Small Ruminant Research 87 (2009) 17–26

Contents lists available at ScienceDirect

Small Ruminant Research

journal homepage: www.elsevier.com/locate/smallrumres

Establishment and characterization of a fibroblast cell line derived from Jining Black Grey goat for genetic conservation

X.C. Li[b,1], H. Yue[a,b,1], C.Y. Li[a], X.H. He[b], Q.J. Zhao[b], Y.H. Ma[b], W.J. Guan[b,*], J.Z. Ma[a,**]

[a] Northeast Forestry University, Harbin, Heilongjiang, 50040, PR China
[b] Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, 100193, PR China

ARTICLE INFO

Article history:
Received 18 February 2009
Received in revised form 6 September 2009
Accepted 22 September 2009
Available online 29 October 2009

Keywords:
Jining Black Grey goat
Fibroblast cell bank
Characteristics
Genetic conservation

ABSTRACT

An ear marginal fibroblast cell bank was established from the Jining Black Grey (JBG) goat using attachment culture and freezing biotechniques. This bank included 32 ear samples (15 males and 17 females) and has stocks of 168 cryogenically preserved vials, each vial contained 4.0×10^6 cells per milliliter. The cells of the bank that were checked for the quality and the biological characteristics showed a typical fibroblast morphology when they cultured in vitro. The growth curve consisted of a growth curve consisting of a latent phase, logarithmic growth phase and stationary phase, cell population doubling time (PDT) of 48 h. The chromosome analysis showed that the frequency of cells having the diploid number of chromosomes (60) was $98.65 \pm 2.89\%$, and no microbe contamination (bacteria, epiphyte, virus or mycoplasma) was detected. In addition, lactate dehydrogenase (LDH) and malate dehydrogenase (MDH) zymography indicated that this cell bank was free of cross-contamination. At 24, 48 and 72 h after transfection, the expression efficiency of pEGFP-C1, pEGFP-N3, pEYFP-N1, pECFP-N1, pECFP-mito and pDsRed1-N1 were between 11.8% and 56.3%. The fluorescence could be observed well-distributed in cytoplasm and nucleus except for some cryptomere vesicles at 24 h after transfection. These newly established cell lines meet all the quality control standards established by the American Type Culture Collection. We have employed a new method for conserving the genetic resources of an important and endangered animal breed. The fibroblast bank that we have established from the JBG goat also provides an invaluable material resource for future studies that will utilize molecular and cell biology applications.

© 2009 Elsevier B.V. All rights reserved.

1. Introduction

The diversity of livestock and poultry genetic resources is an important part of biodiversity, and is the basis for human society to keep living and achieve sustainable development. If these genetic resources have not been preserved in any way before extinction, not only the genetic resources will lost evermore, but also the research on biological mechanisms and on cell cloning will not be completed. Therefore, there is a very urgent need to commence conservation of endangered species (Weijun, 2002). At present, preservation of individual animals, semen, embryos, genomic libraries and cDNA libraries are all practical options. In addition, modern cloning techniques have made somatic cells an attractive resource for conserving animal genetic materials (Changxin, 1999). The establishment of fibroblast banks especially for endangered species has been proposed as a practical approach for this purpose; not only does it preserve precious genetic materials, but also provides an excellent resource for biological research.

* Corresponding author. Tel.: +86 10 6281 5992; fax: +86 10 6281 3463.
** Corresponding author. Tel.: +86 451 8219 1025;
fax: +86 451 8219 0624.
E-mail addresses: wjguan86@iascaas.net.cn (W.J. Guan), jianzhangma@163.com (J.Z. Ma).
[1] These two authors contributed equally to the work.

0921-4488/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.smallrumres.2009.09.028

The Jining Black Grey (JBG) goat inhabits the Heze and Jining areas of Shandong province, China. It is noted for the attractive wavy patterns of its kid-pelt, which is the traditional commodity in international markets. It is characterized by early sexual maturity, high reproductive rates, the ability to breed throughout the year and to give birth twice a year or three kiddings in 2 years. The JBG also has some production of cashmere. The production from the males will range from 50 to 150 g with cashmere fiber being 18–30% of the total fleece. Production in the females is lower, ranging from 25 to 50 g and cashmere comprising 16–20% of the fleece. The fiber diameter in both sexes averages 13.0 μm. These show strong fecundity and are of significant commercial and economic value (Yao, 2004; Jin, 1997). Furthermore, it was among the 138 national protected domestic animals listed by the Chinese government in 2006 (http://www.agri.gov.cn/BLGG/t20060609_626418.htm).

To preserve this valuable genetic resource, establishing fibroblast banks has been proposed as a practical approach; not only does it preserve precious genetic material, but it also provides an excellent resource for biological research.

2. Materials and methods

2.1. Materials

Except where otherwise indicated, all chemicals were obtained from Sigma Chemical Co. (St. Louis, MO).

2.2. Cell isolation and culture

2.2.1. Tissue culture

Ear tissue samples (about 1 cm^2) were isolated from 32 JBG goats (17 males and 15 females) and cultured using a primary explant technique (Freshney et al., 2000). Under sterile conditions, the samples were washed 2–3-times using phosphate buffered saline (PBS) containing penicillin and streptomycin, then the samples were cut into small pieces (1 mm^3). These pieces were cultured in DMEM containing 10% fetal bovine serum, 100 IU/mL penicillin, 100 μg/mL streptomycin and 3.0 μg/mL amphotericin B for 7 days in 95% air/5% CO_2 until near confluence. Fibroblast cultures that reached 85% confluence were digested with 0.25% trypsin and subcultured at an approximately 1:3 ratio.

