09.22.10 MS
10/21/16 MLS

Genotyping Protocol: **MMRRC 14117**

Assay Type: PCR- can distinguish heterozygous animals from homozygous animals.

DNA Extraction: DNA from tail snips was extracted using Sigma's Extract-N-Amp Tissue PCR Kit (Cat#XNAT2R). Kit directions for animal tissues were performed with a few minor modifications as follows: Use only 50 µl of Extraction Solution, 12.5 µl Tissue Preparation Solution and 50 µl of Neutralization Solution B.

Strain Description: A PGK-neo cassette was inserted into Exon 1 of the mouse *Crhbp* gene. This resulted in a deletion of the coding sequence of Exon 1, as well as the deletion of the entirety of exons 2-5 of *Crhbp*. Details can be found in Karolyi, et al (1999) Proc Natl Acad Sci U S A 96(20):11595-600.

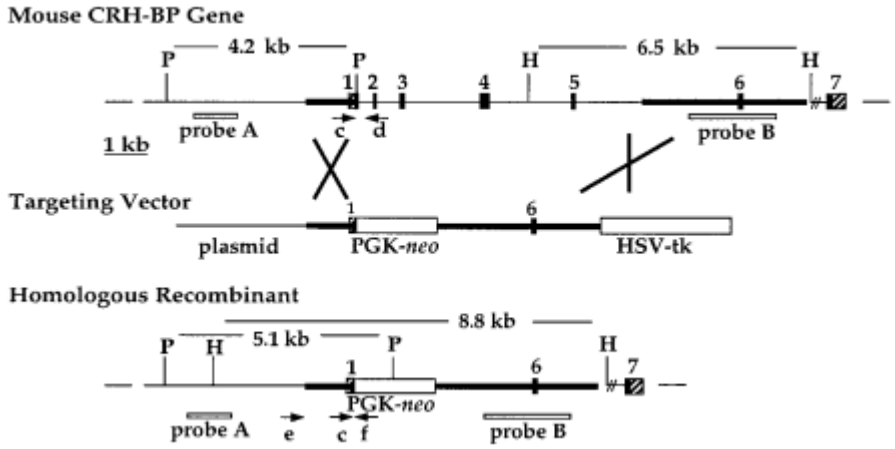


Image from Karolyi, et al (1999) Proc Natl Acad Sci U S A 96(20):11595-600.

Primer Information:
1) Name: Primer C Sequence: 5'-TGG ACC CTC GTC ATT GCC AGG-3'
2) Name: Primer F Sequence: 5'-AGA CTA GTG AGA CGT GCT ACT TCC ATT TGT-3'
3) Name: Primer D Sequence: 5'-CCC GTC GGT ACG GCT GCT CCT CTG CCA GGT-3'

Primer Location: Primer C is located immediately upstream of Exon 1 of *Crhbp*. Primer D is located in Exon 2 of *Crhbp*. Primer F is located in the inserted PGK-neo cassette.

Assay Name: CRH-BP KO PCR

Master Mix Components:

Component	manufacturer	concentration	µl/rxn
Extract-N-Amp PCR Reaction Mix	Sigma (Cat#XNAT2R)	2X	10
Primer C	Sigma or IDT	25µM	0.3
Primer F	Sigma of IDT	25µM	0.3
Primer D	Sigma or IDT	25µM	0.3
sterile water			5.1

PCR Setup:
Final reaction: 16µl master mix & 4µl DNA template (10-20ng/µl)
All reactions were performed in 200µl thin walled PCR tubes and were run in an Applied Biosystems 2700 thermocycler.

Cycle Parameters :

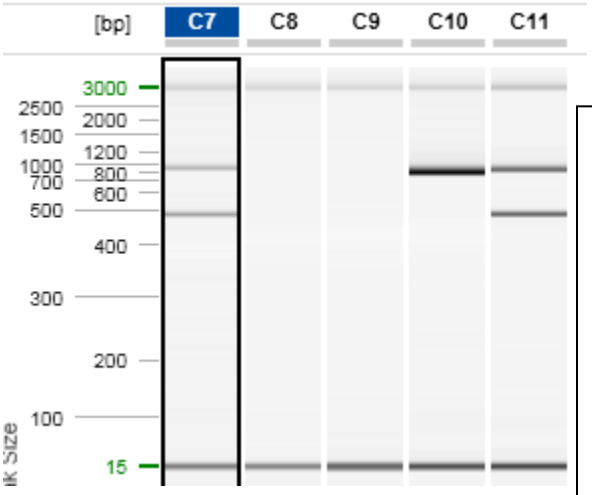
1)	94ºC	3 minutes
2)	94ºC	1 minute
3)	67ºC	1 minute
4)	72ºC	1 minute
5)	Repeat steps 2-4 34 times for a total of 35 cycles	
6)	72ºC	10minutes
7)	4ºC	hold until refrigerate product

Product Analysis:
All products were analyzed on the Qiaxcel (instrument and all supplies from Qiagen) with the Qiaxcel DNA Screening Kit (Cat# 929004).

Alignment Marker: QX Alignment Marker 15bp/3Kb (Cat# 929522)
Size Marker: QX DNA Size Marker 100-3Kb (Cat# 929553)
Method: AM320 Injection: 10s at 5KV
Separation: 320s at 6KV

Expected Products:
WT allele: 722bp
Mutant allele: 440bp
Heterozygote = 440bp & 722bp product

Example gel:



Lane C7 displays a heterozygous sample (440bp and 722bp products)
Lanes C8 and C9 display extraction and PCR blanks, respectively
Lane C10 displays a WT sample (722bp product)
Lane C11 displays a heterozygous sample (440bp and 722bp products)

Please note: the 15bp and 3kb bands are reference markers specific to the QIAxcel method and do not represent expected products.