PCR protocol

Sheet ID PS_07672
Release date : 12/9/2019
Print date : 12/9/2019

RBRC 10292 **Strain name** B6.Cg-Itpr1<tm1.1Kmik>/274D Itpr2<tm2.1Kmik> Itpr3<tm2.1Kmik>

Primer	primer_name	primer_seq	length		Reference
1	Itpr1loxP S-primer	TACATAGATCCAAAGAAGTGCCTCTG	26		J. Neurosci., 33(30) 12186-12196 (2013).
2	Itpr1loxP AS-primer	AGGTTGAGTGATGACTGATTGGAGG	25		J. Neurosci., 33(30) 12186-12196 (2013).
3	Itpr2loxP S-primer	TCTCAGCAACTTCATAATCTC	21		Depositor's primer
4	Itpr2loxP AS-primer	ATCAAAACAAGGCAGGGAAG	20		Depositor's primer
5	Itpr3loxP S-primer	AGTACCAGGTCTGTTGACACC	21		Depositor's primer
6	Itp3loxP AS-primer	TGTGGTCTTGGATGGAAAAGC	21		Depositor's primer

Reaction Components

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

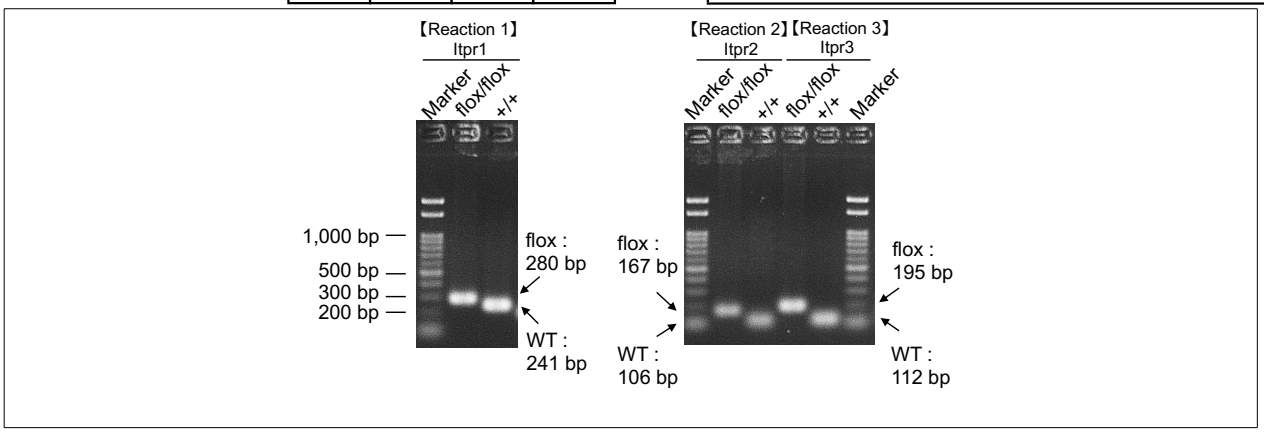
Reagent	Conc	Reaction 1	2	3	4	
H2O		2.8	2.8	2.8		(μL)
PCR buffer	2 X	10.0	10.0	10.0		
dNTP	2.0 mM	4.0	4.0	4.0		
primer 1	10 pmol/μl	1.0	0	0		
primer 2	10 pmol/μl	1.0	0	0		
primer 3	10 pmol/μl	0	1.0	0		
primer 4	10 pmol/μl	0	1.0	0		
primer 5	10 pmol/μl	0	0	1.0		
primer 6	10 pmol/μl	0	0	1.0		
DNA		1.0	1.0	1.0		
Taq	1 U/μl	0.2	0.2	0.2		
	total	20	20	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 100 bp ladder (Invitrogen, Cat. 15628-050)

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



primer_set		product_size	product (allele type)
primer 1	primer 2	241 bp, 280 bp	WT, flox (Itpr1)
primer 3	primer 4	106 bp, 167 bp	WT, flox (Itpr2)
primer 5	primer 6	112 bp, 195 bp	WT, flox (Itpr3)

Reaction 1
+/+ (wild-type): 241 bp
flox/flox (homozygous): 280 bp
Reaction 2
+/+ (wild-type): 106 bp
flox/flox (homozygous): 167 bp
Reaction 3
+/+ (wild-type): 112 bp
flox/flox (homozygous): 195 bp

Comment Standard PCR

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