

PCR protocol

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RBRC 03514

Strain name Hrs(loxP)(Acc.No. CDB0476K)

Primer	primer_name	primer_seq	length	
1	5_loxP_Fw	GATGATGAGATGTTTACCTTAGTC	24	RIKEN BRC
2	5_loxP_Rv	TTGTCCTTTACCTCTTAGCTGGG	23	RIKEN BRC
3	6851F	TTGTTGAATGAGTAACAAGGGTGGT	25	Am. J. Pathol., 173, 1806-1817 (2008).
4	9100R	TGGATCCCATGAAATGGGGAACAGC	25	Am. J. Pathol., 173, 1806-1817 (2008).

Reaction Components

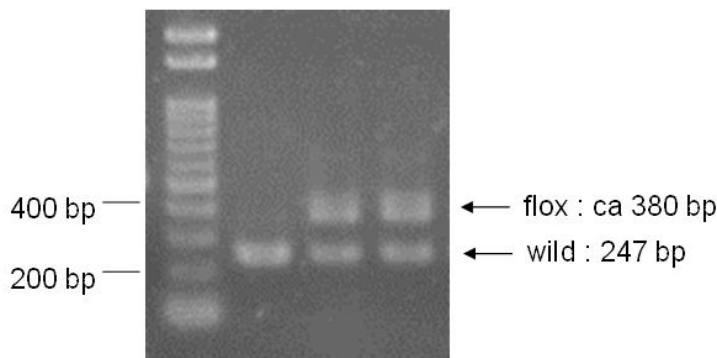
Enzyme TaKaRa Ex Taq (Cat. No. RR001A)

Reaction Conditions

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		13.3	13.3		
PCR buffer	10 X	2.0	2.0		
dNTP	2.5 mM	1.6	1.6		
primer 1	10 pmol/ μ l	1.0	0		
primer 2	10 pmol/ μ l	1.0	0		
primer 3	10 pmol/ μ l	0	1.0		
primer 4	10 pmol/ μ l	0	1.0		
DNA		1.0	1.0		
Taq	5 U/ μ l	0.1	0.1		
total		20	20		

step	temp (°C)	time (sec)	note
1	94	180	
2	94	30*	
3	60	30*	
4	72	60*	*Repeat for 30 cycles
5	72	300	

Marker +/- flox/+ flox/+



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	247 bp	WT
primer 1	primer 2	ca 380 bp	flox
primer 3	primer 4	2250 bp	WT
primer 3	primer 4	ca 300 bp	null

Comment