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

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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.

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.

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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:

arm ratio

= long arm length(q) *vs* short arm length(p) centromere exponent

= short arm length *vs* chromosomal length *vs* (total autosome lengths + X-chromosomes)

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.

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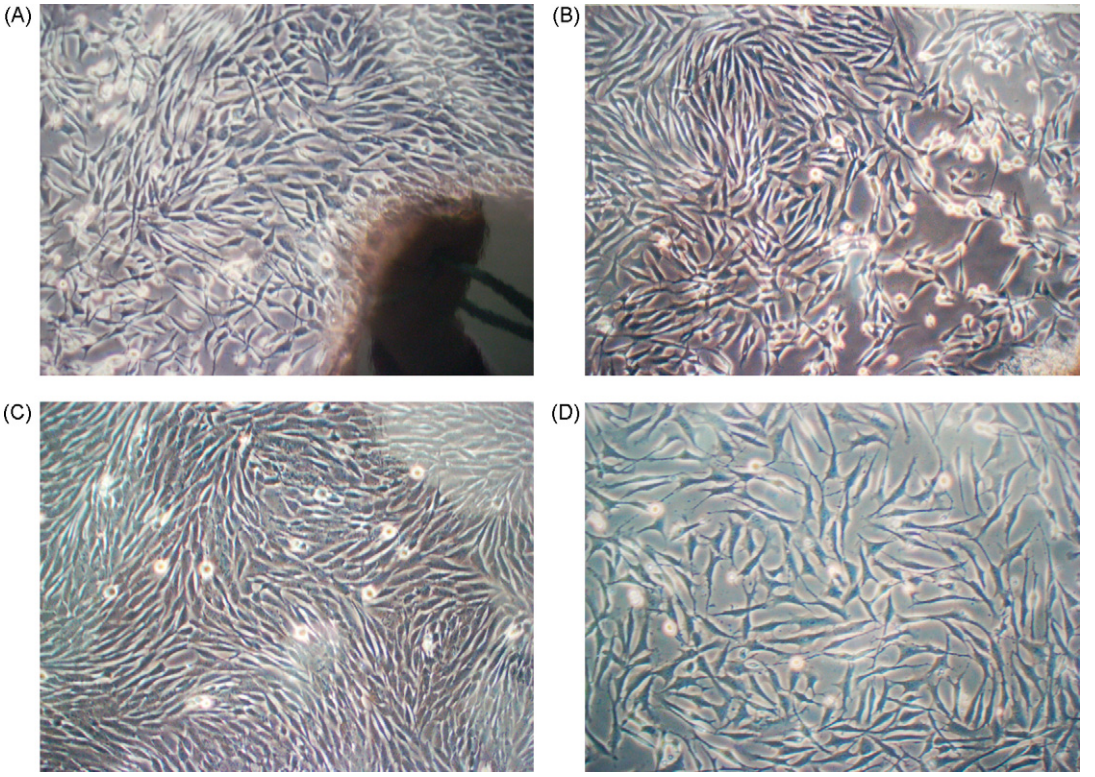

Fig. 1. Morphology of JBG goat fibroblasts cultured in vitro: (A) 5–12 days after explanting; (B) near confluence; (c) JBG goat fibroblasts before cryopreservation, and (d) 24 h after recovery from cryostorage.

The electrophoretic mobilities of lactate dehydrogenase (LDH) and malate dehydrogenase (MDH) were determined using the polyacrylamide gel electrophoresis protocol provided by Marvin and John (1977), with following modifications: the vertical slab polyacrylamide gel electrophoresis apparatus was used and the electrophoretic buffer was changed into Tris–glycin (pH 8.7). The gel buffer was prepared into discontinuous system using two different concentrations of Tris–citric acid buffer, 0.078 mol/L (pH 8.9) and 0.017 mol/L (pH 6.8), respectively. Electrophoretic mobility was defined by numbers and intensity of enzyme bands, as well as the distance of band migrating from the point of origin (Freshney, 2000). Mobility was expressed as the ratio of the distance migrated by the isoenzyme band to the distance migrated by the indicator dye.

2.8. Expression of fluorescent protein gene in JBG goat fibroblastic cell

To obtain the highest transfection efficiency and low cytotoxicity, optimized transfection conditions by varying cell density as well as plasmid DNA (BD Biosciences Clontech product) and Lipofectamine 2000 (Invitrogen) concentrations were discovered, according to lipofectamine media methods of Escriou et al. (2001) and Tsuchiya et al. (2002). The cultured cells were observed 24, 48, 72 h, 96 h, 1-week, 2 weeks and 1-month after six fluorescent protein genes transfect under excitation wavelength of 405, 488 and 543 nm, separately. Each experiment group picked 10 visual fields to take pictures, and statistics all the cells and the positive cells of each field to count the transfection efficiency by confocal microscopy.

