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DETECTION OF C282Y AND H63D MUTATION IN THE FERROPORTIN GENE (SLC11A3)

AMPLI-set- EMO C282Y e H63D Cat. n. 1.320

Haemochromatosis is a common recessive disorder characterised by progressive iron overload. Unfortunately it is little considered and it is often accidentally discovered in periodic check or due to the development of its complications. In Italy and in some European regions there are 2-5 affected individuals in 1000 people and 9-15 carriers in 100. Haemochromatosis is regarded as the most common genetic disorder in the west hemisphere. In 1996 the HFE gene has been detected and two mutations, C282Y and H63D, have been described. Most affected patients with haemochromatosis are homozygous for the C282Y mutation (80-100%), whereas a few are compound heterozygous for the C282Y and H63D .Homozygote for the H63D mutation isn't clearly linked to haemochromatosis. The kit allows the simultaneous detection of C282D and H63D mutations using the Polymerase. Chain reaction PCR and restriction analysis using the PmI restriction enzyme.

Principle of method: A) extraction of genomic DNA B) amplification C)enzymatic digestion D)detection on agarose gel

Applicability: On extracted and purified genomic DNA from whole blood samples.

Numbers of Tests: 45.

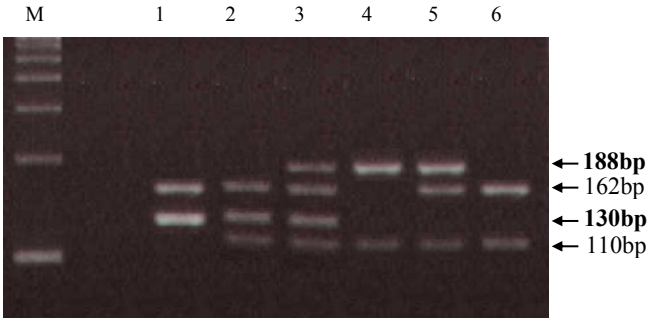
REAGENTS AND STORAGE

AMPLIFICATION and DIGESTION	
PCR mix	-20°C
Water DNase-RNase free	-20°C
Taq Polymerase (5U/µl)	-20°C
Pml I Enzyme (10U/µl)	-20°C
Digestion BUFFER 10X	-20°C
Positive control	-20°C

Stability: over 12 months if correctly stored.

ANALYSIS OF RESULTS

The amplification yield is of 188 bp for the C282Y mutation and 130 bp for the mutation H63D. When the two mutations are present Pml I enzyme isn't able to recognize the specific restriction site. This site is recognized when there isn't the mutation. (wild-type).



M) Marker 100bp ladder
1) wild-type C282Y, homozygote mutation H63D
2) wild-type C282Y, heterozigote H63D
3) heterozygote C282Y ,heterozigote H63D (compound heterozigote)
4) homozigote C282Y, wild-type H63D
5) heterozigote C282Y, wild-type H63D
6) wild-type C282Y e H63D

References:
N. Engl J med 1988; 318: 1355-62; *Br J Haematol* 1990; 74:525-30; *Am J Med* 1995; 98:464-8; *Hepatology* 1997; 25:1439-46; *NZ Med J* 1997; 110:429-32; *Acta Med Scand* 1984; 215:105-12; *Med J Aust* 1996; 164:348-51; *Nat Genet* 1996; 13:339-408; *Cell* 1998; 93:111-23; *Proc Natl Acad Sci* USA 1998; 95:1472-7; *J med Genet* 1997: 34: 275-8; *Gut* 1998: 43: 830-6; *Blood* 1997; 90:4235-6; *Atherosclerosis* 1991; 89:137-41.