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Efficiency of human lactoferrin transgenic donor cell preparation for SCNT

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Abstract

The combination of somatic cell nuclear transfer (SCNT) and transgenic technology leads to the production of transgenic cloned animals, wherein the preparation of competent transgenic donor cells is the pivotal upstream step. The purpose of this study was to establish an efficient procedure to prepare human lactoferrin (hLTF) transgenic donor cells for SCNT. Thus, two cell culture systems were employed: caprine mammary epithelial cells (for evaluation of the hTLF transgenic expression in vitro), and fetal-derived fibroblast cells (for identification of competent transgenic donor cells). Induced by hormonal signals, recombinant hLTF was detected in the supernatant of transfected mammary epithelial cells by Western blot. Reliable hLTF transgenic fibroblast cell clones were identified by screening with multiple PCR amplification, EGFP fluorescence, and chromosomal counting (32.5 ± 2.3%). This study may provide an effective upstream system to prepare SCNT donor cells for the production of human recombinant pharmaceuticals from the milk of transgenic animals.

Keywords: β-Casein gene; Donor cells; EGFP; Human lactoferrin; Multiple PCR amplification

1. Introduction

Lactoferrin (LTF) is an 80-kDa iron-binding glycoprotein of the transferrin family, which consists of two highly homologous lobes, designated the N and C lobes [1–3]. Human lactoferrin (hLTF), speculated to have a crucial role in innate immune defense, has an extensive range of biological activities [4,5]. Numerous applications of hLTF in clinical treatments have resulted in a tremendous demand. However, production of hLTF by purification from human milk has potential safety and supply limitations. Therefore, it may be more practical and cost-effective to produce recombinant hLTF from the mammary gland of transgenic animals, which could overcome many problems associated with microbial bioreactors (e.g. lack of post-translational modifications, improper folding, and high purification costs) or animal cell bioreactors (high capital costs, expensive culture media, and low yield) [6].

Somatic cell nuclear transfer (SCNT) has provided an alternative, efficient pathway for the production of transgenic animals [7,8]. Foreign DNA carried by a

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specific vector can be introduced into somatic cells in vitro and if a selectable marker is included, then cells that incorporate the vector can be selected by adding the appropriate antibiotic. Antibiotic-resistant cells are then identified by Southern blotting, fluorescence in situ hybridization (FISH), or other molecular techniques, and subsequently used as donor cells for SCNT [8–13]. Since the success rate of SCNT is low (<2%) [14], it is important to evaluate tissue-specific expression of the transgenic constructs and to prepare competent transgenic donor cells prior to SCNT. Otherwise, if one transgenic construct is not expressed correctly, it will result in wasted surrogates, labor, and increased costs of for producing transgenic animals. However, few papers described efficient procedures to estimate tissue-specific expression of the transgene in vitro and to identify transgenic donor cell clones for SCNT. Moreover, these methods cannot exclude the possibility that the transgene is integrated in a transcriptionally silent region of chromatin and might be silent in transgenic animals, due to various epigenetic modifications [8–10,14].

The purpose of this study was to determine an effective procedure to prepare hTLF transgenic cells for SCNT, which consists of evaluating the expression of transgenic constructs in mammary epithelial cells in vitro and identifying competent transgenic fibroblast cell clones. In addition to lactoferrin, this procedure could be prospectively applied for production of other human recombinant pharmaceuticals in transgenic animals by SCNT.

2. Materials and methods

2.1. Construction of plasmids

All standard recombinant DNA protocols were followed as described [15,16].

Genomic DNA was extracted from the blood of a Saanen milk goat by using a TIANamp Genomic DNA Kit (TIANGEN Inc., PR China) The promoter region (GBC4.5, 4.5 kb) and 6.5-kb 5′-flanking sequence (GBC6.5, including GBC4.5, exon 1, intron 1 and part of exon 2) of the caprine β-casein gene were amplified from genomic DNA by using LA Taq DNA Polymerase (TaKaRa, Japan). Primers were designed from the caprine β-casein gene in GenBank (AF409096); all primers used in this study are listed in Table 1. The GBC6.5 and GBC4.5 were cloned into pMD18-T vector (TaKaRa) and named pGBC6.5 and pGBC4.5, respec-

Table 1
Primers used in this study

Primer	Sequence	Length (bp) of products
6S	5′-CTTTCACATTTATTATCTGATTACAAGCAGGA-3′	6532 (GBC6.5)
6A	5′-AAGGATGAGGACCTTCAAGGCTCTC-3′	
6BSS	5′-TGC*TCCGGA***TAA**ATTTATTATCTGATTACAAGCAGGA-3′	6546 (GBC6.5 with enzyme recognition sites)
6BSA	5′-TCG*GTCGAC*AAGGATGAGGACCTTCAAG-3′	
4S	5′-TTTCACATTTATTATCTGATTACAAGCAGG-3′	4463 (GBC4.5)
4A	5′-GGATAATGATCTGATTTAGTGGCTGTTT-3′	
4BSS	5′-TGC*TCCGGA***TAA**CACATTTATTATCTGATTACAAGCA-3′	4478 (GBC4.5 with enzyme recognition sites)
4BSA	5′-CGC*GTCGAC***TAA**TGATCTGATTTAGTGGCTGT	
6.5S	5′-TTGCTTCCCTCATTTGCCCTTC-3′	4460[a]
6.5A	5′-TCTGATCTCCTAACCACCGCCAC-3′	
4.5S	5′-CTCAAAGACCCACTGAATACTAAAG-3′	1717[b]
4.5A	5′-ATCTCCTAACCACCGCCAC-3′	
hS	5′-GCC*GTCGAC*CAGACCGCAGACATGAAACT-3′	2275 (hLTF cDNA with enzyme recognition sites)
hA	5′-TGC*GGATCC*AATCCCCACCTTCAGCAG-3′	
HS	5′-CAGACCGCAGACATGAAACT-3′	2257 (hLTF cDNA)
HA	5′-AATCCCCACCTTCAGCAG-3′	
ES	5′-GGTTTAGTGAACCGTCAGATCC-3′	847 (EGFP)
EA	5′-ATCAGTTATCTAGATCCGGTGGAT-3′	
NS	5′-GATCGTTTCGCATGATTGAAC-3′	811 (Neo^R)
NA	5′-CCCGCTCAGAAGAACTCGT-3′	

[a] The fragment spanning hLTF cDNA and GBC6.5.
[b] The fragment spanning hLTF cDNA and GBC4.5.