

RMRC Genotyping Protocol-PCR

No.	RMRC13190	Strain Name	C57BL/6-Trim5 ^{tm1a(GEMMS)Narl}
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A. Primers

No.	Primer name	Sequence
1	Trim5LF	GGTACACGGATCATA CATGCAGG
2	Trim5LR	TGCTTGAATGCTGCTGTGCTC

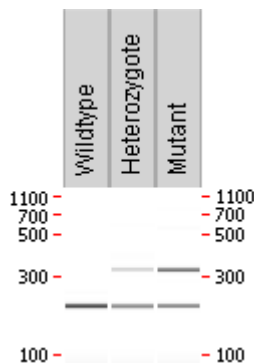
B. Reaction Conditions

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	Trim5LF	Trim5LR	210 bp	Wildtype
2	Trim5LF	Trim5LR	320 bp	Mutant

D. Gel Photo



此法只能區分 wild type 與 knock out mice，無法區分 heterozygotes 與 homozygotes。

E. Primers

No.	Primer name	Sequence
1	Trim5DF	TCCAGGGTCTGCTGTAGTGGGTAG
2	Trim5DR	CTCCATTCAACTGCCCGCTCTCAG
3	Trim5R1F	CCCAGGCGCGTTTGCTATTTACCT
4	Trim5R1R	GACTGCCATGGAAATCTGCTGTCC

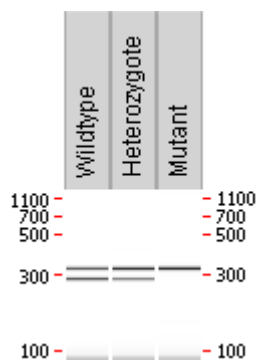
F. Reaction Conditions

Component	Concentration	Volume	Step	Temperature, °C	Time, sec	Number of Cycles
H ₂ O	–	10.2 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 (ρmol/μl)	0.3 μl	5	72	180	1
Primer 2	20 μM (ρmol/μl)	0.3 μl				
Primer 3	20 μM (ρmol/μl)	0.3 μl				
Primer 4	20 μM (ρmol/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	Trim5DF	Trim5DR	282 bp	Mutant (resulting in loss of the product)
2	Trim5R1F	Trim5R1R	334 bp	Wildtype

H. Gel Photo



此法只能區分出 homozygotes，無法區分 wild type 與 heterozygotes。

故要區分 wild type，heterozygotes 與 homozygotes 需兩種 PCR 都做。