

RMRC Genotyping Protocol-PCR

No.	RMRC13438	Strain Name	C57BL/6- <i>Trem2^{em1Narl}</i> /Narl
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A. Primer

	Primer name	Sequence
1	I15F	AGCCTCTGGGTTCTCTTTGTCC-
2	3U3B	AATACCGTGTCCTTCTCCGAG
3	HE23B	AAGTTGTGCGTGCTGACCACAC
4	mE23B	CCAGTGCTTCAAGGCGTCATAAG

B. Genomic extraction :

- Phenol-Chloroform extraction commercial extraction kit _____
 Lysis buffer Allele-In-One ABP-PP-MT01500 Mouse Tail Direct Lysis Buffer other _____

C. Reaction Conditions

Step 1. PCR

Component	Concentration		Volume		Step	Temperature, °C	Time, sec	Number of Cycles
				μl				
H ₂ O	--		8.8	μl	1	95	300	1
PCR buffer	10x		-	μl	2	95	30	35
PCR Dye Marker Mix	2x		10	μl	3	60	30	
Primer 1	10	μM(ρmol/μl)	0.4	μl	4	72	30	
Primer 2	10	μM(ρmol/μl)	0.4	μl				
DNA	50~250ng/ml		0.4	μl	5	72	300	1
Total			20	μl				

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				μl				
H ₂ O	--		8.4	μl	1	95	300	1
PCR buffer	10x		-	μl	2	95	30	35
PCR Dye Marker Mix	2x		10	μl	3	60	30	

Primer 1	10	$\mu\text{M}(\rho\text{mol}/\mu\text{l})$	0.4	μl	4	72	30	
Primer 3	10	$\mu\text{M}(\rho\text{mol}/\mu\text{l})$	0.4	μl				
Primer 4	10	$\mu\text{M}(\rho\text{mol}/\mu\text{l})$	0.4					
DNA	50~250ng/ml		0.4	μl	5	72	300	1
Total			20	μl				

The information of Taq, including company name and catalog number : Taq DNA Polymerase 2x

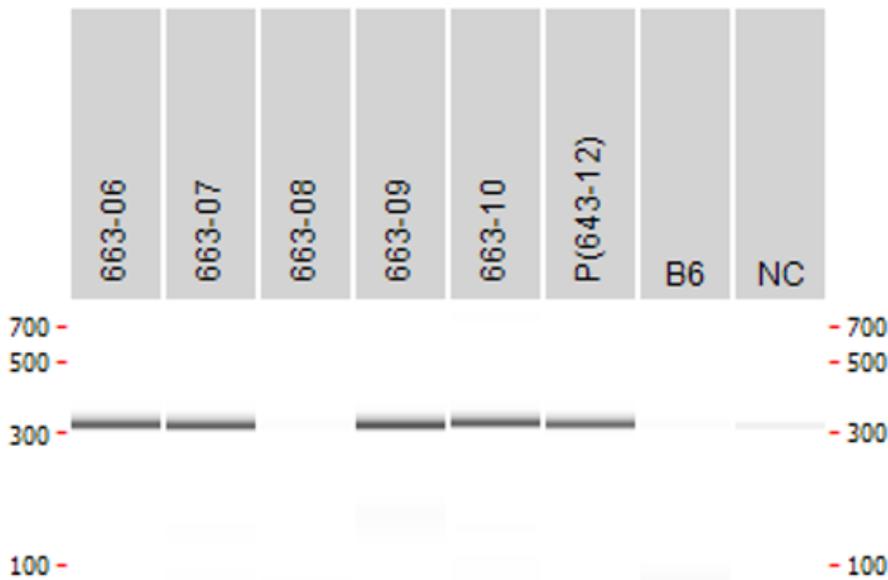
Master Mix RED (Ampliqon, Cat.No.:A180303)

PCR Product

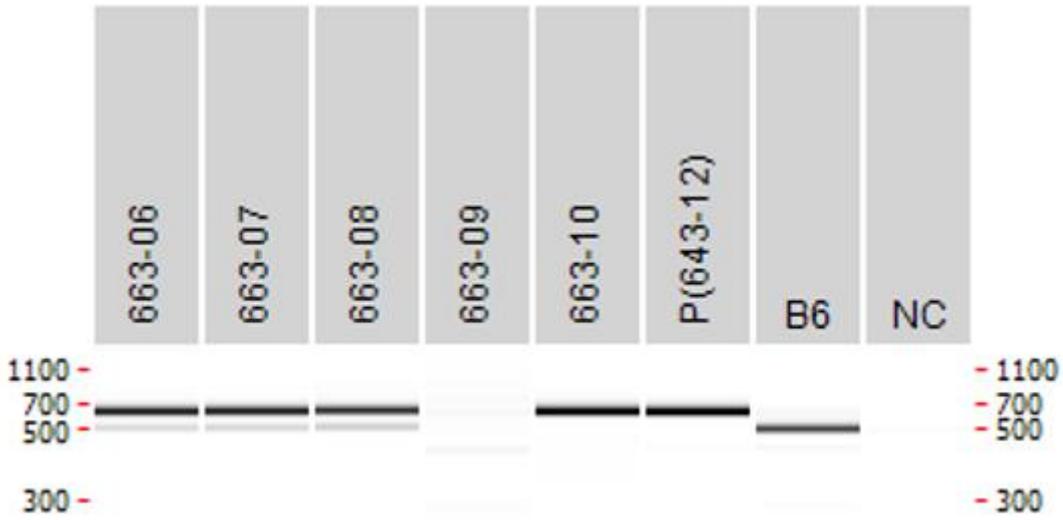
	Primer set		Product size	Target region
1	I15F	3U3B	300 bp	Trem2 knockout
	I15F	HE23B mE23B	460 bp	Trem2 wildtype

D. Gel Photo

I15F+3U3B



I15F + HE23B + mE23B



663-06<+/->; 663-08<+ / +> ; 663-09<- / ->