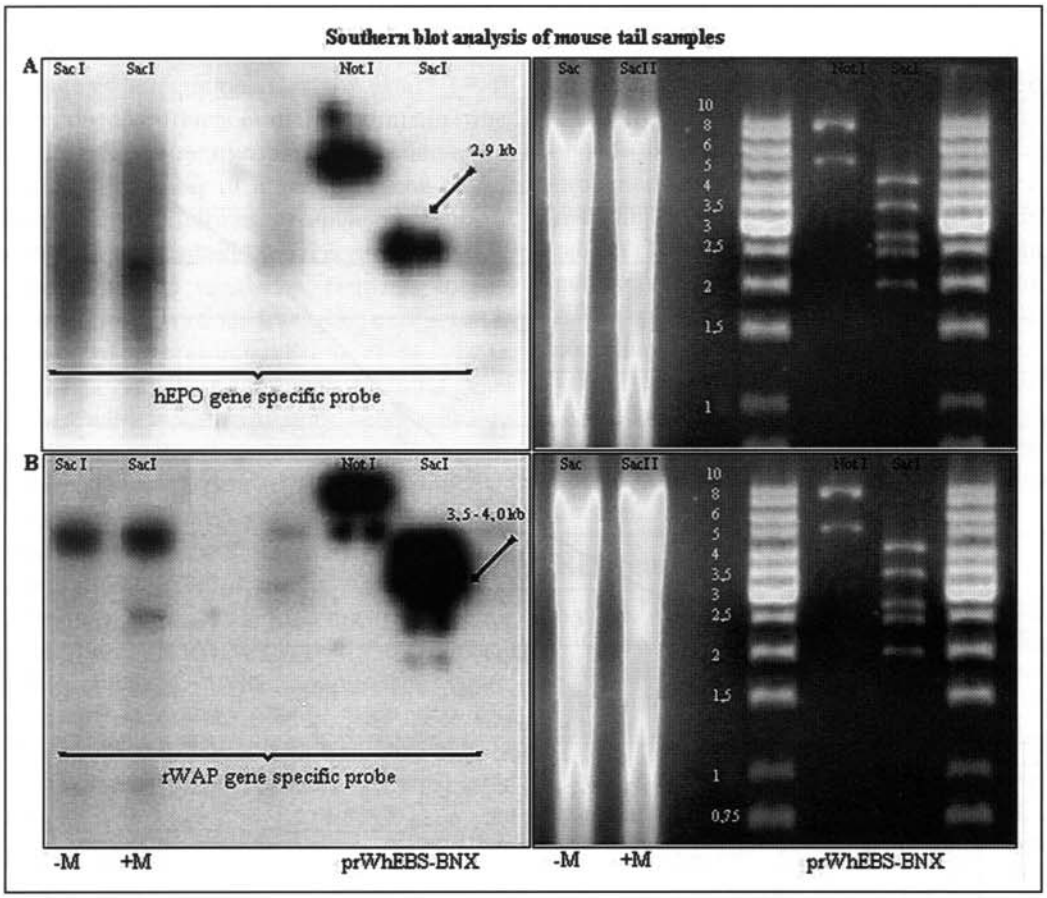

Fig. 4. Southern blot analysis of DNA from transgenic mice. DNA was isolated from tails of PCR-positive mice, digested with *Sac*I and subjected to Southern blot analysis with the fragment ATG-*Kpn*I of the *hEPO* gene (A) and promoter region of the *rWAP* gene (B), both as probes digested from vector prWhEBS-BNX. DNA from positive mouse no. 8 (lane M+) and DNA from non-transgenic mice were analysed. The last two lines correspond to DNA of vector prWhEBS-BNX digested with *Sac*I and *Not*I enzymes.

pups were born and the presence of the *rWAP/hEPO* foreign gene was detected in one female.

Introduction of linear hybrid *rWAP/hEPO* gene into the mouse genome was assayed by PCR with the primers specific for the *hEPO* gene (Fig. 2A) or with the primers specific for the chimaeric gene *rWAP/hEPO* (Fig. 2B).

In addition, the PCR-positive female was also tested by Southern hybridization using two different probes specific for the *hEPO* gene and the *rWAP* gene. First we tested two different fragments of the *hEPO* gene for specificity in hybridization experiments (Fig. 3). We found one specific part of the *hEPO* gene between the ATG start and *Kpn*I site. This fragment (hE1ex/3ex) is specific for the region placed between the first and the third exons. This part of the *hEPO* gene shows 50–60 % homology in the primary nucleotide structure with the mouse *EPO* gene.

The second probe suitable for identification of transgenic animals carrying the hybrid gene *rWAP/hEPO* is the fragment of the promoter of *rWAP* gene adjacent to an upstream-ATG regulatory sequence, called *Kpn*I-ATG probe.

Integration of the hybrid *rWAP/hEPO* gene into the genome of a transgenic mouse and transgenic rabbit was verified in other hybridization experiments (unpublished result) using a probe carrying the full-length coding region of the *hEPO* gene (Fig. 4).

Recombinant human erythropoietin is produced in the milk of transgenic animals

The actual concentration of hEPO samples was obtained from the calibration curve developed for the hEPO assay. Samples of the milk (diluted five times) and blood (diluted ten times) collected from a transgenic mouse carrying the hybrid gene *rWAP/hEPO* showed activities of 1.068 mIU/ml and 0.723 mIU/ml, respectively (Table 1).

We found differences in optical density of samples obtained from the transgenic or non-transgenic animal. It shows a weak activity of the hybrid transgene *rWAP/hEPO* in cells of the mammary gland (Fig. 5).