Genes-4U LightCycler Protocol for Parallel Testing of HFE 282 and 63/65

ONLY For use with Pack Inserts : Prospectus_HFE_6365_March_05 and Prospectus _HFE_282_September_03

You can use the ToolSets HFE 282 and HFE 63/65 in parallel. For this, program the LightCycler with the combined protocol as shown below. Set up your reactions as detailed in the above cited pack inserts. Run the LightCycler PCR program and analyse the melting curves with the corresponding melting programs using the parameter settings as shown in the "Typical Results" further down.

Denaturation	**Value**
Cycles	1
Analysis Mode	None
Temperature Targets	**Segment 1**
Target Temperature (°C)	95
Incubation time (s)	120
Temperature Transition Rate (°/s)	20.0
Acquisition Mode	None

Amplification	**Value**		
Cycles	55		
Analysis Mode	None		
Temperature Targets	**Segment 1**	**Segment 2**	**Segment 3**
Target Temperature (°C)	95	50	72
Incubation time (s)	5	10	15
Temperature Transition Rate (°/s)	20.0	20.0	20.0
Acquisition Mode	None	Single	None

Melting Curve Analysis HFE 282	**Value**		
Cycles	1		
Analysis Mode	Melting Curves		
Temperature Targets	**Segment 1**	**Segment 2**	**Segment 3**
Target Temperature (°C)	95	**40**	85
Incubation time (s)	20	60	0
Temperature Transition Rate (°/s)	20.0	20.0	**0.2**
Acquisition Mode	None	None	Continuous

Melting Curve Analysis HFE 63/65	**Value**		
Cycles	1		
Analysis Mode	Melting Curves		
Temperature Targets	**Segment 1**	**Segment 2**	**Segment 3**
Target Temperature (°C)	95	**53**	85
Incubation time (s)	20	60	0
Temperature Transition Rate (°/s)	20.0	20.0	**0.05**
Acquisition Mode	None	None	Continuous

Cooling	**Value**
Cycles	1
Analysis Mode	None
Temperature Targets	**Segment 1**
Target Temperature (°C)	40
Incubation time (s)	30
Temperature Transition Rate (°/s)	20.0
Acquisition Mode	None

LC Program Version and Fluorescence Display Mode

Developed with LC Program Version 3.5 (automatic gain control).
Display Mode : Use F2/F1 or preferably F2 with colour compensation.

Typical results

HFE 282

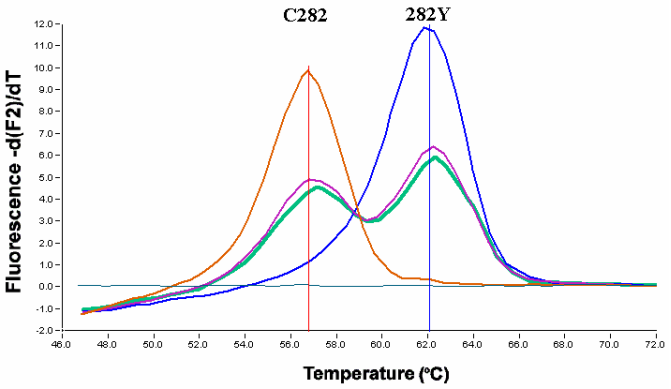

Figure 1 : Melting curve analysis of the three possible genotypes of the HFE sequence.

BLUE : **Homozygote for 282Y**, **RED** : **Homozygous for C282** (wildtype), **PINK** : Heterozygote;
GREEN : The artificial Heterozygote Control contained in the ToolSet, Control HFE 282-16.
Red Cursor : T_m = 56.77 ^{0}C, Blue Cursor : T_m = 62.05 ^{0}C
Conditions : Color compensation and Digital Filter enabled, Calculation Method : Polynomial

HFE 63/65

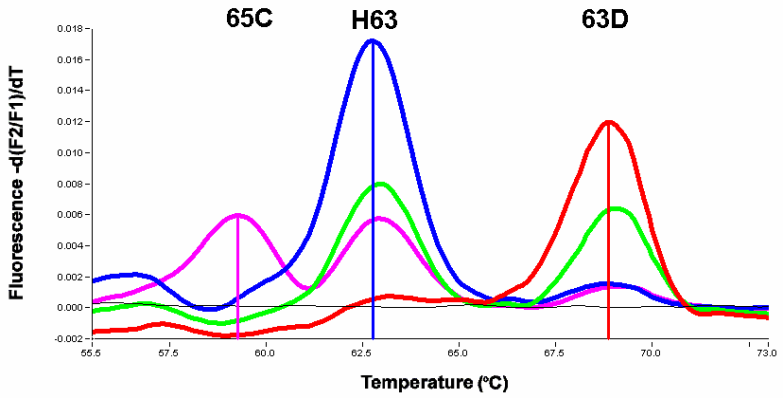

Figure 2 : Melting curve analysis of possible genotypes of the HFE sequence at AA's 63 and 65.

BLUE : Homozygote for H63. RED : Homozygote for 63D (Mutant). Black : No DNA Control.
GREEN: **Control HFE 6365 -16 DNA A = H63D** (Heterozygote for H63D); contained in the ToolSet
PINK: **Control HFE 6365 -16 DNA B = S65C** (Heterozygote for S65C) , contained in the ToolSet
Pink Cursor : T_m = 59.2 ^{0}C, Blue Cursor : T_m = 62.8 ^{0}C, Red Cursor : T_m = 68.9 ^{0}C
Conditions : LC program 3.5, No Color compensation, Digital Filter disabled, Degrees to average : 5.0.

Note ! Calculation Method : Polynomial with Background correction :
Set **Lower** background cursors to **55 / 77.5** ^{0}C, **Upper** background cursors to **67.5 / 85** ^{0}C.
Other calculation modes may display suboptimally.

Note : The values for the respective melting temperatures may vary for +/- 2.5 ^{0}C between different experiments.
The Delta T between the melting peaks for different genotypes may vary +/- 0.5 ^{0}C. The HFE C282Y and H63D+S65C ToolSets™ have been developed for and validated with the LightCycler™ and its original accessory materials and reagents. Performance of the ToolSets with other instruments, accessories and reagents have not been validated by Genes-4U.