

RBRC 10761

Strain name B6.B6CB-Gsdmd<tm1.1Tshir>/Ms

Primer	primer_name	primer_seq	length	Reference
1	gt1F	TTCCAATCCACAGCCTAAGAG	21	genesis 46:418-423 (2008).
2	gt1R	AGCTCAATAAATAACAAGAC	21	genesis 46:418-423 (2008).
3	gt2R	CTGCTGTTGTTTCTACTACTC	21	genesis 46:418-423 (2008).

**Reaction Components**

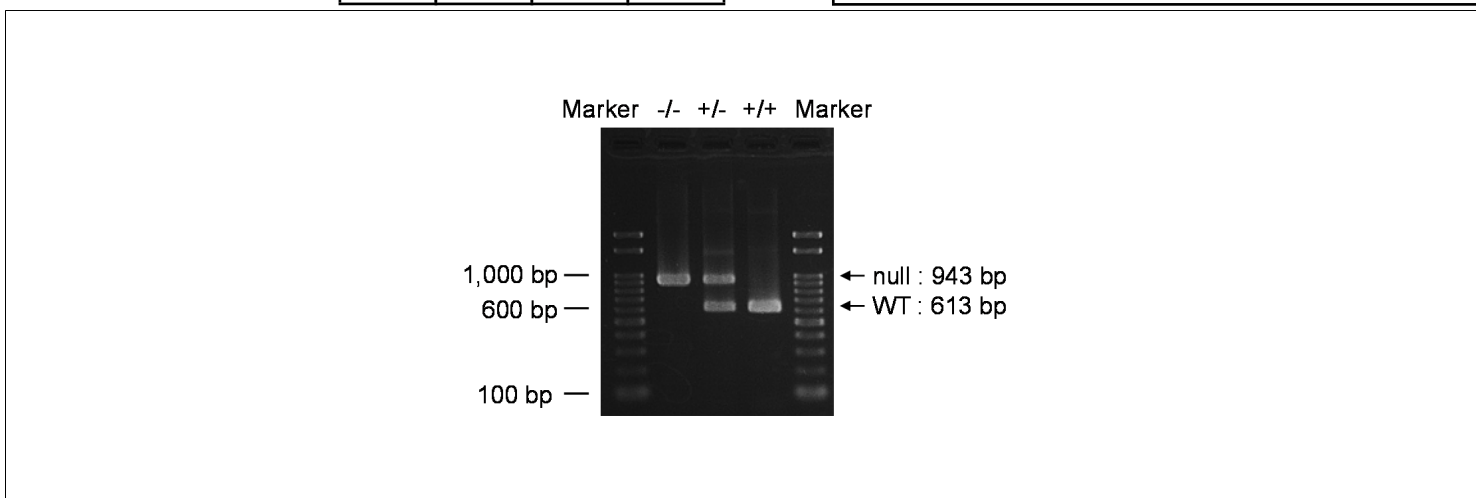
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

**Reaction Conditions**

step	temp (°C)	time (sec)	note
1	94	120	
2	98	10*	
3	55	30*	
4	68	60*	*Repeat for 30 cycles

Reagent	Conc	Reaction 1	2	3	4	( $\mu$ L)
H2O		1.8				
PCR buffer	2 X	10.0				
dNTP	2.0 mM	4.0				
primer 1	10 pmol/ $\mu$ l	1.0				
primer 2	10 pmol/ $\mu$ l	1.0				
primer 3	10 pmol/ $\mu$ l	1.0				
DNA		1.0				
Taq	1 U/ $\mu$ l	0.2				
<b>total</b>		20				

**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	613 bp	WT	-/- (homozygous): 943 bp +/- (heterozygous): 943 bp and 613 bp +/+ (wild-type): 613 bp
primer 1 primer 3	943 bp	null	

**Comment** [Standard PCR](#)

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

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Strain name B6.B6CB-Gsdmd<tm1.1Tshir>/Ms

Primer	primer_name	primer_seq	length	Reference
1	exon 2	GTGATCAAGGAGGTAAGCGGC	21	RIKEN BRC
2	R2	TGGCAACAGAGGCTGAGCATC	21	RIKEN BRC
3	F	GGGACACTCTCAAGAGCCTTACA	23	RIKEN BRC

**Reaction Components**

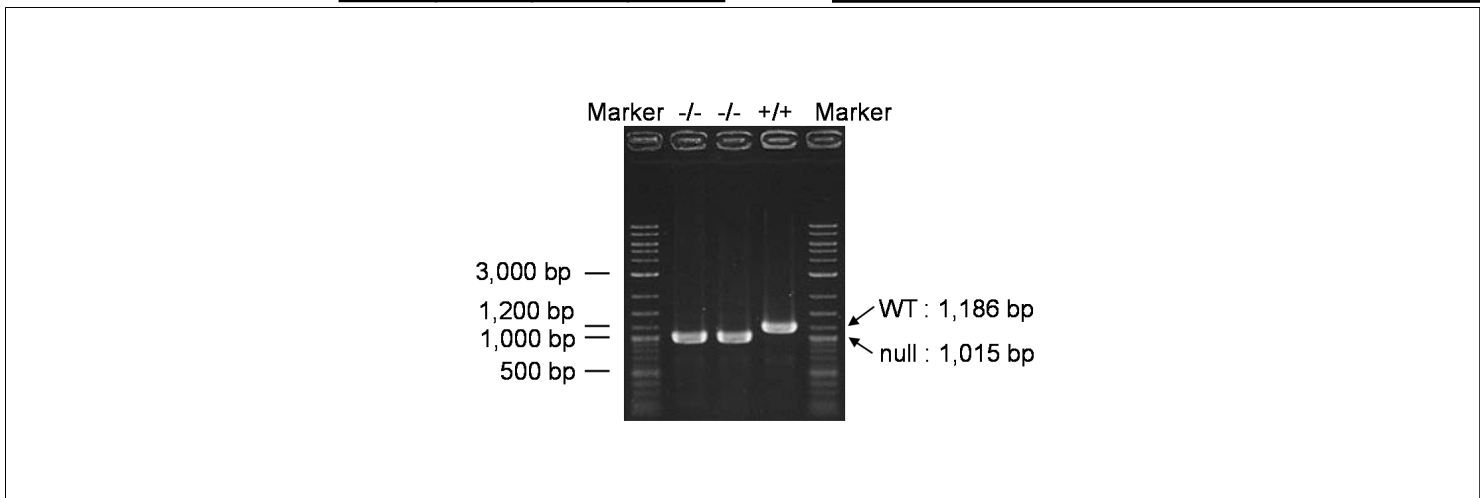
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

**Reaction Conditions**

step	temp (°C)	time (sec)	note
1	94	120	
2	98	10*	
3	60	30*	
4	68	180*	*Repeat for 30 cycles

Reagent	Conc	Reaction 1	2	3	4	(µL)
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				
<b>total</b>		20				

**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	1,186 bp WT
primer 2	primer 3	1,015 bp null

**Comment** Standard PCR

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