3. Results

3.1. Cell morphology

At between 7 and 14 days after the tissue explants had adhered to the culture plates, fibroblastic cells were observed to grow from the margins of these pieces. The cells showed typical fibrous and fusiform morphologies with centered oval shaped nuclei (Fig. 1A). Cells then continued to proliferate and were subcultured when they reached 80–90% confluence. Fibroblasts grew rapidly and gradually replaced the epithelial cells in subcultures. Cells were well spread on the culture surface, forming characteristic multipolar or bipolar shapes (Fig. 1B).

3.2. Cell viability

Cells were harvested and frozen after 3–4 passages in culture. The viability (expressed as mean ± SD) of the cultures before freezing was 96.54 ± 3.22% (Fig. 1C) and 92.36 ± 2.77% after thawing (Fig. 1D), this difference is not significant ($P > 0.05$). These results showed that the cells were healthy under these culture conditions and that freezing had little effect on the viability of the cells.

3.3. Growth curve analysis

The growth curve of JBG goat ear marginal tissue fibroblasts had an obvious “S” shape (Fig. 2) and the population doubling time (PDT) was approximately 48 h. There was a lag time or latency phase of about 24 h after seeding, corresponding to the adaptation and recovery of the cells from protease damage; after that the cells proliferated rapidly and entered exponential phase. As the cell density increased, proliferation was reduced by contact inhibition and the cells began to enter the plateau phase after the 8th day.

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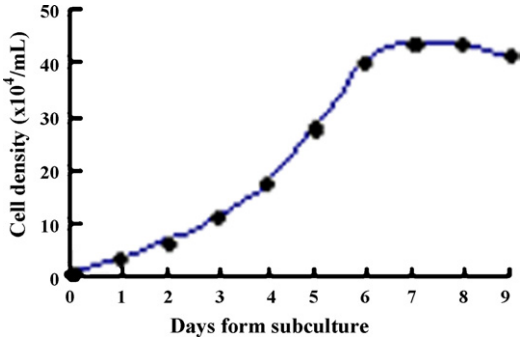

Fig. 2. Growth curve of JBG goat fibroblasts.

3.4. Microbial analysis

3.4.1. Detection of bacteria and fungi

Fungi propagation can be detected by the medium turbidity; the accumulation of acor metabolin made the culture medium flavo-green. Colony or hypha showing silkiness or trees could be easily observed under inverted microscope as well as by naked eyes. The culture medium was clear at all times and no abnormal changes could be observed under the microscope. The results indicated that our JBG goat fibroblasts were free of bacterial contamination. In addition, we did not observe any thallus aggregation indicating that the cultures were free of fungi (Fig. 3A) or in medium collected from cell cultures while there was obvious growth of microorganisms in the positive controls (Fig. 3B).

3.4.2. Mycoplasma detection

It was very difficult to detect mycoplasma contamination at early phase. Later cells collapsed a lot, cell membrane fragments were suspended in the medium, and the cell edge became vague and out of shape, cells were stained with the Hoechst 33258 fluorochrome and tested for mass punctiform and filiform blue fluorescence among the cell nucleoli, indicative of mycoplasma contamination (Barile and Rottem, 1993). Our observations indicated smooth fluorescent nucleoli, and a clear background (Fig. 3A), In positive control, DNA in nucleus showed blue fluorescence while mycoplasma DNA in the cell surface was fluorescein stained, presenting fluorescent grana and dot (Fig. 3C and D).

3.4.3. Virus detection

We performed a hemadsorption test for the presence of viruses which was negative. In addition, no cytopathogenic effects were evident in the cultures.

3.5. Karyotype analysis

In JBG goat the diploid number of chromosomes is 60, consisting of 58 autosomes and two sex chromosomes, XY or XX. The chromosomal properties of relative length, centromere index, and kinetochore type were shown in

