



PCR protocol

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RBRC 00442

Strain name B6.129-Nbl1 <tm1 Saki>/SakiRbrc

Primer	primer_name	primer_seq	length	Reference
1	MDANG1F (597)	ATACCTGCTTCCCCACTCCT	20	Mol. Cell Neurosci., 25(3):504-14 (2004).
2	MDANG1R (331)	GTGTTGGGGACGCTGTAAC	20	Mol. Cell Neurosci., 25(3):504-14 (2004).
3	NeoR(1561)	GAACCTGCGTGCAATCCATCTT	22	Mol. Cell Neurosci., 25(3):504-14 (2004).

Reaction Components

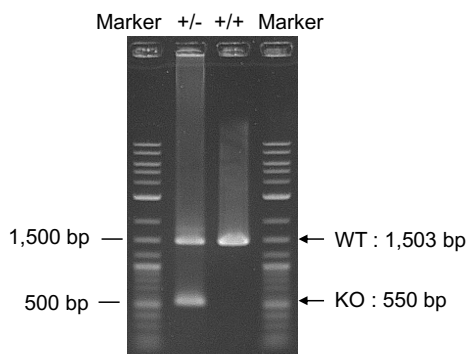
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

Reaction Conditions

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

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			

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	1,503 bp	WT
primer 1	primer 3	550 bp	KO

Comment [Standard PCR](#)

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