## **Reaction Conditions - TR2G5**

				Cocktail Volume (uL)	1130	Use PCF	R Cycles P	ROPDNR	
Reagent	[]	[ ] Units	Desired [ ]	Des'd [ ] Units	Amount to add	5 min	@	94	
Buffer	10			X	113	30 sec	@	94	
dNTP's	2	mM Each	0.2	mM Each	113	1 min	@	58	35x
MgCl2		mM	1.5	mM	33.9	1 min	@	72	
RW34-F-6FAM		uM		uM	28.25	45 min	@	60	
RW34-R		uM		uM	28.25				
GT023-F-HEX		uM	0.3	uM	16.95	Is a 10ul	reaction v	olume	
GT023-R	20	uM	0.3	uM	16.95				
GT271-F-NED		uM		uM	5.65				
GT271-R	20	uM		uM	5.65				
TR2G5-F-NED	10	uM	0.05		5.65				
TR2G5-R		uM	0.05		5.65				
Too	5	U/uL	0.05	U/uL	11.3				
Taq			40	ng/rxn (2 uL/rxn)	226				
DNA	5	ng/uL	10	HIGHTAII (Z UL/IXII)					
	5	ng/uL	to 1130 uL	ng/ixii (2 dL/ixii)	519.8				
DNA		ng/uL		IIgraii (2 dEraii)					
DNA Autoclaved ddH2O		ng/uL		Cocktail Volume (uL)	519.8	Use PCF	R Cycles P	ROPDNR	
DNA Autoclaved ddH2O Don't Dilute Prior to RXN #2 Reagent	Mixing	[ ] Units	to 1130 uL  Desired [ ]	Cocktail Volume (uL) Des'd [ ] Units	519.8 1130	Use PCF 5 min	R Cycles P	94	
DNA Autoclaved ddH2O Don't Dilute Prior to RXN #2 Reagent Buffer	Mixing	[ ] Units	Desired [ ]	Cocktail Volume (uL) Des'd [ ] Units X	519.8 1130 1130 Amount to add	5 min 30 sec	@	94 94	
DNA Autoclaved ddH2O Don't Dilute Prior to RXN #2 Reagent Buffer dJNTP's	Mixing	[ ] Units X mM Each	Desired [ ]	Cocktail Volume (uL) Des'd [ ] Units X mM Each	519.8 1130 1130 Amount to add 113 113	5 min	@	94 94 <b>54</b>	30 X
DNA Autoclaved ddH2O Don't Dilute Prior to RXN #2 Reagent Buffer ddffer MgCl2	Mixing  [ ]  10  2  50	[ ] Units X mM Each mM	Desired [ ] 1 0.2 1.5	Cocktail Volume (uL) Des'd [ ] Units X mM Each mM	519.8 1130 1130 Amount to add 113 113 33.9	5 min 30 sec	@	94 94	30 X
DNA Autoclaved ddH2O Don't Dilute Prior to RXN #2 Reagent Buffer dNTP's MgCl2 TR3G6-F-HEX	Mixing  [ ]  10  2  50  20	[ ] Units X MM Each mM uM	Desired [ ] 1 0.2 1.5 0.08	Cocktail Volume (uL) Des'd [ ] Units X mM Each mM	519.8 1130 1130 Amount to add 113 113 33.9 4.52	5 min 30 sec 1 min	@	94 94 <b>54</b>	30 X
DNA Autoclaved ddH2O Don't Dilute Prior to RXN #2 Reagent Buffer dNTP's MgCi2 TR3G6-F-HEX TR3G6-R		[ ] Units X mM Each mM uM	Desired [ ]	Cocktail Volume (uL) Des'd [ ] Units X mM Each mM uM uM	519.8 1130 1130 Amount to add 113 113 33.9 4.52 4.52	5 min 30 sec 1 min 1 min 45 min	@ @ @	94 94 <b>54</b> 72 60	30 X
DNA Autoclaved ddH2O Don't Dilute Prior to RXN #2 Reagent Buffer dMTP's MgCl2 TR3G6-F-HEX TR3G6-R EV37Mn-F-HEX	D Mixing  [ ] 10 2 50 20 20 20 20 20	[ ] Units X mM Each mM uM uM	Desired [ ]	Cocktail Volume (uL) Des'd [ ] Units X mM Each mM uM uM	519.8 1130 Amount to add 113 113 33.9 4.52 4.52 11.3	5 min 30 sec 1 min 1 min 45 min	<b>.</b> @	94 94 <b>54</b> 72 60	30 X
DNA Autoclaved ddH2O Don't Dilute Prior to RXN #2 Reagent Buffer dNTP's MgCi2 TR3G6-F-HEX TR3G6-R	D Mixing  [ ]  10 22 50 20 20 20 20	[ ] Units X mM Each mM uM uM uM	Desired [ ]  1 0.2 1.5 0.08 0.08 0.02	Cocktail Volume (uL) Des'd [ ] Units X mM Each mM uM uM uM uM	519.8 1130 Amount to add 113 313 33.9 4.52 4.52 11.3 11.3	5 min 30 sec 1 min 1 min 45 min	@ @ @	94 94 <b>54</b> 72 60	30 X
DNA Autoclaved ddH2O Don't Dilute Prior to RXN #2 Reagent Buffer dMTP's MgCl2 TR3G6-F-HEX TR3G6-R EV37Mn-F-HEX EV37Mn-R Tag	Mixing  [ ]  10  2  50  20  20  20  55	[ ] Units X mM Each mM uM uM uM uM	Desired [ ]	Cocktail Volume (uL) Des'd [ ] Units X mM Each mM uM uM UM UM U/UL	1130 Amount to add 113 33.9 4.52 4.52 11.3 11.3 11.3	5 min 30 sec 1 min 1 min 45 min	@ @ @	94 94 <b>54</b> 72 60	30 X
DNA Autoclaved ddH2O Don't Dilute Prior to RXN #2 Reagent Buffer dNTP's MgCi2 TR3G6-F-HEX TR3G6-R EV37Mn-F-HEX EV37Mn-F	Mixing  [ ]  10  2  50  20  20  20  55	[ ] Units X mM Each mM uM uM uM	Desired [ ]	Cocktail Volume (uL) Des'd [ ] Units X mM Each mM uM uM uM uM	519.8 1130 Amount to add 113 313 33.9 4.52 4.52 11.3 11.3	5 min 30 sec 1 min 1 min 45 min	@ @ @ @	94 94 <b>54</b> 72 60	30 X
DNA Autoclaved ddH2O Don't Dilute Prior to RXN #2 Reagent Buffer dMTP's MgCl2 TR3G6-F-HEX TR3G6-R EV37Mn-F-HEX EV37Mn-R Tag	Mixing  [ ]  10  2  50  20  20  20  55	[ ] Units X mM Each mM uM uM uM uM	Desired [ ]	Cocktail Volume (uL) Des'd [ ] Units X mM Each mM uM uM UM UM U/UL	1130 Amount to add 113 33.9 4.52 4.52 11.3 11.3 11.3	5 min 30 sec 1 min 1 min 45 min	@ @ @ @	94 94 <b>54</b> 72 60	30 X

## **TR2G5 Scoring Criteria**

- Allele bins are odd
- Score the +A.
- Is monomorphic for allele 215 bp
- Look out for pull-up form EV37Mn in this allele range!!