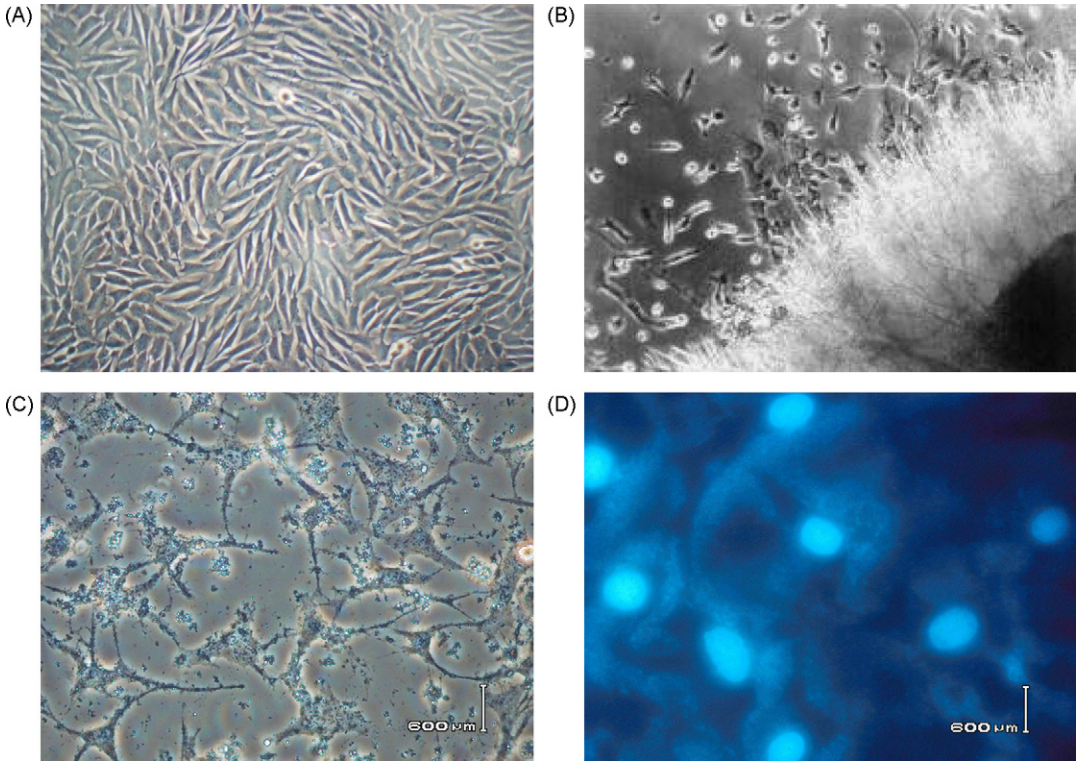

Fig. 3. Test for presence of mycoplasma: (A) fibroblasts without contamination (40×); (B) fibroblasts contaminated by fungi; (C) fibroblasts contaminated by mycoplasma; (D) positive mycoplasma contamination for the fibroblasts stained with Hoechst 33258.

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Table 1
Chromosome parameters of JBG goat fibroblasts (♀).

Chromosome number	Relative length (%)	Kinetochore type	Chromosome number	Relative length (%)	Kinetichore type
1	5.12 ± 0.42	T	17	3.01 ± 0.17	T
2	5.01 ± 0.18	T	18	2.98 ± 0.12	T
3	4.80 ± 0.49	T	19	2.88 ± 0.14	T
4	4.65 ± 0.02	T	20	2.72 ± 0.16	T
5	4.44 ± 0.28	T	21	2.64 ± 0.12	T
6	4.25 ± 0.03	T	22	2.45 ± 0.11	T
7	4.18 ± 0.06	T	23	2.26 ± 0.14	T
8	4.06 ± 0.21	T	24	2.18 ± 0.28	T
9	3.81 ± 0.09	T	25	2.10 ± 0.11	T
10	3.72 ± 0.14	T	26	1.99 ± 0.09	T
11	3.58 ± 0.07	T	27	1.86 ± 0.25	T
12	3.35 ± 0.12	T	28	1.73 ± 0.07	T
13	3.32 ± 0.07	T	29	160 ± 0.13	T
14	3.27 ± 0.12	T	X	5.3 8 ± 0.88	T
15	3.13 ± 0.08	T	X	4.75 ± 0.26	T
16	3.10 ± 0.11	T			

Table 1. These results showed that all somatic chromosomes were acrocentric autosomes and only the two sex chromosomes (XX) were submetacentric (Fig. 4). The chromosome number per spread was counted for 100 spreads of the passage 3–5 cells, and the mean frequency of diploid cells was 98.65 ± 2.89% (mean ± SD). Aberrations in chromosome number tended to increase with increasing number of passages (data not shown) indicating that in vitro culture affected the chromosomal stability of the cells to a limited extent, but supporting the conclusion that the cell line is reproducibly diploid.

