GENOTYPING BY PCR PROTOCOL
MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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Protocol Name: 57BL/6N-Mettl3em1(IMPC)Tcp/Mmucd **MMRRC:** 048590-UCD

Protocol:

Reagent/Constituent	Volume (µL)
Water	5.6
GoTaq® G2 Colorless Master Mix,2X	7.5
Primer 1. (stock concentration is 20µM)	0.45
Primer 2. (stock concentration is 20µM)	0.45
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.0
	TOTAL VOLUME
	15

Comments on protocol:

- Protocol may work with other DNA extraction methods.
- Use Touch-Down cycling protocol-first 10 cycles anneal at 65ºC decreasing in temperature by 1.0ºC; next 30 cycles anneal at 55ºC.

Strategy:

Steps		Temp (ºC)	Time (m:ss)	# of Cycles
1. Initiation/Melting	HOT START? ☐	94	5:00	**1x**
2. Denaturation		94	0:15	
3. Annealing	steps 2-3-4 cycle in sequence	65 (↓1ºC/cycle)	0:30	**10x**
4. Elongation		72	0:40	
5. Denaturation		94	0:15	
6. Annealing	steps 5-6-7 cycle in sequence	55	0:30	**30X**
7. Elongation		72	0:40	

Primers:

Name	Nucleotide Sequence (5' - 3')
1. 48590-F	TTCATGGTGTCTTATCGTCTTCTCC
2. 48590-R	TACGAAATACTCCTTGTGCAAACAG

Electrophoresis Protocol:

Argarose: **1.5%** V: **90**

Estimated Running:Time: **90** min.

Primer Combination	**Band** (bp)	Genotype
1 & 2	378	mutant
1 & 2	517	wildtype

Mouseline - Mettl3_em1_del

Submitted By: *Lauryl Nutter, 2015-10-31*
Last Updated By: *Joanna Joeng, 2016-07-19*
Status: *Modified*

Mouseline Summary

Common Name: *Mettl3_em1_del*
Official Nomenclature: *C57BL / 6N-Mettl3^{em1Tcp}*

Comments: *Please use the following attribution in publications or presentations using this mouse line - The mouse line C57BL/6N-Mettl3<em1Tcp> was made as part of the KOMP2 project at The Centre for Phenogenomics. It was obtained from the Canadian Mouse Mutant Repository.*

Contact Information

Contact Name: *Joanna Joeng*
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Nomenclature

Genetically Modified: *Yes*
Originating PI name: *Lauryl Nutter*
Imported From Institute: *The Centre for Phenogenomics*
Strain Background: *C57BL / 6NCrl*
Strain Type: *Mutant Strain*
Subtype: *Mutant*

Welfare

Welfare Assessment: *No Welfare Concerns*
Potential Welfares:

Mouseline - Mettl3_em1_del

Allele 1: Mettl3^{em1Tcp}

Genetic Modification Type: *Endonuclease Mediated (EM)*
Allele Name: *Mettl3; endonuclease-mediated 1, The Centre for Phenogenomics*
Original Strain Background: *C57BL / 6NCrl*
Originating PI: *KOMP2*
Originating Institute: *The Centre for Phenogenomics*

Description of Mutation: *This allele produced from project TCPR0368 at TCP by injecting Cas9 mRNA and two guide RNAs with the spacer sequences AGGTAGCAGGGACCATCGCA and TATCTCCAGATCAACATCGG. This resulted in a 142 bp deletion from Chr14:52299764 to 52299905 in ENSMUSE00001224053. This mutation is predicted to cause a frameshift with amino acid changes after residue 171 and early truncation 4 amino acids later (I171Mfs*4).*

Comments:

Endonuclease Mediated (EM)

Endonuclease Type: *CAS9-RGN (Cas9 RNA guided nuclease)*
Sequence Recognition Site(s): *AGGTAGCAGGGACCATCGCA*
Sequence Recognition Site(s): *TATCTCCAGATCAACATCGG*
Sequence Recognition Site(s):
Sequence Recognition Site(s):
Sequence Recognition Site(s):
Repair Template Sequence(s):
Repair Template Sequence(s):

Mutation Description & Allele Details

MGI Information:

MGI Gene Name: *methyltransferase like 3*
MGI Gene Symbol: *Mettl3*
MGI Gene Accession ID #: *MGI:1927165*

MGI Allele Name: *Mettl3; endonuclease-mediated 1, The Centre for Phenogenomics*
MGI Allele Symbol: *Mettl3^{em1Tcp}*
MGI Allele Accession ID #: *-*

Genotyping Assay *(1)*

Assay Name: *Mettl3 - Nutter*
Investigator: *Colin McKerlie*

Screening Protocol

Mouseline - Mettl3_em1_del

PCR Protocol... Please see PCR and Primer sections below

PCR

Annealing Temp: 60ÂºC	**Final Mg Conc (mM): KAPA**	**Final dNTP Conc: KAPA**
# Cycles: 35	**Mg Type: KAPA**	**Primer Conc: 0.5 ÂµM**

Comments: ***KAPA HotStart mouse genoytping kit.***
95Â°C 3 min.;
[95Â°C 15 sec., 60Â°C 15 sec., 72Â°C 20sec.] x 35;
72Â°C 1 min.;
hold at 8Â°C
Note: Reaction conditions have not be optimized for multiplex PCR. Each primer pair is run separately.

Primer Set 1
Description: *wild-type*
Band Size: *428 bp*
Sequence Fwd: *AGACCCTGAGTTAGAGAAGAAGTTG*
Sequence Rev: *CAAAGGTGGTCACTGTAGTCAAATC*
Comments: *A: Mettl3_wt_F1; B: Mettl3_wt_R1*
Band present in wild-type and heterozygotes; absent in homozygotes.

Primer Set 2
Description: *em1*
Band Size: *378; 517 bp*
Sequence Fwd: *TTCATGGTGTCTTATCGTCTTCTCC*
Sequence Rev: *TACGAAATACTCCTTGTGCAAACAG*
Comments: *A: Mettl3_em_F1; B: Mettl3_em_R1*
Larger band present in heterozygotes and wild-type; smaller band present in heterozygotes and homozygotes.

Other Information

Any other relevant information: