

Haemochromatosis StripA^{ssay} A

INTENDED USE

The *ViennaLab* Haemochromatosis StripA^{ssay} A provides materials for the isolation of DNA from human whole blood, the *in vitro* amplification of HFE, TFR2 and FPN1 gene sequences, and the subsequent detection of eighteen mutations by reverse-hybridization.

INTRODUCTION

Hereditary haemochromatosis (HH) is a very common autosomal recessive disorder of iron metabolism. Among individuals of Northern European descent the carrier frequency is estimated 1 in 10, resulting in up to 1 in 200 homozygous subjects being predisposed to develop the disease. HH is characterized by progressive accumulation of iron in various organs (liver, heart, pancreas), ultimately leading to liver cirrhosis, diabetes, arthritis, cardiomyopathies and premature death.

A number of point mutations within a novel MHC class I-like gene (HFE) have been identified and related to HH. Homozygosity for C282Y is observed in the majority of Caucasian HH patients, but other HFE mutations have been identified in HH families. More recently, mutations in the genes encoding transferrin receptor-2 (TFR2) and ferroportin (FPN1) have been found in individuals with non-HFE haemochromatosis.

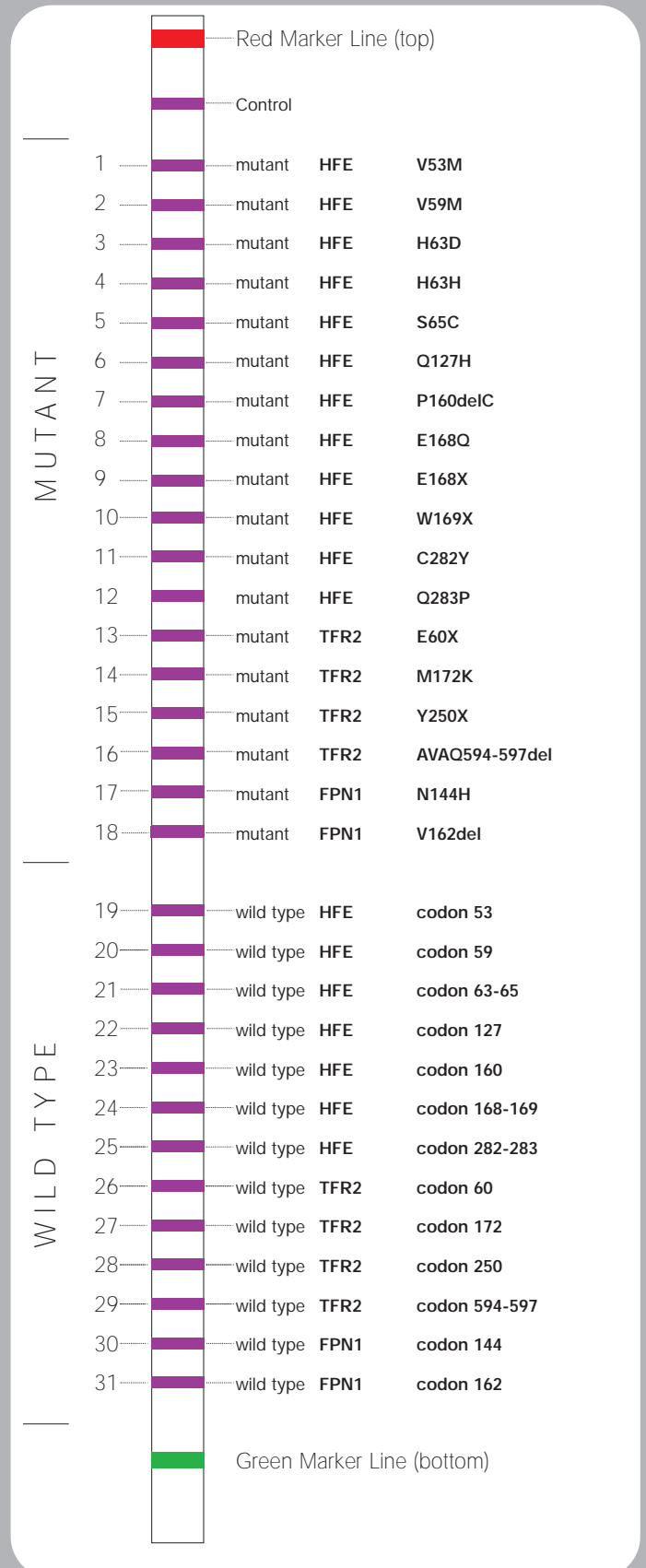
Molecular genetic testing for HH-associated mutations is considered valuable for carrier identification, as well as for pre-symptomatic diagnosis of the disease. With early detection and simple and very effective treatment by therapeutic bleeding (phlebotomy) in order to remove the iron overload, irreversible organ damage can be completely prevented and survival of patients is virtually normal.

