

GENOTYPING BY PCR PROTOCOL
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530-754-MMRRC

NAME OF PCR: B6.129-Kif5a^{tm2Gsn}/Mmucd MMRRC # 000224-UCD

Protocol:

Reagent/ Constituent	Volume (μL)
Water	20.25
10x Buffer (contains 15mM MgCl ₂)	2.5
dNTPs (stock concentration is 10mM)	0.5
Primer 1 (stock concentration is 20μM) 5'Kif5All	0.25
Primer 2 (stock concentration is 20μM) 5'Kif5All	0.25
Taq Polymerase 5Units/μL	0.25
DNA extracted with ☒ NaOH ☐ Proteinase K ☐ Other:	1.0
TOTAL VOLUME OF REACTION:	25μL

Comments on protocol:

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	95	3:00	1
2. Denaturation	94	0:45	} 30x
3. Annealing	60	0:45	
4. Elongation	72	1:00	
5. Amplification	72	10:00	1
6. Finish	4	∞	n/a

Primers:

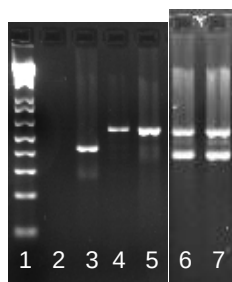
Name	Nucleotide Sequence (5' - 3')
1: 5'Kif5All	CGT TCT GGA CGA GGC AAA GA
2: 5'Kif5All	GAT GGG ACA GCA GCA GTG CA

Electrophoresis Protocol:

Agarose: 2% V: 100

Estimated Running Time: 60 min.

Expected Bands	Genotype
370 bp	WT +/+
370 / 470 bp	HET +/-
470 bp	KO -/-



Lanes:
1: 1 kb+ ladder
(Invitrogen, Cat. #10787-026)
2: H2O
3: B6
4-5: -/-
6-7: +/-