

## Reaction Conditions - RW48

RXN #1 Ta = 55					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	
RW219-F-NED	20	uM	0.18	uM	10.17	
RW219-R	20	uM	0.18	uM	10.17	
TV19-F-6FAM	10	uM	0.1	uM	11.3	
TV19-R	10	uM	0.1	uM	11.3	
RW48-F-HEX	20	uM	0.1	uM	5.65	
RW48-R	20	uM	0.1	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		578.56	
					1130	
Dilute 3 ul of PCR product with 12 ul of water						
RXN #2 Ta = 60					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	
RW18-F-HEX	10	uM	0.08	uM	9.04	
RW18-R	10	uM	0.08	uM	9.04	
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		614.72	
					1130	
Don't dilute prior to mixing						

  

Use PCR Cycles PROPDNR				
5 min	@		94	
30 sec	@		94	
1 min	@		55	30 X
1 min	@		72	
45 min	@		60	
Is a 10ul reaction volume				

  

Use PCR Cycles PROPDNR				
5 min	@		94	
30 sec	@		94	
1 min	@		60	30 X
1 min	@		72	
45 min	@		60	
Is a 10ul reaction volume				

## RW48 Scoring Criteria

- Allele bins are even
- I've only seen one peak, can't tell if it's +A or allele

