## **Reaction Conditions - SAM25**

RXN#1				Cocktail Volume (uL)	1130	Use PCF	Cycles P	ROPDNR	
Reagent	[]	[ ] Units	Desired [ ]	Des'd [ ] Units	Amount to add	5 min	@	94	
Buffer	10		1	X	113	30 sec	@	94	
dNTP's	2	mM Each	0.2	mM Each	113	1 min	@	54.5	35x
MgCl2	50	mM	1.5	mM	33.9	1 min	@	72	
SAM25-F-HEX		uM	1	uM	28.25	45 min	@	60	
SAM25-R		uM	1	uM	28.25				
RW31-F-HEX		uM		uM	70.625	Is a 10ul	reaction v	olume	
RW31-R	40	uM	2.5	uM	70.625				
EV1Pm-F-NED		uM	0.3	uM	8.475				
EV1Pm-R		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	o De-saltir	g							
	o De-saltin	g		Cocktail Volume (uL)	1130	Use PCF	Cvcles P	ROPDNR	
RXN#2	o De-saltin	g [ ] Units	Desired [ ]	Cocktail Volume (uL) Des'd [ ] Units	1130 Amount to add	Use PCF	Cycles P	ROPDNR 94	
RXN#2 Reagent	[]	[ ] Units					@		
RXN#2 Reagent Buffer	[]	[ ] Units	1	Des'd [ ] Units	Amount to add	5 min	@	94 94	30 X
RXN#2 Reagent Buffer BUTP's	[] 10 2	[] Units	1 0.2	Des'd [ ] Units	Amount to add	5 min 30 sec	@ @	94 94	30 X
RXN#2 Reagent Buffer JNTP's MgCl2	[] 10 2 50	[ ] Units X mM Each	1 0.2	Des'd [ ] Units X mM Each mM	Amount to add 113 113	5 min 30 sec 1 min	@	94 94 <b>54</b>	30 X
RXN#2 Reagent Buffer INTP's MgCl2 FR3A1-F-6FAM	[] 10 2 50 40	[ ] Units X mM Each mM	1 0.2 1.5	Des'd [ ] Units X mM Each mM uM	Amount to add 113 113 33.9	5 min 30 sec 1 min 1 min	@ @	94 94 <b>54</b> 72	30 X
RXN#2 Reagent Buffer JNTP's MgCl2 TR3A1-F-6FAM TR3A1-R	10 2 50 40	[ ] Units X mM Each mM uM	1 0.2 1.5 0.45 0.45	Des'd [ ] Units X mM Each mM uM	Amount to add 113 113 33.9 12.7125	5 min 30 sec 1 min 1 min 45 min	@ @	94 94 <b>54</b> 72 60	30 X
RXN#2 Reagent Buffer JNTP'S MgCl2 TR3A1-F-6FAM TR3A1-R TR2F3-F-NED	10 2 50 40 40 20	[ ] Units X mM Each mM uM	0.2 1.5 0.45 0.45	Des'd [ ] Units X mM Each mM uM uM	Amount to add 113 113 33.9 12.7125 12.7125	5 min 30 sec 1 min 1 min 45 min	@ @ @	94 94 <b>54</b> 72 60	30 X
RXN#2 Reagent Buffer dhTP's MgCi2 TR3A1-F-6FAM TR3A1-R TR2F3-F-NED TR2F3-F-NED	[] 10 2 50 40 40 20 20	[ ] Units X mM Each mM uM uM	0.2 1.5 0.45 0.45 0.3 0.3	Des'd [ ] Units X mM Each mM uM uM uM	Amount to add 113 113 33.9 12.7125 12.7125 16.95	5 min 30 sec 1 min 1 min 45 min	@ @ @	94 94 <b>54</b> 72 60	30 X
RXN#2 Reagent Buffer dNTP's MgCl2 TR3A1-F-6FAM TR3A1-R TR2F3-F-NED TR2F3-R	10 2 50 40 40 20 20 5	[ ] Units X mM Each mM uM uM uM	0.2 1.5 0.45 0.45 0.3 0.3 0.3	Des'd [ ] Units X mM Each mM uM uM uM UM UM UM UM UM UM	Amount to add 113 113 33.9 12.7125 12.7125 16.95 16.95	5 min 30 sec 1 min 1 min 45 min	@ @ @	94 94 <b>54</b> 72 60	30 X
RXN#2 Reagent Buffer dhTP's MgCl2 TR3A1-F-6FAM TR3A1-R TR2F3-F-NED TR2F3-R Taq DNA	10 2 50 40 40 20 20 5	[ ] Units X mM Each mM uM uM uM uM	0.2 1.5 0.45 0.45 0.3 0.3 0.3	Des'd [ ] Units X mM Each mM uM uM uM uM	Amount to add  113 113 33.9 12.7125 12.7125 16.95 16.95 11.3	5 min 30 sec 1 min 1 min 45 min	@ @ @	94 94 <b>54</b> 72 60	30 X
RXN#2 Reagent Buffer dNTP's MgCi2 TR3A1-F-6FAM TR3A1-R TR2F3-F-NED TR2F3-R Taq DNA Autoclaved ddH2O	10 2 50 40 40 20 20 5	[ ] Units X mM Each mM uM uM uM uM	1 0.2 1.5 0.45 0.45 0.3 0.3 0.05	Des'd [ ] Units X mM Each mM uM uM uM UM UM UM UM UM UM	Amount to add  113 113 33.9 12.7125 12.7125 16.95 16.95 11.3 226 573.475	5 min 30 sec 1 min 1 min 45 min	@ @ @	94 94 <b>54</b> 72 60	30 X
RXN#2 Reagent Buffer JINTP's MgCl2 TR3A1-F-6FAM FR3A1-F FR3A1-F FR2F3-F-NED FR2F3-R Taq DNA	10 2 50 40 40 20 20 5	[ ] Units X mM Each mM uM uM uM uM	1 0.2 1.5 0.45 0.45 0.3 0.3 0.05	Des'd [ ] Units X mM Each mM uM uM uM UM UM UM UM UM UM	Amount to add  113  113  33.9  12.7125  12.7125  16.95  11.3  226	5 min 30 sec 1 min 1 min 45 min	@ @ @	94 94 <b>54</b> 72 60	30 X

## **SAM25 Scoring Criteria**

- Score the allele, although the +A is still there
- Allele bins are even

