PCR protocol

Sheet ID PS_08433
Release date : 1/13/2023
Print date : 1/13/2023

RBRC 11843 **Strain name** C57BL/6-Tmem158<em1Tfur>

Primer	primer_name	primer_seq	length		Reference
1	Tmem158-CRISPR-51	CCCAATACACTGGCAGAGTTCCGCCTC	27		Depositor's primer
2	wt	GTGAAGGACGCATTGGCCGAGGCGT	25		RIKEN BRC
3	mut	CGGCGGGGCCACGGATGCGCCCCC	24		RIKEN BRC
4	common	GGAGCCTCCTCTGGGCCCGCG	21		RIKEN BRC

Reaction Components

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

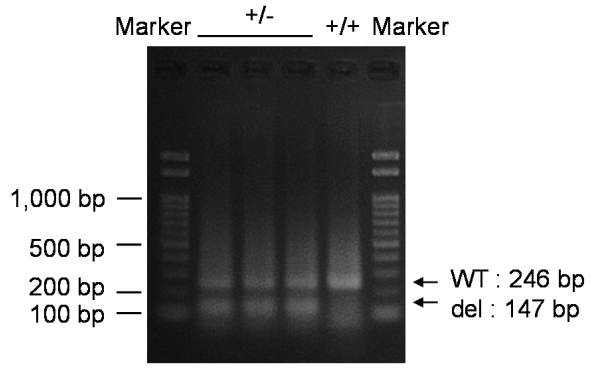
Reagent	Conc	Reaction 1	2	3	4	
H2O		0.8				(μL)
PCR buffer	2 X	10.0				
dNTP	2.0 mM	4.0				
primer 1	10 pmol/μl	1.0				
primer 2	10 pmol/μl	1.0				
primer 3	10 pmol/μl	1.0				
primer 4	10 pmol/μl	1.0				
DNA		1.0				
Taq	1 U/μl	0.2				
	total	20				

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
2	98	10	*
3	65	30	*
4	68	60	* *Repeat for 30 cycles

Marker Gene Ladder 100 (Wako, Cat. 316-06951)

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



primer_set		product_size	product (allele type)
primer 1	primer 2	246 bp	WT
primer 3	primer 4	147 bp	del

Comment Allele specific PCR (ASP)

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