

Reaction Conditions - RW219

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				

RW219 Scoring Criteria

- Score the +A
- Allele bins are even

