PCR protocol

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RBRC 06494 **Strain name** C57BL/6-Grm6<tm1Nak>

Primer	primer_name	primer_seq	length		Reference
1	F	AGCGCAGGAACCCCAGTGATGTTCA	25		Depositor's primer
2	R1	GTGAGCAGGGCAGAGTAGTTGAGCG	25		Depositor's primer
3	3Neo	CGGCCACAGTCGATGAATCC	20		Proc. Natl. Acad. Sci. USA, 94, 13069-13074 (1997).
4	mGluR6-Cre51	GGGTGGGTCCAGAGGAAGAGCTGCCCA	27		Depositor's primer
5	LacZ381L22	GGTTTTCCCAGTCACGACGTTG	22		Depositor's primer

Reaction Components

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

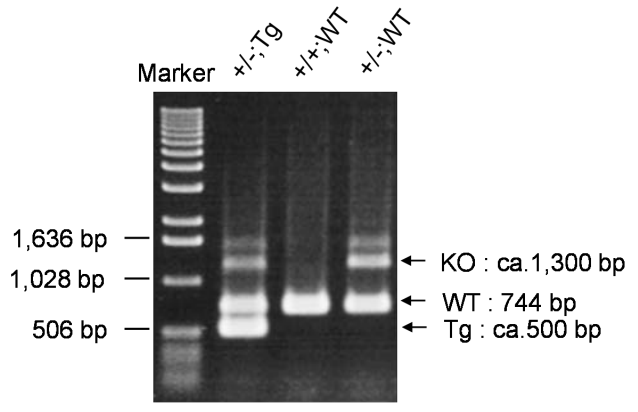
Reagent	Conc	Reaction 1	2	3	4	
H2O		2.3				(μL)
PCR buffer	2 X	10.0				
dNTP	2.0 mM	4.0				
primer 1	10 pmol/μl	0.5				
primer 2	10 pmol/μl	0.5				
primer 3	10 pmol/μl	0.5				
primer 4	10 pmol/μl	0.5				
primer 5	10 pmol/μl	0.5				
DNA		1.0				
Taq	1 U/μl	0.2				
	total	20				

Reaction Conditions

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

Marker 1 kb DNA ladder (Invitrogen, Cat. 15615-024)

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



primer_set		product_size	product (allele type)
primer 1	primer 2	744 bp	WT
primer 1	primer 3	ca. 1,300 bp	KO
primer 4	primer 5	ca. 500 bp	Tg

Comment

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