Fig. 1. Structure of the hEPO gene digested with BspHII from plasmid phEBS-HB (A) and the rWAP/hEPO chimaeric gene derived from prWhEBS-BNX after digestion with BspHII (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, BamHI; H, HindIII; K, KpnI; X, XbaI. Position of the translation initiation is indicated (ATG).

Generation of transgenic mice

As donors of ova, F1 hybrid mice (CBA/CaOla x C57Bl6) were used. Four–six weeks old females were superovulated with intraperitoneal injection of 5 IU of pregnant mare serum gonadotropin (Sergon, Biovolta, Czech Republic); followed 46 h latter i.p. injection of 5 IU of human chorionic gonadotropin (Praedín, 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 HindIII and BamHI) or hybrid rWAP-hEPO gene (digested from prWhEBS-BNX with enzyme BspHII) 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 CCG 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