

## Genotyping Protocol - Malumbres' Lab

malumbreslab.org


 Centro Nacional  
de Investigaciones  
Oncológicas

 Date: \_\_\_\_\_  
 Name: \_\_\_\_\_

## Mouse allele

Allele	<b>CoIA1_miR-203</b>	Type of modification	Inducible knockin
Gene Symbol	<i>mmu-mir203</i>	Modification details	miR-203 mature sequence was subcloned downstream the CoIA1 sequences including tetO
Gene Name and aliases	Mirn203, mmu-mir-203	Comments	To be combined with a tTA or rtTA Doxycycline-inducible alleles
MGI Mouse Locus	MGI:2676878		
Generated by	M <sup>o</sup> José Bueno		
Responsible	María Salazar		
Citation:	Unpublished		
Pubmed ID	Unpublished		
Public Repository:	Not deposited yet		

## Description

Inducible overexpression of miR-203. The mmu-mir203 gene was inserted downstream of tet-responsive CoIA1 sequences using KH2 mouse embryonic stem (ES) cells following the strategy reported by Beard et al. 2006, Genesis 44, 23–28 [PubMed]. Induction is achieved after the activation of the reverse tetracycline transactivator (rtTA; expressed in a different allele in the Rosa26 locus) with the tetracycline derivative doxycycline.

## Oligonucleotides

Primer	Description	Sequence
<b>FW CoIA-F1</b>	<b>Wild-type sequence upstream CoIA1 promoter</b>	<b>5'-GCACAGCATTGCGGACATGC-3'</b>
<b>miR-203-R1</b>	<b>miR-203 in the CoIA1 locus</b>	<b>5'-TACAGAAGCTGTGAACTGTCAAGA-3'</b>
RV CoIB-R2	miR-203 in the CoIA1 locus	5'-CCCTCCATGTGTGACCAAGG-3'
RV CoIC-R3	Wild-type sequence downstream CoIA1 promoter	5'-GCAGAAGCGCGCCGTCTGG-

## Alleles to be detected

Allele	Description	Primers	Size (bp)
<i>CoIA1(+)</i>	Wild-type allele	<b>FW CoIA-F1 x miR-203-R1</b>	no band
<i>CoIA(miR-203)</i>	miR-203 in the CoIA1 locus	<b>FW CoIA-F1 x miR-203 R1</b>	300
<i>CoIA1(+)</i>	Wild-type allele	FW CoIA-F1 x RV CoIB-R2, RV CoIC-R3	300
<i>CoIA(miR-203)</i>	miR-203 in the CoIA1 locus	FW CoIA-F1 x RV CoIB-R2, RV CoIC-R3	500

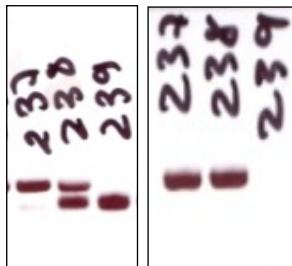
## PCR conditions:

Summary of conditions: Program: 5'-95°; 35 x (30''- 95°, 30''-60°, 45''-72°); 10'-72°

Comments: \_\_\_\_\_

See details for further details

## Representative results



237: CoIA(miR-203)/CoIA(miR-203)  
 238: CoIA1(+)/ CoIA(miR-203)  
 239: CoIA1(+)/CoIA1(+)

## 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		Alleles:		
Oligonucleotides: FW CoIA-F1: GCACAGCATTGCGGACATGC		CoIA1(+)		
miR-203-R1: TACAGAAGCTGTGAACTGTCAAGA		CoIA(miR-203)		
<b>MASTERMIX COMPONENTS</b>				
	STOCK	VOLUME	FINAL CONC.	SPECIFICATIONS
Template gDNA	0.1-1 microgram	1.5		
Buffer	5X	5.0		Go Taq Buffer 5X
MgCl <sub>2</sub>	25 mM	1.5		
dNTPs	10 nM	0.75		
FW primer	10 microM	2.5		
RV primer	10 microM	2.5		
Other primers (Specify)				
Taq		0.5		
Aditive (Specify)		5.0		betain
H <sub>2</sub> O		5.75		
		Total volume		

CYCLING CONDITIONS			
STEP	TEMPERATURE	TIME	NUMBER OF CYCLES
Initial denaturation		95 5 min	1
Denaturation		95 30 sec	35
Annealing		60 30 sec	
Extension		72 45 sec	
Final Extension		72 10 min	1
Soak		4 pause	

**PCR2**

Oligonucleotides: FW CoIA-F1: GCACAGCATTGCGGACATGC  
 RV CoB-R2: CCCTCCATGTGTGACCAAGG  
 RV CoIC-R3: GCAGAAGCGCGGCCGTCTGG

Alleles: CoIA1(+)  
CoIA(miR-203)

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	2.0	
RV primer	10 microM	2.0	
Other primers (Specify)	10 microM	2.0	
Taq		0.5	
Aditive (Specify)		5.0	betain
H2O		4.75	
		Total volume	

CYCLING CONDITIONS			
STEP	TEMPERATURE	TIME	NUMBER OF CYCLES
Initial denaturation		95 5 min	1
Denaturation		95 30 sec	35
Annealing		60 30 sec	
Extension		72 45 sec	
Final Extension		72 10 min	1
Soak		4 pause	