Establishment and characterization of a fibroblast cell line derived from Texel sheep

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Abstract: A Texel sheep ear marginal tissue fibroblast cell line (named TSF19) was successfully established by using a primary explant technique and cell cryoconservation technology. TSF19 cells were adherent, with a population doubling time of 24.9 h. Chromosome analysis showed that >90% of cells were diploid prior to cell passage 4. Isoenzyme analyses of lactate dehydrogenase and malate dehydrogenase showed that the TSF19 cells had no cross-contamination with other species. Tests for cell line contamination with bacteria, fungi, or mycoplasmas were also negative. Plasmids encoding the fluorescent proteins pEGFP-N3, pECFP-N1, pDsRed1-N1, and pEYFP-N1 were transfected into cells to study exogenous gene expression in the cells. The plasmid transfection efficiency was between 21.8% and 46.5%. This newly established cell line will not only preserve the genetic resources of the important Texel sheep at the cell level but will also provide a valuable resource for genomic, postgenomic, somatic cloning research.

Key words: Texel sheep, fibroblast cell line, establishment, identification.

Résumé : Une lignée cellulaire de fibroblastes provenant du tissu marginal de l'oreille de mouton de race Texel (TSF19) a été établie avec succès en utilisant une technique d'explant primaire et une technologie de cryoconservation. Les cellules TSF19 étaient adhérentes, et leur temps de doublage était de 24.9 h. L'analyse chromosomique a montré que >90 % des cellules étaient diploïdes avant le quatrième passage cellulaire. Les analyses des profils des isoenzymes de la lactate déshydrogénase et de la malate déshydrogénase ont montré que les cellules TSF19 n'étaient pas contaminées par d'autres types de cellules. Les tests de dépistage d'une contamination par des bactéries, des champignons ou des mycoplasmes étaient aussi négatifs. Des plasmides codant les protéines fluorescentes pEGFP-N3, pECFP-N1, pDsRed1-N1 et pEYFP-N1 ont été transfectés dans les cellules afin d'étudier l'expression de gènes exogènes. L'efficacité de transfection se situait entre 21.8 % et 46.5 %. Cette nouvelle lignée cellulaire permettra non seulement de préserver à l'échelle cellulaire les ressources génétiques de cette race importante de mouton, le Texel, mais elle fournira aussi une ressource précieuse pour la recherche en génomique, post-génomique et clonage somatique.

Mots-clés : mouton Texel, lignée fibroblastique, établissement, identification.

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Introduction

The genetic diversity of livestock and poultry is an important part of overall biological diversity and also forms the basis for the survival and sustainable development of human societies. Therefore, the preservation of genetic resources from endangered species is of significant scientific importance. The preservation of individual animals, semen, embryos, genomic libraries, and cDNA libraries are all practical approaches. The establishment of cell lines using cryopreservation techniques is another effective approach (Shi 1989). Most cell banks emphasize conservation and utilization of animal resources, in particular animal generative cells and embryos (Ho et al. 1997; Oishi 1997; Simon 1999; Park et al. 2009). In addition to these methods, the development of modern somatic cell cloning techniques has made somatic cells an attractive resource for the conservation of animal genetic materials (Wu 1999; Hong et al. 2005; Yun et al. 2008). There have been a number of recent publications on the development of fibroblast cell lines from various animals, including the Debao pony (Ma et al. 2004), Beijing fatty chicken (Zhou et al. 2005), sheep (Chen et al. 2006), Taihu pig (Zhang et al. 2008), Luxi cattle (Liu et al. 2008), and white ear lobe chicken (Wu et al. 2008).

Texel sheep originated in the Netherlands on Texel Island. In the middle of the 19th century, a local coastal strain of low-wetland sheep with the characteristics of late maturation and fine hair selectively bred with male Lincoln Long-

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wool sheep from the Border Leicester to improve the breed. Thus, Texel sheep is short-tailed breed of mutton and fuzz sheep. This breed has a number of desirable features, including a long life span, resistance to cold, sexual precocity, a strong maternal instinct, high lambing percentage, rapid lamb growth, and good lamb quality, muscularity, and flesh production, high cutability, powerful marketplace competition, and strong breeding potential for hybridization with other species. These qualities have become a driving force for in-depth investigations of Texel sheep. Collection of genetic information for a species establishes the richness of genetic diversity and confirms the degree of uniqueness of specific genetic resources through genetic markers.

