PCR protocol

Sheet ID PS_07151
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RBRC 09808 **Strain name** C57BL/6-Rnf31<tm2.1Kiwa>

Primer	primer_name	primer_seq	length		Reference
1	fl-f4	ACACTAAGCAAGTAGCAGCCAC	22		Depositor's primer
2	fl-r5	GCTCTGGCCTTTCTGATTGCTG	22		Depositor's primer
3	HOIPC R1	CCTGAAGAATTGCCAACTTTGACC	24		Depositor's primer

Reaction Components

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

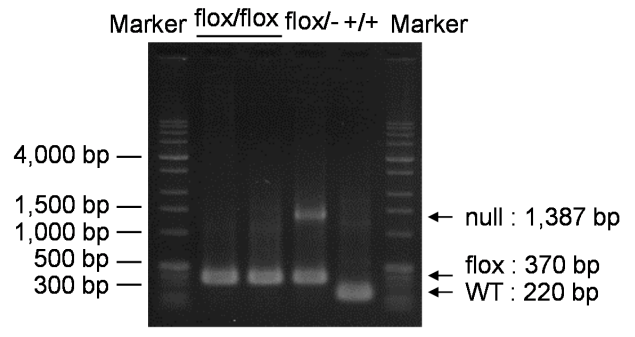
Reagent	Conc	Reaction 1	2	3	4	
H2O		1.8				(μL)
PCR buffer	2 X	10.0				
dNTP	2.0 mM	4.0				
primer 1	10 pmol/μl	1.0				
primer 2	10 pmol/μl	1.0				
primer 3	10 pmol/μl	1.0				
DNA		1.0				
Taq	1 U/μl	0.2				
total		20				

Reaction Conditions

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

Marker DNA Ladder,1 kb Plus (Affymetrix, Cat. 76714 500 UL)

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



primer_set		product_size	product (allele type)
primer 1	primer 2	220 bp	WT
primer 1	primer 2	370 bp	flox
primer 1	primer 3	1,387 bp	null

Comment

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