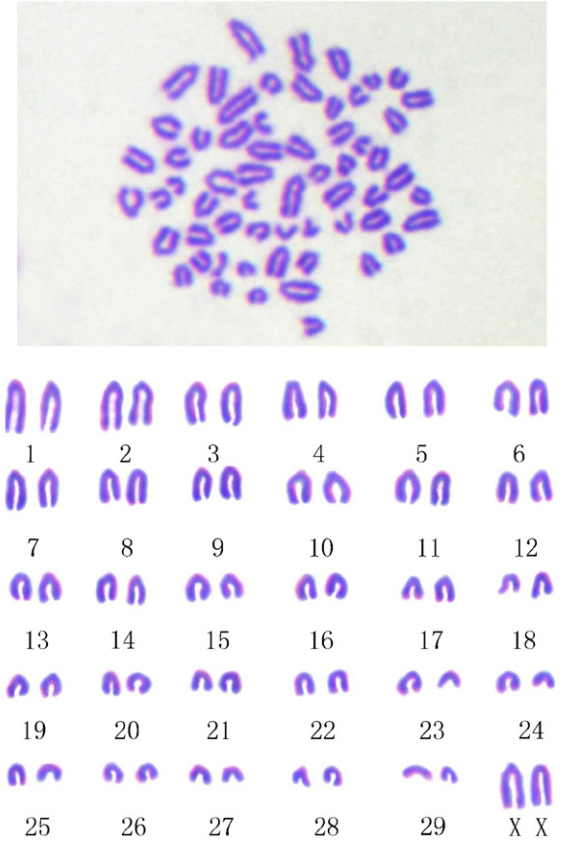

Fig. 4. Karyotype of JBG goat fibroblasts (♀).

3.6. Isoenzyme analysis

The LDH bands obtained from JBG goat fibroblasts were compared with those from other species. The isoenzyme patterns showed five isoenzyme bands (LDH-1, -2,-3,-4,-5) (Fig. 5A). The enzymatic mobilities were in the order LDH-3, LDH-2, LDH-1, LDH-4, LDH-5; three MDH isoenzyme bands

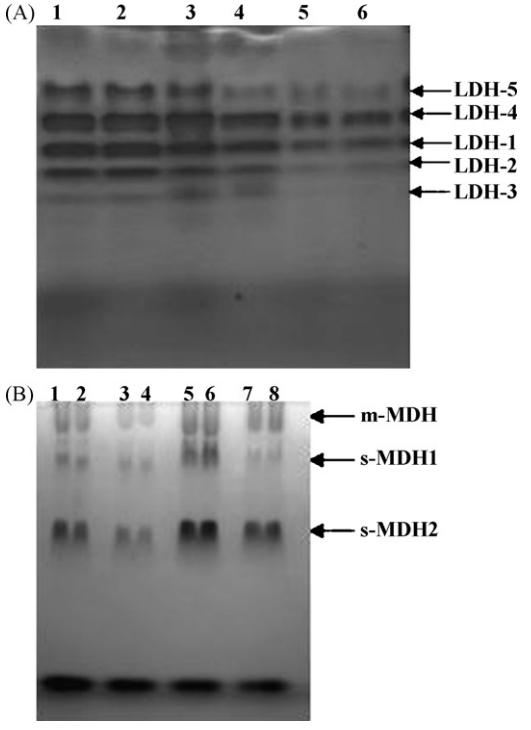

Fig. 5. Isoenzyme patterns of LDH and MDH in the JBG goat. (A) Isoenzyme patterns of LDH in the JBG goat. Lane 1 and Lane 2: JBG goat; Lane 3 and Lane 4: Mongolian sheep; Lane 5 and Lane 6: Dorset sheep. (B) Isoenzyme patterns of MDH in the JBG goat. Lane 1 and Lane 2: Taihang Black goat; Lane 3 and Lane 4: Texel sheep; Lane 5 and Lane 6: JBG goat; Lane 7 and Lane 8: Jining White goat.

