCCAG	22		Depositor's primer
2	mafK cod2u	GCTCGGCAGATTTGACGATG	20		Depositor's primer
3	LacZ4	CCTGTAGCCAGCTTTCATCAAC	22		Depositor's primer

Reaction Components

Enzyme TOYOBO KOD FX (Code.No : KFX-101)

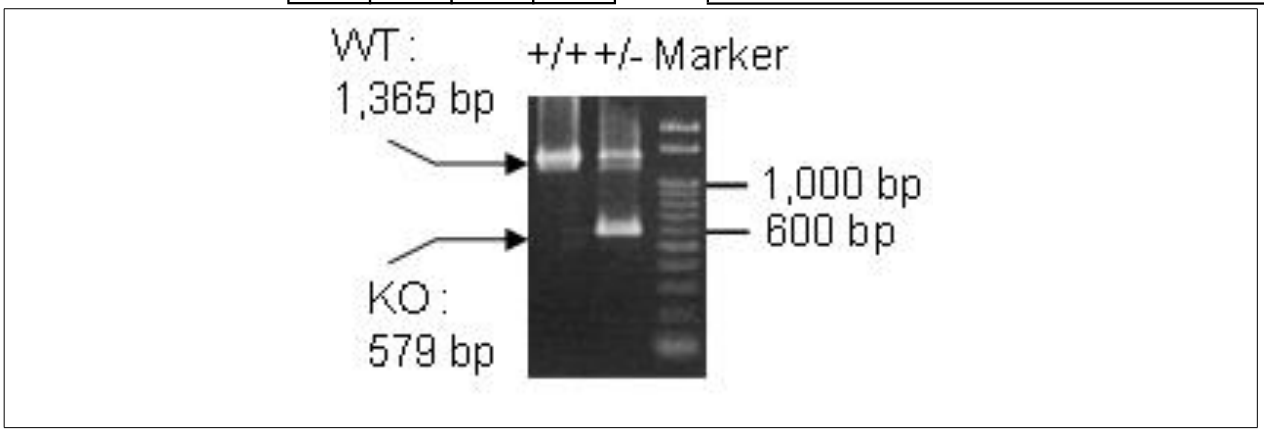
Reagent	Conc	Reaction 1	2	3	4	
H2O		1.8				(μL)
PCR buffer	2 X	10.0				
dNTP	2.0 mM	4.0				
primer 1	10 pmol/μl	1.0				
primer 2	10 pmol/μl	1.0				
primer 3	10 pmol/μl	1.0				
DNA		1.0				
Taq	1 U/μl	0.2				
	total	20				

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
2	98	10	*
3	60	30	*
4	68	180	* *Repeat for 30 cycles

Marker DNA Ladder,1 kb Plus (Affymetrix, Cat. 76714 500 UL)

Gel 1% agarose in 1 X TAE (100 V, 30 min)



primer_set		product_size	product (allele type)
primer 1	primer 2	1365 bp	WT
primer 1	primer 3	579 bp	KO

+/+: 1365 bp
+/-: 1365 bp, 579 bp

Comment Standard PCR

Novagen KOD Xtreme™ Hot Start DNA Polymerase can be purchased from EMD.