

## Genotyping protocol

EMMA ID: EM:10856

Gene symbol: Mirc40

Strain designation: C57BL/6N-A<sup>tm1Brd</sup> Mirc40<sup>tm1Hmpr</sup>/WtsiOulu

### Primers:

Primer name	Primer sequence (5'- 3')
Pr1	TAT TGG GAT GTG ATG GGA AAC TCT G
Pr2	TAG CAG AAG GCT AGA CCC CAA AGA C

### Products:

Allele	Primer 1	Primer 2	Product size (bp)
Wild-type	Pr1	Pr2	841
Mutant	Pr1	Pr2	645

### Reaction mix:

Component	Volume (µl)
Genomic DNA (~50-100 ng)	1.0
Primer 1 (10 µM)	1.0
Primer 2 (10 µM)	1.0
10x Buffer (15 mM MgCl <sub>2</sub> )	3.0
dNTPs (25 mM)	0.25
DNA polymerase (5 U/µl)	0.1
H <sub>2</sub> O	23.65
<b>Total</b>	<b>30.0</b>

### PCR program:

Time	Temperature (+ °C)	Cycles
4 min	94	
1 min	94	30
1 min	62	
1 min	72	
10 min	72	
∞	10	