

Reaction Conditions - GT271

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	
RW34-F-6FAM		20 uM		0.5 uM	28.25	
RW34-R		20 uM		0.5 uM	28.25	
GT023-F-HEX		20 uM		0.3 uM	16.95	
GT023-R		20 uM		0.3 uM	16.95	
GT271-F-NED		20 uM		0.1 uM	5.65	
GT271-R		20 uM		0.1 uM	5.65	
TR2G5-F-NED		10 uM		0.05 uM	5.65	
TR2G5-R		10 uM		0.05 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		519.8	
					1130	
Don't Dilute Prior to Mixing						

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	
TR3G6-F-HEX		20 uM		0.08 uM	4.52	
TR3G6-R		20 uM		0.08 uM	4.52	
EV37Mn-F-HEX		20 uM		0.2 uM	11.3	
EV37Mn-R		20 uM		0.2 uM	11.3	
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		601.16	
					1130	
Don't dilute prior to mixing						

Use PCR Cycles PROPDNR				
5 min	@			94
30 sec	@			94
1 min	@			54
1 min	@			72
45 min	@			60
Is a 10ul reaction volume				

Use PCR Cycles PROPDNR				
5 min	@			94
30 sec	@			94
1 min	@			54
1 min	@			72
45 min	@			60
Is a 10ul reaction volume				

GT271 Scoring Criteria

- Allele bins are even
- Score the +A
- Locus is monomorphic for 102 bp, so if you don't get that then your ladder is screwed up.
- Sometimes the allele (rather than the +A) is preferentially amplified

