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

2795 2nd Street, Suite 400, Davis, CA 95618
mmrrc@ucdavis.edu
530-754-MMRRC

NAME OF PCR: B6.129-*B2m*^{tm1Jae} *Tap1*^{tm1Hpl}/Mmcd MMRRC# 032245-UCD

DNA Extraction Method: NaOH _____ Proteinase K __X___ Other_____

Protocol: Wild-type

Reagent/ Constituent	Volume (uL)
DNA Sample	2
Invitrogen Platinum Blue PCR SuperMix	22
1:10 Primer mix (stock concentration is 100 uM)	1
ddH2O	1
TOTAL VOLUME OF REACTION:	**26 ul**

Comments on protocol **:** Make 100 uM solutions of each primer and store these as "stocks" @ -20˚C. Make 1:10 working solutions for use in the master mix as follows:

B2m WT: 50 ul each primer + 400 ul ddH_2O
B2m KO: 50 ul oIMR183 + 50 ul oIMR184 + 400 ul ddH_2O
Tap1: 50 ul each primer + 350 ul ddH_2O.

Three separate master mixes are made in order to distinguish band sizes on a 1.2% agarose gel, though they are run on the same cycling protocol.

Strategy:

Steps	Temp (^oC)	Time (min)	# of Cycles
1. Initiation/Melting HOT START?..CHECK HERE [X]	94	5	**1**
2. Denaturation	94	0.5	
3. Annealing } steps 2-3-4 will cycle in sequence	62	0.5	} **10x**
4. Elongation	72	1	
5. Denaturation	94	0.5	
6. Annealing } steps 5-6-7 will cycle in sequence	59	0.5	} **35x**
7. Elongation	72	1	
8. Amplification (i.e., 72^{o}C, 10 min)	72	7	**1**
9. Finish (i.e., 4^{o}C, indefinite)	4	indefinite	n/a

Primers:

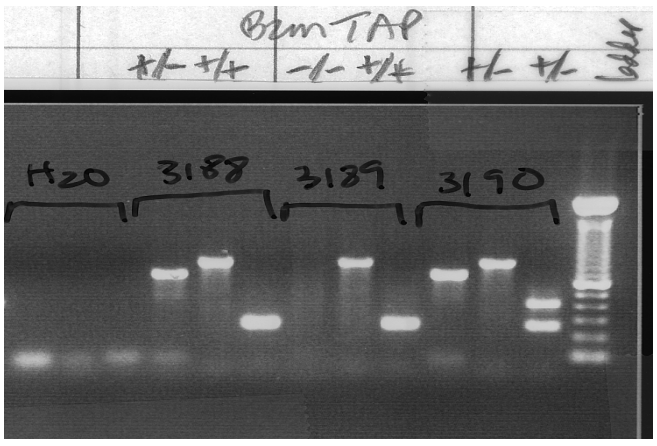
Primer Name	Nucleotide Sequence (5$^-$ - 3$^-$)
1: B2m WT1	5'-GTC AGA TAT GTC CTT CAG CAA G-3'
2: B2m WT2	5'-GAT GCT GAT CAC ATG TCT CG-3'

Electrophoresis Protocol:

% Agarose: 1.2 **V: 124**

Estimated Running Time (min): 150

Primer combination	Band (kB)	Genotype
(i.e. 1&2)	657	WT



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NAME OF PCR: B6.129-*B2m*^{tm1Jae} *Tap1*^{tm1Hpl}/Mmcd MMRRC# 032245-UCD

DNA Extraction Method: NaOH _____ Proteinase K __X___ Other_____

Protocol: Knockout

Reagent/ Constituent	Volume (uL)
DNA Sample	2
Invitrogen Platinum Blue PCR SuperMix	22
1:10 Primer mix (stock concentration is 100 uM)	1.5
ddH2O	0.5
TOTAL VOLUME OF REACTION:	**26 ul**

Comments on protocol **:** Make 100 uM solutions of each primer and store these as "stocks" @ -20˚C. Make 1:10 working solutions for use in the master mix as follows:

B2m WT: 50 ul each primer + 400 ul ddH_2O
B2m KO: 50 ul oIMR183 + 50 ul oIMR184 + 400 ul ddH_2O
Tap1: 50 ul each primer + 350 ul ddH_2O.

Three separate master mixes are made in order to distinguish band sizes on a 1.2% agarose gel, though they are run on the same cycling protocol.

