GENOTYPING BY PCR PROTOCOL
MUTANT MOUSE REGIONAL RESOURCE CENTER

sacoord@mmrrc.org

800-910-2291 North America, +1-530-757-5710 International

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.

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

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Lab Contact **Dr. Talat Afroze, PhD**		
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Telephone **416-581-7491**	FAX **NA**	
Strain Name B6.Cg-Tg(tetO/CMV-ATP2B4,-lacZ)1Husa Tg(Myh6-tTA)6Smbf/Mmjax		MMRRC Stock Number MMRRC:43951

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NAME OF PCR: human PMCA4b Genomic PCR **MMRRC:** 043951

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

Reagent/Constituent	Volume (µL)
Water autoclaved; MilliQ brand reverse osmosis water	12.5
10x Buffer NH4SO4 based Buffer; ThermoFisher	2.5
$MgCl_2$ (stock concentration is 25mM) 2.5mM working concentration	2.0
Betaine (stock concentration is 5M) *Optional* N.A.	0
dNTPs (stock concentration is 10mM) 0.2mM working concentration	0.5
DMSO *Optional* N.A.	0
Human PMCA4b Forward Primer (stock concentration= 20µM)	0.5
Human PMCA4b Reverse Primer (stock concentration is 20µM)	0.5
	0
	0
Taq Polymerase 1Units/µL use 1.5Units per 25uL PCR reaction	1.5
DNA (5uL of crude tail digest using Proteinase K overnight; volume 400uL per tail)	5
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:**	**25.000 µL**

Comments on protocol:

- We use ThermoFisher recombinant Taq DNA Polymerase 1U/uL Cat. # EP0404

Strategy: human PMCA4b Genomic PCR

Steps	Temp (ºC)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? Yes X	94	3 min	**1**
2. Denaturation	94	30 sec	
3. Annealing steps 2-3-4 cycle in sequence	**60**	**45 sec**	**45x**
4. Elongation	72	60 sec	
5. Amplification	72	7 min	**1**
6. Finish	4	∞	n/a

Primers: huPMCA4b

Name	Nucleotide Sequence (5' - 3')
1. huP4 For	GGCTCCCTGAGTGTACTCCC
2. huP4 Rev	CCTGATGACGGTGCTCATTG
3.	
4.	
5.	

Electrophoresis Protocol:

Argarose: 2% in V:110

Estimated Running:Time: 60 min.

Primer Combination	Band	Genotype
huP4 For + Rev	**554 bp**	**huPMCA4b**
	bp	
	bp	

NAME OF PCR: Tet O (Tet-sensitive TransActivator; tTA) Genomic PCR **MMRRC:** 043951

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

Reagent/Constituent	Volume (µL)
Water autoclaved; MilliQ brand reverse osmosis water	12.5
10x Buffer NH4SO4 based Buffer; ThermoFisher	2.5
$MgCl_2$ (stock concentration is 25mM) 2.5mM working concentration	2.0
Betaine (stock concentration is 5M) *Optional* N.A.	0
dNTPs (stock concentration is 10mM) 0.2mM working concentration	0.5
DMSO *Optional* N.A.	0
Tet O (tTA) Forward Primer (stock concentration= 20µM)	0.5
Tet O (tTA) Reverse Primer (stock concentration is 20µM)	0.5
	0
	0
Taq Polymerase 1Units/µL use 1.5Units per 25uL PCR reaction	1.5
DNA (5uL of crude tail digest using Proteinase K overnight; volume 400uL per tail)	5
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:**	**25.000 µL**

Comments on protocol:

- We use ThermoFisher recombinant Taq DNA Polymerase 1U/uL Cat. # EP0404

Strategy: Tet O (Tet-sensitive TransActivator; tTA) Genomic PCR

Steps	Temp (ºC)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? Yes X	94	3 min	**1**
2. Denaturation	94	30 sec	
3. Annealing steps 2-3-4 cycle in sequence	**50**	**45 sec**	**45x**
4. Elongation	72	60 sec	
5. Amplification	72	7 min	**1**
6. Finish	4	∞	n/a

Primers:tTA PCR

Name	Nucleotide Sequence (5' - 3')
1. tTA For	GCTGCTTAATGAGGTCGG
2. tTA Rev	CTCTGCACCTTGGTGATC
3.	
4.	
5.	

Electrophoresis Protocol:

Argarose: 2% in V:110

Estimated Running:Time: 60 min.

