PCR protocol

Sheet ID PS_08289
Release date : 3/16/2022
Print date : 3/16/2022

RBRC 11572 **Strain name** B6-Pum3<em1Nkn>

Primer	primer_name	primer_seq	length		Reference
1	Pum3 del-F	CACCAAGGCTTCCCAGGTTA	20		Depositor's primer
2	Pum3 del-R	AAAACCTTGTGTGCTTCCTTCC	22		Depositor's primer

Reaction Components

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

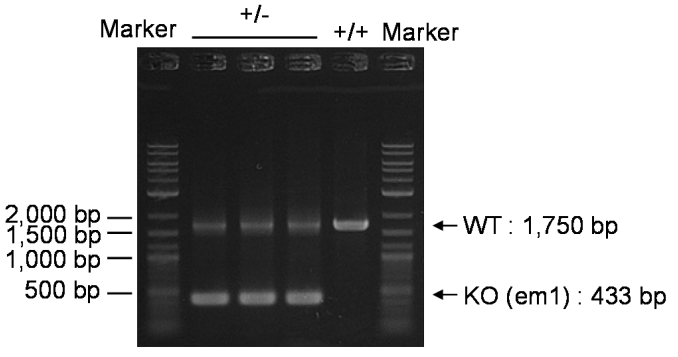
Reagent	Conc	Reaction 1	2	3	4	
H2O		2.8				(μL)
PCR buffer	2 X	10.0				
dNTP	2.0 mM	4.0				
primer 1	10 pmol/μl	1.0				
primer 2	10 pmol/μl	1.0				
DNA		1.0				
Taq	1 U/μl	0.2				
	total	20				

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
2	98	10	*
3	60	30	*
4	68	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	1,750 bp	WT	+/+ (wild-type): 1,750 bp +/- (heterozygous): 1,750 bp and 433 bp
primer 1	primer 2	433 bp	KO (em1)	

Comment Standard PCR

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