PCR protocol

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RBRC 11399

Strain name B6.Cg-Arntl<tm1.1Shbi>

Primer	primer_name	primer_seq	length		Reference
1	503-45	GGGGATTTCCATCTGTGTTTAC	22		PLoS One. 6(9):e25231 (2011).
2	503-7R	GGCTAATGAAAGCACTGACAGACA	24		PLoS One. 6(9):e25231 (2011).
3	intron 5	GAGAAGTATCTGAGCTCATCTGC	23		RIKEN BRC

Reaction Components

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

Reagent	Conc	Reaction 1	2	3	4
H2O		2.8			
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	0			
DNA		1.0			
Taq	1 U/μl	0.2			
total		20			

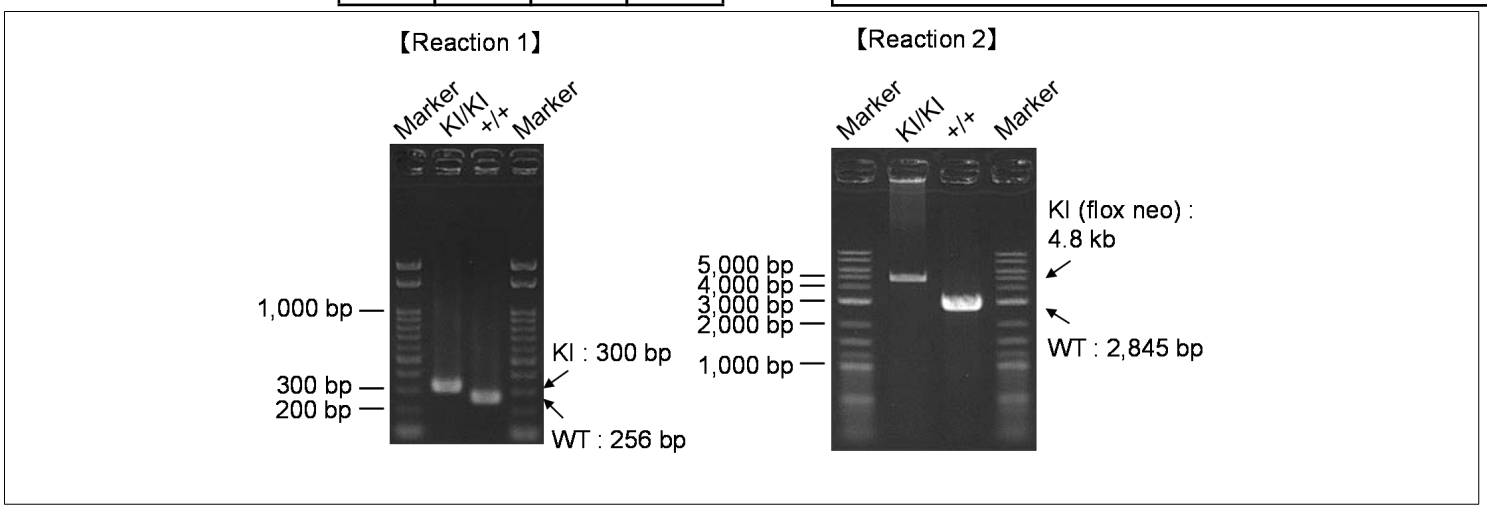
(μL)

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	[Reaction 1]
2	98	10	*
3	60	30	*
4	68	60	* *Repeat for 30 cycles
1	94	120	[Reaction 2]
2	98	10	*
3	60	30	*
4	68	360	* *Repeat for 30 cycles

Marker: marker Run1: Gene Ladder 100 (Wako, Cat. 316-06951), marker Run2: 2-Log DNA Ladder (NEB, Cat. N3200)

Gel: Run1:2% agarose in 1 X TAE (100 V, 30 min)Run2:1% agarose in 1 X TAE (100 V, 30 min)



primer_set		product_size	product (allele type)
primer 1	primer 2	256 bp	WT
primer 1	primer 2	300 bp	KI
primer 1	primer 3	2,845 bp	WT
primer 1	primer 3	4.8 kb	KI(flox neo)

Reaction 1
+/+ (wild-type): 256 bp
KI/KI (homozygous): 300 bp
Reaction 2
+/+ (wild-type): 2,845 bp
KI/KI (homozygous): 4.8 kb

Comment Standard PCR

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