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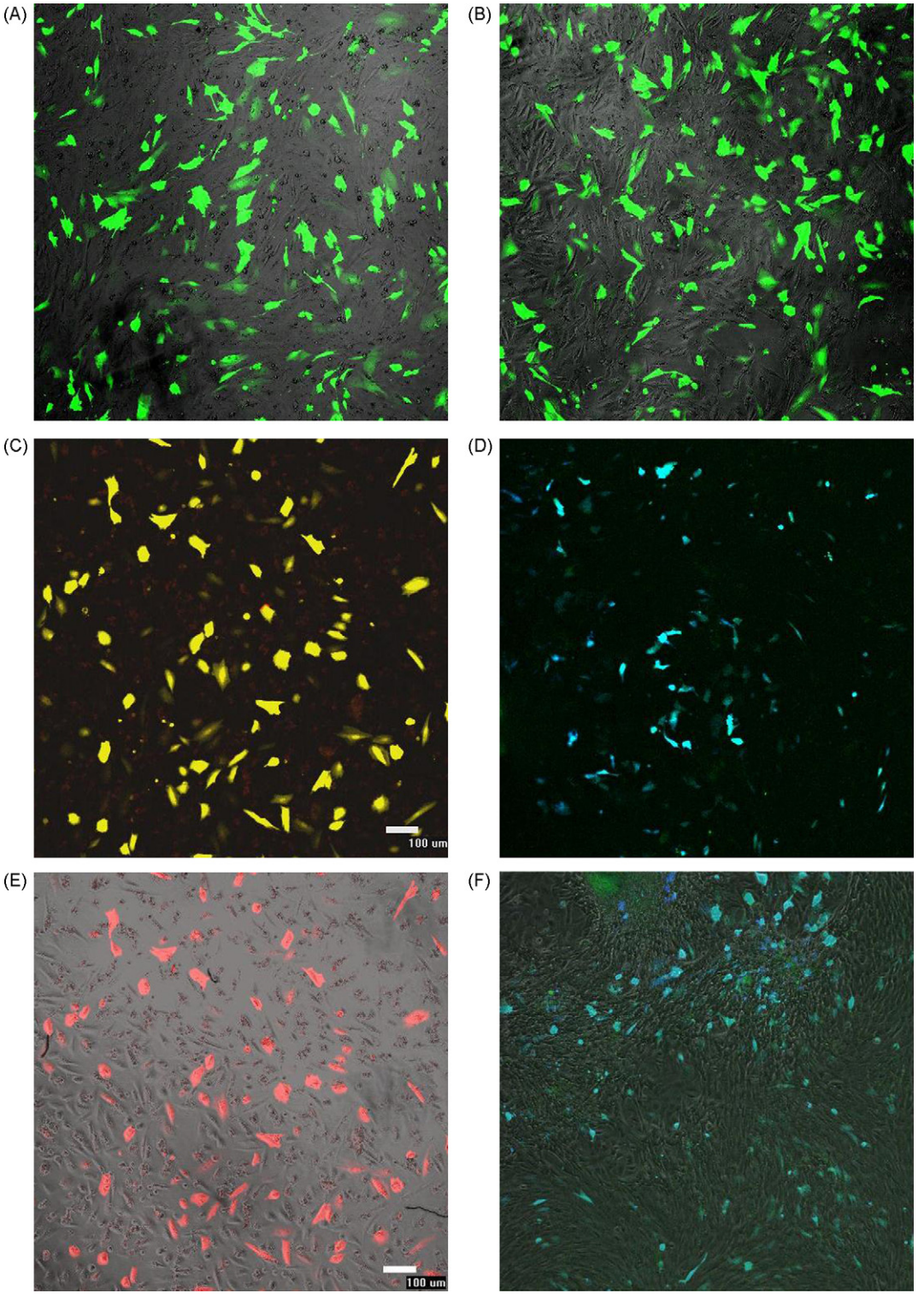

Fig. 6. Transfection photos of six fluorescent protein genes. These photos were taken at 48 h after transfection using a Nikon TE-2000-E confocal fluorescence microscope with excitation wavelengths of 405, 488 and 543 nm to determine the transfection efficiency (10×); (A–F) were the transfection results of pEGFP-C1, pEGFP-N3, pEYFP-N1, pECFP-N1, pDsRed1-N1 and pECFP- mito, respectively.

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Table 2
Transfection efficiencies of six fluorescent proteins in JBG goat.

Time (h)	Transfection efficiency of six fluorescent proteins					
	pEGFP-C1	pEGFP-N3	pEYFP-N1	pDsRed1-N1	pECFP-N1	pECFP-mito
24	29.7	28.9	23.5	16.1	12.9	11.8
48	46.6	42.8	36.1	29.6	25.7	20.4
72	56.3	51.7	42.9	26.9	22.2	16.3

Cells were examined 24, 48 and 72 h after transfection using a Nikon TE-2000-E inverted microscope with excitation wavelengths of 405, 488 and 543 nm to determine the transfection efficiency. The numbers of total cells and signal-positive cells in 10 visual fields of every well were counted to calculate the transfection efficiencies.

(m-MDH, s-MDH1, s-MDH2) were observed in the JBG goat cells (Fig. 5B). The results indicated that there was no cross-contamination with cells from other species.

