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

mmrrc@ucdavis.edu
530-754-MMRRC

NAME OF PCR: 129S(Cg)-Tg(Hoxb7-Ret)LCos **MMRRC #** 036719-UCD

Protocol:

(PCR protocol provided by Donating Investigator)

Reagent/ Constituent	Volume (µL)
Water	41
10x Buffer (15mM $MgCl_2$)	5
dNTPs (stock concentration is 10mM)	0.5
Primer 1 (stock concentration is 10µM)	1
Primer 2 (stock concentration is 10µM)	1
Primer 3 (stock concentration is µM)	
Primer 4 (stock concentration is µM)	
Taq Polymerase	0.5
Additives / Other (if applicable):	
DNA sample extracted ☐ NaOH ☒ Proteinase K ☐ Other:	1
TOTAL VOLUME OF REACTION: *(auto-calculated based on volumes entered above, right click the total and update field to show/recalculate total)*	**50 µL**

Comments on protocol: *(e.g.,* different concentration of $MgCl_2$, etc)

Primer "Abra" is located within the Ret cDNA sequence. Primer Kadabra is located in the 3' beta-globin sequence.

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐			
2. Denaturation	94	40ss	
3. Annealing } steps 2-3-4 will cycle in sequence	55	40ss	} **32**
4. Elongation	72	1m10ss	
5. Amplification (i.e., 72°C, 10 min)	72	7m	**1**
6. Finish (i.e., 4°C, indefinite)	4	indefinite	n/a

Primers:

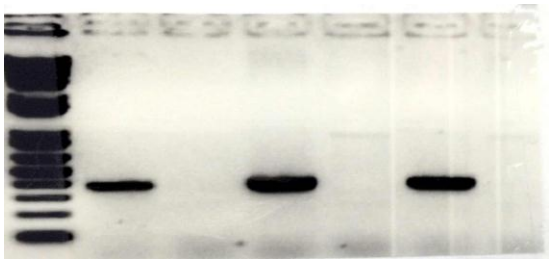
Name	Nucleotide Sequence (5' - 3')
1. Abra	TTC GGA CTC ACT GCT GTA TGA C
2. Kadabra	ACG ATC CTG AGA CTT CCA CAC T

Electrophoresis Protocol:

Agarose: 1% **V:** 24 ul

Estimated Running Time: 35 **min.**

Primer Combination	Expected Bands	Genotype
P1+P2	350 bp	transgenic



Lane 1 – markers
Lane 2 – positive control
Lane 3 – negative control
Lanes 4-7, experimental samples