

Reaction Conditions - TR3A1

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	
SAM25-F-HEX		40 uM		1 uM	28.25	
SAM25-R		40 uM		1 uM	28.25	
RW31-F-HEX		40 uM		2.5 uM	70.625	
RW31-R		40 uM		2.5 uM	70.625	
EV1Pm-F-NED		40 uM		0.3 uM	8.475	
EV1Pm-R		40 uM		0.3 uM	8.475	
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		418.1	
					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	
TR3A1-F-6FAM		40 uM		0.45 uM	12.7125	
TR3A1-R		40 uM		0.45 uM	12.7125	
TR2F3-F-NED		20 uM		0.3 uM	16.95	
TR2F3-R		20 uM		0.3 uM	16.95	
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		573.475	
					1130	
Don't Dilute Prior To De-salting						

Use PCR Cycles PROPDNR

5 min	@	94	
30 sec	@	94	
1 min	@	54.5	35x
1 min	@	72	
45 min	@	60	

Is a 10ul reaction volume

Use PCR Cycles PROPDNR

5 min	@	94	
30 sec	@	94	
1 min	@	54	30 X
1 min	@	72	
45 min	@	60	

Is a 10ul reaction volume

TR3A1 Scoring Criteria

- Allele bins are even
- Peaks have a weird patter, with smaller peaks on either side of the large leak, but it is still clear what to score

