PCR protocol

Sheet ID PS_05774
Release date : 1/30/2017
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RBRC 06047 **Strain name** B6.Cg-Hes1<tm1Imayo>

Primer	primer_name	primer_seq	length		
1		CAGCCAGTGTCAACACGACACCGGACAAAC	30		Development, 135(15):2531-2541 (2008).
2		TGCCCTTCGCCTCTTCTCCATGATA	25		Development, 135(15):2531-2541 (2008).
3		CGCACTCGGCGCTGGCCTGTG	21		RIKEN BRC

Reaction Components

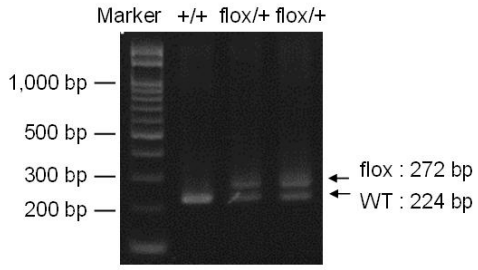
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

	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			

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
2	98	10*	
3	60	30*	
4	68	240*	*Repeat for 30 cycles

Standard PCR



Marker

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

Gel

3% agarose in 1 X TAE (100 V, 40 min)

PCR products	primer_set	product_size	product
primer 1	primer 2	224 bp	WT
primer 1	primer 2	272 bp	flox
primer 1	primer 3	1.2 kb	null (After cre mediated excision)

Comment

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