

# GENOTYPING PROTOCOL-MARCOS MALUMBRES'S LAB

## MOUSE LINE INFORMATION

Name (CNIO nomenclature)	<u>TMI</u>	Type of modification	<u>Knock in for miR-203; transgen MMTV_PyMT</u>
Alleles	<u>ColA miR-203; Rosa 26 rtTA; MMTV_PyMT</u>	Modification details	<u></u>
Created by	<u>M<sup>a</sup> Jose Bueno, María Salazar</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></u>
Gene Name and aliases	<u>Mirn203, mmu-mir-203</u>	<u></u>	<u></u>

## MOUSE LINE DESCRIPTION

Knock-in of miR-203, inducible by DOXYCYCLINE treatment, through the Rosa 26 rtTA 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 transgenes has been targeted into the ROSA26 locus.  
 Transgen PyMT, constitutively active (not inducible), under the promoter of MMTV, only expressed in breast. Tumoral invasive lesions are observed in breast at 15 -16 weeks of age. Long latency model of breast cancer

## 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 Colagenase A miR-203	KI	500	
2	WT	400	
3 miR-203	KI	300	
4	WT	no band	
5 Rosa 26 rtTA	KI	400	
6	WT	600	
7 PyMT	transgen	250	
8	WT	no band	
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.				
Colagenase A miR-203				
Allele (Variants):				
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	2.0		GCACAGCATTGCGGACATGC
RV primer	10 microM	2.0		CCCTCCATGTGTGACCAAGG
Other primers (Specify)	10 microM	2.0		GCAGAAGCGCGCCGCTCTGG
Taq		0.5		
Aditive (Specify)		5.0		betain
H2O		4.75		
Total volume		25		
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
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**PCR2.**

Allele (Variants): miR-203 KI

**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		GCACAGCATTGCCGACATGC
RV primer	10 microM	2.5		TACAGAACTGTTGAACTGTCAAGA
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		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	

**PCR3.**

Allele (Variants): Rosa 26 rtTA

**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		AAAGTCGCTCTGAGTTGTAT
RV primer	10 microM	2.0		GCGAAGAGTTTGTCTCAACC
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): PyMT (controlled by MMTV promoter)

**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	TGCCGGGAACGTTTATTAG
RV primer	10 microM	2.0	GGAACAAGTACCCAGCTCA
Other primers (Specify)			
Taq		0.5	
Aditive (Specify)		5.0	betain
H2O		4.75	
Total volume		25	

**CYCLING CONDITIONS**

STEP	TEMPERATURE	TIME	NUMBER OF CYCLES
Initial denaturation		95 15 min	1
Denaturation		94 30 sec	30
Annealing		59 45 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