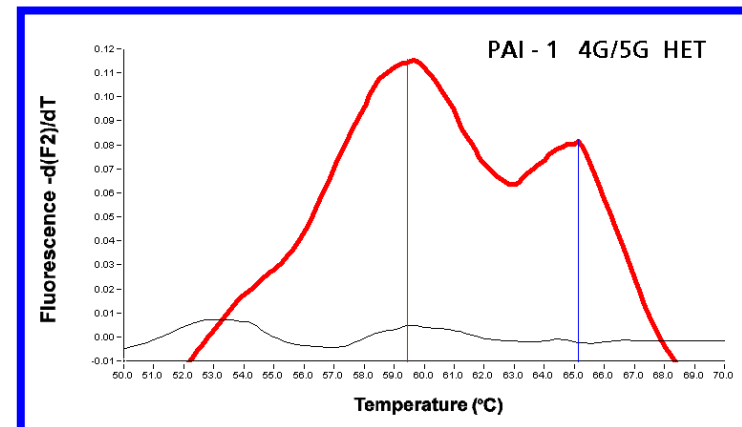
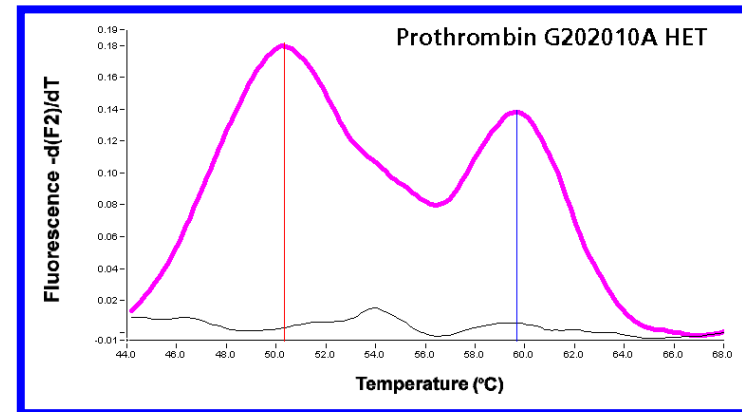
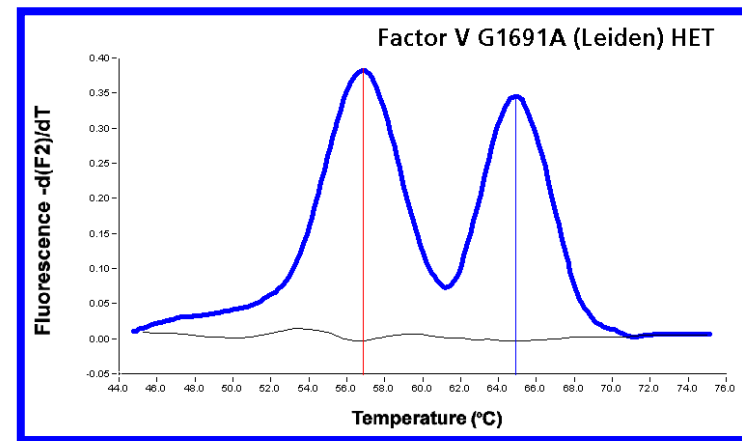
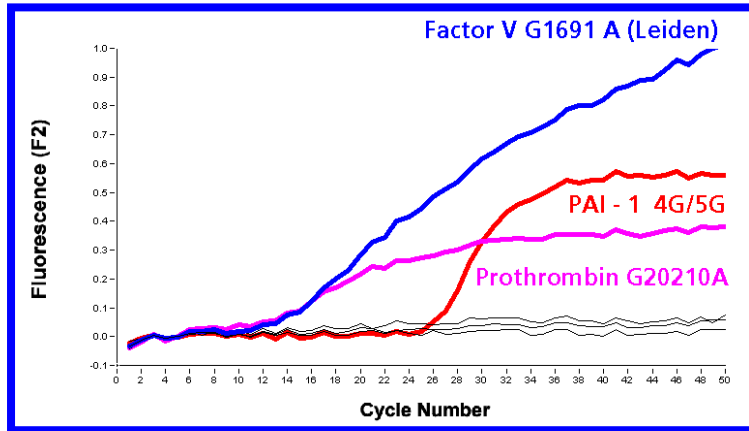


Simultaneous Genotyping of Factor V, Prothrombin and PAI-1



Factor V : Roche Diagnostics

Prothrombin : Roche Diagnostics

PAI-1 4G/5G : **Genes-4U**

Application Note : Simultaneous Genotyping of Factor V G1691A (Leiden), Prothrombin G20210A and Plasminogen-Activator-Inhibitor-1 (PAI-1) 4G/5G

The Factor V Leiden, Prothrombin G20210A and Plasminogen-Activator-Inhibitor-1 4G / 5G mutations each cause a pro-coagulant state and act in a synergistic manner, thus increasing the risk of thromboembolism and related disorders if more than one mutation is present. To genotype for these mutations in parallel, set up individual reactions as described in the respective Roche or Genes-4U package inserts and perform Lightcycler PCR simultaneously with the following ONE protocol :

Denaturation

Cycle Program Data	Value
Cycles	1
Analysis Mode	None
Temperature Targets	Segment 1
Target Temperature (°C)	95
Incubation time (s)	60
Temperature Transition Rate (%/s)	20
Acquisition Mode	None

Amplification

Cycle Program Data	Value		
Cycles	50		
Analysis Mode	None		
Temperature Targets	Segment 1	Segment 2	Segment 3
Target Temperature (°C)	95	57	72
Incubation time (s)	1	5	3
Temperature Transition Rate (%/s)	20	20	3
Acquisition Mode	None	Single	None

Melting Curve Analysis

Cycle Program Data	Value		
Cycles	1		
Analysis Mode	Melting Curves		
Temperature Targets	Segment 1	Segment 2	Segment 3
Target Temperature (°C)	95	40	80
Incubation time (s)	60	60	0
Temperature Transition Rate (%/s)	20	20	0.1
Acquisition Mode	None	None	Continuous

Cooling

Cycle Program Data	Value
Cycles	1
Analysis Mode	None
Temperature Targets	Segment 1
Target Temperature (°C)	40
Incubation time (s)	30
Temperature Transition Rate (%/s)	20
Acquisition Mode	None

Fluorescence display mode and Data analysis

Use LC program 3.3 with gains: F1=1; F2=15 and readout of F2 with color compensation, or F2/F1 without color compensation. Use automatic gain setting with LC program 3.5.

Perform melting curve data analysis as indicated in the individual original package inserts.