



PCR protocol

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RBRC 10154

Strain name C57BL/6N-Etv6^{em1}Rbrc

Primer	primer_name	primer_seq	length	Reference
1	ck_MGE144-R	CACTAGACCCCTTAAAGTTCCAGTG	25	RIKEN DNA Bank
2	WT	AAATGAGTTTTCTTTAAGGCCCATTTGATA	29	RIKEN BRC
3	MT	AAATGAGTTTTCTTTAAGGCCCATTTGATC	29	RIKEN BRC

Reaction Components

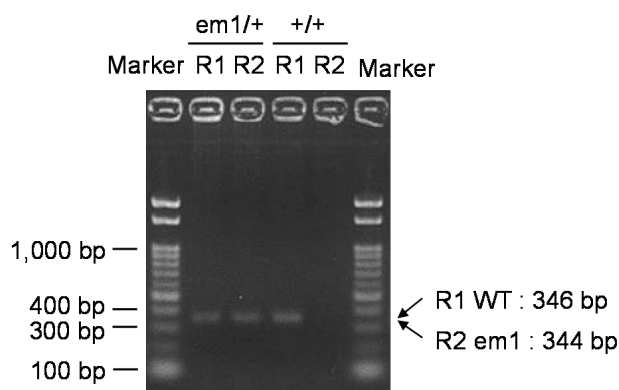
Enzyme	QIAGEN Multiplex PCR Kit (Cat. No. 206143)
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Reaction Conditions

step	temp (°C)	time (sec)	note
1	95	900	
2	94	30*	
3	60	90*	
4	72	60*	*Repeat for 25 cycles

Reagent	Conc	Reaction 1	2	3	4
H2O		2.0	2.0		
primer 1	10 pmol/ μ l	1.0	1.0		
primer 2	10 pmol/ μ l	1.0	0		
primer 3	10 pmol/ μ l	0	1.0		
DNA		1.0	1.0		
Master Mix	2 X	5.0	5.0		
total		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)
primer 1	primer 2	346 bp	WT
primer 1	primer 3	344 bp	em1

Comment Allele specific PCR (ASP)