

RMRC Genotyping Protocol-PCR

No.	RMRC13349	Strain Name	B6.129S6-Zfyve9 ^{tm1Yty} /Narl
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A. Primer

	Primer name	Sequence
1	WT1	tactgtatagatttagcaaa
2	WT2	ggcagtgggtgcatgctc
3	KO1	tttcacttcaggctcccaag
4	KO2	catgccctgctgtaagttgg

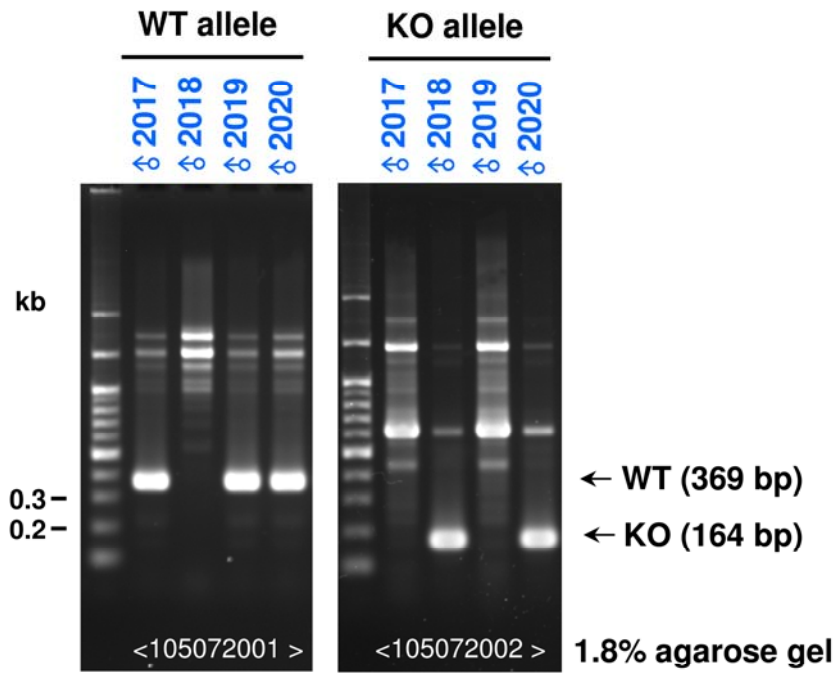
B. Reaction Conditions

Component	Concentration		Volume		Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	--		8.7	μl	1	95	60	
MyTaq HS mix [BIOLINE]	2x		10	μl	2	95	15	36
dNTP		mM		μl	3	51.2	15	36
Primer 1	10	μM(μmol/μl)	0.4	μl	4	72	10	36
Primer 2	10	μM(μmol/μl)	0.4	μl	5	72	180	
DNA	--		0.5	μl	6	4	pause	
Taq		U/μl		μl	7			
Mg ²⁺		mM		μl	8			
Total			20	μl	9			

C. PCR Product

	Primer set		Product size	Target region
1	WT1	WT2	369 bp	Exon2
2	KO1	KO2	164 bp	

D. Gel Photo



Result

- ♂ 2017 (WT)
- ♂ 2018 ($Sara^{KO/KO}$)
- ♂ 2019 (WT)
- ♂ 2020 ($Sara^{KO/+}$)