GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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530-754-MMRRC

NAME OF PCR: B6;129X1-*Hyal1*^{tm1Stn}/Mmucd **MMRRC #** 000086-UCD

Protocol:

Reagent/ Constituent	Volume (µL)
Water	10.775
10x Buffer (contains 15mM $MgCl_2$)	2.5
$MgCl_2$ (stock concentration is 25mM)	1.7
Betaine (stock concentration is 5M) *Optional*	6.5
dNTPs (stock concentration is 10mM)	0.5
DMSO *Optional*	0.325
Primer 1 (stock concentration is 10µM) MHY1-KO For	0.5
Primer 2 (stock concentration is 10µM) MHY1-KO Rev	0.5
Primer 3 (stock concentration is 10µM) KO-Neo-Rev Hetero	0.5
Taq Polymerase 5Units/µL	0.2
DNA extracted with ☐ NaOH ☒ Proteinase K ☐ Other:	1.0
TOTAL VOLUME OF REACTION:	**25µL**

Comments on protocol:

- Use Touch-Down cycling protocol-first 10 cycles anneal at 65° C decreasing in temperature by 1.0 ° C; next 30 cycles anneal at 55° C.
- Betaine/DMSO is standardized due to high GC content in promoter regions and protocol may be tested without. Also, may adjust $MgCl_2$ to increase reaction or decrease non specific amplifications.

Strategy:

Steps		Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐		94	5:00	**1**
2. Denaturation	steps 2-3-4 will cycle in sequence	94	0:15	**40x**
3. Annealing		65 - 55 (↓1°C/cycle)	0:30	
4. Elongation		72	0:40	
5. Amplification		72	5:00	**1**
6. Finish		4	∞	n/a

Primers:

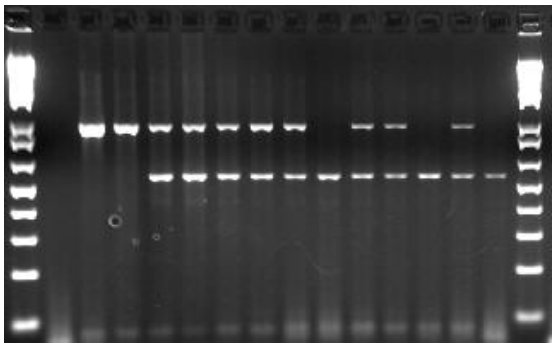
Name	Nucleotide Sequence (5' - 3')
1: MHY1-KO For	GAG ACA TGC CTT GAA CTC TGC CTC C
2: MHY1-KO Rev	ACT CAC CAG GGG CAG AAG ATA ATC TGT C
3: KO-Neo-Rev Hetero	TCG CTA TCA GGA CAT AGC GTT GGC TAC C

Electrophoresis Protocol:

Agarose: 2% **V:** 80

Estimated Running Time: 90 **min.**

Expected Bands	Genotype
1000 bp	WT +/+
500 / 1000 bp	HET +/-
500 bp	KO -/-



L to R:
1 Kb+ ladder
Water
2 wild type controls
2 het controls
3 het samples
1 knockout sample
2 het samples
1 knockout sample
1 het sample
1 knockout sample
1 Kb+ ladder

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NAME OF PCR: B6;129X1-*Hyal1*^{tm1Stn}/Mmucd **MMRRC #** 000086-UCD

DNA Extraction Method: ☐ NaOH ☒ Proteinase K ☐ Other: _____

Protocol: (Neo Tcrd Duplex)

Reagent/ Constituent	Volume (µL)
Water	16.675
10x Buffer (contains 15mM $MgCl_2$)	2.5
dNTPs (stock concentration is 10mM)	0.5
Primer 1 (stock concentration is 20µM) Neo TD F	1.0
Primer 2 (stock concentration is 20µM) Neo TD R	1.0
Primer 3 (stock concentration is 20µM) Tcrd F	0.6
Primer 4 (stock concentration is 20µM) Tcrd R	0.6
Taq Polymerase (5 Units/µL)	0.125
DNA Sample	2.0
TOTAL VOLUME OF REACTION:	**25µL**

Comments on protocol:

Only indicates the presence or absence of internal Neo vector; does not distinguish heterozygous vs. knockout, nor is it specific to any single construct. TCRD is an internal control to verify DNA is present.

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	3:00	**1**
2. Denaturation	94	0:20	
3. Annealing } steps 2-3-4 will cycle in sequence	64	0:30	} **12x**
4. Elongation	72	0:35	
5. Denaturation	94	0:20	
6. Annealing } steps 5-6-7 will cycle in sequence	58	0:30	} **25x**
7. Elongation	72	0:35	
8. Amplification	72	2:00	**1**
9. Finish	10	∞	n/a

Primers:

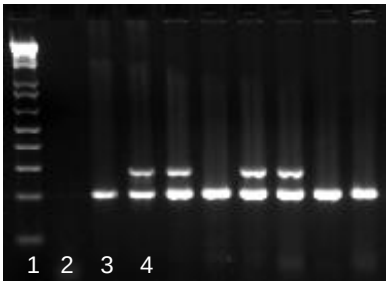
Primer Name	Nucleotide Sequence (5' - 3')
1: Neo TD F	CTT GGG TGG AGA GGC TAT TC
2: Neo TD R	AGG TGA GAT GAC AGG AGA TC
3: Tcrd F	CAA ATG TTG CTT GTC TGG TG
4: Tcrd R	GTC AGT CGA GTG CAC AGT TT

Electrophoresis Protocol:

% Agarose: 2 **mV:** 80

Estimated Running Time (min): 90

Expected Bands	Genotype
200 bp	WT +/+
280 bp	Neo +



Lanes:
1. 1Kb+ ladder
2. H_2O
3. Wild-type +/+
4. Neo +