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Identification of plasma hemostasis mutations by enzyme extension of the allele specific primer with dual bioluminescent detection (PED-Biolume)

E E Bashmakova, V V Krasitskaya and **L A Frank**
Russian Academy of Science, Russia

We developed the method to identify single nucleotide polymorphisms (SNP) based on primer extension reaction (PEXT) with the subsequent bioluminescent solid-phase microassay named PED-Biolume. Two variants of the Ca^{2+}-regulated photoprotein obelin distinctly different in bioluminescence were successfully applied for simultaneous detection of two analytes in a single well. The flash-type signals were triggered by single injection of Ca^{2+} and were discriminated by the spectral and time resolutions. The technique was developed in a high-throughput format, and applied to simultaneous detection of two gene allelic variants at single nucleotide polymorphisms genotyping. The study was performed with SNP genotyping of genetic polymorphisms of clotting factors genes FV (1691 G>A; rs6025) or Leiden mutation, prothrombin (F2 20210 G>A; rs1799963), proconvertin (F710976 G>A; rs 6046) as well as methylenetetrahydrofolate reductase gene (MTHFR, 677 C>T; rs1801133) as the examples. The 213 samples, kindly provided by Hematology Research Center (Krasnoyarsk Branch of RAMS) were studied. Genotypes of all samples were previously detected by conventional method (RT-PCR technique). For comparison, the commercially available kit of "SNP-express-RT" (Litech, Russia) was used. The analyzed results on all the above mentioned polymorphisms showed no divergence, and completely coincided with those received by RT-PCR method. The PED-Biolume and RT-PCR methods demonstrate equal capability of detecting mutant allele and similar economic parameters at employment. At that, the developed technique was shown to be simple, effective, and inexpensive.

Biography

E E Bashmakova is a postgraduate student of the Institute of Biophysics of SB RAS (Russia). She graduated from the Siberian Federal University in 2013 and got the diploma in biochemistry (equivalent of MS Degree). Her current research is mainly focused on the use of Ca2+-regulated photoproteins to detect clinically significant SNPs of human DNA.

jeyn_a@bk.ru