

GENOTYPING BY PCR PROTOCOL FORM

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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530-754-MMRRC

NAME OF PCR: C57BL/6-*Tbx21*^{duane}/Mmcd (*Duane*), MMRRC # 031679-UCD

Protocol:

Reagent/ Constituent	Volume (µL)
ddH ₂ O	1.58
dNTPs (2.5mM each –amplifluor kit)	0.4
20x amplifluor SNP FAM Primer (amplifluor kit)	0.25
20x amplifluor SNP JOE Primer (amplifluor kit)	0.25
20x Duane primer mix (allele 1&2 are 0.5µM, reverse is 7.5µM)	1.0
10x Reaction Mix S Plus	0.5
Platinum Taq (5u/µl)	0.05
DNA Sample (cleaned ear punch in dH ₂ O)	1.0
TOTAL VOLUME OF REACTION:	5µl

Comments on protocol: Perform reactions at room temperature with reagents added in order.

Strategy: program Amp

Steps	Temp (°C)	Time (min)	# of Cycles
1. Initiation/Melting HOT START? [<input checked="" type="checkbox"/>]	94	4:00	1
2. Denaturation	94	0:15	
3. Annealing } steps 2-3-4 will cycle in sequence	55	0:20	35x
4. Elongation	72	0:30	
5. Amplification	72	3:00	1
6. Finish	10	hold	hold

Primers: Nucleotide sequence (5' - 3')

Primer Name	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
1: Duane-1	G	A	A	G	G	T	G	A	C	C	A	A	G	T	T	C	A	T	G	C	T	C	A	T	A	A	G	C	G	G	T		
	T	C	C	C	T	G	T	G	G	T																							
2: Duane-2	G	A	A	G	G	T	C	G	G	A	G	T	C	A	A	C	G	G	A	T	T	C	A	T	A	A	G	C	G	G	T		
	T	C	C	C	T	G	T	G	G	A																							
3: Duane-R	G	T	G	T	C	T	G	G	T	C	G	A	T	A	C	T	T	G	A	C	A	A											

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