

GENOTYPING BY PCR PROTOCOL
MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS
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 530-754-MMRRC

NAME OF PCR: 129S6-Nrcam^{tm1Gmt}/Mmcd MMRRC # 000370-UCD

Protocol:

Reagent/ Constituent	Volume (μL)
Water	11.475
10x Buffer (contains / without 15mM MgCl ₂)	2.5
MgCl ₂ (stock concentration is 25mM)	1.5
Betaine (stock concentration is 5M) <i>Optional</i>	6.5
dNTPs (stock concentration is 10mM)	0.5
DMSO <i>Optional</i>	0.325
Primer 1 (stock concentration is 20μM) neoF1	0.5
Primer 2 (stock concentration is 20μM) neoL	0.5
Primer 3 (stock concentration is 20μM) MP-1	0.5
Primer 4 (stock concentration is 20μM) INT3	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:

- Betaine/DMSO is standardized due to high GC content in promoter regions and protocol may be tested without. Also, may adjust MgCl₂ to increase reaction or decrease non specific amplifications.

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	95	15:00	1
2. Denaturation	94	0:45	} 35x
3. Annealing	55	1:00	
4. Elongation	72	1:00	
5. Amplification	72	5:00	1
6. Finish	4	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1: neoF1	TGG AGA GGC TAT TCG GCT ATG AC
2: neoL	AAG GTG AGA TGA CAG GAG ATC
3: MP-1	CTT CCT GTG CCA GAT GAT CA
4: INT3	GCT CAG GAT GGT TGC GCC

Electrophoresis Protocol:

Agarose: 2% V: 100 Estimated Running Time: 60 min

Expected Bands	Genotype
340 bp	WT +/+
340 / 260 bp	HET +/-
260 bp	KO -/-