

RMRC Genotyping Protocol-PCR

種原編號	RMRC13003	品系名稱	B6;Cg-Tg(SMN2)Hung <i>SMN1^{tm1Hung}</i>
	RMRC13004		FVB;Cg-Tg(SMN2)Hung <i>SMN1^{tm1Hung}</i>

Part I: Tg(SMN2)Hung

A. Primer

	Primer name	Sequence
1	F	AAACCAGTCGGGCACAATACCTAGC
2	R	TATGCTGATTGAAGGGAGGGGTGC

B. Reaction Conditions

Component	Concentration		Volume		Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	--		15.4	μl	1	94	180	1
PCR buffer	10x		2	μl	2	94	30	30
dNTP	10	mM	0.4	μl	3	56	30	
Primer 1	10	μM(ρmol/μl)	0.5	μl	4	72	30	
Primer 2	10	μM(ρmol/μl)	0.5	μl	5	72	180	1
DNA	--		1	μl	6			
Taq	5	U/μl	0.2	μl	7			
Mg ²⁺		mM		μl	8			
Total			20	μl	9			

C. PCR Product

	Primer set		Product size	Target region
1	F	R	719 bp	SMN2
2			bp	

D. Gel Photo

Part II: *SMN1^{tm1Hung}*

A. Primer

	Primer name	Sequence
1	P1	ATAACACCACCACTCTTACTC
2	P2	GTAGCCGTGATGCCATTGTCA
3	P3	AGCCTGAAGAACGAGATCAGC

B. Reaction Conditions

Component	Concentration		Volume	Step	Temperature, °C	Time, sec	Number of Cycles	
H ₂ O	--		15.4	μl	1	94	180	1
PCR buffer	10x		2	μl	2	94	30	30
dNTP	10	mM	0.4	μl	3	56	30	
Primer 1	10	μM(ρmol/μl)	0.5	μl	4	72	30	
Primer 2	10	μM(ρmol/μl)	0.5	μl	5	72	180	1
DNA	--		1	μl	6			
Taq	5	U/μl	0.2	μl	7			
Mg ²⁺		mM		μl	8			
Total			20	μl	9			

C. PCR Product

	Primer set		Product size	Target region
1	P1	P2	1080 bp	<i>Smn1</i>
2	P1	P3	950 bp	mutant <i>Smn1</i>

D. Gel Photo