

## Reaction Conditions - RW34

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
RW34-F-6FAM	20	uM	0.5	uM	28.25
RW34-R	20	uM	0.5	uM	28.25
GT023-F-HEX	20	uM	0.3	uM	16.95
GT023-R	20	uM	0.3	uM	16.95
GT271-F-NED	20	uM	0.1	uM	5.65
GT271-R	20	uM	0.1	uM	5.65
TR2G5-F-NED	10	uM	0.05	uM	5.65
TR2G5-R	10	uM	0.05	uM	5.65
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		519.8
					1130
Don't Dilute Prior to Mixing					

  

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
TR3G6-F-HEX	20	uM	0.08	uM	4.52
TR3G6-R	20	uM	0.08	uM	4.52
EV37Mn-F-HEX	20	uM	0.2	uM	11.3
EV37Mn-R	20	uM	0.2	uM	11.3
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		601.16
					1130
Don't dilute prior to mixing					

  

Use PCR Cycles PROPDNR	
5 min	@ 94
30 sec	@ 94
1 min	@ 58
1 min	@ 72
45 min	@ 60

  

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

## RW34 Scoring Criteria

- Allele bins are odd
- Score the +A, although it is difficult to tell, because the +A is often the only peak you see.

