GENOTYPING BY PCR PROTOCOL
MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

mmrrc@ucdavis.edu
530-754-MMRRC

Please provide the following information required for genetic analysis of your mutant mice.

Note to MAC users: to ensure your graphic can be viewed on a PC please follow the steps below when inserting the graphic into this document. DO NOT drag and drop or copy/paste the graphic into this document.

- *Open the original graphic in the program that created it*
- *Choose File, Save As*
- *Select No Compression in the save options.*
- *Save as JPG or PNG or similar format that's compatible with both PC and Mac Word versions.*
- *Switch to Word, choose Insert, Picture, From File and choose the newly saved picture.*

These instructions are very generic. The menu options for your graphics program may be different.

Donating Investigator/PI **Joseph Napoli**		
Email **jna@berkeley.edu**		
Institution **University of California, Berkeley**		
Address **119 Morgan Hall**		
City **Berkeley**	State **CA**	Zip **94720-3104**
Lab Contact **Charles Krois**		
Email **ckrois@berkeley.edu**		
Telephone **(510) 643-0460**	FAX **(510) 642-0535**	
Strain Name **B6.129S2-Rbp1tm1Ipc/Mmucd**		MMRRC Stock Number **041471**

GENOTYPING BY PCR PROTOCOL
MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

mmrrc@ucdavis.edu
530-754-MMRRC

NAME OF PCR: **CRBP1 KO Genotyping PCR** **MMRRC:** **0-UCD**

Protocol: ***(PCR protocol provided by Donating Investigator)***

Reagent/Constituent	Volume (µL)
Water	14.8
10x Buffer	2.0
$MgCl_2$ (stock concentration is 50mM)	0.6
Betaine (stock concentration is 5M) *Optional*	0.0
dNTPs (stock concentration is 10mM)	0.4
DMSO *Optional*	0.0
Primer 1. (stock concentration is 20µM)	0.4
Primer 2. (stock concentration is 20µM)	0.4
Primer 3. (stock concentration is 20µM)	0.0
Primer 4. (stock concentration is 20µM)	0.0
Taq Polymerase 5Units/µL	0.2
DNA (50-200ng/ µL) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.2
The total volume is auto-calculated based on volumes entered, right click the total and update field to show/recalculate the total volume. **TOTAL VOLUME OF REACTION:**	**20.000 µL**

Comments on protocol:

- DNA from tail lysis:
  Cut off ~2mm of tail specimen into 1.5mL capped tube; add 500µL of 0.05M NaOH solution into tube.
  Place on heat block at 95°C for 20min. Add 50µL of 1M TRIS/EDTA solution. Vortex till disintegration.

Strategy:

Steps	Temp (ºC)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	5:00	**1**
2. Denaturation	94	0:40	
3. Annealing steps 2-3-4 cycle in sequence	61	0:40	**36x**
4. Elongation	72	0:40	
5. Amplification	72	10:00	**1**
6. Finish	4	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1. WT F14	ATTGGTGGCAAGTGTCCGAT
2. WT R14	CAGGTGACGCTAAGGAGTCG
3. KO F	GCC TTC TAT CGC CTT CTT GAC GAG TTC
4. KO R	GCA CTT GCG GTC GTC TAT GC
5.	
6.	
7.	

Agarose: **2.5%** V: **120**
Estimated Running Time: **30** min.

Primer Combination	Band (bp)	Genotype
1 & 2	503	WT
3 & 4	593	KO

GENOTYPING BY PCR PROTOCOL
MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

mmrrc@ucdavis.edu
530-754-MMRRC

Protocol / Gel Comments:

In the “Wild Type” section, primers 1 and 2 were used. In CRBP KO, we used primers 3 and 4.
“+” indicates tail lysate from a WT mouse was used as template, “-“ a KO, and “nt” water (no template).

Gel pictures: