PCR protocol

Sheet ID PS_07721
Release date : 11/16/2018
Print date : 11/16/2018

RBRC 10392 **Strain name** B6.Cg-Ebf3<tm1(cre/ERT2)Tng>

Primer	primer_name	primer_seq	length		Reference
1	exon 16	AGCACAGGAGGAGCAAGCTG	20		RIKEN BRC
2	exon 16	CCATCAGCACATCTTAACACACTAC	25		RIKEN BRC
3	cre 2	TCGACCAGTTTAGTTACCC	19		Depositor's primer
4	ERT2	CTTACTGACCAACCTGGCAGAC	22		RIKEN BRC

Reaction Components

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

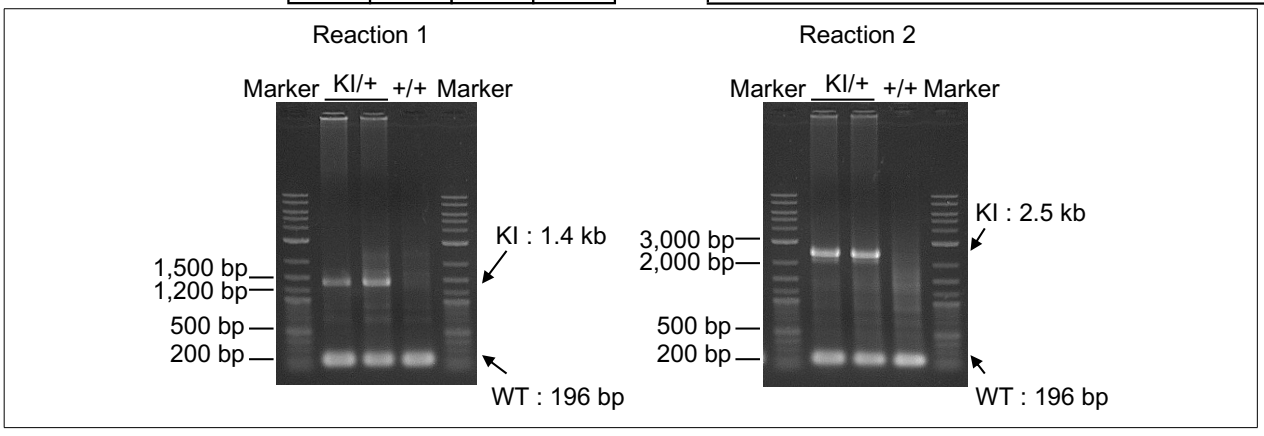
Reagent	Conc	Reaction 1	2	3	4	
H2O		1.8	1.8			(μL)
PCR buffer	2 X	10.0	10.0			
dNTP	2.0 mM	4.0	4.0			
primer 1	10 pmol/μl	1.0	1.0			
primer 2	10 pmol/μl	1.0	1.0			
primer 3	10 pmol/μl	1.0	0			
primer 4	10 pmol/μl	0	1.0			
DNA		1.0	1.0			
Taq	1 U/μl	0.2	0.2			
	total	20	20			

Reaction Conditions

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

Marker 2-Log DNA Ladder (NEB, Cat. N3200)

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



primer_set		product_size	product (allele type)
primer 1	primer 2	196 bp	WT
primer 1	primer 3	1.4 kb	KI
primer 2	primer 4	2.5 kb	KI

Reaction 1
+/+ : 196 bp
KI/+ : 196 bp, 1.4 kb
Reaction 2
+/+ : 196 bp
KI/+ : 196 bp, 2.5 kb

Comment Standard PCR

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