PCR protocol

Sheet ID PS_08320
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RBRC 09679

Strain name C57BL/6-Crem<tm1.1Endo>

Primer	primer_name	primer_seq	length		Reference
1	Target F	CTTCATGAGCTACAACTCTG	20		J. Neurosci., 28, 6459-6472 (2008).
2	Target R	AGATCACTGCCTTAGTCAAC	20		J. Neurosci., 28, 6459-6472 (2008).

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			
DNA		1.0			
Taq	1 U/μl	0.2			
	total	20			

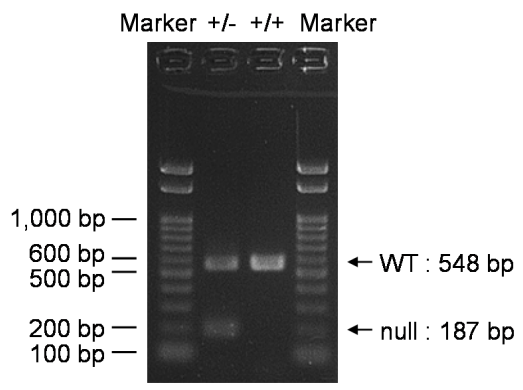
(μL)

Reaction Conditions

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

Marker: Gene Ladder 100 (Wako, Cat. 316-06951)

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



primer_set		product_size	product (allele type)
primer 1	primer 2	548 bp	WT
primer 1	primer 2	187 bp	null

+/+ (wild-type): 548 bp
+/- (heterozygous): 548 bp and 187 bp

Comment Standard PCR

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