

PCR protocol

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RBRC 05760

Strain name B6.Cg-Spred1<tm1.1Ayo>

Primer	primer_name	primer_seq	length		
1	Spred1-flox(A13)	CTTCCTAGTTCTGAGGTAGATGTGC	25		Depositor's primer
2	Spred1-flox(A12)	ACAACTTGCTGGCTGAATACCACAG	25		Depositor's primer
3		CGAAGTATGGAATATGTACAGCGGC	25		RIKEN BRC

Reaction Components

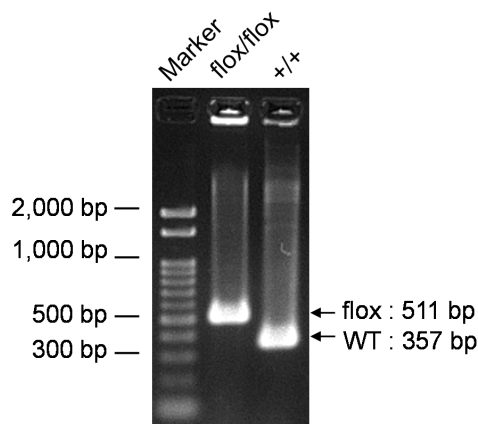
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

Reaction Conditions

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

	Conc	Run 1	Run 2	Run 3	Run 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			

Standard PCR



Marker

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

Gel

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

Comment

PCR products

primer_set	product_size	product
primer 1	357 bp	WT
primer 1	511 bp	flox
primer 2	2 kb	null (After cre mediated excision)

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