

RMRC Genotyping Protocol-PCR

No.	RMRC13094	Strain Name	C57BL/6-Tg(Zfp503-UREA/Hbb-LacZ)22SLYC
	RMRC13095		C57BL/6-Tg(Zfp503-UREA/Hbb-LacZ)26SLYC
	RMRC13096		C57BL/6-Tg(Zfp503-UREB/Hbb-LacZ)28SLYC
	RMRC13097		C57BL/6-Tg(Zfp503-UREB/Hbb-LacZ)81SLYC
	RMRC13098		C57BL/6-Tg(Zfp503-UREB/Hbb-LacZ)96SLYC

A. Primer

	Primer name	Sequence
1	HS4-31	ATg Tgg CAC TgA ggg ACA Tgg C
2	HS4-51	CCT CCT Tgg gCA ACC TgT TCA g

B. Reaction Conditions

Component	Concentration		Volume		Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	--		9.08	μl	1	94	3'	
PCR buffer	10x (contain 20mM MgCl ₂)		1.2	μl	2	94	20"	
dNTP	2.5	mM	0.24	μl	3	66	20"	35
Primer 1	10	μM	0.24	μl	4	72	40"	
Primer 2	10	μM	0.24	μl	5	72	5'	
DNA	--		1	μl	6	4	--	
Taq	5	U/μl	0.05	μl	7			
Mg ²⁺	--	mM	x	μl	8			
Total			12	μl	9			

C. PCR Product

	Primer set		Product size	Target region
1	HS4-51	HS4-31	600 bp	chicken hypersensitive site 4 (cHS4)
2				

D. Gel Photo

