PCR protocol

Sheet ID PS_04142
Release date : 4/5/2018
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RBRC 04832 **Strain name** B6.129P2(CD1)-Id4<tm1Fsky>

Primer	primer_name	primer_seq	length		
1	Common fwd	AAGGCGGCCGAGGCGGCGGCCGATGA	26		Depositor's primer
2	Wild type Rev	TGTCTCAGCAAAGCAGGGTGAGTC	24		Depositor's primer
3	Mutation Rev	ATTAAGTTGGGTAACGCCAGGGTT	24		Depositor's primer

Reaction Components

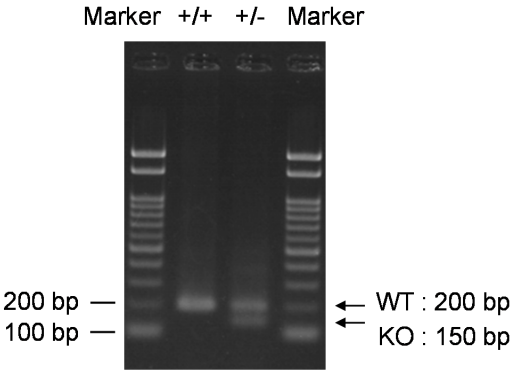
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		1.6			
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	0.4			
	total	20			

Reaction Conditions

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

Standard PCR



Marker

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

Gel

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

PCR products	primer_set	product_size	product
primer 1	primer 2	200 bp	WT
primer 1	primer 3	150 bp	KO

Comment

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