

## Reaction Conditions - RW212

RXN#1											
Reagent	[ ]	[ ] Units	Desired [ ]	Des'd [ ] Units	Amount to add	Use PCR Cycles PROPDNR					
Buffer	10	X		1 X	113	5 min	@	94			
dNTP's	2	mM Each		0.2 mM Each	113	30 sec	@	94			
MgCl2	50	mM		1.5 mM	33.9	1 min	@	49	30 X		
TV17-F-6FAM	20	uM		0.3 uM	16.95	1 min	@	72			
TV17-R	20	uM		0.3 uM	16.95	45 min	@	60			
Taq	5	U/uL		0.05 U/uL	11.3	Is a 10ul reaction volume					
DNA	5	ng/uL		10 ng/rxn (2 uL/rxn)	226						
Autoclaved ddH2O			to 1130 uL		598.9						
					1130						
Dilute by 3 ul of PCR product with 15 ul of water											
RXN#2						Use PCR Cycles PROPDNR					
Reagent	[ ]	[ ] Units	Desired [ ]	Des'd [ ] Units	Amount to add						
Buffer	10	X		1 X	113	5 min	@	94			
dNTP's	2	mM Each		0.2 mM Each	113	30 sec	@	94			
MgCl2	50	mM		1.5 mM	33.9	1 min	@	58	30 X		
RW417-F-6FAM	20	uM		0.4 uM	22.6	1 min	@	72			
RW417-R	20	uM		0.4 uM	22.6	45 min	@	60			
TV20-F-HEX	20	uM		0.07 uM	3.955	Is a 10ul reaction volume					
TV20-R	20	uM		0.07 uM	3.955						
RW45-F-NED	20	uM		0.11 uM	6.215						
RW45-R	20	uM		0.11 uM	6.215						
RW212-F-HEX	20	uM		0.085 uM	4.8025						
RW212-R	20	uM		0.085 uM	4.8025						
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		557.655						
					1130						
Don't Dilute Prior To De-salting											

## RW212 Scoring Criteria

- This one is tricky. For the most part the allele is preferentially amplified over the +A, however it is the +A that is the 121 allele that we've been scoring in the past. Therefore, I propose that we score the allele, but call it 121. This means that the bins will have to be shifted a bit.

