

# GENOTYPING PROTOCOL-MARCOS MALUMBRES'S LAB

## MOUSE LINE INFORMATION

Name (CNIO nomenclature) <u>AYB</u>	Type of modification _____
Alleles <u>CDC20_lox, Pol2a_IRES-CreERT2</u>	Modification details _____
Created by _____	Public Repository code and link _____
Responsible at CDC <u>Guillermo de Cárcer</u>	References _____
MGI Mouse Locus _____	Comments or additional notes _____
Gene Name and aliases _____	_____
_____	_____

## MOUSE LINE DESCRIPTION

Knock out for CDC20, inducible by tamoxifen treatment through Pol2a\_IRES-CreERT2

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

## 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>CDC20_lox</b>	lox	620	
2		wt	500	
3		(D)	350	
4	<b>RERT-CreERT2</b>	wt	464	
5		KI	390	
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): **CDC20\_lox**

**MASTERMIX COMPONENTS**

	STOCK	VOLUME	FINAL CONC.	SPECIFICATIONS
Template gDNA	0,1-1 microgram	1		
Buffer	5X	5		Go Taq Buffer 5X
MgCl2	25 mM	1,5		
dNTPs	10 mM	0,5		
FW primer	10 microM	2,5		Primer - FWend1:GATTTGCACTCACTGCTTCAACTGG
RV primer	10 microM	2,5		Primer - Ex3Rv: CTTTCTGATGCTCCTGAAATACAG
Other primers (Specify)	10 microM			
Taq		0,5		
Aditive (Specify)	betaine (5M)	5		
H2O		6,5		
Total volume		25		

**CYCLING CONDITIONS**

STEP	TEMPERATURE	TIME	NUMBER OF CYCLES
Initial denaturation		95 5 min	1
Denaturation		95 30 sec	40
Annealing		55 30 sec	
Extension		72 45 sec	
Final Extension		72 10 min	1
Soak		4 pause	

**PCR2.**

Allele (Variants):

**RERT-CreERT2**

**MASTERMIX COMPONENTS**

	<b>STOCK</b>	<b>VOLUME</b>	<b>FINAL CONC.</b>	<b>SPECIFICATIONS</b>
Template gDNA	0,1-1 microgram	1		
Buffer	5X	5		
MgCl2	25 mM	1,5		
dNTPs	10 mM	0,5		
FW primer	10 microM	1		Pol2aF: CCAGATGACAGCGATGAGGA
RV primer	10 microM	1		Pol2aR: CCTCTGTGACCTCAATTAAGCAG
Other primers (Specify)	10 microM	1		ESFR: TGAGTAACAAAGGCATGGAGCA
Taq		0,4		
Aditive (Specify)	betaine (5M)	5		
H2O		8,6		
		<b>Total volume</b>	<b>25</b>	

**CYCLING CONDITIONS**

<b>STEP</b>	<b>TEMPERATURE</b>	<b>TIME</b>	<b>NUMBER OF CYCLES</b>
Initial denaturation	95 °C	5min	
Denaturation	95 °C	30s	40
Annealing	50 °C	30S	
Extension	72 °C	60s	
Final Extension	72 °C	10min	
Soak	4	pause	

**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				
		<b>Total volume</b>		

**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			