

Primer	primer_name	primer_seq	length	Reference
1	intron 1	AGTAAGGGAGCTGCAGTGGAG	21	RIKEN BRC
2	intron 1	TTACATAGTCTAACTCGCGACTG	25	RIKEN BRC
3	F2AR(RM)	TGGCGGCCGCTCGAGATGAATC	22	Cell Cycle, 13, 2681-2696 (2014).
4	olMR0214	CCGGAGAACCTGCGTGCAATCC	22	JAX Genotyping Protocol

Reaction Components

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

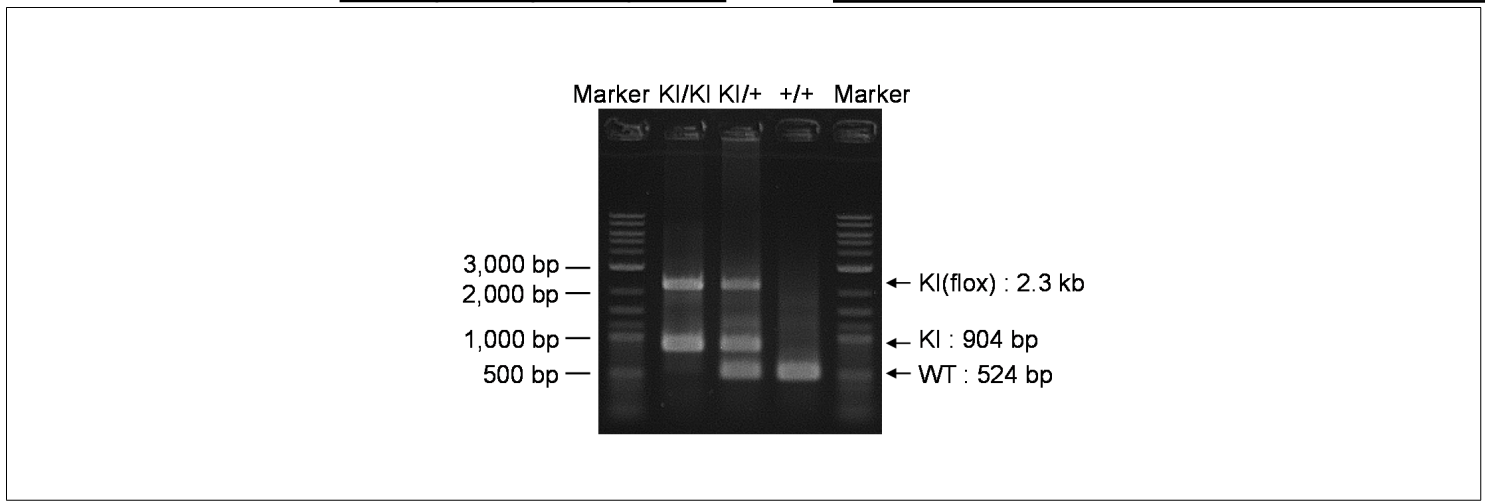
Reaction Conditions

step	temp (°C)	time (sec)	note
1	94	120	
2	98	10*	
3	60	30*	
4	68	240*	*Repeat for 30 cycles

Reagent	Conc	Reaction 1	2	3	4
H2O		0.8			
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			
primer 4	10 pmol/μl	1.0			
DNA		1.0			
Taq	1 U/μl	0.2			
total		20			

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	524 bp	WT
primer 1	primer 3	904 bp	KI
primer 2	primer 4	2.3 kb	KI(flox neo)

+/+ (wild-type): 524 bp
 KI/KI (homozygous): 904 bp and 2.3 kb
 KI/+ (heterozygous): 524 bp and 904 bp and 2.3 kb

Comment [Standard PCR](#)

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