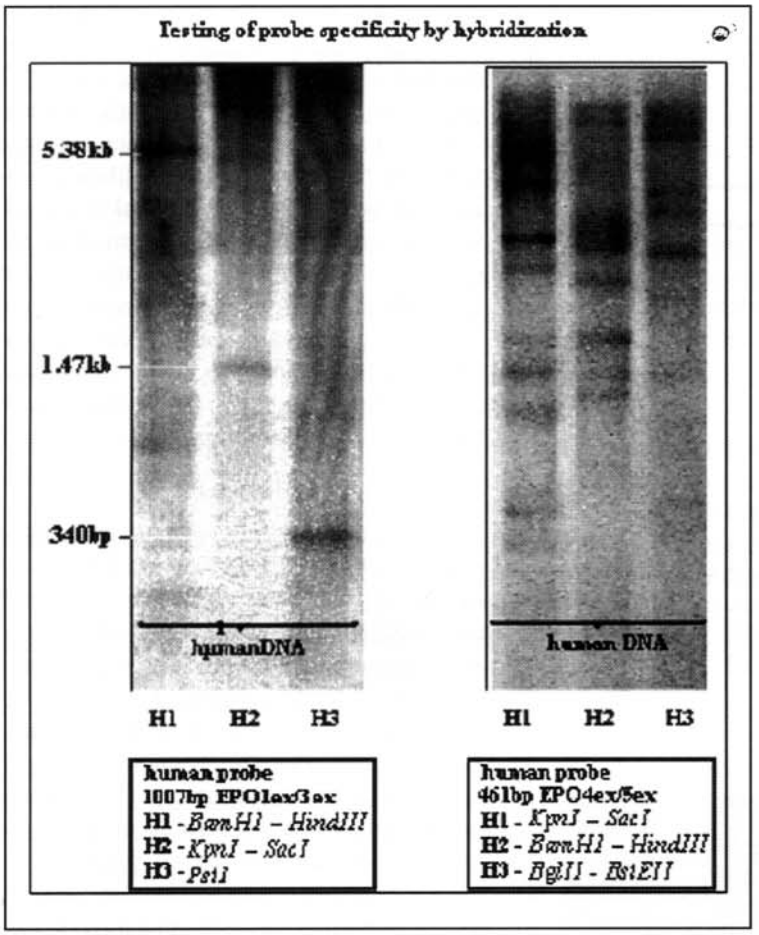

Fig. 3. In order to choose a suitable probe for identification of transgenic animals carrying the hybrid gene *rWAP/hEPO* we tested diferent parts of the *hEPO* gene in hybridization experiments for their specifity. The region encompassing the first two introns and second exon shows 55% homology with the mouse *EPO* gene and is able to distinguish the human gene for hEPO.

samples were digested with the *Sac*I restriction enzyme, fractionated on 0.9% agarose gels and transferred to nylon Hybon N+ membrane. The filters were hybridized at 42°C with radioactively labelled probes according to Sambrook et al. (1989). The filters were finally washed at 60°C for 20 min to the stringency of 0.2% SSC/0.5% (w/v) SDS and exposed to X-ray films for one week.

Milk collection and ELISA of hEPO

Milk samples from transgenic mice carrying the hybrid gene *rWAP/hEPO* or from control mice were obtained on the 14th day of lactation. Samples were isolated from the stomachs of sucking pups. Milk clots were weighed and resuspended in 4 volumes of sample buffer (100 mM NaCl, 10 mM Na2HPO4, 0.02% Tween 20, 0.1% lactose, pH 7.0). Fat and precipitate were removed by centrifugation (10 000 × *g*) for 20 min. Blood was diluted 10 times with the same buffer and centrifuged (10 000 × *g*) for 20 min. These samples (100 µl) were assayed for hEPO by commercial ELISA (R&D Systems Inc., Minneapolis, MN).

Results

Generation and identification of transgenic mice carrying the hEPO gene

A total of 1124 ova were microinjected with several hundred phEBS-BH fragments (Fig. 1A) and transferred into 146 suitable recipients. In sum, 133 mice were born. Introduction of the *hEPO* gene into the mouse genome was assayed in PCR with the primers specific for the *hEPO* gene. The presence of the foreign gene was verified by PCR in 121 mice and 11 transgenic mice were detected. These results correspond well with the results of Rodriguez et al. (1995), where a similar construct was injected.

Generation and identification of transgenic mice carrying the hybrid rWAP/hEPO gene

A total of 577 ova were microinjected with several hundred copies of the *rWAP/hEPO* chimaeric gene (12-kb DNA) released from prWhEBS-BNX by restriction enzyme digestion. A total of 445 surviving 2-cell embryos were transferred into 51 recipients. In sum, 47

Table 1. Determination of the hEPO activity in the milk and serum of a transgenic mouse female carrying the hybrid gene rWAP/hEPO and of a normal control mouse. The quantikine IVD Epo ELISA uses a monoclonal antibody and polyclonal antibody conjugate in sandwich ELISA. The minimum detectable dose is typically less than 0.6 mIU/ml.

Activity of hEPO in the milk and serum of a transgenic mouse carrying the hybrid gene *rWAP/hEPO*						
Technique of measurement	**Sandwich ELISA**					
	Levels of hEPO mIU.ml^{-1}				**Absorbance/450 nm**	
Detection limit	0.6 mIU.ml^{-1}					
Dilution factor of the sample	10x	1x	5x	1x	10x	5x
Sample	serum		milk		serum	milk
Transgenic mouse female	0.723	7.23*	1.068	5.34**	0.053	0.046
Normal control mouse female	0.0	–	0.0	–	0.037	0.029

*theoretical level of hEPO in the serum multiplied by dilution factor 10x
**theoretical level of hEPO in the milk multiplied by dilution factor 5x