Reaction Conditions - RW48

RXN #1 Ta = 55				Cocktail Volume (uL)	1130	Use PCR	R Cycles	PROPDNR		
Reagent	[]	[] Units	Desired []	Des'd [] Units	Amount to add	5 min	@		94	
Buffer	10	X	1	X	113	30 sec	@		94	
dNTP's	2	mM Each	0.2	mM Each	113	1 min	@		55 30 X	
MgCl2	50	mM	1.5	mM	33.9	1 min	@		72	
RW219-F-NED	20	uM	0.18	uM	10.17	45 min	@		60	
RW219-R		uM	0.18	uM	10.17					
TV19-F-6FAM		uM	0.1	uM	11.3	Is a 10ul	reaction	volume		
TV19-R	10	uM	0.1	uM	11.3					
RW48-F-HEX	20	uM	0.1	uM	5.65					
RW48-R		uM	0.1	uM	5.65					
Taq	5	U/uL	0.05	U/uL	11.3					
DNA	5	ng/uL		ng/rxn (2 uL/rxn)	226					
Autoclaved ddH2O			to 1130 uL		578.56					
					1130					
Dilute 3 ul of PCR p	product	with 12 ul	of water							
•	product	with 12 ul	of water	Cocktail Volume (ul.)	1120	lies DCE	Cycles	PROPINIP		
RXN #2 Ta = 60	product			Cocktail Volume (uL)	1130		R Cycles	PROPDNR	94	
RXN #2 Ta = 60 Reagent	[]	[] Units	Desired []	Des'd [] Units	Amount to add	5 min	@		94	
RXN #2 Ta = 60 Reagent Buffer	[]	[] Units	Desired []	Des'd [] Units	Amount to add	5 min 30 sec	@		94 94 60 30 X	
RXN #2 Ta = 60 Reagent Buffer dNTP's	[] 10 2	[] Units X mM Each	Desired [] 1 0.2	Des'd [] Units X mM Each	Amount to add 113 113	5 min 30 sec 1 min	@		60 30 X	
RXN #2 Ta = 60 Reagent Buffer dNTP's MgCl2	[] 10 2 50	[] Units X mM Each mM	Desired [] 1 0.2 1.5	Des'd [] Units X mM Each mM	Amount to add 113 113 33.9	5 min 30 sec 1 min 1 min	@		60 30 X 72	
RXN #2 Ta = 60 Reagent Buffer dNTP's MgCl2 RW18-F-HEX	[] 10 2 50	[] Units X mM Each mM uM	Desired [] 1 0.2 1.5 0.08	Des'd [] Units X mM Each mM	Amount to add 113 113 33.9 9.04	5 min 30 sec 1 min	@ @		60 30 X	
RXN #2 Ta = 60 Reagent Buffer dNTP's MgCl2 RW18-F-HEX RW18-R	[] 10 2 50 10	[] Units X mM Each mM uM	Desired []	Des'd [] Units X mM Each mM uM uM	Amount to add 113 113 33.9 9.04 9.04	5 min 30 sec 1 min 1 min 45 min	@ @ @		60 30 X 72	
RXN #2 Ta = 60 Reagent Buffer dNTP's MgCI2 RW18-F-HEX RW18-R	10 2 50 10 10 5	[] Units X mM Each mM uM uM	Desired [] 1 0.2 1.5 0.08 0.08 0.05	Des'd [] Units X mM Each mM uM uM U/uL	Amount to add 113 113 33.9 9.04	5 min 30 sec 1 min 1 min 45 min	@ @		60 30 X 72	
RXN #2 Ta = 60 Reagent Buffer dNTP's MgCl2 RW18-F-HEX	10 2 50 10 10 5	[] Units X mM Each mM uM	Desired [] 1 0.2 1.5 0.08 0.08 0.05	Des'd [] Units X mM Each mM uM uM	Amount to add 113 113 33.9 9.04 9.04 11.3	5 min 30 sec 1 min 1 min 45 min	@ @ @		60 30 X 72	
RXN #2 Ta = 60 Reagent Buffer dhTP's MgCi2 RW18-F-HEX RW18-R Taq DNA	10 2 50 10 10 5	[] Units X mM Each mM uM uM	Desired [] 1 0.2 1.5 0.08 0.08 0.08 1005	Des'd [] Units X mM Each mM uM uM U/uL	Amount to add 113 113 33.9 9.04 9.04 11.3 226 614.72	5 min 30 sec 1 min 1 min 45 min	@ @ @		60 30 X 72	
RXN #2 Ta = 60 Reagent Buffer dNTP's MgCi2 RW18-F-HEX RW18-R Taq DNA	10 2 50 10 10 5	[] Units X mM Each mM uM uM	Desired [] 1 0.2 1.5 0.08 0.08 0.08 1005	Des'd [] Units X mM Each mM uM uM U/uL	Amount to add 113 113 33.9 9.04 9.04 11.3 226	5 min 30 sec 1 min 1 min 45 min	@ @ @		60 30 X 72	
RXN #2 Ta = 60 Reagent Buffer dNTP's MgCi2 RW18-F-HEX RW18-R Taq DNA	[] 10 2 50 10 10 5 5	[] Units X mM Each mM uM uM U/uL ng/uL	Desired [] 1 0.2 1.5 0.08 0.08 0.08 1005	Des'd [] Units X mM Each mM uM uM U/uL	Amount to add 113 113 33.9 9.04 9.04 11.3 226 614.72	5 min 30 sec 1 min 1 min 45 min	@ @ @		60 30 X 72	

RW48 Scoring Criteria

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

