

RBRC 11849

Strain name C57BL/6-Itgb2<sup><em>1Nsas</em></sup>

Primer	primer_name	primer_seq	length	Reference
1	wt	GGTTGTCCAGCCGATGATATCATG	24	RIKEN BRC
2	Itgb2-R	ACCTGGTCGCAAGTAAAGTGTC	22	Depositor's primer
3	Itgb2-F	GACTACCGCATCCCACAGAG	20	Depositor's primer
4	mut	AGCGATGCTCCTGGGGTCCATCGG	24	RIKEN BRC

**Reaction Components**

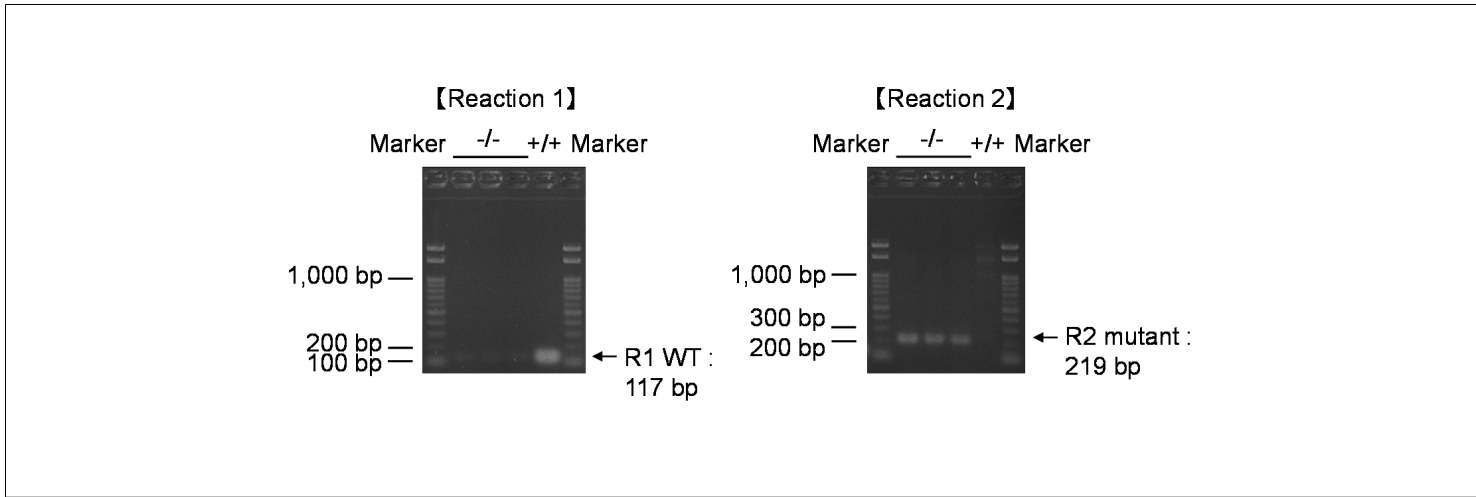
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

**Reaction Conditions**

step	temp (°C)	time (sec)	note
1	94	120	
2	98	10*	
3	65	30*	
4	68	60*	*Repeat for 35 cycles

Reagent	Conc	Reaction 1	2	3	4	(μL)
H2O		2.8	2.8			
PCR buffer	2 X	10.0	10.0			
dNTP	2.0 mM	4.0	4.0			
primer 1	10 pmol/μl	1.0	0			
primer 2	10 pmol/μl	1.0	0			
primer 3	10 pmol/μl	0	1.0			
primer 4	10 pmol/μl	0	1.0			
DNA		1.0	1.0			
Taq	1 U/μl	0.2	0.2			
<b>total</b>		20	20			

<b>Marker</b>	Gene Ladder 100 (Wako, Cat. 316-06951)
<b>Gel</b>	2% agarose in 1 X TAE (100 V, 30 min)



primer_set	product_size	product (allele type)
primer 1 primer 2	117 bp	WT
primer 3 primer 4	219 bp	mutant (del)

**Comment** Allele specific PCR (ASP)

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