3.7. Exogenous expression of fluorescent proteins

Expression of pEGFP-C1, pEGFP-N3, pEYFP-N1, pDsRed1-N1, pECFP-N1 and pECFP-mito in the JBG goat cells was observed 24, 48, and 72 h, 1-week and 2 weeks after transfection. The results indicated that all six fluorescent proteins were expressed in most positive cells, and the strongest fluorescence intensity and the highest transfection efficiency of the exogenous genes appeared 48 h after transfection (Fig. 6). The transfection efficiencies of the six fluorescent proteins 24, 48 and 72 h after transfer were between 11.8% and 56.3% (Table 2). While the transfection efficiencies of 1-week and 2 weeks after transfection were less than 5% which may ascribe to the degradation of the interest proteins. The highest transfection efficiency reached 56.3% at 72 h after transfection, which was higher than that of 32.6% in the study of Lei et al. (2007). Confocal fluorescence microscopy was

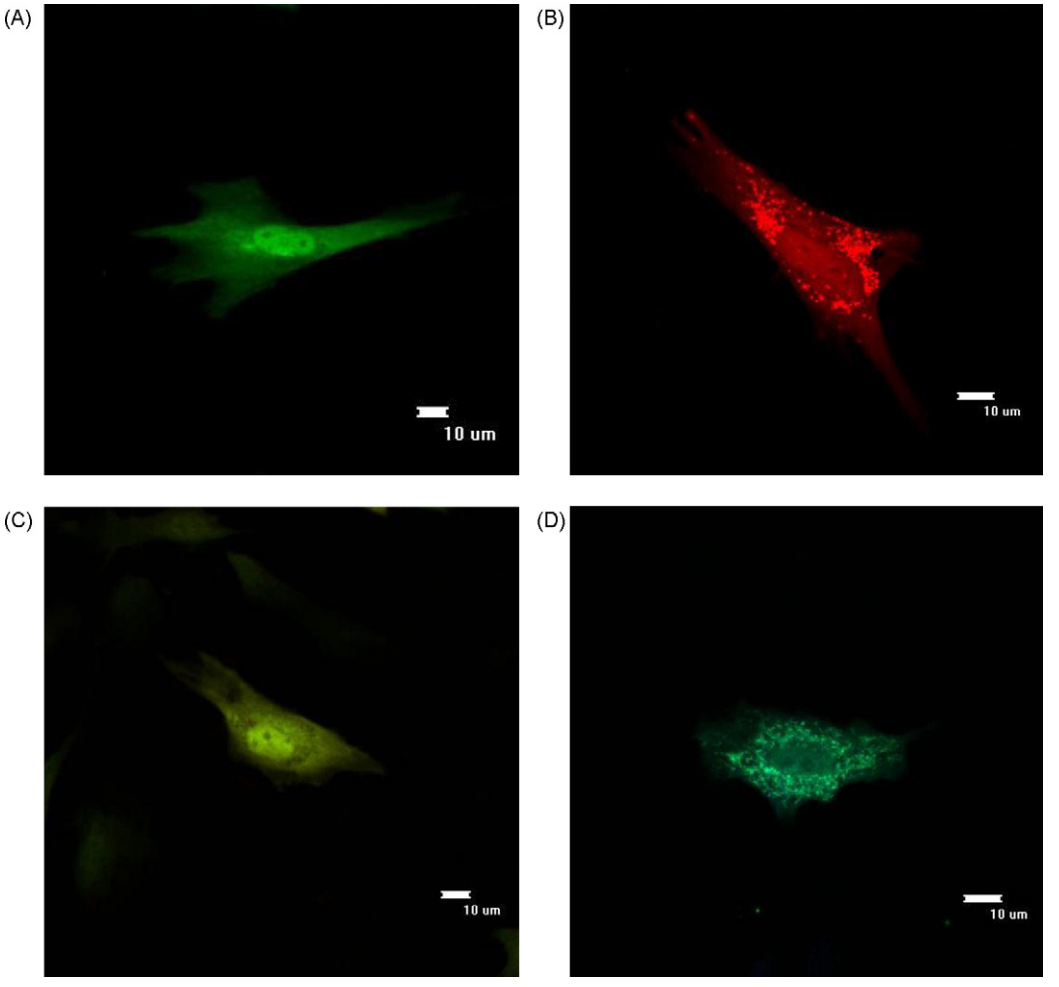

Fig. 7. The expression and distribution of pEGFP-C1, pDsRed1-N1, pEYFP-N1 and pECFP- mito in JBG goat cell (40×); (A–D) were the expression patterns of pEGFP-C1, pDsRed1-N1, pEYFP-N1 and pECFP-mito at 48 h after transfection.

