PCR protocol

Sheet ID PS_07799
Release date : 12/9/2019
Print date : 12/9/2019

RBRC 10613 **Strain name** B6.Cg-Usp15<tm1Tchi>

Primer	primer_name	primer_seq	length		Reference
1	Ubp109 in8	GATTTAAGAAGGGATACAGAACGAAAGAC	29		Depositor's primer
2	Ubp109out2(-)	GACATACTTACCACGATTTGGTCTTCAG	28		Depositor's primer
3	Neo 5F	GATCGGCCATTGAACAAGAT	20		Depositor's primer

Reaction Components

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

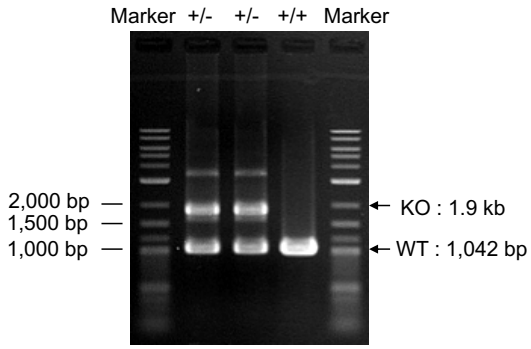
Reagent	Conc	Reaction 1	2	3	4	
H2O		1.8				(μL)
PCR buffer	2 X	10.0				
dNTP	2.0 mM	4.0				
primer 1	10 pmol/μl	1.0				
primer 2	10 pmol/μl	1.0				
primer 3	10 pmol/μl	1.0				
DNA		1.0				
Taq	1 U/μl	0.2				
	total	20				

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	1,042 bp	WT
primer 3	primer 2	1.9 kb	KO

+/+ (wild-type): 1,042 bp
+/- (heterozygous): 1,042 bp and 1.9 kb

Comment Standard PCR

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