

# GENOTYPING PROTOCOL-MARCOS MALUMBRES´S LAB

## MOUSE LINE INFORMATION

Name (CNIO nomenclature) <u>DAB</u>	Type of modification _____
Alleles <u>Mastl_KO</u>	Modification details _____
Created by _____	Public Repository code and link _____
Responsible at CDC <u>Mónica Álvarez</u>	References _____
MGI Mouse Locus _____	Comments or additional notes _____
Gene Name and aliases _____	_____
_____	_____

## MOUSE LINE DESCRIPTION

Constitutive Mastl knock-out (whole body). Embryonically lethal.

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

## 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	<b>Mastl-KO</b>	<b>WT</b>	<b>783</b>	
2		<b>NULL</b>	<b>289</b>	
3				
4				
5				
6				
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): Mastl-KO

**MASTERMIX COMPONENTS**

	STOCK	VOLUME	FINAL CONC.	SPECIFICATIONS
Template gDNA	<u>0,1-1 microgram</u>	<u>1</u>		
Buffer	<u>5X</u>	<u>5</u>		<u>Go Taq Buffer 5X</u>
MgCl2	<u>25 mM</u>	<u>1,5</u>		
dNTPs	<u>10 mM</u>	<u>0,5</u>		
FW primer	<u>10 microM</u>	<u>1</u>		<u>MASTL KO-1-Fw:TCTTTCCACTCCCATACCACA</u>
RV primer	<u>10 microM</u>	<u>1</u>		<u>MASTL KO-2-Rv:GGCTGGCAGTTCCATTTTTA</u>
Other primers (Specify)	<u>10 microM</u>			
Taq		<u>0,4</u>		<u>Go Taq</u>
Aditive (Specify)				
H2O		<u>14,6</u>		
Total volume		<u>25</u>		

**CYCLING CONDITIONS**

STEP	TEMPERATURE	TIME	NUMBER OF CYCLES
Initial denaturation	95 °C	5min	
Denaturation	95 °C	30s	40
Annealing	58 °C	30s	
Extension	72 °C	60s	
Final Extension	72 °C	10min	
Soak	4	pause	

**PCR2.**

Allele (Variants): \_\_\_\_\_

**MASTERMIX COMPONENTS**

	<b>STOCK</b>	<b>VOLUME</b>	<b>FINAL CONC.</b>	<b>SPECIFICATIONS</b>
Template gDNA	-----	-----	-----	-----
Buffer	-----	-----	-----	-----
MgCl2	-----	-----	-----	-----
dNTPs	-----	-----	-----	-----
FW primer	-----	-----	-----	-----
RV primer	-----	-----	-----	-----
Other primers (Specify)	-----	-----	-----	-----
Taq	-----	-----	-----	-----
Aditive (Specify)	-----	-----	-----	-----
H2O	-----	-----	-----	-----
Total volume				

**CYCLING CONDITIONS**

<b>STEP</b>	<b>TEMPERATURE</b>	<b>TIME</b>	<b>NUMBER OF CYCLES</b>
Initial denaturation			
Denaturation	-----	-----	-----
Annealing	-----	-----	-----
Extension	-----	-----	-----
Final Extension			
Soak			

**PCR3.**

Allele (Variants): \_\_\_\_\_

**MASTERMIX COMPONENTS**

	<b>STOCK</b>	<b>VOLUME</b>	<b>FINAL CONC.</b>	<b>SPECIFICATIONS</b>
Template gDNA	-----	-----	-----	-----
Buffer	-----	-----	-----	-----
MgCl2	-----	-----	-----	-----
dNTPs	-----	-----	-----	-----
FW primer	-----	-----	-----	-----
RV primer	-----	-----	-----	-----
Other primers (Specify)	-----	-----	-----	-----
Taq	-----	-----	-----	-----
Aditive (Specify)	-----	-----	-----	-----
H2O	-----	-----	-----	-----
Total volume				

**CYCLING CONDITIONS**

<b>STEP</b>	<b>TEMPERATURE</b>	<b>TIME</b>	<b>NUMBER OF CYCLES</b>
Initial denaturation			
Denaturation	-----	-----	-----
Annealing	-----	-----	-----
Extension	-----	-----	-----
Final Extension			
Soak			

**PCR4.**

Allele (Variants): \_\_\_\_\_

**MASTERMIX COMPONENTS**

	<b>STOCK</b>	<b>VOLUME</b>	<b>FINAL CONC.</b>	<b>SPECIFICATIONS</b>
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			