

RBRC 06449

Strain name C57BL/6-H3mmT&lt;em1Osb&gt;

Primer	primer_name	primer_seq	length	Reference
1	common	CGTTGGGTTCATGAGCGGTGC	21	RIKEN BRC
2	wt	AGGAGCAAGGGAGGCGGACGA	21	RIKEN BRC
3	mut	AGGAGCAAGGGAGGCGGACACG	22	RIKEN BRC

**Reaction Components**
**Enzyme** Roche Taq (Cat. No. BM1418432)

**Reaction Conditions**

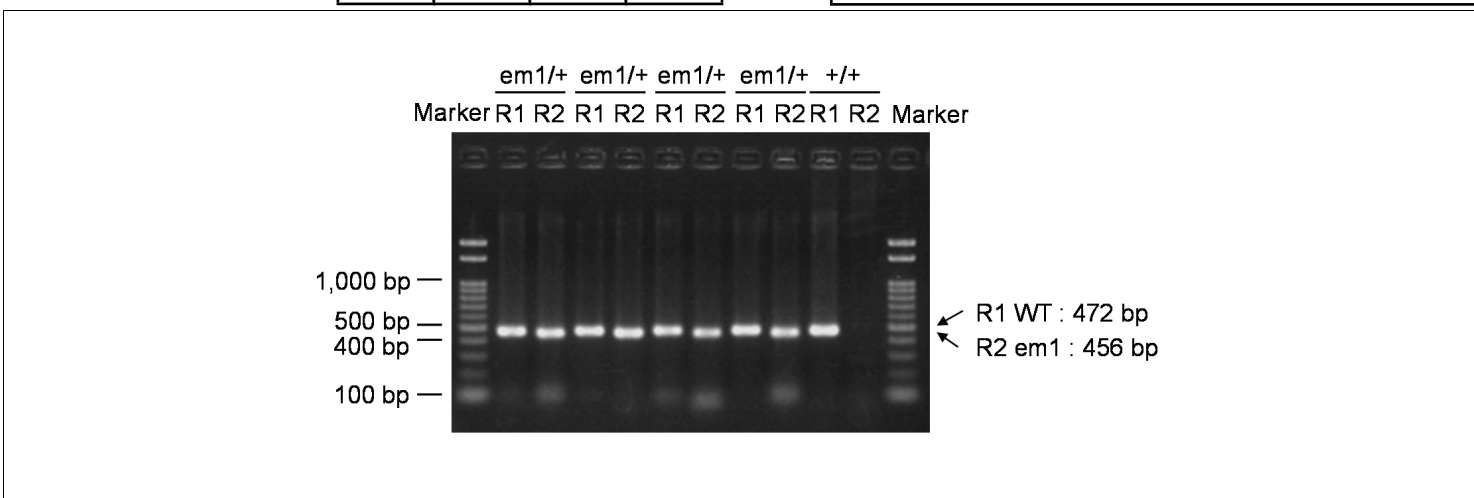
step	temp (°C)	time (sec)	note
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1	94	180	
2	94	30*	
3	60	30*	
4	72	60*	*Repeat for 35 cycles
5	72	300	

Reagent	Conc	Reaction 1	2	3	4
H <sub>2</sub> O		13.3	13.3		
PCR buffer	10 X	2.0	2.0		
dNTP	2.5 mM	1.6	1.6		
primer 1	10 pmol/μl	1.0	1.0		
primer 2	10 pmol/μl	1.0	0		
primer 3	10 pmol/μl	0	1.0		
DNA		1.0	1.0		
Taq	5 U/μl	0.1	0.1		
<b>total</b>		20	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	472 bp WT
primer 1	primer 3	456 bp em1
		<b>Reaction 1</b> +/+ (wild-type): 472 bp em1/+ (heterozygous): 472 bp
		<b>Reaction 2</b> +/+ (wild-type): none em1/+ (heterozygous): 456 bp

**Comment** Allele specific PCR (ASP)