

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

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530-754-MMRRC

NAME OF PCR: B6;C-Adap1^{Gt(IST11672B9)Tigm}/Mmucd

MMRRC # 034374-UCD

Protocol: *(PCR protocol provided by Donating Investigator)*

Reagent/ Constituent	Volume (μL)	
Water	11.9	
10x Buffer (contains 2mM MgCl ₂)	2.5	
dNTPs (stock concentration is 10mMmM)	0.5	
Primer 1 (stock concentration is 10μM)	1.0	
Primer 2 (stock concentration is 10μM)	1.0	
Taq Polymerase (Qiagen cat no. 201205)	0.1	
Additives / Other (if applicable): Q buffer	5.0	
DNA sample extracted ☐ NaOH ☒ Proteinase K ☐ Other:	1.0	
TOTAL VOLUME OF REACTION:		23μL

Comments on protocol:

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	2min	1
2. Denaturation	94	45sec	} 35
3. Annealing } steps 2-3-4 will cycle in sequence	70	45sec	
4. Elongation	72	1min	
5. Amplification	72	10min	1
6. Finish	4	n/a	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1: CentaF	GAAGCATCTTGATCTGTAGGTAGCCTAAGCTAGCCTG
2: CentaR	GGTGCCACACAGGACCAAGCCCTGGGAGACAGACAGCTGGAAGGA
3: V76F	CCATGCCTTGCAAAATGGCGTTACTTAAGCTAGCTTGCC
4: V76R	CCCGTGTATCCAATAAACCCTCTTGCAGTTGCATCCGAC

Electrophoresis Protocol:

Agarose: 1% V: 200 Estimated Running Time: 30 min

Primer Combination	Band	Genotype
CentaF+V76R	~340 bp	Genetrap +
CentaR+V76F	~375 bp	Genetrap +
CentaF + CentaR	~545 bp	Wt band

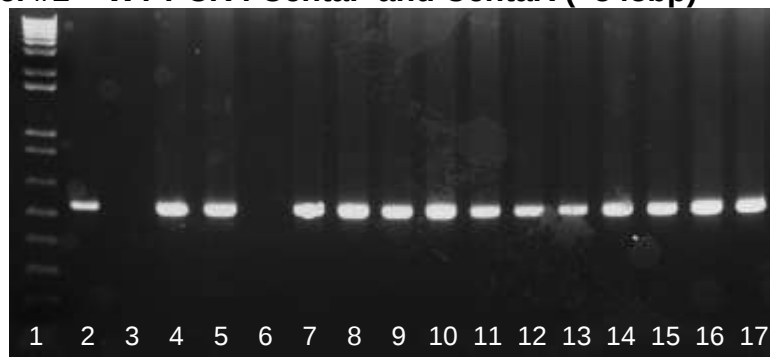
Gels displayed on page 2.

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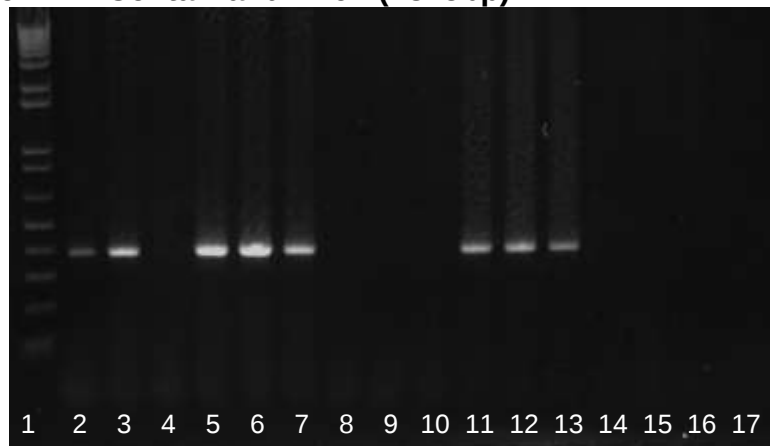
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Gel #1 = WT PCR : CentaF and CentaR (~545bp)



Lane #	WT Band	Genetrap Band	Genotype
1	Marker	Marker	
2	+	+	HET
3	-	+	HOM
4	+	-	WT
5	+	+	HET
6	-	+	HOM
7	+	+	HET
8	+	-	WT
9	+	-	WT
10	+	-	WT
11	+	+	HET
12	+	+	HET
13	+	+	HET
14	+	-	WT
15	+	-	WT
16	+	-	WT
17	+	-	WT

Gel #2 = CentaR and V76F (~375bp)



Gel #3 = CentaF and V76R (~340bp)

