PCR protocol

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RBRC 06217 **Strain name** B6;129-Slc39a13<tm1Thir>

Primer	primer_name	primer_seq	length		
1	ex5_Fw	CACTGCTGCCGCGCTCAATG	20		RIKEN BRC
2		TGGTGGTCAGAAGCCCGATCT	21		PLoS One, 3(11):e3642 (2008).
3	Neo	GCAATCCATCTTGTTCAATGGCC	23		
4	ex9_Rv	CACGGGTCGTCTTCTTCCAAG	21		RIKEN BRC
5	WT/Hetero Forward	CACCATAGACAACTTCACTCA	21		Depositor's primer
6	PTN-Reverse	GCGAGGATCTCGTCGTGACCCATG	24		

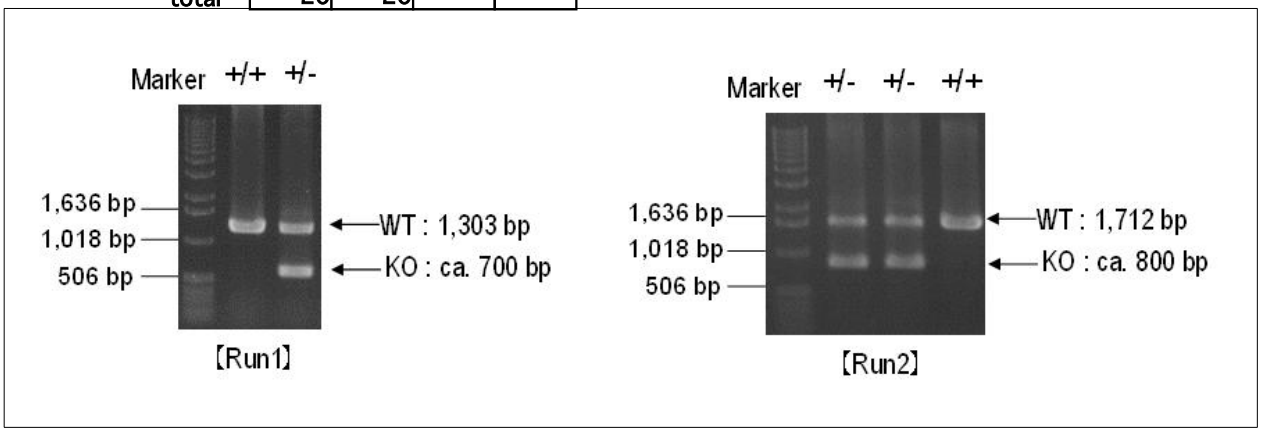
Reaction Components

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

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		1.8	1.8		
PCR buffer	2 X	10.0	10.0		
dNTP	2.0 mM	4.0	4.0		
primer 1	10 pmol/μl	1.0	0		
primer 2	10 pmol/μl	1.0	0		
primer 3	10 pmol/μl	1.0	0		
primer 4	10 pmol/μl	0	1.0		
primer 5	10 pmol/μl	0	1.0		
primer 6	10 pmol/μl	0	1.0		
DNA		1.0	1.0		
Taq	1 U/μl	0.2	0.2		
	total	20	20		

Reaction Conditions

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



Marker

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

Gel

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

PCR products

primer_set		product_size	product
primer 1	primer 2	1,303 bp	WT
primer 1	primer 3	ca. 700 bp	KO
primer 4	primer 5	1,712 bp	WT
primer 4	primer 6	ca. 800 bp	KO

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