

Reaction Conditions - RW45

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
MgCl ₂	50	mM	1.5	mM	33.9
TV17-F-6FAM	20	uM	0.3	uM	16.95
TV17-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 ddH ₂ O			to 1130 uL		598.9
					1130
Dilute by 3 ul of PCR product with 15 ul of water					

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
MgCl ₂	50	mM	1.5	mM	33.9
RW417-F-6FAM	20	uM	0.4	uM	22.6
RW417-R	20	uM	0.4	uM	22.6
TV20-F-HEX	20	uM	0.07	uM	3.955
TV20-R	20	uM	0.07	uM	3.955
RW45-F-NED	20	uM	0.11	uM	6.215
RW45-R	20	uM	0.11	uM	6.215
RW212-F-HEX	20	uM	0.085	uM	4.8025
RW212-R	20	uM	0.085	uM	4.8025
Taq	5	U/uL	0.05	U/uL	11.3
DNA	5	ng/uL	10	ng/rxn (2 uL/rxn)	226
Autoclaved ddH ₂ O			to 1130 uL		557.655
					1130
Don't Dilute Prior To De-salting					

Use PCR Cycles	PROPDNR	
5 min	@	94
30 sec	@	94
1 min	@	49
1 min	@	72
45 min	@	60

Is a 10ul reaction volume

Use PCR Cycles	PROPDNR	
5 min	@	94
30 sec	@	94
1 min	@	58
1 min	@	72
45 min	@	60

Is a 10ul reaction volume

RW45 Scoring Criteria

- Score the +A
- Allele bins are even

