GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

mmrrc@ucdavis.edu
530-754-MMRRC

NAME OF PCR: C3.Cg-*Foxq1*$^{sa\text{-}e1}$/Mmucd, **MMRRC #** 000058-UCD

Protocol: ***sa*** **deletion mutation**

Reagent/ Constituent	Volume (µL)
Water	
10x Buffer	2.5
MgCl2 (stock concentration is 50mM)	0.75
Betaine (stock concentration is 4M)	6.25
dNTPs (stock concentration is 1mM)	5
Primer 1 (stock concentration is µM)	
Primer 2 (stock concentration is µM)	
Use primers 1 & 2 **or** primers 2 & 3	
Primer 3 (stock concentration is µM)	
Taq Polymerase	0.25
DNA Sample	1.0
TOTAL VOLUME OF REACTION:	**25µL**

Comments on protocol:

This satin mutant is a compound heterozygote for the intragenic deletion (*sa*) and a novel ENU-induced point mutation (*sa*^{e1}). The radiation-induced intragenic deletion is detected by PCR analysis using primers SaF2 and SaR5 or SaF8 and SaR5. The T to G transversion is detected in the ENU-induced allele at bp 383. This mutation is detected by PCR analysis using primers SaF3 and SaR4 followed by restriction enzyme digestion with *Fok*1. Electrophoresis resolves two fragments of 141 and 143 bp from the wild-type allele and 284 bp from the ENU-mutant allele. The compound heterozygote satin shows all three bands.

Strategy:

Steps		Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting	HOT START? ☐	96	4:00	**1**
2. Denaturation	} steps 2-3-4 will cycle in sequence	94	0:45	} **34x**
3. Annealing		58	0:45	
4. Elongation		72	1:00	
5. Amplification		72	10:00	**1**
6. Finish		4	n/a	n/a

Primers:

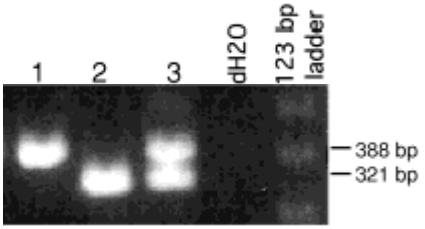
Primer Name	Nucleotide Sequence (5' - 3')
1: SaF2	GAG ATC AAC GAG TAC CTC ATG GG
2: SaR5	CGA AGG AGC TGG AGA ACT TG
3: SaF8	CCC AAC AGC GAA TAC ACC TT

Electrophoresis Protocol:

% Agarose: 2-2.5 **V:** 96, 60

Estimated Running Time (min) 5 min, 150 min

Primer Combination	Band	Genotype
SaF2 + SaR5	388 bp	wild-type
SaF2 + SaR5	321 bp	SB/Le deletion
SaF8 + SaR5	226 bp	wild-type
SaF8 + SaR5	159 bp	SB/Le deletion



Lanes:
1. wild-type SB/Le (SB/Le - ++/++)
2. radiation-induced satin (SB/Le-*bg sa*/*bg sa*)
3. ENU-induced satin in a compound *sa*$_{e1}$/*sa* heterozygote

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NAME OF PCR: C3.Cg-*Foxq1*$^{sa\text{-}e1}$/Mmcd, **MMRRC #** 000058-UCD

Protocol: ***sa-e1*** **transversion mutation**

Reagent/ Constituent	Volume (µL)
Water	
10x Buffer	2.5
MgCl2 (stock concentration is 50mM)	0.75
Betaine (stock concentration is 4M)	6.25
dNTPs (stock concentration is 1mM)	5
Primer 1 (stock concentration is µM)	
Primer 2 (stock concentration is µM)	
Taq Polymerase	0.25
DNA Sample	1.0
TOTAL VOLUME OF REACTION:	**25µL**

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5. Amplification	72	10:00	**1**
6. Finish	4	n/a	n/a

Primers:

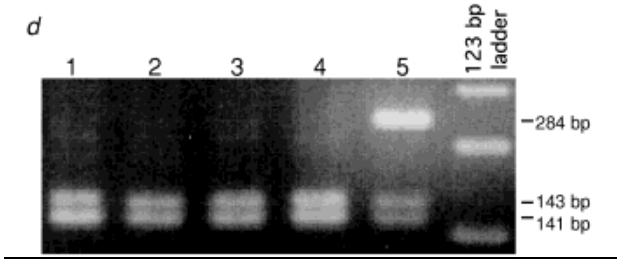
Primer Name	Nucleotide Sequence (5' - 3')
4: SaF3	GGC AAC TGA TGA CAG CAG AA
5: SaR4	TGA CGA AAC AGT CGT TGA GC

Electrophoresis Protocol:

% Agarose: 2-2.5 **V:** 96, 60

Estimated Running Time (min) 5 min, 150 min

Primer Combination	Band	Genotype
SaF2 + SaR5	388 bp	wild-type
SaF2 + SaR5		



Restriction Digest Cutting overnight at 37 °C

Steps	Volume (µL)
PCR Product	10.0
Water	19.25
NEB Buffer 4	2.5
100x BSA } supplied with enzyme	1.25
BccI Enzyme (NEB)	1.5
TOTAL VOLUME:	**27µL**