Genotyping Protocol: MMRRC Line 17218, 17219, 17220 (Creb PCR)

Assay Type: PCR (can not distinguish heterozygous animals from homozygous animals) to determine if animal carries a CREB transgene and restriction digest to distinguish whether the transgene is wildtype or carries a point mutation.

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.

Primer Information:

Name: Creb B1 Sequence: 5'- GCT GCA TTG GTC ATG GTT AAT GTC -3'
Name: Creb F1 Sequence: 5'- CAG CCA TCA GTT ATT CAG TCT CCA -3'
Name: GdfUPF1M Sequence: 5'- AAG CCC TCA GTC AGT TGT GC -3'
Name: GdfUPR2M Sequence: 5'- AAA ACC ATG AAA GGA GTG GG -3'

Creb transgene sequence:

CAGCCATCAGTTATTCAGTCTCCACAAGTCCAAACAGTTCAG
TCTTCCTGTAAGGACTTAAAAAGACTTTTCTCCGGAACTCAGATTTCAACTATTGCAGAA
AGTGAAGATTCACAGGAGTCTGTGGATAGTGTAACTGATTCCCAAAAACGAAGGGAAATC
CTTTCAAGGAGGCCTGCCTACAGGAAAATTTTGAATGACTTATCTTCTGATGCACCAGGG
GTGCCAAGGATTGAAGAAGAAAAATCAGAAGAAGAGACTTCAGCCCCTGCCATCACCACT
GTAACAGTGCCAACCCCGATTTACCAAACTAGCAGTGGGCAGTATATTGCCATTACCCAG
GGAGGAGCAATACAGCTGGCTAACAATGGTACCGATGGGGTACAGGGCCTGCAGACATTA
ACCATGACCAATGCAGC

Primer binding site: Creb primers are underlined above in the Creb transgene sequence in red font. GdfU primers are primers to housekeeping gene that should be present in all mouse DNA samples.

Assay Name: Creb PCR

PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
Extract-N-Amp PCR Reaction Mix	Sigma (Cat#XNAT2R)	2X	10
Creb B1	Sigma	25 µM	0.3
Creb F1	Sigma	25 µM	0.3
GdfUPF1M	Sigma	25 µM	0.3
GdfUPR2M	Sigma	25 µM	0.3
sterile water			4.8

PCR Setup:

Final Reaction: 16µl master mix & 4µl DNA template (10-20 ng/µl)

All reactions were performed in 200µl thin walled PCR tubes and were run in Perkin Elmer 2400 thermocycler or Applied Biosystems 2700 thermocycler.

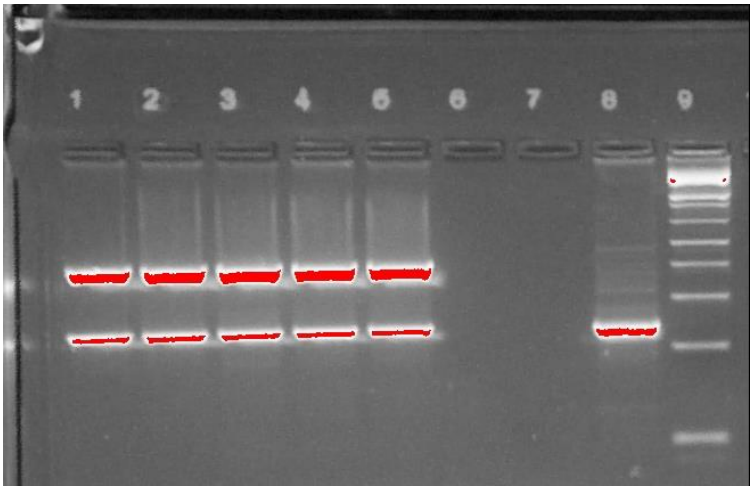
Cycle Parameters:

1) 94ºC 3 minutes
2) 94ºC 1 minute
3) 58ºC 1 minute
4) 72ºC 1 minute
5) Repeat steps 2-4 34 times for a total of 35 cycles
6) 72ºC 10 minutes
7) 4ºC hold until refrigerate product

Product Analysis:
All products were analyzed on a 3% agarose gel with ethidium bromide staining
Positive: 419 bp and 250 bp
Negative: 250 bp only (represents housekeeping gene)

Example of Gel:

419 bp →
250 bp →



Wells 1-5 are positive. Well 8 is negative. Used a 1Kb Plus DNA ladder(Invitrogen Cat. # 10787-018).

