

RMRC Genotyping Protocol-PCR

No.	RMRC13153	Strain Name	C57BL/6-Gskip<tm1c(GEMMS)Narl>
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A. Primers

No.	Primer name	Sequence
1	GskipNF	ATCCTCAGCTACAGAGGATACGGGAGGTC
2	emGFP159R	GGTGGTGCAGATGAACTTCAGG

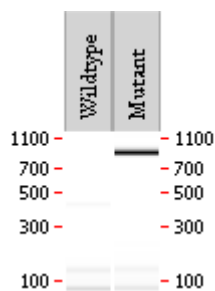
B. Reaction Conditions

Component	Concentration	Volume	Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	–	10.8 μl	1	94	180	1
PCR buffer	10 x	1.5 μl	2	94	30	35
Formamide	–	0.5 μl	3	60	30	35
dNTP	10 mM	0.3 μl	4	72	30	35
Primer 1	20 μM (μmol/μl)	0.3 μl	5	72	180	1
Primer 2	20 μM (μmol/μl)	0.3 μl				
gDNA	50-250 ng/μl	1 μl				
Taq	5 U/μl	0.3 μl				
Total		15 μl				

C. PCR Product

No.	Primer set		Product size	Target region
1	GskipNF	emGFP159R	900 bp	Mutant

D. Gel Photo



此方法只能確定有 mutant, 無法判斷是 heterozygous 或是 homozygous. 因 deletion 區域太大, 所以 wild type 片段無法 P 出來. 如要判斷是否為 homozygous, 要再加做下方的 PCR, 如下方的 PCR 沒有 product 才是 homozygous.

E. Primers

No.	Primer name	Sequence
1	GskipDF	GCACAGGCAGACCTCTATCCTAAG
2	GskipDR	TTTTGGAGCCCCTTTCAAGTG

F. Reaction Conditions

Component	Concentration	Volume	Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	–	10.8 µl	1	94	180	1
PCR buffer	10 x	1.5 µl	2	94	30	35
Formamide	–	0.5 µl	3	60	30	35
dNTP	10 mM	0.3 µl	4	72	30	35
Primer 1	20 µM (pmol/µl)	0.3 µl	5	72	180	1
Primer 2	20 µM (pmol/µl)	0.3 µl				
gDNA	50-250 ng/µl	1 µl				
Taq	5 U/µl	0.3 µl				
Total		15 µl				

G. PCR Product

No.	Primer set		Product size	Target region
1	GskipDF	GskipDR	318 bp	Wildtype

H. Gel Photo