Primer Combination	Band	Genotype
tTA For + Rev	**514 bp**	**Tet O (tTA)**
	bp	
	bp	

Please size gel images to fit in this space

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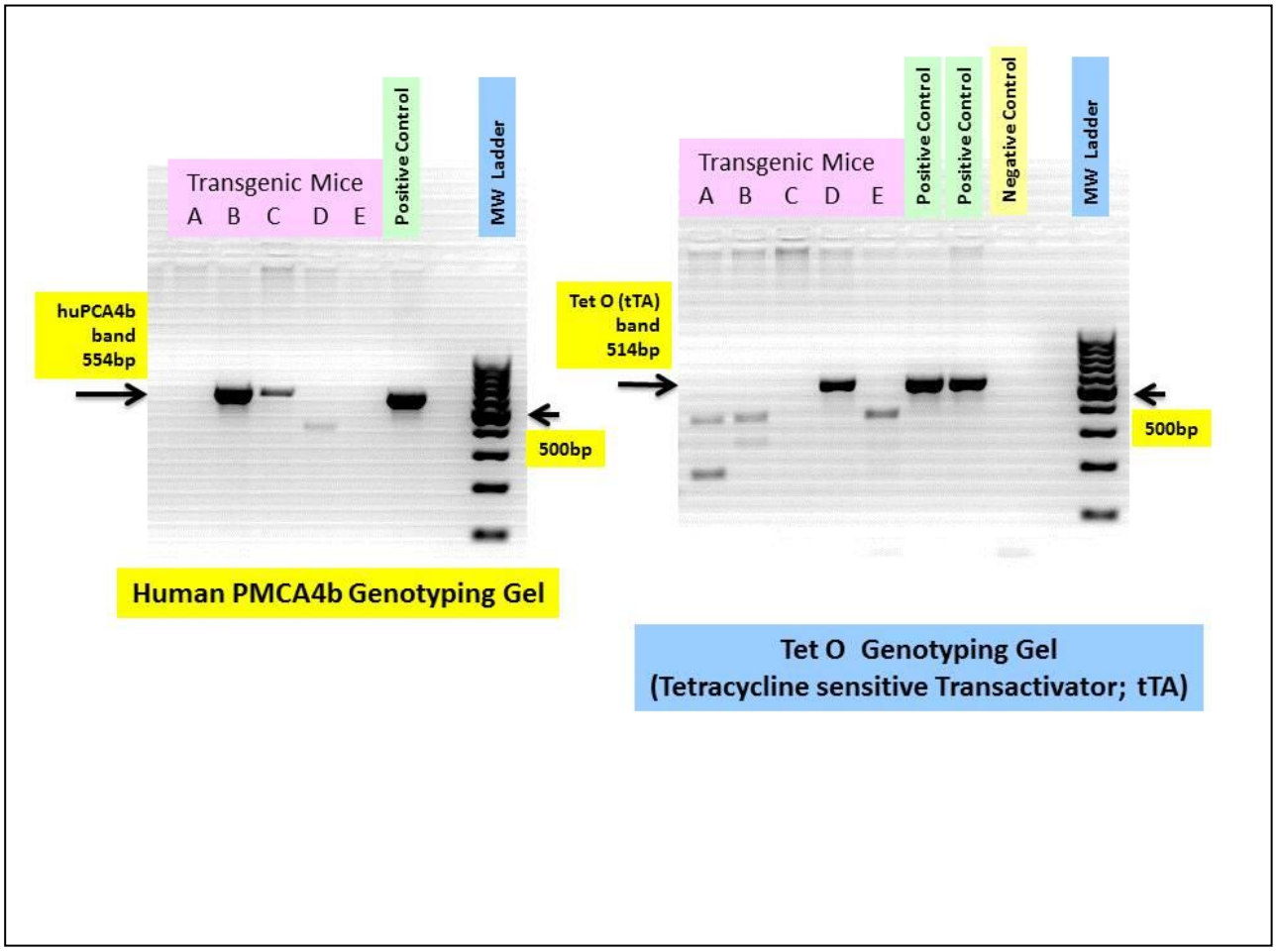
Protocol / Gel Comments:

We digest mouse tails with Proteinase K overnight at 55 centigrade in a volume of 40uL, inactivate the Prot. K at 95 centigrade for 10 min. and then add 10mM TRIS, pH 8.0 to get a final volume of 400uL. We use 5uL of this crude genomic DNA for all our genotyping.
We run a separate 25uL genomic PCR reaction for huPMCA4b transgenic locus and another separate 25uL genomic PCR reaction for the Tet O (Tetracycline sensitive TransActivator transgenic locus. We use positive control tail DNA from previously genotyped mouse tail DNA as well as a 100bp-1,000 bp DNA ladder where the 500bp band is more intense than the rest of the ladder bands to assign genotypes.

Gel pictures: These are the results of our latest genotyping (2018-05-25).



PCR protocol provided by Donating Investigator