

## RMRC Genotyping Protocol-PCR

No.	RMRC13409	Strain Name	B6.Cg- <i>Mcl1</i> <sup>tm5Yen</sup> /Narl
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### A. Primer

	Primer name	Sequence
1	mMcl1-E2-202(p5)	5'-TTCTTGTAAGGACGAAACGGGACT-3'
2	mMcl1-In2-171-rev(p6)	5'-TTTCTCTGGACCTACCACCTACAA-3'

### B. Genomic extraction :

- Phenol-Chloroform extraction       commercial extraction kit \_\_\_\_\_  
 Lysis buffer \_\_\_\_\_       other \_\_\_\_\_

### C. Reaction Conditions: for Mcl-1 wt and Mcl-1-floxed allele

Component	Concentration		Volume		Step	Tempera- ture, °C	Time, min	Number of Cycles
H <sub>2</sub> O			5.25	μl	1	94	5	1
Ampliqon Taq DNA Polymerase 2x Master Mix RED	2X		12.5	μl	2	94	1	40
						55	1	
						72	1	
Primer 1	mMcl1-E2-202(p5)	10 μM	0.5	μl				
Primer 2	mMcl1-In2-171-rev(p6)	10 μM	0.5	μl	3	72	10	
DMSO			1.25	μl	4	4	pause	
DNA	--		5	μl				
Total			25	μl				

The information of Taq, including company name and catalog number :

Taq DNA Polymerase 2x Master Mix RED, 2 mM MgCl<sub>2</sub>; Ampliqon #A190303

### D. PCR Product

	Product size		Target region
1	Mcl-1 wt allele	220bp	
2	Mcl-1-floxed allele	320bp	

E. Gel Photo