PRINCIPLES OF THE ASSAY

The Haemochromatosis StripA^{ssay} A is based on the reverse-hybridization principle, and includes three successive steps: DNA is isolated from anticoagulated blood by a rapid and convenient procedure. Then, HFE, TFR2 and FPN1 gene sequences are simultaneously *in vitro* amplified and biotin-labelled in a single ("multiplex") amplification reaction. Finally, the amplification products are selectively hybridized to a test strip, which contains oligonucleotide probes (wild type- and mutant-specific) immobilized as parallel lines. Bound biotinylated sequences are detected using streptavidin-alkaline phosphatase and color substrates.

The assay covers 12 mutations in the HFE gene (V53M, V59M, H63D, H63H, S65C, Q127H, P160delC, E168Q, E168X, W169X, C282Y, Q283P), four mutations in the TFR2 gene (E60X, M172K, Y250X, AVAQ594-597del) and 2 mutations in the FPN1 gene (N144H, V162del).

References: Feder, J.N., Gnirke, A., Thomas, W., et al. (1996), *Nature Genetics* 13, 399-408. Oberkanins, C., Moritz, A., De Villiers, J.N.P., et al. (2000), *Genet. Testing* 4, 121-124. Camaschella C, Roetto A, Cali A, et al. (2000), *Nature Genetics* 25, 14-15.



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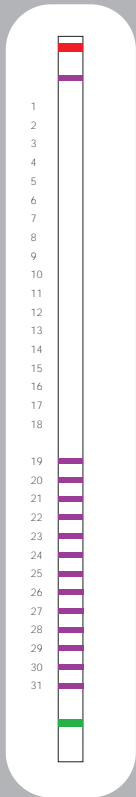
TEST RESULTS:

For each polymorphic position, one of three possible staining patterns may be obtained:

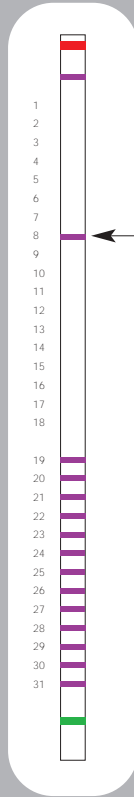
1. wild type probe only: *normal genotype*
2. wild type and mutant probe: *heterozygous genotype*
(«carrier» individual)
3. mutant probe only: *homozygous mutant genotype*
(«affected» individual)

EXAMPLES:

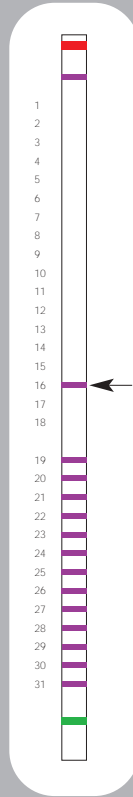
(A.) normal



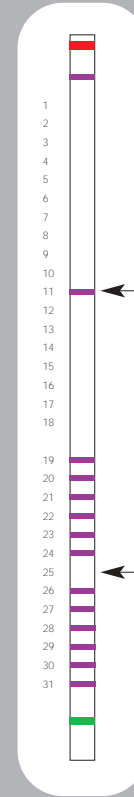
(B.) E168Q heterozygous



(C.) TFR2: AVAQ594-597del heterozygous



(D.) HFE: C282Y homozygous



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