In the present study, we culture fibroblasts from the ear margin tissues of Texel sheep to develop a new fibroblast cell line. A combination of methods, including assessment of cell viability, microorganism detection, chromosome analysis, isoenzyme analysis, and transfection of genes encoding fluorescent proteins, was used to establish and characterize this new cell line. To study exogenous gene expression, fluorescent protein genes were transfected into the cells as reporter genes. Our overall objective was to cryopreserve cells from Texel sheep as a genetic resource for possible future revival of the breed using somatic cloning techniques. As part of this effort, we also aimed to develop a convenient and useful resource for future genomic research on breed qualities and breed enhancement. Moreover, with the development of modern somatic cell cloning techniques, limited cell lines will have increasingly prominent roles in research and may be useful in currently unforeseen applications.

Materials and methods

Ear margin tissue collection

Ear margin tissue samples (approximately 1 cm^2 in size) were removed from Texel sheep and placed into separate tubes containing DMEM with ampicillin (100 U/mL) and streptomycin (100 μg/mL). The samples were immediately transported to the laboratory for further analysis.

Primary culture and subculture

Each ear margin tissue sample was rinsed and then cut into approximately 1 mm^3 pieces. Individual tissue pieces were seeded on the bottom of a tissue culture flask and then DMEM with 10% fetal bovine serum was added. The flasks were inverted in a 37 °C incubator with 5% CO_2 for 2 h and then turned over (Ma et al. 2004). The medium was changed as needed and the cultures were observed daily for substantial outgrowth of cells from the tissue pieces. The cells were harvested at 80%–90% confluence, rinsed twice with phosphate-buffered saline to remove all traces of trypsin inhibitors, and then incubated in a 0.05% trypsin solution in an inverted position at 37 °C for 3 min. Flasks were then turned over, shaken gently to detach the cells, and medium added to the cell suspensions to block trypsinization. The cell volume was split into new culture flasks and incubated at 37 °C in a 5% CO_2 atmosphere.

Cryogenic preservation and recovery

Prior to freezing, the cultures were maintained in an actively growing state (log phase or exponential growth) to ensure optimum health and good cell recovery. The culture medium was changed 24 h prior to harvesting. The subculture protocol described above was then used for harvesting the cells. The cells were counted with a hemocytometer and cell viability was assessed by trypan blue staining. The cells were centrifuged at 1000 rpm for 8 min, the supernatant was removed, and then the cell pellet was resuspended in sufficient media for freezing (10% DMSO plus 50% fetal bovine serum plus 40% DMEM) to reach a final cell density of 3×10^6 to 5×10^6 viable cells/mL. Subsequently, 1 mL of the cell suspension was allocated into one sterile plastic cryogenic vial labeled with the animal name, gender, cell line, passage number, and date. The vials were sealed and placed into boxes filled with the proper amount of isopropyl alcohol. Then, these boxes were placed in a –80 °C freezer overnight and the cells were transferred to a liquid nitrogen storage system (Werners et al. 2004). To recover and reseed the cells, the frozen tubes were removed from liquid nitrogen and quickly thawed in a 42 °C water bath, and then the cells were transferred into a flask with complete DMEM. The cells were cultured at 37 °C in a 5% CO_2 atmosphere and the medium was renewed 24 h later (Freshney 1992).

Estimation of cell viability

Assays of cell viability before freezing or after recovery were carried out using the trypan blue vital stain method. The cells were seeded in a six-well plate and 1000 cells were counted for viability (Qi et al. 2007).

Growth curve of TSF19

Following the method of Gu et al. (2006), 2.5×10^4 cells/mL were seeded onto 24-well plates. After culturing for 7 d, three wells were monitored on a daily basis for cell concentration until a plateau phase was reached. The cell growth curves were plotted and the population doubling time was calculated based on the growth curve.

Microorganism detection

The Doyle and Freshney method (Doyle et al. 1990) was followed to detect potential bacteria and fungi contamination. The cultured cells were stained with Hoechst 33258 according to the DNA fluorescent staining protocol to detect mycoplasma contamination (Guan et al. 2005). An ELISA mycoplasma detection kit (Roche Diagnostics Corp., Indianapolis, Indiana) that could detect the four most common mycoplasma species (*Mycoplasma arginini*, *Mycoplasma hyorhinis*, *Acholeplasma laidlawii* and *Mycoplasma orale*) was used to confirm the results of DNA staining for mycoplasma.

Chromosome analysis

The cells were harvested at 80%–90% confluence. Microslide preparation and chromosome staining were performed according to the method of Suemori et al. (2006). Chromosome numbers were counted for 50–100 individual chromosome spreads as previously described (Kawarai et al. 2006). This method focuses on three important parameters: relative chromosome length, arm ratio, and centromere index.

Isoenzyme analysis

Isoenzyme patterns of lactate dehydrogenase (LDH) and malate dehydrogenase (MDH) were determined using a vertical slab noncontinuous PAGE assay. Texel sheep cells were harvested, and protein extraction and gel electrophoresis were conducted as previously described (He and Zhang 1999).

