

RBRC 06251 Strain name B6.Cg-Ret<tm1Mat> Ptch1<tm1Mps>

Primer	primer_name	primer_seq	length	
1	Y1062F 19F	ACTACTTGGACCTGGCTGCATCCA	24	Depositor's primer
2	ex12_Rv	GCCAGTTCGGGTCTGACATGC	21	RIKEN BRC
3	Neo10	ATCAGAGCAGCCGATTGTCTGTTG	24	
4	ex1_up	CTCACGCGCAATGTGGCAATG	21	RIKEN BRC
5	WT4	AGGGCTTCTCGTTGGCTACAAG	22	Depositor's primer
6	lacZ-R	TCTGCTCAATCAGCGTGCC	20	Genes Cells, 1:59-72 (1996).

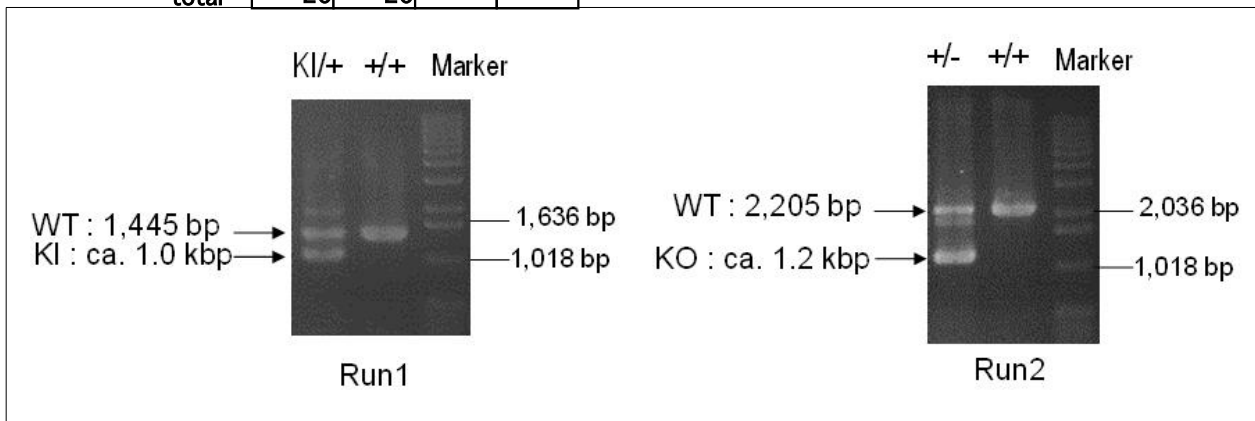
Reaction Components

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

Reaction Conditions

	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/ μ l	1.0	0		
primer 2	10 pmol/ μ l	1.0	0		
primer 3	10 pmol/ μ l	1.0	0		
primer 4	10 pmol/ μ l	0	1.0		
primer 5	10 pmol/ μ l	0	1.0		
primer 6	10 pmol/ μ l	0	1.0		
DNA		1.0	1.0		
Taq	1 U/ μ l	0.2	0.2		
total		20	20		

step	temp (°C)	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	1,445 bp	WT (Ret)
primer 1	ca. 1.0 kbp	KI (Ret)
primer 4	2,205 bp	WT (Ptch1)
primer 4	ca. 1.2 kbp	KO (Ptch1)

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