PCR protocol

Sheet ID PS_07193
Release date : 6/20/2017
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RBRC 09393 **Strain name** B6-Kcnj11<tm2Sse>

Primer	primer_name	primer_seq	length		
1	promoter	ATGGCCTGGCTGAGGACAGTG	21		RIKEN BRC
2	Kir6.2-loxP 11606Rwt	CCAAGACGGGCTTTGAGACC	20		Depositor's primer
3		GCAGCGCATCGCCTTCTATCGC	22		RIKEN BRC

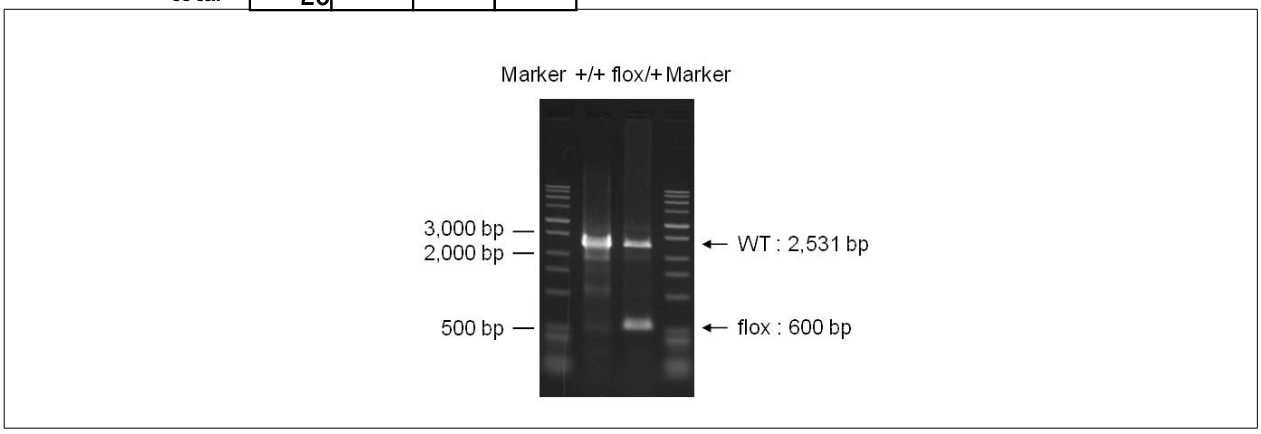
Reaction Components

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



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

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

PCR products	primer_set	product_size	product
primer 1	primer 2	2,531 bp	WT
primer 2	primer 3	600 bp	flox
primer 1	primer 2	1 kb	null (After cre mediated excision)

Comment

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