

RBRC 11406

Strain name B6.Cg-Klh8<em1Tfur>

Primer	primer_name	primer_seq	length	Reference
1	Klh8-PCR-51	CTACCAATGGGAAACAAGCTAGGAGTC	27	Depositor's primer
2	Klh8-PCR-32	CCGTGAAAGTCCTTCCAGGCCTCGTTG	27	Depositor's primer
3	Klh8-8delKO-31	CCGTGAAAGTCCTTCCAGGCCTTCGAA	27	Depositor's primer

**Reaction Components**

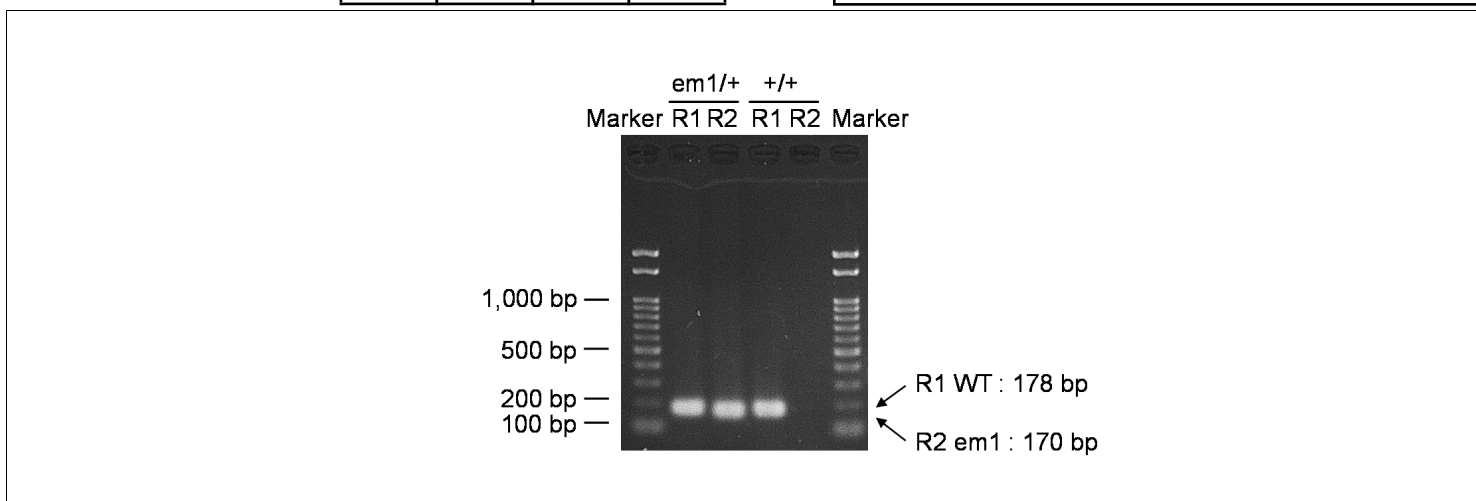
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

**Reaction Conditions**

step	temp (°C)	time (sec)	note
1	94	120	
2	98	10*	
3	65	30*	
4	68	60*	*Repeat for 30 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	1.0			
primer 2	10 pmol/μl	1.0	0			
primer 3	10 pmol/μl	0	1.0			
DNA		1.0	1.0			
Taq	1 U/μl	0.2	0.2			
<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	178 bp	WT
primer 1	primer 3	170 bp	em1

Reaction 1  
+/+ (wild-type): 178 bp  
em1/+ (heterozygous): 178 bp  
Reaction 2  
+/+ (wild-type): none  
em1/+ (heterozygous): 170 bp

**Comment** Standard PCR

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