

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

NAME OF PCR: B6.129-Kif5a^{tm1Gsn}/Mmcd **MMRRC #** 000223-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'Kif5AI	0.125
Primer 2 (stock concentration is 20μM) 3'Kif5AI	0.125
Primer 3 (stock concentration is 20μM) 3'loxP5AI	0.125
Taq Polymerase 5Units/μL	0.25
DNA extracted with ☒ NaOH ☐ Proteinase K ☐ Other:	1.0
TOTAL VOLUME OF REACTION:	25μL

Comments on protocol:

- Homozygous mutant is embryonic lethal.
- Number of cycles to use should be based on optimizations in each laboratory.

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	95	3:00	1
2. Denaturation	94	0:45	} 30-35x
3. Annealing } steps 2-3-4 will cycle in sequence	60	0:45	
4. Elongation	72	1:00	
5. Amplification	72	10:00	1
6. Finish	4	∞	n/a

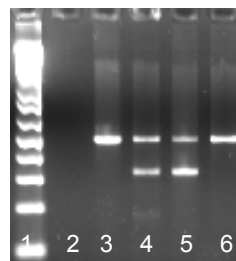
Primers:

Name	Nucleotide Sequence (5' - 3')
1: 5'Kif5AI	GAT ACT CCA AGG CTG GGA ACA TA
2: 3'Kif5AI	TGT GGA GGT CAG AGG TCA AGT
3: 3'loxP5AI	CGG TAC CCG GGG ATC AAT TCG AG

Electrophoresis Protocol:

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

Expected Bands	Genotype
450 bp	WT +/+
300 / 450 bp	HET +/-
300 bp	KO -/-



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