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used to observe the distribution of green, yellow, cyan, and red fluorescence in the JBG goat cells to determine the subcellular location of six fluorescent proteins. The results showed that the fluorescence could be observed throughout the cytoplasm and nuclei of control cells except in the cryptomere vesicle. DsRed and pECFP-mito were mostly shown in cytoplasmic (with a punctuate pattern), whereas EGFP and EYFP showed an intense nuclear signal (Fig. 7). The numbers of fluorescent cells reduced at 1-week, however, there remained a few dispersed positive cells after 2 weeks even 1-month and 2 months. The viabilities of cells transfected with pEGFP-C1, pEGFP-N3, pECFP-N1, pECFP-mito, pEYFP-N1 and pDsRed1-N1 were 88.2%, 87.5%, 86.3%, 87.5%, 88.3% and 89.6%, respectively. Among them, no one was significantly different from that of the control group (90.3%, $P > 0.05$).

4. Discussion

Animal genetic resources are considered as a part of the present biodiversity. Unfortunately; our native animal genetic resources have not been appreciated as it should have been; some are already gone extinct and some are at the edge of extinction. Moreover, animal genetic resources must be considered as the insurance of the future, since they may have an important potential to improve social and economic life.

Traditionally, in vitro conservation comprises material like semen, embryos and oocytes. Keeping in liquid nitrogen allows indefinite storage, while recovery is relatively straight forward. There are however a number of reasons, why these techniques cannot generally be used for endangered breeds world wide. Deep freezing of semen and embryos can only be done for a number of species, and furthermore requires species specific techniques. Moreover, the need for substantial infrastructure has not been met by many countries which cannot afford considerable costs (Woolliams and Wilmut, 1999). Storage of somatic cells may open an option as pointed out by Corley-Smith and Brandhorst (1999).

For every animal in a cheap and fast way, tissue samples can be cryo-conserved in liquid nitrogen easily. The collection of a sufficient large number of samples is thus also possible in countries with little infrastructure. Somatic cell cryo conservation is also proposed as the method of choice for the rapid creation of emergency gene banks.

JBG goat are mainly from Jining city and Heze city of southwest of Shandong province, China. The 32 goats were chosen randomly from Jiaxiang, Liangshan, Jinxiang, Rencheng, Yutai and Wenshang which are the main habitats of Jining Black and Grey goat, so the samples are representative and capture as much genetic variation as possible. We established successfully a JBG goat cell bank from 32 samples by adherent culture. In order to ensure the cell quality, cells of each individual were tested using the same method. We concluded that the cell quality is the same among the cell lines. The biological characteristics, especially the genetic characteristics, of the cells may be changed by in vitro culture conditions after many passages, so a minimal number of passages is recommended to conserve them. The JBG goat cells were frozen within 10 passages.

Morphology, as the most important qualitative parameter of epidermal tissue reconstitution, is evaluated by light and electron microscopy. In our study the cells had fibrous characteristics with turgor vitalis cytoplasm, and during growth they showed typical fibroblast-like morphology as radiating, flame-like or whirlpool migrating shapes. Using phase-contrast microscopy Changqing observed in the Luxi cattle fibroblasts, fibroblast-like or epithelial-like cells could be seen migrating from the tissue pieces 5–12 day after explanting (Changqing et al., 2008). When explanting tissues to derive new primary cultures, epithelial cells and fibroblasts will initially grow together. Fibroblasts adhere more easily to flasks and can be trypsinized more readily, whereas epithelial cells do not adhere in a short time and are easily shed using gentle mechanical agitation (Ren, 2002). Because of these differences, fibroblasts will quickly outgrow their epithelial counterparts. In this manner the cells were treated with trypsin over 2–3 passages, then a pure fibroblast culture was obtained (Zhou et al., 2005; Li et al., 2003).

From the growth curve of the 8th passage, we found that cells entered the exponential phase at 48 h, and from the 8th day cells grew more slowly. These results indicated that the 8th passage JBG goat ear cells cultured in vitro had the biological characteristics of normal fibroblasts.

The genetic stability of cell line is critical to preserve the genetic resources, the fibroblasts must maintain the same diploid character as cells in vivo. Cells cultured in vitro vary still keeping the division capability; after successive cell divisions a differentiation of the cell lines it appears. Those cell lines cannot be used in breed conservation. A $2n = 60$ frequency of 98.6% indicated our JBG goat fibroblast cultures were stably diploid in accordance with the reports of Liu et al. (1993) and Zhan et al. (1994). In addition, the frequency of diplont chromosomes was above 98%, further validating the stability of these cells. Although hypodiploid and hyperdiploid cells, and some polyploid cells did emerge in the cultures with increased passaging (Men et al., 2002), the incidence of such cells was still very small in our study (below 2%). Hence, chromosomal structure variation was rare in our JBG goat fibroblasts.