Strategy:

Steps	Temp (°C)	Time (min)	# of Cycles
1. Initiation/Melting HOT START?..CHECK HERE [X]	94	5	**1**
2. Denaturation	94	0.5	
3. Annealing } steps 2-3-4 will cycle in sequence	62	0.5	} **10x**
4. Elongation	72	1	
5. Denaturation	94	0.5	
6. Annealing } steps 5-6-7 will cycle in sequence	59	0.5	} **35x**
7. Elongation	72	1	
8. Amplification (i.e., 72°C, 10 min)	72	7	**1**
9. Finish (i.e., 4°C, indefinite)	4	indefinite	n/a

Primers:

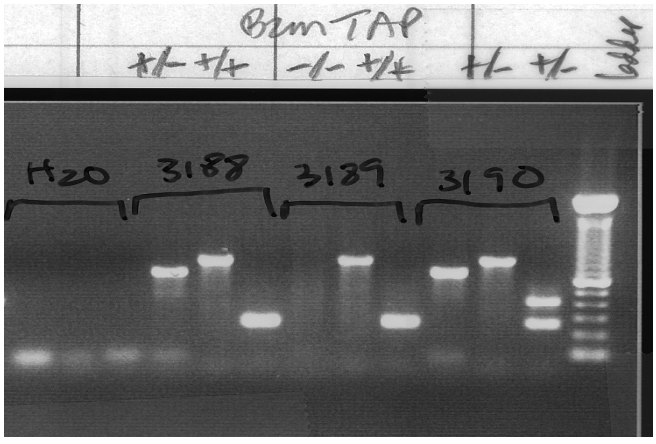
Primer Name	Nucleotide Sequence (5- - 3-)
1: oIMR183	5'-GCT ATT CGG CTA TGA CTG GG-3'
2: oIMR184	5'-TAT CAG TCT CAG TGG GGG TG-3'

Electrophoresis Protocol:

% Agarose: 1.2 **V: 124**

Estimated Running Time (min): 150

Primer combination	Band (kB)	Genotype
1&2	768	KO



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NAME OF PCR: B6.129-*B2m*^{tm1Jae} *Tap1*^{tm1Hpl}/Mmcd MMRRC# 032245-UCD

DNA Extraction Method: NaOH _____ Proteinase K __X___ Other_____

Protocol: Tap1

Reagent/ Constituent	Volume (uL)
DNA Sample	2
Invitrogen Platinum Blue PCR SuperMix	23
1:10 Primer mix (stock concentration is 100 uM)	1
ddH2O	1
TOTAL VOLUME OF REACTION:	**27 ul**

Comments on protocol **:** Make 100 uM solutions of each primer and store these as "stocks" @ -20˚C. Make 1:10 working solutions for use in the master mix as follows:

B2m WT: 50 ul each primer + 400 ul ddH_2O
B2m KO: 50 ul oIMR183 + 50 ul oIMR184 + 400 ul ddH_2O
Tap1: 50 ul each primer + 350 ul ddH_2O.

Three separate master mixes are made in order to distinguish band sizes on a 1.2% agarose gel, though they are run on the same cycling protocol.

Strategy:

Steps		Temp (°C)	Time (min)	# of Cycles
1. Initiation/Melting HOT START?..CHECK HERE [X]		94	5	**1**
2. Denaturation		94	0.5	
3. Annealing	steps 2-3-4 will cycle in sequence	62	0.5	**10x**
4. Elongation		72	1	
5. Denaturation		94	0.5	
6. Annealing	steps 5-6-7 will cycle in sequence	59	0.5	**35x**
7. Elongation		72	1	
8. Amplification (i.e., 72°C, 10 min)		72	7	**1**
9. Finish (i.e., 4°C, indefinite)		4	indefinite	n/a

Primers:

Primer Name	Nucleotide Sequence (5- - 3-)
1: TAP 912-931	5'-TCC CAG TGC ACA CTA AGC AG-3'
2: TAP 1179-1160	5'-ACC TAG GTT AGC TGC GTG GA-3
3: oIMR423	5'-TTC TGG ATT CAT CGA CTG TGG-3'

Electrophoresis Protocol:

% Agarose: 1.2 **V: 124**

Estimated Running Time (min): 150

Primer combination	Band (kB)	Genotype
1&2	250	WT
2&3	500	KO