

GENOTYPING PROTOCOL-MARCOS MALUMBRES'S LAB

MOUSE LINE INFORMATION

Name (CNIO nomenclature)	TAL	Type of modification	Knock in for miR-203 and OSKM
Alleles	miR-203 KO; Rosa 26_rTA; OSKM	Modification details	
Created by	M ^a José Bueno, María Salazar	Public Repository code and link	
Responsible at CDC	María Salazar	References	
MGI Mouse Locus	MGI:2676878	Comments or additional notes	
Gene Name and aliases	Mirn203, mmu-mir-203		

MOUSE LINE DESCRIPTION

Knock-out of miR-203 and Knock-in for OSKM (Oct4-Sox2-Klf4-Myc). The OSKM inducible strain was kindly provided by Manuel Serrano's lab in 2014. We crossed the OSKM strain with our BED strain, knock out for miR-203. OSKM transgen is inducible by DOXYCYCLINE treatment, using Rosa 26_rTA allele.

Reverse tetracycline transactivator (rtTA)/tetracycline-responsive element (tet-O)-driven transgenes. To ensure reliable rtTA expression in a broad range of cell types, the rtTA

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	miR-203 lox	754	PCR performed with primers 1 and 4
2		miR-203 wt	665	
3		miR-203 ko	322	
4	miR-203	miR-203 lox	327	PCR performed with primers 3 and 4
5		miR-203 wt	227	
6		miR-203 ko	no band	
7	Rosa 26_rTA	KI	700	
8		WT	400	
9	OSKM	transgen	350	
10		WT	no band	

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	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		CCTGAAAAACCGGTCAG
RV primer	10 microM	2.5		GAGAGCTCCCGAGAAGAAT
Other primers (Specify)				
Taq		0.5		
Aditive (Specify)		5.0		betain
H2O		5.75		
		Total volume	25	
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.

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

MASTERMIX COMPONENTS

	<u>STOCK</u>	<u>VOLUME</u>	<u>FINAL CONC.</u>	<u>SPECIFICATIONS</u>
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		GAGAGCTCCCGAGAAGAAT
Other primers (Specify)				
Taq		0.5		
Aditive (Specify)		5.0		betain
H2O		5.75		
Total volume		25		

CYCLING CONDITIONS

<u>STEP</u>	<u>TEMPERATURE</u>	<u>TIME</u>	<u>NUMBER OF CYCLES</u>
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): Rosa 26 rTA

MASTERMIX COMPONENTS

	<u>STOCK</u>	<u>VOLUME</u>	<u>FINAL CONC.</u>	<u>SPECIFICATIONS</u>
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	2.0		AAAGTCGCTCTGAGTTGTTAT
RV primer	10 microM	2.0		GCGAAGAGTTTGCCTCAACC
Other primers (Specify)	10 microM	2.0		GGAGCGGGAGAAATGGATATG
Taq		0.5		
Aditive (Specify)		5.0		betain
H2O		4.75		
Total volume		25		

CYCLING CONDITIONS

<u>STEP</u>	<u>TEMPERATURE</u>	<u>TIME</u>	<u>NUMBER OF CYCLES</u>
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	

PCR4.

Allele (Variants): OSKM

MASTERMIX COMPONENTS

	<u>STOCK</u>	<u>VOLUME</u>	<u>FINAL CONC.</u>	<u>SPECIFICATIONS</u>
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	
RV primer	10 microM	2.5	
Other primers (Specify)	10 microM		
Taq		0.5	
Aditive (Specify)		5.0	betain
H2O		5.75	
Total volume		25	

CYCLING CONDITIONS

STEP	TEMPERATURE	TIME	NUMBER OF CYCLES
Initial denaturation		94 5 min	1
Denaturation		94 30 sec	35
Annealing		60 30 sec	
Extension		72 1 min	
Final Extension		72 10 min	1
Soak		4 pause	

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

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