

Genotyping Protocol - Malumbres' Lab

malumbreslab.org


 Centro Nacional
de Investigaciones
Oncológicas

 Date:
 Name:

Mouse allele

 Allele **miR-203 knock out**
 Gene Symbol *mmu-mir-203*
 Gene Name and aliases Mirn203, mmu-mir-203
 MGI Mouse Locus MGI:2676878
 Generated by M^o José Bueno
 Responsible María Salazar

Type of modification Knockout

Modification details

Comments Constitutive knockout model

 Citation: Unpublished
 Pubmed ID Unpublished
 Public Repository: Not deposited yet

Oligonucleotides

Primer	Description	Sequence
FW miR-203 F1		5'-CCTGGAAAAACCGGTCAG-3'
FW miR-203 F3		5'-CACAGCAGCCTGCATTTTC-3'
RV miR-203 R4		5'-GAGAGCTCCCGCAGAAGAAT-3'

Alleles to be detected

Allele	Description	Primers	Size (bp)
<i>miR-203 (+)</i>	Wild-type allele	FW miR-203 F1 X RV miR-203 R4	665
<i>miR-203 (-)</i>	miR-203 knockout	FW miR-203 F1 X RV miR-203 R4	322
<i>miR-203 (lox)</i>	miR-203 lox allele	FW miR-203 F1 X RV miR-203 R4	754
<i>miR-203 (+)</i>	Wild-type allele	FW miR-203 F3 X RV miR-203 R4	227
<i>miR-203 (-)</i>	miR-203 knockout	FW miR-203 F3 X RV miR-203 R4	no band
<i>miR-203 (lox)</i>	miR-203 lox allele	FW miR-203 F3 X RV miR-203 R4	327

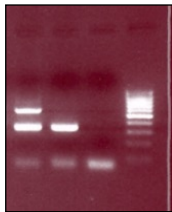
PCR conditions:

Summary of conditions: Program: 5'-94°; 35 x (30''- 94°, 30''-55°, 30''-72°); 10'-72°

Comments:

See details for further details

Representative results


 1: miR-203 (+)/ miR-203 (-)
 2: miR-203 (-)/ miR-203 (-)
 3: negative control
 4: MW marker

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

Oligonucleotides:		Alleles:	
FW miR-203 F1		<i>miR-203 (+)</i>	
RV miR-203 R4		<i>miR-203 (-)</i>	
		<i>miR-203 (lox)</i>	
MASTERMIX COMPONENTS			
	STOCK	VOLUME	FINAL CONC.
Template gDNA	0.1-1 microgram	1.5	
Buffer	5X	5.0	Go Taq Buffer 5X
MgCl ₂	25 mM	1.5	
dNTPs	10 nM	0.75	
FW primer	10 microM	2.5	
RV primer	10 microM	2.5	
Taq		0.5	
Aditive (Specify)		5.0	betain
H ₂ O		5.75	
Total volume			
CYCLING CONDITIONS			
STEP	TEMPERATURE	TIME	NUMBER OF CYCLES
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	

PCR2

Oligonucleotides:	FW miR-203 F3	Alleles:	miR-203 (+)
	RV miR-203 R4		miR-203 (-)
			miR-203 (lox)

MASTERMIX COMPONENTS

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

CYCLING CONDITIONS

STEP	TEMPERATURE	TIME	NUMBER OF CYCLES
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	