PCR protocol

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RBRC 05968 **Strain name** C57BL/6N-Efcab1<tm1a(KOMP)Osb>/19

Primer	primer_name	primer_seq	length		Reference
1	int3_Fw	GCAGAATCTGACATAATGATGAGTTTG	27		RIKEN BRC
2	ex5_Rv	CATCATGGTCATGGTCCTGTGAG	23		RIKEN BRC
3		GGCACATGGCTGAATATCGACGG	23		RIKEN BRC

Reaction Components

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

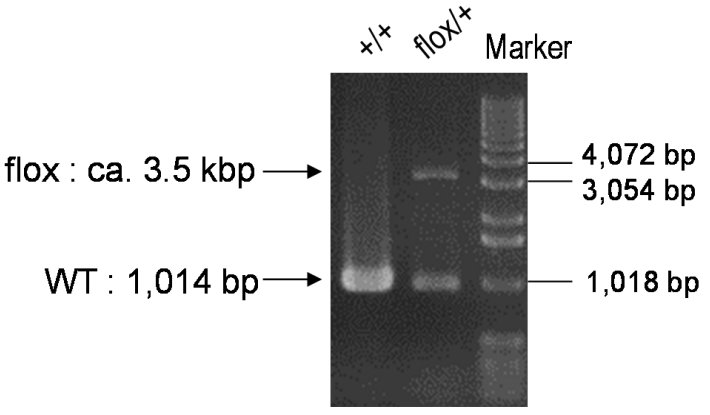
Reagent	Conc	Reaction 1	2	3	4	
H2O		1.8				(μL)
PCR buffer	2 X	10.0				
dNTP	2.0 mM	4.0				
primer 1	10 pmol/μl	1.0				
primer 2	10 pmol/μl	1.0				
primer 3	10 pmol/μl	1.0				
DNA		1.0				
Taq	1 U/μl	0.2				
	total	20				

Reaction Conditions

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

Marker 1 kb DNA ladder (Invitrogen, Cat. 15615-024)

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



primer_set		product_size	product (allele type)	
primer 1	primer 2	1,014 bp	WT	
primer 2	primer 3	ca. 3.5 kbp	flox	

Comment Standard PCR

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