

# GENOTYPING PROTOCOL-MARCOS MALUMBRES'S LAB

## MOUSE LINE INFORMATION

Name (CNIO nomenclature)	<u>BED</u>	Type of modification	<u>Knock out for miR-203</u>
Alleles	<u>miR-203 KO</u>	Modification details	<u></u>
Created by	<u>M<sup>a</sup> José Bueno</u>	Public Repository code and link	<u></u>
Responsible at CDC	<u>María Salazar</u>	References	<u></u>
MGI Mouse Locus	<u>MGI:2676878</u>	Comments or additional notes	<u>Chromosome 12, 61.14 cM</u>
Gene Name and aliases	<u>Mirn203, mmu-mir-203</u>		<u></u>

## MOUSE LINE DESCRIPTION

Knock out for microRNA 203

---



---



---



---

## GENERAL PCRS INFORMATION

Here you should list as many PCRs as needed to fully genotype the line, specifying the variants you detect on each PCR. One PCR setup per line

Allele	Variants detected	Size (bp)	Notes:
1 miR-203	<b>miR-203 lox</b>	<b>754</b>	PCR performed with primers 1 and 4
2	<b>miR-203 wt</b>	<b>665</b>	
3	<b>miR-203 ko</b>	<b>322</b>	
4 miR-203	<b>miR-203 lox</b>	<b>327</b>	PCR performed with primers 3 and 4
5	<b>miR-203 wt</b>	<b>227</b>	
6	<b>miR-203 ko</b>	<b>no band</b>	
7			
8			Both PCRs are complementary
9			
10			

## PCRS DETAILS

The following two tables should be repeated as needed to include the detailed information of all PCRs needed to genotype the referred mouse line

**PCR1.**

Allele (Variants): miR-203 KO: PCR combining primers 1&4

**MASTERMIX COMPONENTS**

	STOCK	VOLUME	FINAL CONC.	SPECIFICATIONS
Template gDNA	<u>0.1-1 microgram</u>	<u>1.5</u>		
Buffer	<u>5X</u>	<u>5.0</u>		<u>Go Taq Buffer 5X</u>
MgCl2	<u>25 mM</u>	<u>1.5</u>		
dNTPs	<u>10 mM</u>	<u>0.75</u>		
FW primer	<u>10 microM</u>	<u>2.5</u>		<u>CCTGGAAAAACCGGTCAG</u>
RV primer	<u>10 microM</u>	<u>2.5</u>		<u>GAGAGCTCCCGCAGAAGAAT</u>
Other primers (Specify)				
Taq		<u>0.5</u>		
Aditive (Specify)		<u>5.0</u>		<u>betain</u>
H2O		<u>5.75</u>		
Total volume		<u>25</u>		

**CYCLING CONDITIONS**

STEP	TEMPERATURE	TIME	NUMBER OF CYCLES
Initial denaturation		<u>94 5 min</u>	<u>1</u>
Denaturation		<u>94 30 sec</u>	<u>35</u>
Annealing		<u>55 30 sec</u>	
Extension		<u>72 30 sec</u>	
Final Extension		<u>72 10 min</u>	<u>1</u>
Soak		<u>4 pause</u>	

**PCR2.**

Allele (Variants): miR-203 KO: PCR combining primers 3&4 \_\_\_\_\_

**MASTERMIX COMPONENTS**

	<b>STOCK</b>	<b>VOLUME</b>	<b>FINAL CONC.</b>	<b>SPECIFICATIONS</b>
Template gDNA	0.1-1 microgram	1.5		
Buffer	5X	5.0		Go Taq Buffer 5X
MgCl2	25 mM	1.5		
dNTPs	10 mM	0.75		
FW primer	10 microM	2.5		CACAGCAGCCTGCACTTTC
RV primer	10 microM	2.5		GAGAGCTCCCGCAGAAGAAT
Other primers (Specify)				
Taq		0.5		
Aditive (Specify)		5.0		betain
H2O		5.75		
Total volume		25		

**CYCLING CONDITIONS**

<b>STEP</b>	<b>TEMPERATURE</b>	<b>TIME</b>	<b>NUMBER OF CYCLES</b>
Initial denaturation		94 5 min	1
Denaturation		94 30 sec	35
Annealing		55 30 sec	
Extension		72 30 sec	
Final Extension		72 10 min	1
Soak		4 pause	

**PCR3.**

Allele (Variants): \_\_\_\_\_

**MASTERMIX COMPONENTS**

	<b>STOCK</b>	<b>VOLUME</b>	<b>FINAL CONC.</b>	<b>SPECIFICATIONS</b>
Template gDNA				
Buffer				
MgCl2				
dNTPs				
FW primer				
RV primer				
Other primers (Specify)				
Taq				
Aditive (Specify)				
H2O				
Total volume				

**CYCLING CONDITIONS**

<b>STEP</b>	<b>TEMPERATURE</b>	<b>TIME</b>	<b>NUMBER OF CYCLES</b>
Initial denaturation			
Denaturation			
Annealing			
Extension			
Final Extension			
Soak			

**PCR4.**

Allele (Variants): \_\_\_\_\_

**MASTERMIX COMPONENTS**

	<b>STOCK</b>	<b>VOLUME</b>	<b>FINAL CONC.</b>	<b>SPECIFICATIONS</b>
Template gDNA				
Buffer				
MgCl2				
dNTPs				
FW primer				
RV primer				
Other primers (Specify)				
Taq				

Aditive (Specify) \_\_\_\_\_  
 H2O \_\_\_\_\_  
 Total volume \_\_\_\_\_

**CYCLING CONDITIONS**

STEP	TEMPERATURE	TIME	NUMBER OF CYCLES
Initial denaturation			
Denaturation			
Annealing			
Extension			
Final Extension			
Soak			

**PCR5.**

Allele (Variants): \_\_\_\_\_

**MASTERMIX COMPONENTS**

	STOCK	VOLUME	FINAL CONC.	SPECIFICATIONS
Template gDNA				
Buffer				
MgCl2				
dNTPs				
FW primer				
RV primer				
Other primers (Specify)				
Taq				
Aditive (Specify)				
H2O				
Total volume _____				

**CYCLING CONDITIONS**

STEP	TEMPERATURE	TIME	NUMBER OF CYCLES
Initial denaturation			
Denaturation			
Annealing			
Extension			
Final Extension			
Soak			

**PCR6.**

Allele (Variants): \_\_\_\_\_

**MASTERMIX COMPONENTS**

	STOCK	VOLUME	FINAL CONC.	SPECIFICATIONS
Template gDNA				
Buffer				
MgCl2				
dNTPs				
FW primer				
RV primer				
Other primers (Specify)				
Taq				
Aditive (Specify)				
H2O				
Total volume _____				

**CYCLING CONDITIONS**

STEP	TEMPERATURE	TIME	NUMBER OF CYCLES
Initial denaturation			
Denaturation			
Annealing			
Extension			
Final Extension			
Soak			