Optimized strategy for rapid cytochrome P450 2D6 genotyping by real-time long PCR.

BACKGROUND: Because of genetic polymorphisms, cytochrome P450 2D6 (Cyp2D6) activity in humans varies widely and alters the metabolism of commonly used drugs such as antidepressants, neuroleptics, and cardioactive agents. Severe adverse effects or resistance to therapy may result. METHODS: We performed long PCR on the LightCycler(TM) and used the product as a template for a previously validated multiplex PCR that examines the *3, *4, *6, *7, and *8 alleles of Cyp2D6. We used real-time PCR to identify the *5 null allele and duplication of Cyp2D6 with detection by either hybridization probes or SYBR Green((R)). The *2 -1584 C/G polymorphism and the *35 allele were identified by PCR with detection by hybridization probes. Products of all PCRs were visualized with gel electrophoresis using a 0.7-1.5% agarose gel and ethidium bromide. Samples containing the *35 allele were analyzed in parallel by digestion with NlaIII, MslII, and BstXI and Smal. We analyzed samples from volunteers and patients (105 samples for deletion and duplication and 116 samples for preamplification). Of those samples, 59 were from depressive inpatients taking part in a trial not yet published.

RESULTS: Identical genotyping results for both real-time and conventional PCR were obtained and verified by gel electrophoresis. Use of long-PCR methods on the LightCycler enabled comprehensive analysis of all relevant polymorphisms of the
Cyp2D6 gene in 1 working day with a hands-on time of approximately 3-4 h. CONCLUSIONS: This is the first description of a successful long-PCR application on the LightCycler and the fastest technique for amplification and specific detection of a PCR product of comparable length. The method appears suitable for large clinical and epidemiologic studies.