PCR: San Roque Mutant Screening PCR (MSPCR)

Amplifluor: No

Primers

Nucleotide sequence (5` --> 3`)

Primer	Sequence
SRMsFa	CATCAGGCTGGCCTCTAGTT
SRRWT	AAGACCAGCTTCAGAGCTTCCTCCAGCA
SRRMut	GAACCATCTTCTAAGGCCAGCGTGACCAGCTTCAGAGCTTCCTCCTACC

Reagents

Reagents/Constituents	Commercial name	Stock Concentration	Volume (µL)
DNA Sample			1.0
10x Buffer		25.0 mM MgCl2	4.0
dNTPs		10.0 mM	0.2
Primer: **SRMsFa**		10.0 µM	
Primer: **SRRWT**		10.0 µM	
Primer: **SRRMut**		10.0 µM	
Water			29.8
Taq Polymerase			0.5
Reagent: Tritonx100 10%			0.5

Comments on protocol: Need to do a primer titration to find the optimal combination of primer amounts to amplify the two products at equal intensity. The above volumes are what currently works for us. A non specific product is sometimes observed beneath the lower band.

Strategy

Steps	Temp (°C)	Time (seconds)
Hot start: **No**		
Initiation/Melting	94.0	120
Denaturation	94.0	20
Annealing	62.0	20
Elongation	72.0	30
Number of cycles (repeat denature, anneal & elgonate): **35**		
Strand completion (i.e. 72°C, 10 min)	72.0	300
Finish (i.e. 4°C, indefinite)	10.0	Hold

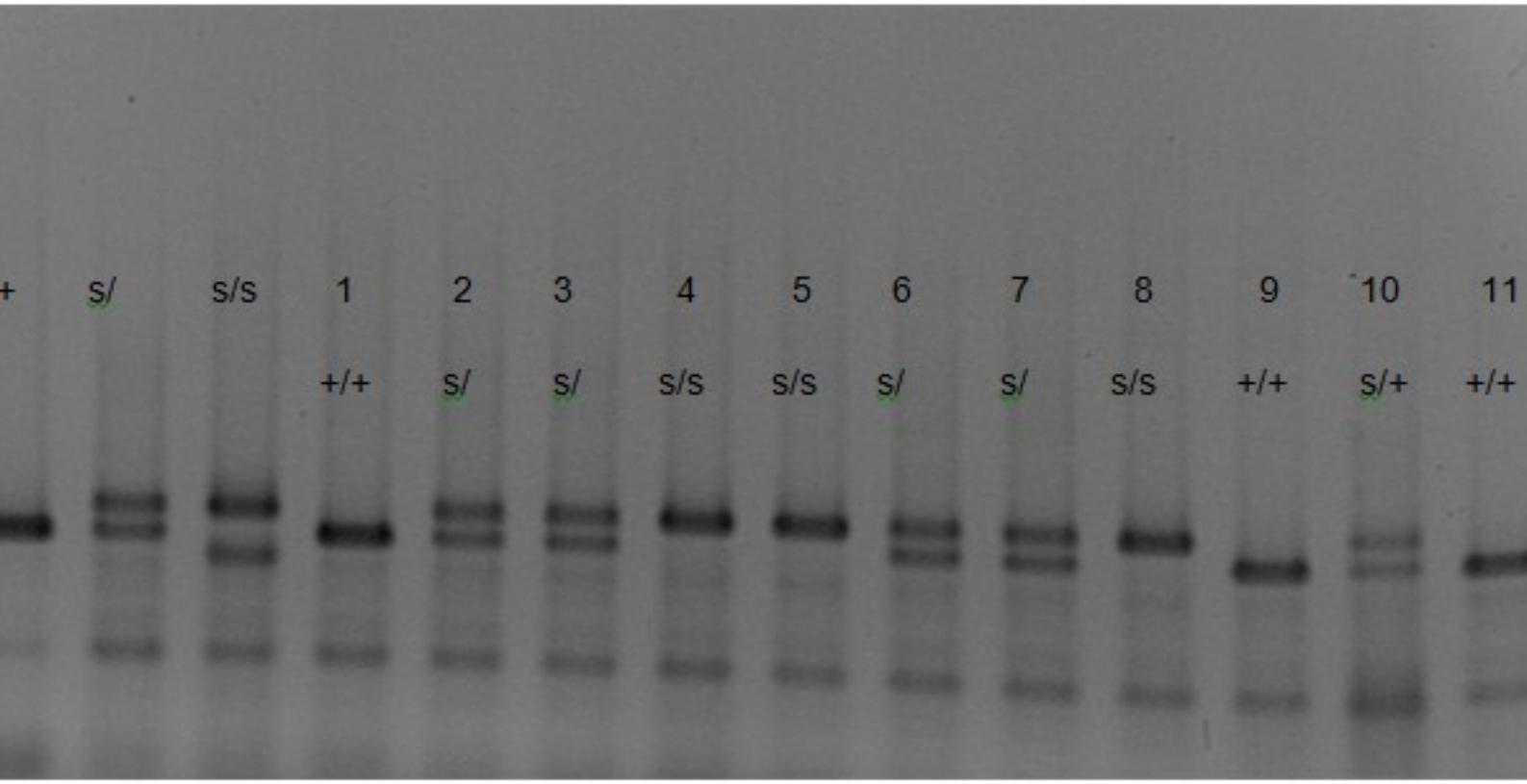
Electrophoresis

Running buffer:	
% Agarose:	3.0
Volt:	140.0
Estimated running time (min):	60.0

Image

Bands

Number	Band (bp)	Genotype
1.	194	Wild Type (+/+)
2.	215	Mutant (s/s)


+
S/
S/S
1
2
3
4
5
6
7
8
9
10
11
+/+
S/
S/
S/S
S/S
S/
S/
S/S
+/+
S/+
+/+