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DETECTION OF H1299R MUTATION IN FV GENE

AMPLI FV H1299R REAL TIME Cat 1.315RT

The heterogeneity of clinic phenotype and the variability of thrombotic events showed by patients with familiarity for thrombotic disease have led to the hypothesis that the predisposition to these type of disorders may be due to many genetic factors. Recently, a complex haplotype of Factor V (HR2), which includes 13 different polymorphisms, has been reported. Among them, 7 cause an amino acid substitution and a functional modification of the protein, leading to an excess of plasmatic isoform FV1 concentration, more thrombogenic.
It isn't clear if haplotype HR2 alone could be a factor of thrombotic risk. It is sure that the risk of clinical thrombotic events in subjects carriers of the F V Leiden mutation is increased.
The detection of the polymorphism involves performing a PCR with specific primers and a probe which anneals between primer sites).
The probe is is labeled with a fluorescent reporter dye bound to the 5' and quencher on the 3' end. Due to the 5' nuclease activity of Taq pol during extension, the cleavage of the probe causes an increase of the reporter dye signal and the fluorescent intensity is proportional to the amount of amplicon produced (real time quantitative PCR)
In the kit for the detection of H1299R polymorphism, the probe matching the wild type sequence (allele A) is labeled to the FAM dye reporter, whereas the probe matching the mutation sequence (allele G) is labeled to the VIC dye reporter.

Principle of method: A) extraction of genomic DNA B) amplification C) detection using real time PCR instrument
Applicability: Genomic DNA extracted and purified by whole blood samples
Number of reactions: 96.

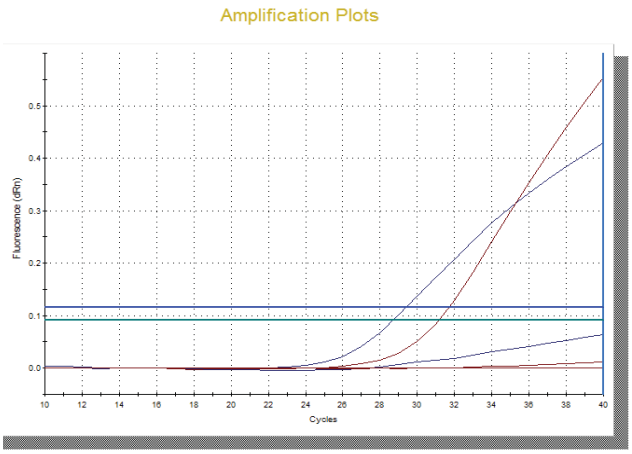
REAGENTS AND STORAGE

AMPLIFICATION	
PCR mix 2X	+4°C
H_2O sterile	-20°C
Primer-probe mix 20X	-20°C in the dark
WT Control	-20°C
Eterozygosis positive control	-20°C

Stability: over 18 months if correctly stored.

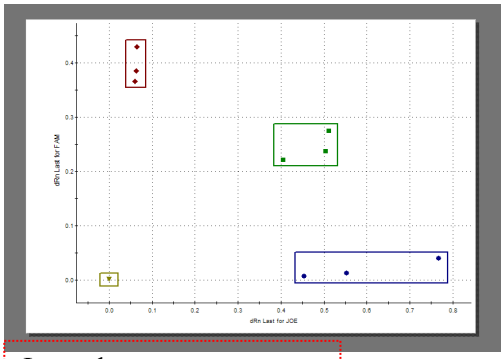
ANALYSIS OF RESULTS

After an AD post-read run, the software analyzes raw data using the AD specific program. Anywhere it is useful analyzing the amplification plots, in order to check the amplification reaction.



Amplification plot: eterozigousis sample, both probes indicate a product of PCR.
Red: probe labelled with VIC fluorescent reporter dye.
Blue: probe labelled with FAM fluorescent reporter dye.

Allelic discrimination FV H1299R



Legend:
Red: allele A
Green: A and G alleles
Blue: allele G

References:
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