It is not uncommon for cells to cease growth, show altered biological characteristics or even lose their diplont characteristics with time in culture due to a variety of stimuli and other factors. Effective measures are thus required to ensure diploid stability in cultures of cells that are used to preserve valuable genetic resources. The pH must therefore be controlled strictly, the culture medium must be changed at appropriate intervals, and a split ratio of between 1:2 and 1:3 should be used. Importantly also, the passage intervals should not be too great and the cells should be maintained at subconfluence to avoid deleterious effects from contact inhibition. Explanted cells should be passaged quickly and with care and high quality reagents should always be used. Finally, cells should be cryopreserved with the utmost care as required to maintain a high quality cell bank that is viable over a long-term.

Apart from the need to determine the tissue of origin of a culture, it is also important to avoid cross-contamination

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from other cell lines. Isoenzyme analysis is an extremely reliable and straightforward technique, which provide rapid and reliable identification of species of origin. This technique is based on visualization of certain enzyme activities, which exhibit interspecies polymorphism in their electrophoretic mobility, thus showing characteristic profiles for the same species cells. Different isoenzyme profiles represent the products of different gene alleles. While the species of origin of a cell line can usually be determined with only two isoenzyme tests (lactate dehydrogenase and glucose-6-phosphate dehydrogenase), specific identification of a cell Line would require a larger battery of tests (Halton et al., 1983). A system in which eight isoenzyme activities were investigated offers a useful compromise (O'Brien et al., 1980). This procedure retained the advantage of rapid testing while also giving a useful level of specificity for identification purposes.

The JBG goat fibroblast LDH pattern showed five bands that differed from other sheep and goat breeds. In contrast, the MDH profile produced three bands for this species. Analysis of these genes in this way have shown both interspecies and intraspecies isoenzyme polymorphisms (O'Brien et al., 1977; Nims et al., 1998). Therefore, these tests can be used to identify the relationship between species, and also heredity distribution, in addition to confirming the origin and purity of explanted cells. In this study, the isoenzyme bands of LDH and MDH of JBG goat fibroblasts were clear, indicating that the genetic characteristics were stable and there was no contamination with cells of other species.

The researches about fluorescent protein are mainly focused on tumor cell, nerve cell and stem cell (Jung et al., 2001). DNA concentration, lipofectine concentration, the incubate time of the DNA and lipofectine combination, and serum all can affect the transfect efficiency which is identical with the Vero cell research, Hela cell and some other cell lines (Tseng et al., 1999; Escriou et al., 2001; Rong et al., 2006). In our experiment, the transfer efficiency of six fluorescent proteins could achieve 36.2% with optimized ratio of plasmid and lipofactamine. The numbers of fluorescent cells reduced at 1-week, however, there remained a few dispersed positive cells after 2 weeks even 1-month and 2 months. Through G418 resistance screening and monoclone culture during 1-month, we gained three positive cell strains which can express EGFP, EYFP and DsRed stably.

At 24 and 48 h after transferring, the six fluorescences could be observed in cytoplasm and nucleus well-distributed except cryptomere vesicle. At 72 h after transferring, EGFP and EYFP gene still expressed steadily in cytoplasm and nucleus, which were nearly unchanged, but some cells morphous were irregular and semilism. But in 72 h, DsRed gene mainly expressed some granula-expression product surrounding nuclear membrane, and formed a red ring profile.

We could see that the transfected cells at the state of reduplication and different dividing and growth phases exhibited no obvious differences with control groups. The result showed that the transfected cells had not been affected by fluorescin under certain range. Moreover, some researches indicated that GFP did not obviously affect cell doubling time in tumor and human cell lines, which were identical with ours. Fluorescins were not distributed homogeneously in all cell lines, for example GFP mainly distributed in nucleus in COS cell and DsRed expressed some granula-expression product surrounding nuclear membrane and formed a red ring profile. Due to these exceptions, it was quite important for researcher to determine the distribution of reporter gene before we analyzed the location of fusion protein.

A fibroblast cell bank was established from explanted ear marginal tissue of the JBG goat using standard tissue attachment culture and continuous passaging following trypsinization. We contend that our cell bank makes a valuable contribution to the preservation of the genetic resources of the JBG goat and provides useful biomaterial for future studies in cell biology, medicine, genomics, post-genomics, and both genetic and embryonic engineering.

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