Restriction Digest:

PCR any positive samples with only the Creb primers, and then digest them with *Cac8*I to confirm the correct version of the Creb transgene is present. M17218- carries wild type CREB transgene , M17219 & M17220 carry a point mutation in the CREB transgene.

Assay Name: Creb/Cac8I

CAGCCATCAGTTATTCAGTCTCCACAAGTCCAAACAGTTCAG
TCTTCCTGTAAGGACTTAAAAAGACTTTTCTCCGGAACTCAGATTTCAACTATTGCAGAA
AGTGAAGATTCACAGGAGTCTGTGGATAGTGTAACTGATTCCCAAAAACGAAGGGAAATC
CTTTCAAGGAG***GCCTGCC***TACAGGAAAATTTTGAATGACTTATCTTCTGATGCACCAGGG
GTGCCAAGGATTGAAGAAGAAAAATCAGAAGAAGAGACTTCAGCCCCTGCCATCACCACT
GTAACAGTGCCAACCCCGATTTACCAAACTAGCAGTGGGCAGTATATTGCCATTACCCAG
GGAGGAGCAATACA**GCTGGC**TAACAATGGTACCGATGGGGTACAGG**GCCTGC**A**GACATTA**
ACCATGACCAATGCAGC

*Cac8*I sites = GCNNGC
Point mutation= T to G (underlined green base)

PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
Fast start buffer	Roche	10X	2
dNTP	Promega (Cat# U1515)	1.25 mM	3.2
Creb B1	Sigma	25 µM	0.3
Creb F1	Sigma	25 µM	0.3
FastStart *Taq*	Roche (Cat#12032953001)	5 U/µl	0.2
sterile water			13

PCR Setup:

Final Reaction: 19µl master mix & 1µl DNA template (10-20 ng/µl)

All reactions were performed in 200µl thin walled PCR tubes and were run in Perkin Elmer 2400 thermocycler or Applied Biosystems 2700 thermocycler.

Cycle Parameters:
1) 95ºC 5 minutes
2) 94ºC 1 minute
3) 58ºC 1 minute
4) 72ºC 1 minute
5) Repeat steps 2-4 34 times for a total of 35 cycles
6) 72ºC 5 minutes
7) 4ºC hold until refrigerate product

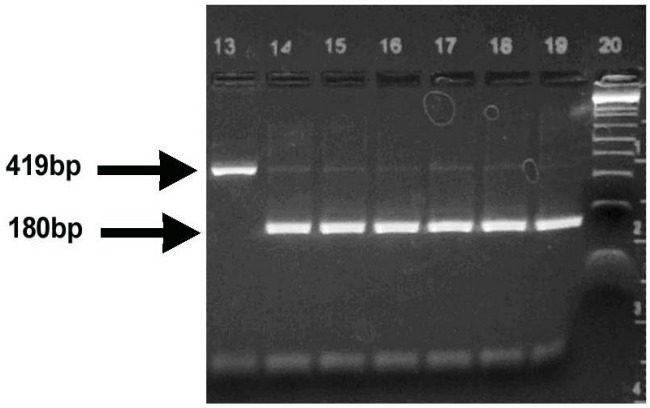
Restriction Digest:
10 µl PCR product from above reaction
0.4 µl *Cac8*I (NEB – 4U/µl Cat#R0579S)
2 µl NEB Buffer 3 (NEB)
7.6µl water
20 µl reaction

Incubate overnight at 37ºC

Product Analysis:
All products were analyzed on a 3% agarose gel with ethidium bromide staining

M17218 transgene positive mice: should carry WT Creb transgene which is not cut by *Cac8*I- 419 bp product.

M17219 & M17220 transgene positive mice: should carry point mutation of Creb transgene which is cut into 4 fragments by *Cac8*I enzyme= 182bp & 176bp fragment (these may run as single band on gel ~180bp), 31bp & 27bp fragment (these may run as single band on gel ~30bp and look more like primer dimer form than distinct products).



Restriction digest gel image:
Lane 13= M17218- uncut PCR product 419bp
Lanes 14-19 = M17219- cut product ~180bp
Molecular weight marker = 1Kb Plus DNA ladder (Invitrogen Cat. # 10787-018)