

RBRC 05926

Strain name B6.Cg-Map3k5<tm1Hijo> Map3k6<tm1Hijo>

Primer	primer_name	primer_seq	length	Reference
1	promoter	ACGATAACAGCGTAAGCAACTGG	23	RIKEN BRC
2	GENO2	GTGAGGCGTGATGTAAATAGGAAGC	25	Depositor's primer
3	olMR1416	TCCTTGAAGAAGATGGTGCG	20	JAX Genotyping Protocol
4	195S	ACTGGATCCACACCTTCAGC	20	Depositor's primer
5	961AS	CAAGAGTCTGTGGGTGTAGCCT	22	Depositor's primer
6	lacZ-R	CGCATCGTAACCGTGCATCTGCC	23	RIKEN BRC

Reaction Components

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

Reaction Conditions

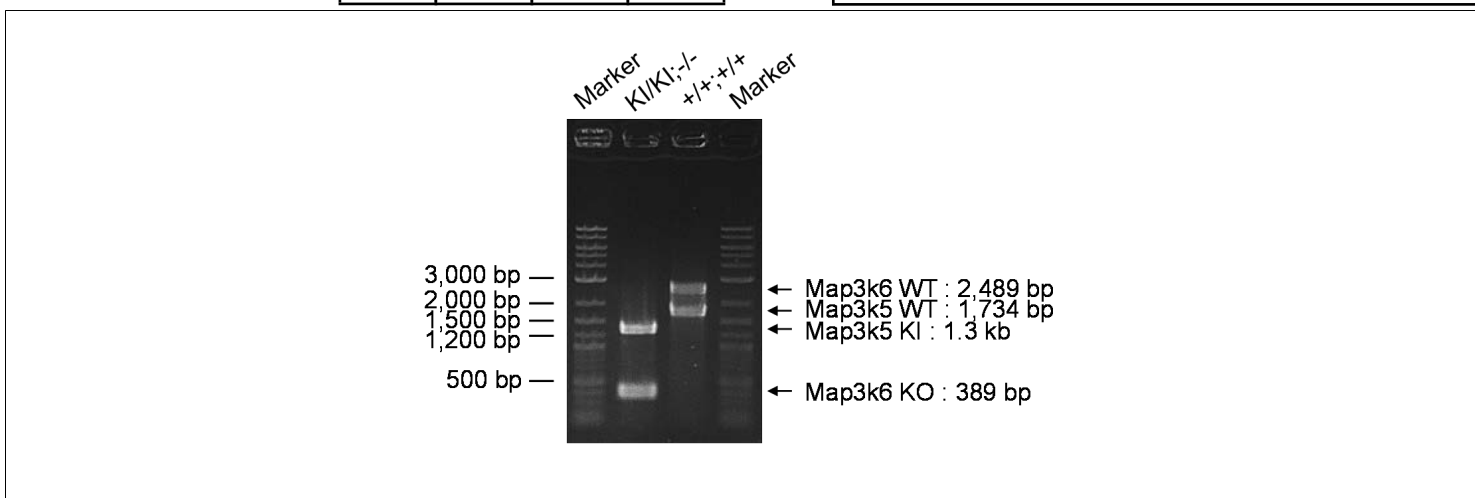
step	temp (°C)	time (sec)	note
1	94	120	
2	98	10*	
3	60	30*	
4	68	180*	*Repeat for 30 cycles

Reagent	Conc	Reaction 1	2	3	4
H2O		1.8			
PCR buffer	2 X	10.0			
dNTP	2.0 mM	4.0			
primer 1	10 pmol/μl	0.5			
primer 2	10 pmol/μl	0.5			
primer 3	10 pmol/μl	0.5			
primer 4	10 pmol/μl	0.5			
primer 5	10 pmol/μl	0.5			
primer 6	10 pmol/μl	0.5			
DNA		1.0			
Taq	1 U/μl	0.2			
total		20			

(μL)

Marker 2-Log DNA Ladder (NEB, Cat. N3200)

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



primer_set	product_size	product (allele type)	
primer 1	primer 2	1,734 bp	WT (Map3k5)
primer 1	primer 3	1.3 kb	KI (Map3k5)
primer 4	primer 5	2,489 bp	WT (Map3k6)
primer 4	primer 6	389 bp	KO (Map3k6)

+/+;+/+ (wild-type): 1,734 bp and 2,489 bp
KI/KI;-/- (homozygous): 1.3 kb and 389 bp

Comment Standard PCR

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