## **Reaction Conditions - IGF**

RXN#1				Cocktail Volume (uL)	1130	Use PCR	Cycles PR	OPDNR		
Reagent	[]	[ ] Units	Desired [ ]	Des'd [ ] Units	Amount to add	5 min	@	9	1	
Buffer	10	X	1	X	113	30 sec	@	94	1	
dNTP's	2	mM Each	0.2	mM Each	113	1 min	@	5	2 30 X	
MgCl2	50	mM	1.5	mM	33.9	1 min	@	73	2	
GATA028-F-NED	40	uM	1	uM	28.25	45 min	@	6	)	
GATA028-R	40	uM	1	uM	28.25					
TR3G11-F-6FAM	20	uM	0.52	uM	29.38	Is a 10ul reaction volume				
TR3G11-R	20	uM	0.52	uM	29.38					
TV14-F-NED	20	uM	0.1	uM	5.65					
TV14-R	20	uM	0.1	uM	5.65					
IGF-F-HEX	40	uM	0.675	uM	19.06875					
IGF-R		uM	0.675		19.06875					
Taq		U/uL		U/uL	11.3					
DNA	5	ng/uL	10	ng/rxn (2 uL/rxn)	226					
DNA					100 1005					
Autoclaved ddH2O			to 1130 uL		468.1025					
Autoclaved ddH2O  Don't Dilute Prior To	o De-salting		to 1130 uL		1130					
Autoclaved ddH2O  Don't Dilute Prior To	De-salting		to 1130 uL	Cocktail Volume (uL)		Use PCR	R Cycles PR	OPDNR		
Autoclaved ddH2O  Don't Dilute Prior To  RXN#2	De-salting	[ ] Units	to 1130 uL  Desired [ ]	Cocktail Volume (uL) Des'd [ ] Units	1130	Use PCR	R Cycles PR	OPDNR 9		
Autoclaved ddH2O  Don't Dilute Prior To  RXN#2  Reagent  Buffer	[]		Desired [ ]		1130		@	OPDNR 9		
Autoclaved ddH2O  Don't Dilute Prior To  RXN#2  Reagent  Buffer	[]	[ ] Units	Desired [ ]	Des'd [ ] Units	1130  1130  Amount to add	5 min	@	94	1 1 1 0 30 X	
Autoclaved ddH2O  Don't Dilute Prior To  RXN#2  Reagent  Buffer  dhTP's  MgCl2	10 2 50	[ ] Units X mM Each	Desired [ ] 1 0.2 1.5	Des'd [ ] Units X mM Each mM	1130 Amount to add 113 113 33.9	5 min 30 sec	@	94	30 X	
Autoclaved ddH2O  Don't Dilute Prior To  RXN#2  Reagent  Buffer  dNTP's  MgCl2  TR3G13-F-NED	[] 10 2 50 20	[ ] Units X mM Each mM	Desired [ ] 1 0.2 1.5 0.4	Des'd [ ] Units X mM Each mM uM	1130 Amount to add 113 113 33.9 22.6	5 min 30 sec 1 min	@ @	94 60	30 X	
Autoclaved ddH2O  Don't Dilute Prior To  RXN#2  Reagent  Buffer dNTP's MgCi2  TR3G13-F-NED  TR3G13-R	[] 10 2 50 20	[ ] Units X mM Each	Desired [ ] 1 0.2 1.5 0.4 0.4	Des'd [ ] Units X mM Each mM uM uM	1130 Amount to add 113 113 33.9	5 min 30 sec 1 min 1 min	@ @ @ @	94 60	30 X	
Autoclaved ddH2O  Don't Dilute Prior To  RXN#2  Reagent  Buffer  dNTP's  MgCl2  TR3G13-F-NED  TR3G13-R  TR3G10-F-6FAM	[] 10 2 50 20 20	[ ] Units X mM Each mM	Desired [ ]	Des'd [ ] Units X mM Each mM uM uM uM	1130 Amount to add 113 113 33.9 22.6	5 min 30 sec 1 min 1 min 45 min	@ @ @ @	94 60 77 61	30 X	
Autoclaved ddH2O	[] 10 2 50 20 20 40 40	[ ] Units X mM Each mM uM uM uM	Desired [ ] 1 0.2 1.5 0.4 0.4 0.76 0.76	Des'd [ ] Units X mM Each mM uM uM uM uM	1130 Amount to add 113 33.9 22.6 22.6 21.47 21.47	5 min 30 sec 1 min 1 min 45 min	@ @ @ @	94 60 77 61	30 X	
Autoclaved ddH2O  Don't Dilute Prior To  RXN#2  Reagent  Buffer dNTP's MgCi2  TR3G13-F-NED  TR3G13-R  TR3G10-F-6FAM  TR3G10-R	10 2 50 20 20 40 40	[ ] Units X mM Each mM uM uM uM uM	Desired [ ]	Des'd [ ] Units X mM Each mM uM uM uM UM UM UM UM UM UM	1130  Amount to add  113  113  33.9  22.6  22.6  21.47  21.47  11.3	5 min 30 sec 1 min 1 min 45 min	@ @ @ @	94 60 77 61	30 X	
Autoclaved ddH2O  Don't Dilute Prior To  RXN#2  Ragent Buffer dNTP's MgCI2  TR3G13-F-NED  TR3G13-R  TR3G10-F-6FAM  TR3G10-R  Taq  DNA	10 2 50 20 20 40 40	[ ] Units X mM Each mM uM uM uM	Desired [ ]	Des'd [ ] Units X mM Each mM uM uM uM uM	1130 Amount to add 113 113 33.9 22.6 22.6 21.47 21.47 11.3 226	5 min 30 sec 1 min 1 min 45 min	@ @ @ @	94 60 77 61	30 X	
Autoclaved ddH2O  Don't Dilute Prior To  RXN#2  Reagent  Buffer  dNTP's  MgCl2  TR3G13-F-NED  TR3G13-R  TR3G10-F-6FAM  TR3G10-R	10 2 50 20 20 40 40	[ ] Units X mM Each mM uM uM uM uM	Desired [ ]	Des'd [ ] Units X mM Each mM uM uM uM UM UM UM UM UM UM	1130  Amount to add  113  113  33.9  22.6  22.6  21.47  21.47  11.3	5 min 30 sec 1 min 1 min 45 min	@ @ @ @	94 60 77 61	30 X	

## **IGF Scoring Criteria**

- Score the +A
- Allele bins are even
- Beware of pull-up from GATA028 in size range!!

