

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

mmrrc@ucdavis.edu

530-754-MMRRC

Protocol Name: C57BL/6N-Pcyox1em1(IMPC)/J/Mmucd **MMRRC:** 049020-UCD

Protocol:

Reagent/Constituent	Volume (μL)
Water	5.6
GoTaq® G2 Colorless Master Mix, 2X	7.5
Primer 1. (stock concentration is 20μM)	0.45
Primer 2. (stock concentration is 20μM)	0.45
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.0
TOTAL VOLUME	
	15

Comments on protocol:

- Protocol may work with other DNA extraction methods.
- Use Touch-Down cycling protocol-first 10 cycles anneal at 65°C decreasing in temperature by 1.0°C; next 30 cycles anneal at 55°C.
- The mutant PCR is a general LacZ PCR. The wild type is specific for this strain.

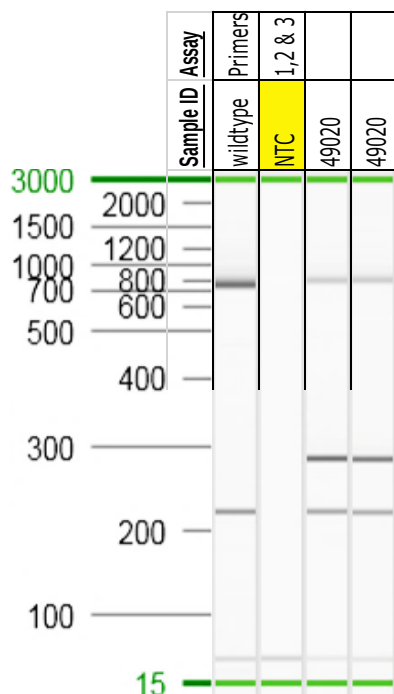
Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	2:00	1x
2. Denaturation	94	0:10	
3. Annealing steps 2-3-4 cycle in sequence	65 (↓1°C/cycle)	0:30	10x
4. Elongation	68	2:00	
5. Denaturation	94	0:15	
6. Annealing steps 5-6-7 cycle in sequence	55	0:30	25x
7. Elongation	68	2:00 (↑20sec/cycle)	

Primers:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Argarose: 1.5%	V: 90
1. 49020-mutF2	AACACTTCCCTCACTCTTGGCTAAGC	Estimated Running Time: 90 min.	
2. 49020-comR2	CCTGCCACCCTCTTACTCGTCG	Primer Combination	Band (bp)
3. 49020-wtF	ACAGTTCAATGCAGGCTCAG	1 & 2	~280
		2 & 3	227
			Genotype
			mutant
			wildtype





Welcome to The Genotyping Protocol System

Master Protocol

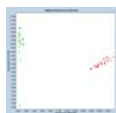
Strain Name: C57BL/6NJ-Pcyox1^{em1J}/J
Stock Number: 029570
Allele: Pcyox1^{em1J}
Protocol Name: Pcyox1^{em1J}
Method: Probe
Created: 12-September-2016 (LUCASM) **Updated:** 03-December -2016 (ESCHAAB)

Notes

Notes: Taqman qPCR protocols are run on a real time PCR instrument. Use an appropriate instrument specific Fluorophore/Quencher combination. The transgene genotype is determined by comparing ΔC_t values of each unknown sample against known homozygous and hemizygous controls, using appropriate endogenous references.

Expected Results: Mutant= 89 bp
 Wild Type = 102 bp

Attachments



View pcyox11 JW.jpg

- View Pcyox1 genomic.gcs
- View Pcyox1em1j_in progress 8-16-16.docx

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
29638	-	ACA GTT CAA TGC AGG CTC AG	-	Wild type Forward	A
29639	-	GGT GGG AGG AAA GAA TGG AG	-	Common	A
29640	-	TCG CTA GGA AGA GCA AAC TTT C	-	Mutant Forward	A
29641	Hex	CGG ATG AGT GTG TGG GAC A	Black Hole Quencher 1	WT Probe	-
29642	6-FAM	TGC ATT AGA GTG CTG CTT ACA GA	Black Hole Quencher 1	MUT Probe	-

Number Of Reactions 1

Reaction A (Endpoint 3 primer)		Cycling (EndPoint)			
Component	Final Concentration	Step #	Temp°C	Time	Note
Kapa Probe Fast QPCR	1 X	1	95	3min	-
ddH2O		2	95	5sec	-
29638	.4 uM	3	60	30sec	-
29639	.4 uM	4	-	-	repeat steps 2-3 for 40 cycles
29640	.4 uM	5	4	-	Forever
Wt Probe	.15 uM				

Reaction A (Endpoint 3 primer)

Mutant Probe	.15 uM
DNA	

Version 3.2