

Reaction Conditions - TR3F2

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
TR3G1-6FAM	20	uM		0.255 uM		14.4075
TR3G1-R	20	uM		0.255 uM		14.4075
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			603.985
						1130
Don't dilute prior to de-salting						
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
Q-Solution	5	X		1 X		226
TR3F2-F-HEX	40	uM		0.255 uM		7.20375
TR3F2-R	40	uM		0.255 uM		7.20375
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			392.3925
						1130
Don't Dilute Prior To De-salting						

PCR Cycles			
5 min	@	94	
30 sec	@	94	
30 sec	@	68	5X
1 min	@	72	
30 sec	@	94	
30 sec	@	64	5X
1 min	@	72	
30 sec	@	94	
30 sec	@	61	5X
1 min	@	72	
30 sec	@	94	
30 sec	@	58	5X
1 min	@	72	
30 sec	@	94	
30 sec	@	55	10X
1 min	@	72	
10 min	@	72	

Is a 10ul reaction volume			
Use PCR Cycles PROPDNR			
5 min	@	94	
30 sec	@	94	
1 min	@	58	30 X
1 min	@	72	
45 min	@	60	

Is a 10ul reaction volume			
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TR3F2 Scoring Criteria

- Allele bins are even
- Score the +A, although you don't really see the allele

