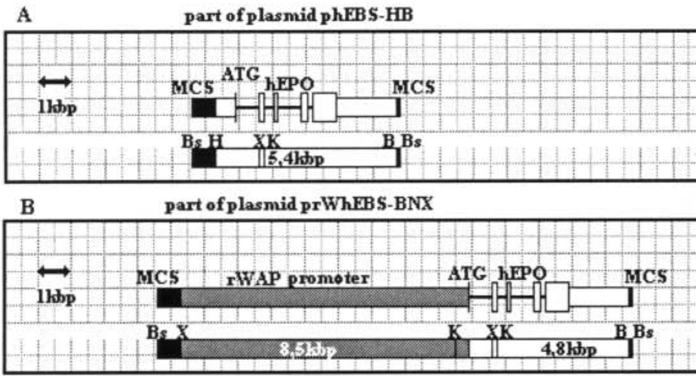


*Fig. 1.* Structure of the *hEPO* gene digested with *Bss*HII from plasmid phEBS-HB (A) and the *rWAP/hEPO* chimaeric gene derived from prWhEBS-BNX after digestion with *Bss*HII (B). The dark box represents the upstream fragment (8.5-kb) of the rabbit *WAP* gene. The white big boxes indicate *hEPO* exons and the thin line indicates introns of *hEPO*. The main restriction sites are represented as follows: B, *Bam*HI; Bs, *Bss*HII; H, *Hind*III; K, *Kpn*I; X, *Xba*I. Position of the translation initiation is indicated (ATG).

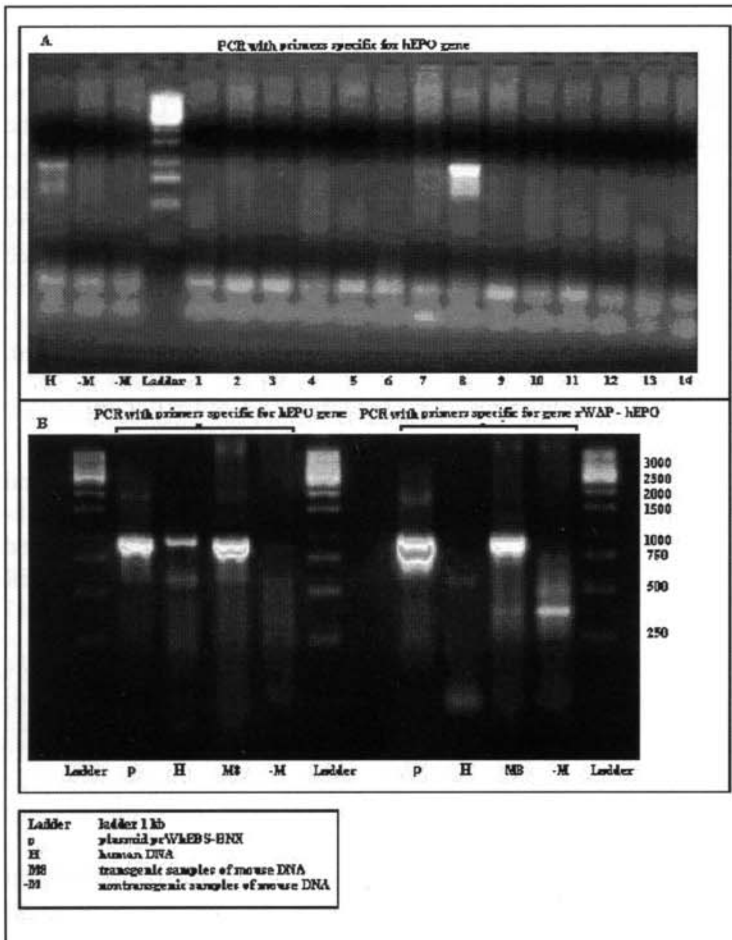


*Fig. 2.* Samples of the DNA isolated from the mouse tails in PCR reaction with primers specific for human *EPO* (Fig. 2A) and verifying of positive sample no. 8 with primers specific for the sequence of plasmid vector prWhEBS-BNX (Fig. 2B).

difference between Caucasians and Blacks in amino acid 40. In our case, this change matches the recently presented *EPO-EST* sequence cloned from a black woman (Jacobs et al., 1985). As we cloned our gene from the Stratagene genomic library constructed from the human material of a black woman, our point mutation seems to be the result of a polymorphic difference in the *EPO* allele rather then a PCR error. For the purpose of DNA microinjections, the *rWAP/hEPO* transgene (Fig. 1B) was released from the vector by digestion with the *Bss*HII restriction enzyme.

## *Generation of transgenic mice*

As donors of ova, F1 hybrid mice (CBA/CaOla x C57B6 ) were used. Four–six weeks old females were superovulated with intraperitoneal injection of 5 IU of pregnant mare serum gonadotropin (Sergon, Bioveta, Czech Republic); followed 46 h latter i.p. injection of 5 IU of human chorionic gonadotropin (Praedin, Léčiva a.s., Czech Republic). Microinjection of DNA into the pronucleus of ova was done according to the procedure formerly published (Hogan et al., 1994). Two linear constructs, carrying the *hEPO* gene (digested from phEBS-HB with enzymes *Hind*III and *Bam*HI) or hybrid *rWAP-hEPO* gene (digested from prWhEBS-BNX with enzyme *Bss*HII) were microinjected into the male pronucleus. The released DNA inserts were separated on agarose gel and clean DNA was diluted in injection buffer. Enlargement of pronuclei during microinjection indicated a successful transfer of approximately 500 copies of DNA molecules into ova. Two-cell-stage embryos, developed from injected ova, were transferred into the oviducts of BALB/c/Ola pseudo-pregnant mouse recipients.

## *Screening of transgenic mice by PCR and Southern blot*

Two sets of primers were used in PCR to identify positive transgenic mice. The first set of primers, specific for *hEPO* gene amplification, were called hE+1 (forward primer 5'ATG GGG GTG CAC GGT GAG TAC TCG CGG 3') and hE+998 (reverse primer 5'CAA GCT GCA GTG TTC AGC ACA GCC 3'). The second primer set specific for chimaeric *rWAP-hEPO* included rW-26 (forward primer 5'CCA CCA CCA GCC TAC CAG CGG CCG CCA) and hE+998 (the same as above). Positive DNA