PCR protocol

Sheet ID PS_07999
Release date : 4/15/2020
Print date : 4/15/2020

RBRC 09245 **Strain name** B6.Cg-Ehmt2<tm2Yshk>

Primer	primer_name	primer_seq	length		Reference
1	mG125F	CTGCACGCTGCCTAGATGGAGCATG	25		Depositor's primer
2	mG9a-10205R	CTGGGTGGAAAGTTGCCAGGCTTAG	25		Depositor's primer
3	GE28R	GCTCCAGGGCGATGGCCTCCGCTGAATGC	29		EMBO J., 26, 3346-3359 (2007).

Reaction Components

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

Reagent	Conc	Reaction 1	2	3	4
H2O		2.8	2.8		
PCR buffer	2 X	10.0	10.0		
dNTP	2 mM	4.0	4.0		
primer 1	10 pmol/μl	1.0	1.0		
primer 2	10 pmol/μl	1.0	0		
primer 3	10 pmol/μl	0	1.0		
DNA		1.0	1.0		
Taq	1 U/μl	0.2	0.2		
total		20	20		

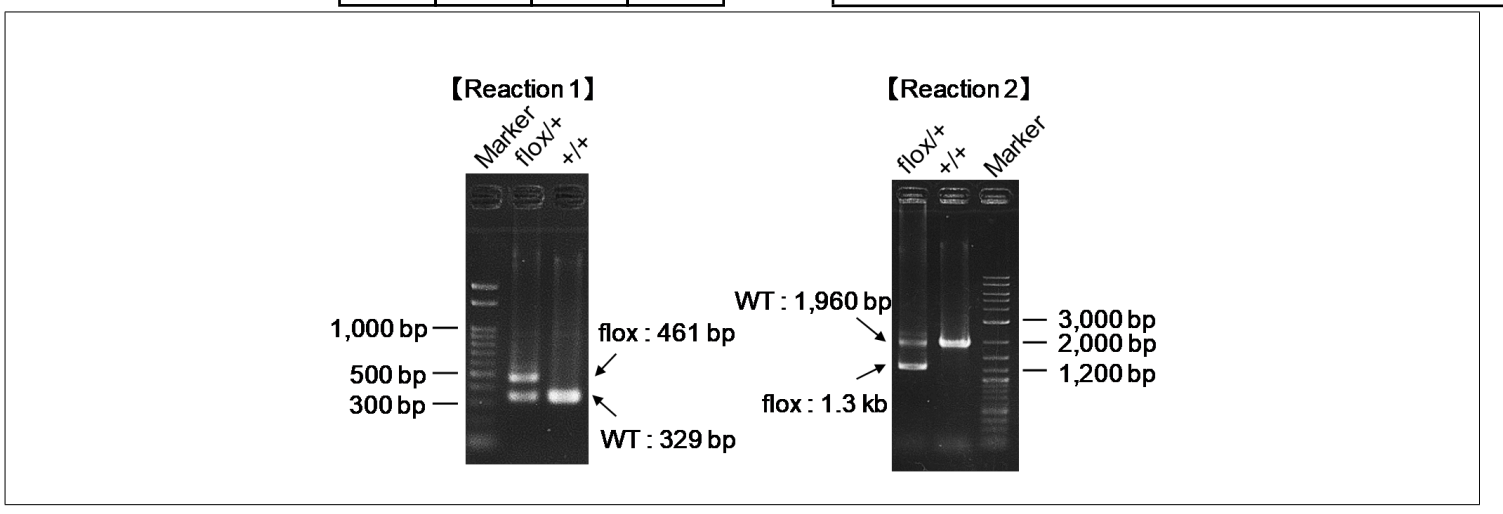
(μL)

Reaction Conditions

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

Marker marker Run1: Gene Ladder 100 (Wako, Cat. 316-06951), marker Run2: 2-Log DNA Ladder (NEB, Cat. N3200)

Gel Run1:2% agarose in 1 X TAE (100 V, 30 min) Run2:1% agarose in 1 X TAE (100 V, 30 min)



primer_set		product_size	product (allele type)	
primer 1	primer 2	329 bp	WT	[Reaction 1] +/+ (wild-type): 329 bp flox/+ (heterozygous):329 bp, 461 bp [Reaction 2] +/+ (wild-type): 1,960 bp flox/+ (heterozygous): 1,960 bp, 1.3 kb
primer 1	primer 2	461 bp	flox	
primer 1	primer 3	1,960 bp	WT	
primer 1	primer 3	1.3 kb	flox	

Comment Standard PCR

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