

GENOTYPING BY PCR PROTOCOL
MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS
 2795 2nd Street, Suite 400, Davis, CA 95618
mmrrc@ucdavis.edu
 530-754-MMRRC

NAME OF PCR: B6.129X1-Serpind1^{tm1Toll}/Mmcd MMRRC # 034621-UCD

Protocol: (PCR protocol provided by Donating Investigator)

Reagent/ Constituent	Volume (μL)
Water	5.36
Tissue extract (Sigma REDEExtract-N-Amp Tissue PCR kit)	4.00
Primer 1 (stock concentration is 50 μM) mHC-5'F4	0.32
Primer 2 (stock concentration is 50 μM) mHC-E1R2	0.16
Primer 3 (stock concentration is 50 μM) Neo-R1	0.16
Sigma REDEExtract-N-Amp Reaction Mix	10.00
TOTAL VOLUME OF REACTION:	20.00 μL

Comments on protocol:

- Tail extracts are prepared following the Sigma's instructions.
- REDEExtract-N-Amp Reaction Mix contains buffer, salts, dNTPs, Taq polymerase, REDTaq dye and JumpStart Taq antibody.

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	3:00	1
2. Denaturation	94	1:00	} 35
3. Annealing } steps 2-3-4 will cycle in sequence	62	1:00	
4. Elongation	72	1:00	
5. Amplification	72	10:00	1
6. Finish		n/a	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1: mHC-5'F4	5'-CCT GTG GGT CAC CCA GAT AAC CTG AAT-3'
2: mHC-E1R2	5'-GCT CAG CAA GGC CTT TGC TCC CAA TA-3'
3: Neo-R1	5'-GCC TAC CGG TGG ATG TGG AAT-3'

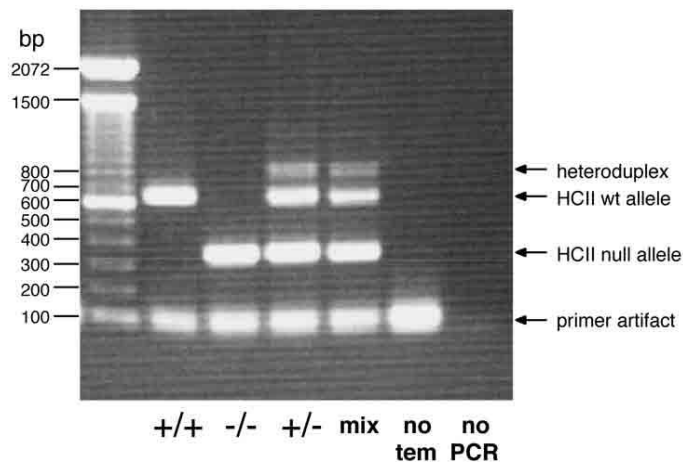
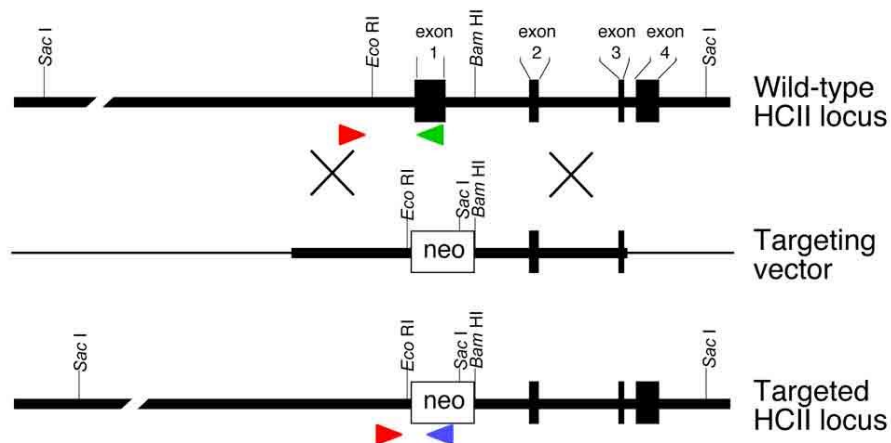
Electrophoresis Protocol:

Agarose: 1% V: 100 Estimated Running Time: 30 min

Primer Combination	Band	Genotype
1 & 2	~600 bp	Wild-type
1 & 3	~300 bp	Knockout

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

	mHC5 ⁺ F ₄ primer: 5'-CCT GTG GGT CAC CCA GAT AAC CTG AAT-3'
	mHCE ₁ R ₂ primer: 5'-GCT CAG CAA GGC CTT TGC TCC CAA TA-3'
	Neo-R ₁ primer: 5'-GCC TAC CGG TGG ATG TGG AAT-3'



Notes:

- (1) The band at ~100 nt is present in PCR reactions lacking a DNA template ("no tem") but containing the 3 primers only.
- (2) The "heteroduplex" band appears in PCR reactions containing heterozygous DNA (" +/- ") or an equimolar mixture ("mix") of DNA from homozygous +/+ and -/- animals.
- (3) Primer bands are not visible on the gel ("no PCR").