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Identifi Cation of Fviii Gene Mutations in Patients Withhemophilia a Using New Combinatorial Sequencing by Hybridization

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Standard methods of mutation detection are time consuming in Hemophilia A (HA) rendering their application unavailable in some analysis such as prenatal diagnosis. To evaluate the feasibility of combinatorial sequencing-by-hybridization (cSBH) as an alternative and reliable tool for mutation detection in FVIII gene. We have applied a new method of cSBH that uses two different colors for detection of multiple point mutations in the FVIII gene. The 26 exons encompassing the HA gene were analyzed in 7 newly diagnosed Italian patients and in 19 previously characterized individuals with FVIII deÞ ciency. Data show that, when solution-phase TAMRA and QUASAR labeled 5-mer oligonucleotide sets mixed with unlabeled target PCR templates are co-hybridized in the presence of DNA ligase to universal 6-mer oligonucleotide probe-based arrays, a number of mutations can be successfully detected. The technique was reliable also in identifying a mutant FVIII allele in an obligate heterozygote. A novel missense mutation (Leu1843Thr) in exon 16 and three novel neutral polymorphisms are presented with an updated protocol for 2-color cSBH.cSBH is a reliable tool for mutation detection in FVIII gene and may represent a complementary method for the genetic screening of HA patients.