

## Reaction Conditions - RW31

RXN#1				Cocktail Volume (uL)	1130
Reagent	[ ]	[ ] Units	Desired [ ]	Des'd [ ] Units	Amount to add
Buffer	10	X		1 X	113
dNTP's	2	mM Each	0.2	mM Each	113
MgCl2	50	mM	1.5	mM	33.9
SAM25-F-HEX	40	uM		1 uM	28.25
SAM25-R	40	uM		1 uM	28.25
RW31-F-HEX	40	uM	2.5	uM	70.625
RW31-R	40	uM	2.5	uM	70.625
EV1Pm-F-NED	40	uM	0.3	uM	8.475
EV1Pm-R	40	uM	0.3	uM	8.475
Taq	5	U/uL	0.05	U/uL	11.3
DNA	5	ng/uL		10 ng/rxn (2 uL/rxn)	226
Autoclaved ddH2O			to 1130 uL		418.1
					1130
Don't Dilute Prior To De-salting					

  

RXN#2				Cocktail Volume (uL)	1130
Reagent	[ ]	[ ] Units	Desired [ ]	Des'd [ ] Units	Amount to add
Buffer	10	X		1 X	113
dNTP's	2	mM Each	0.2	mM Each	113
MgCl2	50	mM	1.5	mM	33.9
TR3A1-F-6FAM	40	uM	0.45	uM	12.7125
TR3A1-R	40	uM	0.45	uM	12.7125
TR2F3-F-NED	20	uM	0.3	uM	16.95
TR2F3-R	20	uM	0.3	uM	16.95
Taq	5	U/uL	0.05	U/uL	11.3
DNA	5	ng/uL		10 ng/rxn (2 uL/rxn)	226
Autoclaved ddH2O			to 1130 uL		573.475
					1130
Don't Dilute Prior To De-salting					

  

Use PCR Cycles PROPDNR			
5 min	@		94
30 sec	@		94
1 min	@	54.5	35x
1 min	@	72	
45 min	@	60	

Is a 10ul reaction volume

  

Use PCR Cycles PROPDNR			
5 min	@		94
30 sec	@		94
1 min	@	54	30 X
1 min	@	72	
45 min	@	60	

Is a 10ul reaction volume

## RW31 Scoring Criteria

- Score the +A
- Allele bins are even
- This locus is pretty easy to score, but look out for pull-up from EV1Pm in the same size range.

