PCR protocol

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RBRC 10293 **Strain name** C57BL/6-Itpr2<tm2.1Kmik>

Primer	primer_name	primer_seq	length		Reference
1	Itpr2loxP S-primer	TCTCAGCAACTTCATAATCTC	21		Depositor's primer
2		CAGCCGCGCGCTGAGAACGG	20		RIKEN BRC
3		GAACCCAGTTACCCTCACTTGTG	23		RIKEN BRC

Reaction Components

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

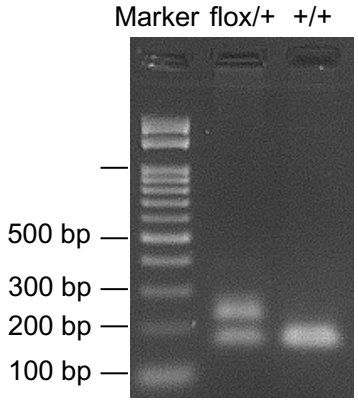
Reagent	Conc	Reaction 1	2	3	4	
H2O		1.8				(μL)
PCR buffer	2 X	10.0				
dNTP	2.0 mM	4.0				
primer 1	10 pmol/μl	1.0				
primer 2	10 pmol/μl	1.0				
primer 3	10 pmol/μl	1.0				
DNA		1.0				
Taq	1 U/μl	0.2				
	total	20				

Reaction Conditions

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

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

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



primer_set		product_size	product (allele type)
primer 1	primer 2	161 bp	WT
primer 1	primer 2	220 bp	flox
primer 2	primer 3	~900 bp	null (After cre mediated excision)

+/+ : 161 bp
flox/+ : 161 bp, 220 bp

Comment Standard PCR

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