2.2.2. Cryopreservation and reseeding

Cells were supplemented with fresh medium 24 h prior to freezing to make sure the nutrition was sufficiently absorbed by the cells. The monoplast cell suspension was acquired by digesting cells in 0.25% Trypsin. The suspension was centrifuged at 1000 rpm for 8 min and supernatant was abandoned, and collect the cells to make sure cell density approaches 4×10^6/mL. The cells were preserved in 10% dimethyl sulfoxide (DMSO), 30% FBS and 60% DMEM at a density of 4.0×10^6/mL, and then subpackaged in freezing tubes labelled with breeding names, sex and numbers. The tubes were placed at 4 °C for 20–30 min to enable the DMSO to permeate efficiently, and then placed in liquid nitrogen for long-term storage (Ren et al., 2002). For reseeding, the tubes were warmed at 42 °C to thaw and the cells suspension placed in DMEM and centrifuged at 1000 rpm for 10 min to remove the DMSO. The cells were then resuspended in fresh DMEM and seeded into petri dishes, and cultured under 5% CO_2 at 37 °C. Medium needs to be changed after 24 h (Freshney, 1994).

2.3. Cell viability

Cell viability was determined using trypan blue staining as described previously (Xue, 2001; Weingartl et al., 2002). The number of dead cells was determined from a field of 1000 cells.

2.4. Growth curve analysis

Cells of the eighth passage were seeded in 24-well plates at a density of approximately 1.5×10^5 cells/well and cultured for 9 days counted every day (three wells each time). The average cell counts at each time point were then plotted against time and the PDT was determined based on this curve (Weingartl et al., 2002; Costa et al., 2005; Kim et al., 2005).

2.5. Microbial analysis

2.5.1. Detection of bacteria and fungi

The cells were cultured in DMEM containing 10% fetal bovine serum without antibiotics and tested for the presence of microbes 3 days after subculture. The explanted fibroblasts were cultured and analyzed 3 days after subculture according to the method of Doyle et al. (1990).

2.5.2. Mycoplasma detection

Cells were cultured in medium free of antibiotics for at least 1-week and then fixed and stained with Hoechst dye 33258 according to the American Type Culture Collection protocol for fluorescence staining of DNA Masover and Becker (1998) and Freshney's method (2000). Results of DNA staining were confirmed by ELISA using the ELISA Mycoplasma Detection kit (Roche, Lewes, East Sussex, UK.) This kit identifies the four most common mycoplasma species: *M. arginini*, *M. hyorhinis*, *A. laidlawii*, and *M. orale*.

2.5.3. Virus detection

Routine examination for cytopathogenic effects using phase-contrast microscopy was performed using Hay's hemadsorption protocol (Hay, 1992).

2.6. Karyotype analysis

Metaphase spreads were prepared from cells at the exponential phase of growth following treatment with 0.1 μg/mL colcemid (Gibco/BRL). The cells were treated with a hypotonic KCl/HEPES/EDTA solution and harvested according to standard cytogenetic procedures. Slides of fixed cells were trypsin-Giemsa banded to identify individual metaphase chromosomes. Representative chromosome sets were photographed and analyzed. Diploid percentage was determined by counting 100 cells. Karyotypes were prepared following the protocol described in the Reading Conference report (Ford et al., 1980).

These parameters were calculated using the formulas:

$$\begin{aligned}\text{arm ratio} \\ &= \text{long arm length}(q)\ vs\ \text{short arm length}(p)\ \text{centromere exp onent} \\ &= \text{short arm length}\ vs\ \text{chromosomal length}\ vs\ (\text{total autosome lengths} \\ &\quad + \text{X-chromosomes})\end{aligned}$$

2.7. Isoenzyme analysis

Enzyme protein polymorphism, evidenced by the existence of isoenzymes, occurs among species and sometimes among races, as well as among tissues within an organism (O'Brien et al., 1977). Isoenzymes can be separated chromatographically or electrophoretically, revealing distribution patterns characteristic of a species or tissue. Biochemical analysis of isoenzyme polymorphism is currently considered to be the standard method for quality control for cell line identification and detection of interspecies contamination, and is routinely used by the leading biological resource centers around the world (i.e., American Type Culture Collection, European Collection of Cell Cultures) (Parodi et al., 2002.) However, this test requires dedicated equipment and expensive reagents, as well as a high level of technical expertise. We have used a modified apparatus and conditions for polyacrylamide gel electrophoresis, and successfully determined the mobility of the isoenzymes MDH and LDH isolated from cultured JBG goat fibroblasts.

The cells were collected by digesting in 0.25% Trypsin, cleansed in PBS 3-times, and then centrifuged with supernatant abandoned. The cells were resuspended in protein extract which is made by Triton × 100 to make sure cell density approaches 5×10^7/mL, and then centrifuged at 1000 rpm for 2 min. The supernatant was analyzed by the polyacrylamide gel electrophoresis.

Download English Version:

https://daneshyari.com/en/article/2457672

Download Persian Version:

https://daneshyari.com/article/2457672

Daneshyari.com