TRANSGENIC CATTLE FROM TRANSGENE-ANALYZED AND SEXED EMBRYOS

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Introduction

Although the production of transgenic livestock still is its infancy and our expectations regarding their commercial application may be too optimistic, several examples of transgenic farm animals indicate that the approach is viable. In comparison with transgenic rodents, the generation of transgenic livestock, especially transgenic cattle, is extremely labor-intensive and expensive. This is because of the rather inefficient production of bovine embryos *in vitro* and poor transgene integration rate resulting in the need of a large number of recipient animals for embryo transfer. Thus embryo screening for transgene integration prior to their transfer into recipient animals is of utmost importance. We have generated a transgenic dairy cattle from embryos produced *in vitro* and subjected to transgene integration analysis and sexing before their transfer into recipient animals.

Experimental

Bovine oocytes were isolated from slaughterhouse-derived ovaries, maturated and fertilized *in vitro*. Fertilized oocytes were microinjected with a transgene construct containing human erythropoietin encoding genomic fragment operationally fused with bovine alphaS1-casein promoter. The gene construct was propagated in dam+ *E.coli* strain TG-1. After microinjection the embryos were grown in culture for 7 to 8 days after which they were biopsied. The biopsies, representing about one third of the embryo, were subjected to transgene integration analysis and sexing. Embryonic DNA was released with proteinase K and the samples were digested with DpnI-Bal31 mixture before PCR amplification of the transgene, bovine alphaS1-casein and bovine Y chromosome-specific fragments in the same reaction. The digestion removed up to 50 fg of unintegrated or contaminating dam-methylated transgene copies and reduced the number of transgene-positive embryos as compared with direct PCR analysis. The analysis revealed a transgenesis rate of 19 % which is almost identical to the transgenesis rate in mice with the same gene construct.

Results

Pregnancy was achieved with a heifer receiving three transgene-positive female embryos. The presence of the transgene was confirmed after two months with amniotic fluid PCR analysis. All the analyzed samples of the subsequently born calf were transgene-positive in PCR and Southern blot analysis. Apparently, a single copy of the transgene was integrated. The transgenic calf was healthy and developed normally with no signs of increased number of erythrocytes.

Discussion

As random transfer of microinjected bovine embryos requires a large number of hormonally synchronized recipient animals owing to the low transgene integration rate, a reliable screening of embryos for the integration of the transgene prior to the embryo transfers is of utmost importance. A direct PCR analysis of the embryos gives a large number (up to 70 % of the embryos) of false-positive and is thus of limited value. Our transgene analysis is based to the fact that the dam-methylated (methylated adenine at the sequence GATC) transgene is efficiently cleaved with DpnI (cleaving the same sequence providing that adenine is methylated). However, if the transgene integrates and subsequently replicates the adenine methylation is lost as eukaryotic cells do not possess a maintenance methylation system for adenine. Hence an integrated transgene becomes DpnI-insensitive upon replication.

The inclusion of Bal31 exonuclease in the assay system appears to improve the results providing that the digestion conditions have been properly determined for each individual gene construct. Our primer for the subsequent PCR were designed in such a way that altogether 6 DpnI cleavage sites were located within the amplified fragment. The absolute reliability of our embryo screen for integrated transgene is on present experience still impossible to judge However, any enrichment of transgene-positive embryos prior to the transfer will reduce the number of recipient animals and hence decrease the costs and labor involved.