PCR protocol

Sheet ID PS_07155
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RBRC 09862 **Strain name** C57BL/6-Pgam5<tm1Hijo>

Primer	primer_name	primer_seq	length		Reference
1	WT-f1	CTGCCTTCAGGAACTACATCCACCG	25		EBioMedicine, 5, 82-92 (2016).
2	v1	ACTTCTCTGACCAGGCTTCACCAGC	25		EBioMedicine, 5, 82-92 (2016).
3	mt-f1	TTAAGGGCCAGCTCATTCCTCCCAC	25		EBioMedicine, 5, 82-92 (2016).

Reaction Components

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

Reagent	Conc	Reaction 1	2	3	4
H2O		1.8			
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			

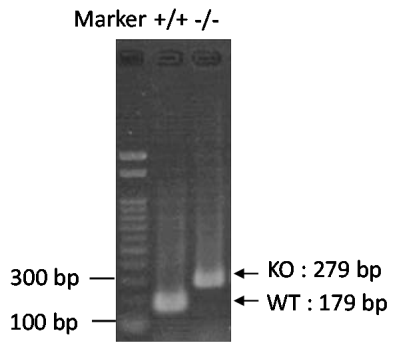
(μL)

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
2	98	10	*
3	60	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	179 bp	WT
primer 2	primer 3	279 bp	KO

Comment

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