

RMRC Genotyping Protocol-PCR

種原編號	RMRC13057	品系名稱	B6.129-Arl4d ^{tm1Lj}
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A. Primer

	Primer name	Sequence
1	CU	accacatcggtgctcttac
2	FD	taggggccatttcagtcaag
3	JD	caagactcccacccagaaag

B. Reaction Conditions

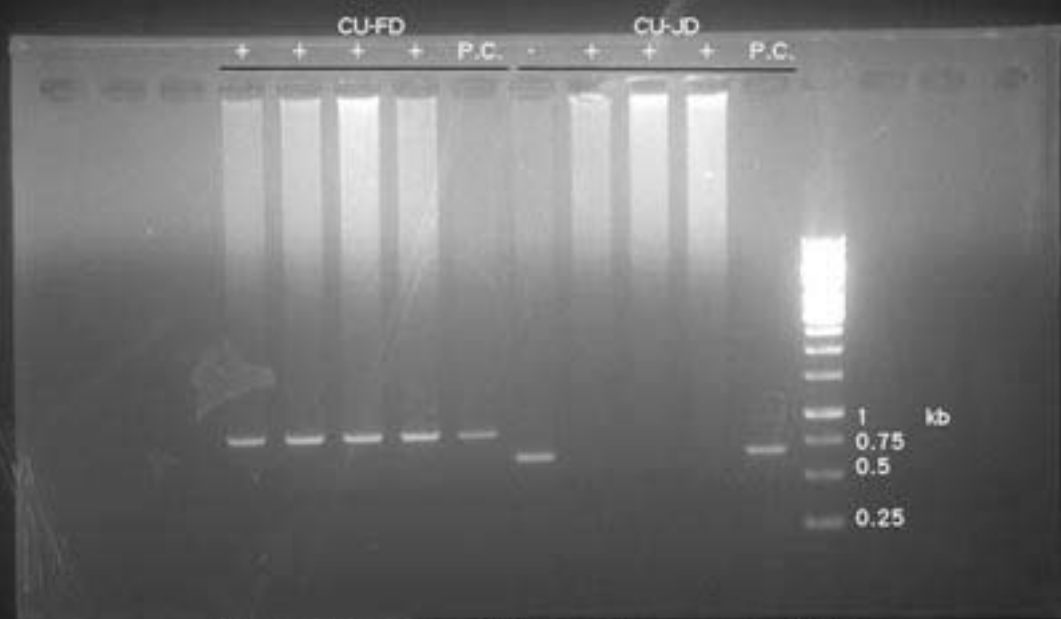
Component	Concentration		Volume		Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	--		15-17	μl	1	95	300	1
PCR buffer	10x		2.5	μl	2	95	30	30
dNTP	2.5 each	mM	2	μl	3	55	30	30
Primer 1	5	μM(μmol/μl)	1	μl	4	72	60	30
Primer 2	5	μM(μmol/μl)	1	μl	5	72	600	1
DNA	--		1-3	μl	6	4	--	--
Taq	5	U/μl	0.5	μl	7			
Mg ²⁺	15	mM	included in 10x PCR buf.	μl	8			
Total			25	μl	9			

C. PCR Product

	Primer set		Product size		Target region
1	CU	FD	“+” allele	863 bp	CU: upstream of LexA 5'-flanking of exon2 of Arl4d
			“-” allele	No	FD: downstream of LexA 5'-flanking of exon2 of Arl4d CU-FD: amplifying LexA upstream of Arl4d exon2 in wide type mice; failed to amplify in KO mice
2	CU	JD	“+” allele	3000* bp	JD: downstream of LexA 3'-flanking of exon2 of Arl4d
			“-” allele	670 bp	CU-JD: amplifying the region from upstream of Arl4d exon2 to downstream in wide type mice; amplifying the recombined LexA in KO mice.

* the 3000-kb product is not seen all the time; mostly there is no band on gel.

D. Gel Photo



P.C., positive control for PCR reaction, showing success of PCR with the genotype "+" in CU-FD amplification and "-" in CU-JD.