

GENOTYPING BY PCR PROTOCOL
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 530-754-MMRRC

NAME OF PCR: B6;120-Kif3c^{tm1Gsn}/Mmucd MMRRC # 000214-UCD

Protocol:

Reagent/ Constituent	Volume (μL)
Water	11.275
10x Buffer (contains 15mM MgCl ₂)	2.0
dNTPs (stock concentration is 10mM)	0.4
Primer 1 (stock concentration is 20μM) B51820	0.2
Primer 2 (stock concentration is 20μM) B5 RVR	0.2
Primer 3 (stock concentration is 20μM) Floxkpn	0.2
Taq Polymerase 5Units/μL	0.2
DNA extracted with ☒ NaOH ☐ Proteinase K ☐ Other:	1.0
TOTAL VOLUME OF REACTION:	20μL

Comments on protocol:

Strategy:

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

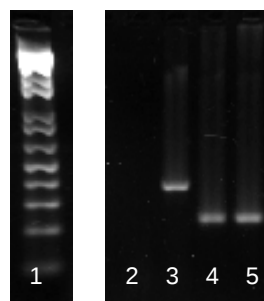
Primers:

Name	Nucleotide Sequence (5' - 3')
1: B51820	GGA GAA TTA CCT GCA GGA CC
2: B5 RVR	TGT AAG TGC ACC TAG CTA CC
3: Floxkpn	CGG TAC CCG GGG ATC AAT TCG AG

Electrophoresis Protocol:

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

Expected Bands	Genotype
350 bp	WT +/+
200 bp	KO -/-



Lanes:
 1. 1Kb+ ladder
 2. H₂O
 3. Wild-type +/+ sample
 4-5. Hom -/- samples