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 **Dr. David L Nelson**		
Email **Nelson@bcm.edu**		
Institution **Baylor College of Medicine**		
Address **Moursund Street, Suite 1050.01**		
City **Houston**	State Texas	Zip 77030
Lab Contact **Yanghong Gu**		
Email ygu@bcm.edu		
Telephone **832-824-8936**	FAX **832-825-1269**	
Strain Name Name: B6.Cg-*Aff4*^{tm1Dln}	MMRRC Stock Number 041408	

NAME OF PCR:	**WT1/WT2 or CK/KT**	**MMRRC:**	**0-UCD** **-UCD**

Protocol:	***(PCR protocol provided by Donating Investigator)***
Reagent/Constituent	**Volume (µL)**
Water	42.375
10x Buffer	5
$MgCl_2$ (stock concentration is 25 mM)	0.5
Betaine (stock concentration is 5M) *Optional*	0
dNTPs (stock concentration is 10mM)	0.125
DMSO *Optional*	0
Primer 1. (stock concentration is 20µM)	0.25 (S1 or Kt)
Primer 2. (stock concentration is 20µM)	0.25 (S2, or S2, or KT30
Primer 3. (stock concentration is 20µM)	
Primer 4. (stock concentration is 20µM)	
Taq Polymerase 5Units/µL	0.5
DNA (50-200ng/ µL) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1
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:** **50.000 µL**

Comments on protocol:

•	

Strategy:

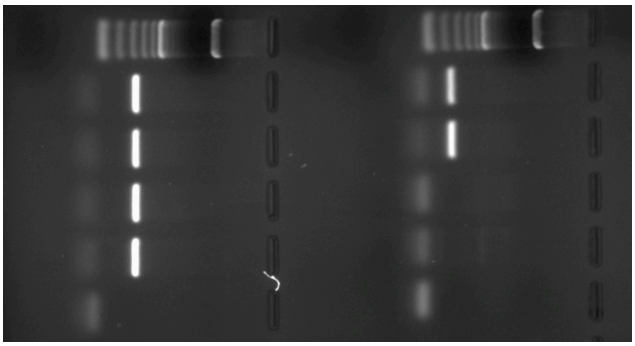
Steps	**Temp (^{O}C)**	Time (m:ss)	**# of Cycles**
1. Initiation/Melting HOT START?	95	5:00	**1**
2. Denaturation	95	0:30	
3. Annealing steps 2-3-4 cycle in sequence	55	0:20	**35x**
4. Elongation	72	1:20	
5. Amplification	72	7:00	**1**
6. Finish	4	∞	n/a

Name	**Nucleotide Sequence (5' - 3')**	Argarose:	**1.5%**	V:	**90**
1. WT1	GAATGTGCTGCGCATGAAAG		Estimated Running:Time:	**90**	min.
2.WT2	GGCTCAAAGCTGCTTGTAAC		**Primer Combination**	**Band** (bp)	**Genotype**
3.CK	GGAAACACAGCATCTGTGAC		WT1/WT2	316	Aff4 wild type
4.KT	GGTGTCATTCTATTCTGGGG		KT/CK	217	Aff4 Knockout
5.					
6.					
7.					

Primers:

Please size gel images and comments to fit within this space

WT1/WT2 for Aff4 wild type ***CK/KT for Aff4 knockout***



100 bp marker

1

2

3

4

5

1:Aff4 heterozygous, 2: Aff4 heterozygous, 3: Aff4 wild type
4: Aff4 wild type, 5: no DNA control

This page is not protected and freely editable.

Protocol / Gel Comments:

Gel pictures:

Please size gel images and comments to fit within this space