

GENOTYPING PROTOCOL-MARCOS MALUMBRES'S LAB

MOUSE LINE INFORMATION

| | | | |
|--------------------------|----------------------------------|---------------------------------|--|
| Name (CNIO nomenclature) | <u>BGC</u> | Type of modification | <u>Knock in for MIR-203, inducible by DOX treatment through Rosa 26_rTA allele</u> |
| Alleles | <u>ColA_mir-203; Rosa 26_rTA</u> | Modification details | <u></u> |
| Created by | <u>M^a 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></u> |
| Gene Name and aliases | <u>Mirn203, mmu-mir-203</u> | | <u></u> |

MOUSE LINE DESCRIPTION

Knock-in of miR-203, inducible by DOXYCYCLINE treatment, through the Rosa 26_rTA allele.
 Reverse tetracycline transactivator (rtTA)/tetracycline-responsive element (tet-O)-driven transgenes. To ensure reliable rTA expression in a broad range of cell types, the rTA transgenes has been targeted into the ROSA26 locus.

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_rTA | KI | 400 | |
| 6 | | WT | 600 | |
| 7 | | | | |
| 8 | | | | |
| 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): Colagenase A_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 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 | | |
| Additive (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 _____

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 | | GCACAGCATTGCGGACATGC |
| RV primer | 10 microM | 2.5 | | TACAGAACTGTTGAAGTGTCAAGA |
| 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 | | 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): _____

MASTERMIX COMPONENTS

| | <u>STOCK</u> | <u>VOLUME</u> | <u>FINAL CONC.</u> | <u>SPECIFICATIONS</u> |
|---------------|--------------|---------------|--------------------|-----------------------|
| 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