PCR protocol

Sheet ID PS_08244
Release date : 6/2/2022
Print date : 6/2/2022

RBRC 11405 **Strain name** B6;Cg-Ipp<em1Tfur>

Primer	primer_name	primer_seq	length		Reference
1	Ipp-PCR-52	CCAGGTGGGGAAGGAAACCTTCCAAGT	27		Depositor's primer
2	Ipp-PCR-31	CACAGCAACAGAAAACCAATACATGGA	27		Depositor's primer

Reaction Components

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

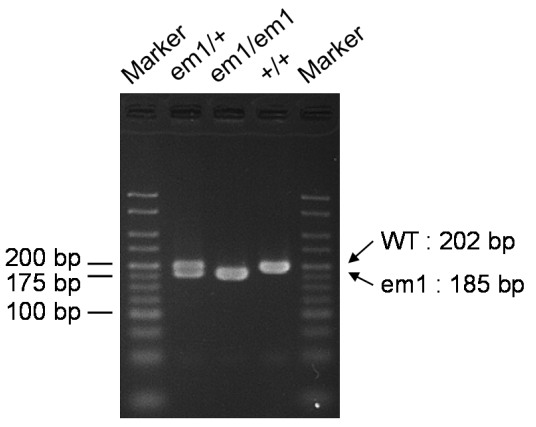
Reagent	Conc	Reaction 1	2	3	4	
H2O		2.8				(μL)
PCR buffer	2 X	10.0				
dNTP	2.0 mM	4.0				
primer 1	10 pmol/μl	1.0				
primer 2	10 pmol/μl	1.0				
DNA		1.0				
Taq	1 U/μl	0.2				
	total	20				

Reaction Conditions

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

Marker 25bp DNA Step Ladder (Wako, Cat. 292-69001)

Gel 4% Nusieve in 0.5 X TBE (100 V, 50 min)



primer_set		product_size	product (allele type)	
primer 1	primer 2	202 bp	WT	+/+ (wild-type): 202 bp em1/em1 (homozygous): 185 bp em1/+ (heterozygous): 202 bp and 185 bp
primer 1	primer 2	185 bp	em1	

Comment Standard PCR

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