

RBRC 11320

Strain name B6J.D2-Gpnmb<R150X>/Jwada

Primer	primer_name	primer_seq	length	Reference
1	WT_exon 4	TGACTGGGAAGATGGCACCAGTC	23	RIKEN BRC
2	GpnmbAS	CTCATTGTATGCCTGTGGTGTCTCG	25	Sci. Rep., 5:16920 (2015).
3	GpnmbS	CTTGGCTGTGATAACCAGTGAGTGAG	25	Sci. Rep., 5:16920 (2015).
4	mut intron 4	CGGGAACCTGAGATGCTGGCTTAA	24	RIKEN BRC

**Reaction Components**

**Enzyme** QIAGEN Multiplex PCR Kit (Cat. No. 206143)

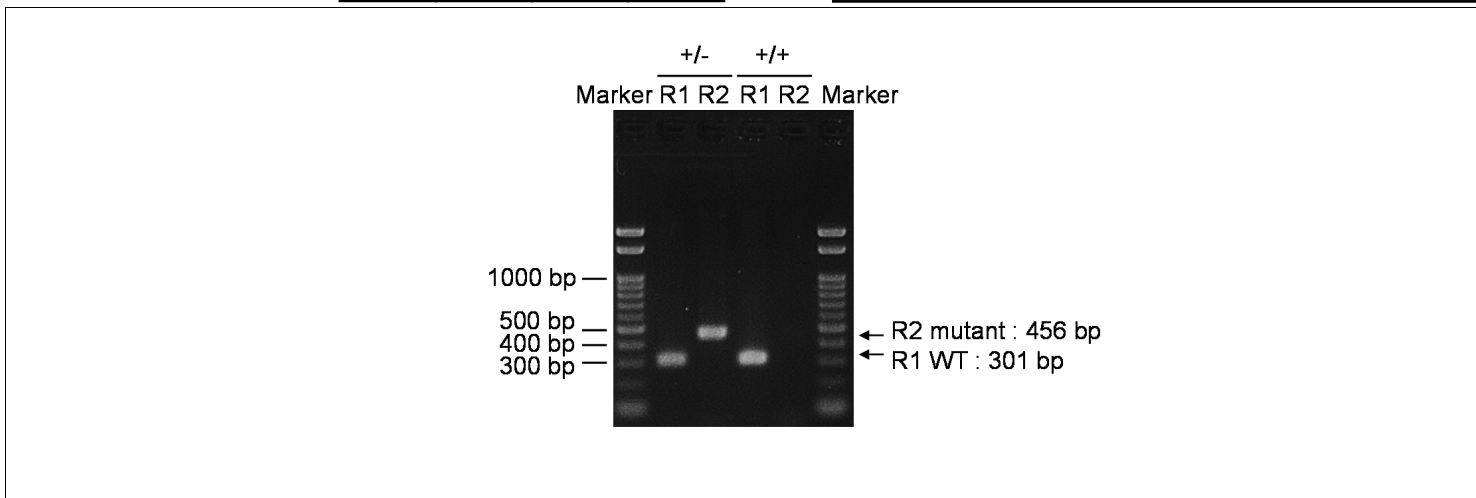
**Reaction Conditions**

step	temp (°C)	time (sec)	note
1	95	900	
2	94	30*	
3	60	90*	
4	72	60*	*Repeat for 30 cycles

Reagent	Conc	Reaction 1	2	3	4	(μL)
H2O		2.0	2.0			
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			
Master Mix	2 X	5.0	5.0			
DNA		1.0	1.0			
<b>total</b>		10	10			

**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)	Reaction 1
primer 1	301 bp	WT	Reaction 1 +/+ (wild-type): 301 bp +/- (heterozygous): 301 bp
primer 3	456 bp	mutant	
			Reaction 2
			+/+ (wild-type): none
			+/- (heterozygous): 456 bp

**Comment** Allele specific PCR (ASP)