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# A1298C polymorphism of the methylenetetrahydrofolate reductase (MTHFR) gene

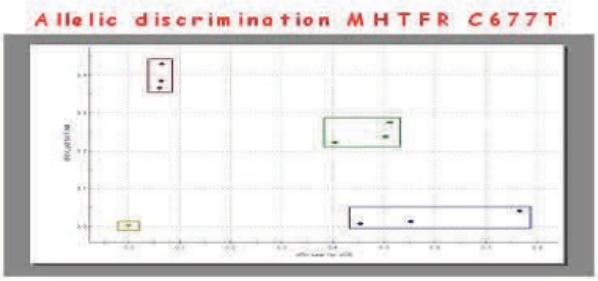
## AMPLI-MTHFR Cat. n. 1.301RT

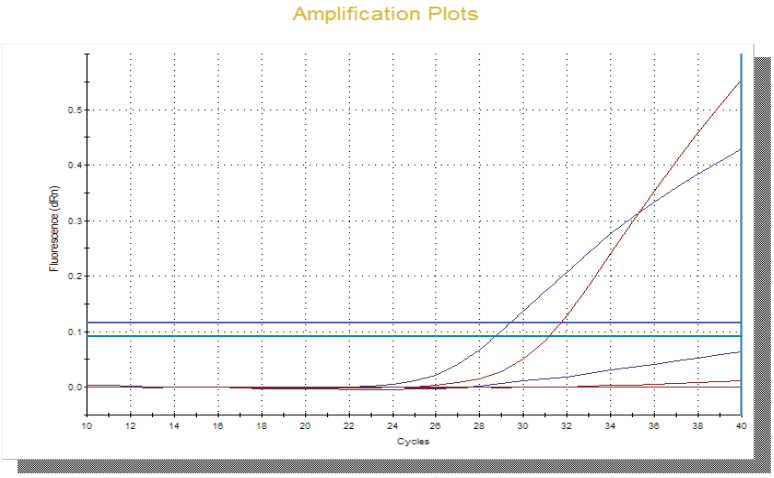
The increase of the level of homocysteine may depend on a metabolic block of the transforma- tion of homocysteine in cystathionine or on the unsuccessful methilation of homocysteine in methionine.The classic form of hyper-homocysteinemia is due to a deficiency of the cystathionine sintetase that catalyzes the production of cystathionine from homocysteine and serine. The enzyme MTHFR catalyses the reduction of 5,10-methylentetrahydrofolate in 5- metyltetrahydrofolate, the predominant form of circulating folate and donor of carbon in the process of re-methylation of thr homocysteine in methionine.A mutation A-G,that inserts a Valine instead of a Alanine, is associated with a reduced activity and an increased thermolability of this enzyme. Homozigote subjects for the mutation show a significative increase of the plasmatic level of circulating homocysteine, due to the unsuccessful conversion in methionine.The grave deficiency of 5,10-metylentetrahydrofolate Reduttase (MTHFR) is the most common defect linked to metabolic processes of folates which causes iper-homocysteinemia ,homocysteinemia, homocystinuria and low levels of methionine. The subjects that carry this pathology show during childhood or adolescence disorders of the motor development, mental disorders and other neurological abnormality. Fasting hyper-homocysteinemia is associated to an increased risk of vascular cerebral, peripheral and coronary diseases. As shown in fig 1, the detection of the polymorphism involves performing a PCR with specific primers and a probe which anneals between primer sites (1). The probe is labeled with a fluorescent reporter dye bound to the 5’ and quencher on the 3’ end. (2) . Due to the 5’ nuclease activity of Taq pol during extension, the cleavage of the probe causes an increase of the reporter dye signal (3), and the fluorescent intensity is proportional to the amount of amplicon produced.(4) (real time quantitative PCR). In the kit for the detection of A1298G polymorphism, the probe matching the wild type sequence (allele C) is labeled to the VIC dye reporter, whereas the probe matching the mutation sequence (alleleT) is labeled to the FAM dye reporter.

## 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

Legend: Red: Allele C Green: Alleles C and T Blu: Allele T



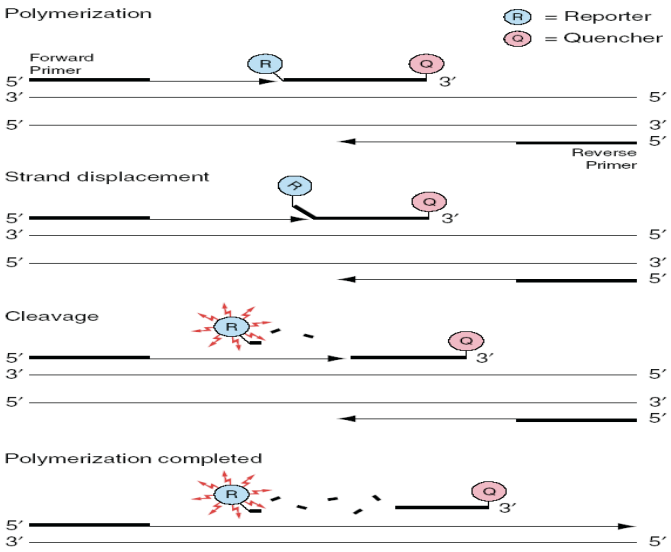




**Amplification plot:** eterozigosis sample, both probes indicate a product of PCR.

**Red:** probe labelled with VIC fluorescent reporter dye.

**Blue**: probe labelled with FAM fluorescent reporter dye.

## Real-Time Quantitative PCR (RQ-PCR




## References

*A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reduc- tase.* ***Nat Genet*** *1995 May;10(1):111-3. Frosst P et al.The methylenetetrahydrofolate reductase TT677 genotype is associated with venous thrombosis independent- ly of the coexistence of the FV Leiden and the prothrombin A20210 mutation.* ***Thromb Haemost*** *1998*

# Reagents and Storage

| AMPLIFICATION | |
|---|---|
| PCR mix 2X | +4°C |
| $H_2O$ sterile | -20°C |
| Primer-probe mix 20 X | -20°C in dark |
| Eterozygosis positive control | -20°C |
| WT Control (CC) | -20°C |
| Mutant control (TT) | -20°C |