## **Reaction Conditions - RW31**

RXN#1				Cocktail Volume (uL)	1130	Use PCF	R Cycles F	PROPDNR	
Reagent	[]	[ ] Units	Desired [ ]	Des'd [ ] Units	Amount to add	5 min	@	94	
Buffer	10			X	113	30 sec	@	94	
dNTP's	2	mM Each	0.2	mM Each	113	1 min	@	54.5	35x
MgCl2	50	mM	1.5	mM	33.9	1 min	@	72	
SAM25-F-HEX		uM	1	uM	28.25	45 min	@	60	
SAM25-R		uM	1	uM	28.25				
RW31-F-HEX		uM		uM	70.625	Is a 10ul	reaction	volume	
RW31-R		uM	2.5	uM	70.625				
EV1Pm-F-NED	40	uM	0.3	uM	8.475				
EV1Pm-R		uM	0.3	uM	8.475				
Taq		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				
TOTOGRAFOO GOLLEO					4400				
	De-saltin	ıg			1130				
Don't Dilute Prior To	De-saltin	g		Cocktail Volume (uL)		Use PCF	R Cycles F	PROPDNR	
Don't Dilute Prior To	De-saltin	g [ ] Units	Desired [ ]	Cocktail Volume (uL)	1130 Amount to add	Use PCF	R Cycles F	PROPDNR 94	
Don't Dilute Prior To RXN#2 Reagent		[ ] Units		Cocktail Volume (uL) Des'd [ ] Units	1130		@		
Don't Dilute Prior To RXN#2 Reagent Buffer	[]	[ ] Units	1	Des'd [ ] Units	1130 Amount to add	5 min	@	94 94	30 X
Don't Dilute Prior To RXN#2 Reagent Buffer dNTP's	10 2	[] Units	0.2	Des'd [ ] Units	1130 Amount to add	5 min 30 sec	@	94 94	30 X
Don't Dilute Prior To RXN#2 Reagent Buffer dhTP's MgCl2	[] 10 2 50 40	[ ] Units X mM Each mM uM	0.2	Des'd [ ] Units X mM Each mM	1130 Amount to add 113	5 min 30 sec 1 min	@ @ @	94 94 <b>54</b>	30 X
Don't Dilute Prior To RXN#2 Reagent Buffer dNTP's MgCl2 TR3A1-F-6FAM	10 2 50 40 40	[ ] Units X mM Each mM uM	1 0.2 1.5	Des'd [ ] Units X mM Each mM uM	1130 Amount to add 113 113 33.9	5 min 30 sec 1 min 1 min	@	94 94 <b>54</b> 72	30 X
Don't Dilute Prior To RXN#2 Reagent Buffer dNTP's MgCl2 TR3A1-F-6FAM TR3A1-R TR2F3-F-NED	10 20 50 40 40 20	[ ] Units X mM Each mM uM uM uM	1 0.2 1.5 0.45 0.45	Des'd [ ] Units X mM Each mM uM uM uM	1130 Amount to add 113 113 33.9 12.7125	5 min 30 sec 1 min 1 min 45 min	@	94 94 <b>54</b> 72 60	30 X
Don't Dilute Prior To RXN#2 Reagent Buffer dhTP's MgCl2 TR3A1-F-6FAM TR3A1-R TR2F3-F-NED	10 2 50 40 40 20 20	[ ] Units X mM Each mM uM uM uM	0.2 1.5 0.45 0.45 0.3 0.3	Des'd [ ] Units X mM Each mM uM uM uM uM	1130 Amount to add 113 113 33.9 12.7125 12.7125	5 min 30 sec 1 min 1 min 45 min	@ @ @	94 94 <b>54</b> 72 60	30 X
Don't Dilute Prior To RXN#2 Reagent Buffer dNTP's MgCl2 TR3A1-F-6FAM TR3A1-R	10 2 50 40 40 20 20	[ ] Units X mM Each mM uM uM uM	0.2 1.5 0.45 0.45 0.3 0.3	Des'd [ ] Units X mM Each mM uM uM uM	1130 Amount to add 113 113 33.9 12.7125 12.7125	5 min 30 sec 1 min 1 min 45 min	@ @ @	94 94 <b>54</b> 72 60	30 X
Don't Dilute Prior To RXN#2 Reagent Buffer dNTP's MgCl2 TR3A1-F-6FAM TR3A1-R TR2F3-F-NED TR2F3-R	[] 10 2 50 40 40 20 20	[ ] Units X mM Each mM uM uM uM	0.2 0.2 1.5 0.45 0.45 0.3 0.3	Des'd [ ] Units X mM Each mM uM uM uM uM	1130 Amount to add 113 113 33.9 12.7125 12.7125 16.95 16.95	5 min 30 sec 1 min 1 min 45 min	@ @ @	94 94 <b>54</b> 72 60	30 X
Don't Dilute Prior To RXN#2 Reagent Buffer dNTP's MgCI2 TR3A1-F-6FAM TR3A1-R TR2F3-F-NED TR2F3-R Taq DNA	[] 10 2 50 40 40 20 20	[ ] Units X mM Each mM uM uM uM uM	0.2 0.2 1.5 0.45 0.45 0.3 0.3	Des'd [ ] Units X mM Each mM uM	1130 Amount to add 113 113 33.9 12.7125 12.7125 16.95 16.95 11.3	5 min 30 sec 1 min 1 min 45 min	@ @ @	94 94 <b>54</b> 72 60	30 X
Don't Dilute Prior To RXN#2 Reagent Buffer dNTP's MgCI2 TR3A1-F-6FAM TR3A1-R TR2F3-F-NED TR2F3-R	[] 10 2 50 40 40 20 20	[ ] Units X mM Each mM uM uM uM uM	1 0.2 1.5 0.45 0.45 0.3 0.3 0.05	Des'd [ ] Units X mM Each mM uM	1130 Amount to add 113 113 33.9 12.7125 12.7125 16.95 16.95 11.3 226	5 min 30 sec 1 min 1 min 45 min	@ @ @	94 94 <b>54</b> 72 60	30 X

## **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.

