PCR protocol

Sheet ID PS_07924
Release date : 2/3/2021
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RBRC 01048 **Strain name** B6.Cg-Atf7<tm1Sis>/Rbrc

Primer	primer_name	primer_seq	length		Reference
1	intron 10	CTGCCATTGACTTCATGACTTACC	24		RIKEN BRC
2	intron 11	CTGATCTCTAGCAGAGTATCAGATC	25		RIKEN BRC
3	Neo_F	GGTGGAGAGGCTATTCGGCTATG	23		RIKEN BRC

Reaction Components

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

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	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			

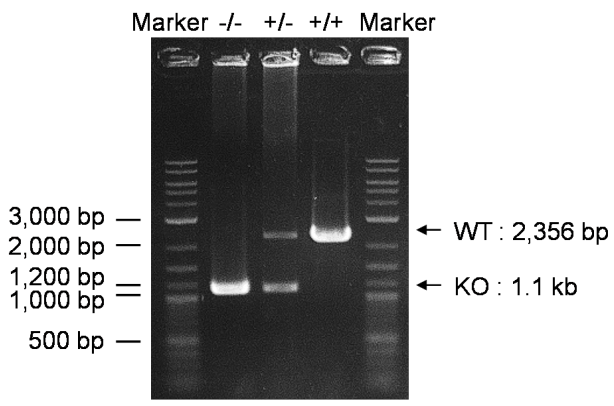
(μL)

Reaction Conditions

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

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	2,356 bp	WT
primer 2	primer 3	1.1 kb	KO

+/+ (wild-type): 2,356 bp
+/- (heterozygous): 2,356 bp and 1.1 kb
-/- (homozygous): 1.1 kb

Comment Standard PCR

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