Transfection of fluorescent proteins in TSF19

Using the method of Tsuchiya et al. (2002), the same quantity of the fluorescent protein vectors pEGFP-N3, pECFP-N1, pDsRed1-N1, and pEYFP-N1 were transfected into cells in serum-free medium using Lipofectamine 2000 transfection reagent (Invitrogen Corp., Carlsbad, California). The plasmid DNA (micrograms) to Lipofectamine 2000 (microlitres) ratio was 1:3. After 8 h, the nonseum medium was removed and replaced with medium that contained serum. The cells were observed 24, 48, and 72 h after transfection to estimate the transfection efficiency. Cell morphologies were also observed using confocal microscopy (Nikon TE-2000-E, Japan). The relative fluorescence intensity of the different fluorescent proteins was evaluated in the cell nucleus and cytoplasm and then positive cells were screened for G418 resistance as a selection marker.

Results

Cell morphology and viability

A few epithelial-like and fibroblast-like cells grew from the different tissues, after the cells had been attached in culture for approximately 2 weeks. When observed over time, fibroblasts were seen to move out of the vicinity of the tissue and then multiply quickly. The cells then assumed a typical long spindle shape and the primary cell growth slowed. The growth medium was changed whenever it began to turn yellow. The cells were subcultured when they reached 90% confluence. After passage, growth accelerated and plateaued after 3–4 d. The average viability before freezing was 97.6% and after thawing was 93.2%, which demonstrated that the cells were healthy and the culture conditions were optimal (Figs. 1*a*–1*d*).

Growth curve (cell dynamics)

The growth curve of the TSF19 cells appeared as a typical "S" shape (Fig. 1*e*). There was a lag time or latency phase of about 72 h after the cells were seeded, corresponding to the adaptation of primary cells in recovery that were repairing protease damage. The cells then proliferated rapidly and entered an exponential growth phase until a stationary phase was reached after about 3 d. The population doubling time calculated from the curve data was approximately 24.9 h.

Microbial analysis of the TSF19

Upon analysis, the culture medium being tested did not become turbid or display other visible changes, whereas the positive test control was visibly turbid with precipitation. These results demonstrated that the newly established TSF19 cell line was not contaminated by bacteria or fungi. After staining with Hoechst 33258, fluorescence microscopy revealed that the nucleus of the fibroblasts appeared as a blue ellipse, which showed that our established cell line was mycoplasma negative (data not shown). These results were confirmed by DNA staining with an ELISA mycoplasma detection kit (Roche Diagnostics Corp.) and the results of the two tests confirmed that TSF19 was negative for mycoplasma.

Chromosomal analysis of TSF19 cells

Number of diploid chromosomes

Because Texel sheep chromosomes are small and difficult to differentiate, only the hypodiploid, diploid, and hyperdiploid numbers were calculated for 50 cells. The results are presented in Table 1. The majority of the cells were diploid, but the cell chromosome number changed over time, with an increasing frequency of hyperdiploid occurrences. The diploid proportion dropped to approximately 76% by the 13th generation in culture. Thus, preparation of these cells for frozen storage should occur during the early generations in culture.

Chromosome morphology

The sheep 2*n* chromosome number is 54, which includes three pairs of metacentric chromosomes and 23 pairs of telocentric chromosomes. The X chromosome is the longest acrocentric chromosome and the Y chromosome is the shortest submetacentric chromosome. TSF19 exhibited this pattern, with a karyotype composition of 3 m + 1 sm + 1 st + 49 t, that is, chromosomes 1–3 were metacentric chromosomes, 4–26 were telocentric chromosomes, the X chromosome was subtelocentric, and the Y chromosome was submetacentric. These results are in agreement with those reported by Men et al. (1984) and Shen and Zheng (1983). However, Chao et al. (1986) stated that Ujmuqin sheep chromosomes 1 and 2 are submetacentric chromosomes, that the X and Y chromosomes are metacentric, and that the other chromosomes are telocentric, possibly owing to a chromosome translocation or distortion. Further analysis is required to establish a concrete basis for this discrepancy. A representative karyotype is presented in Fig. 1*f*.

Isoenzyme analysis of TSF19

The distribution patterns of isoenzyme polymorphisms are regarded as characteristic of a species or a tissue (MacLeod et al. 1999). Polymorphism analysis of isoenzymes is currently the standard method for the quality control of cell line identification and detection of interspecies contamination. Isoenzyme patterns for LDH and MDH in TSF19 cells were obtained and compared with white grey goat, Angus, large Yorkshire, and Mongolian horse fibroblasts. The patterns of LDH shown in Fig. 2*a* reveal clear band differences between the different livestock species. For the same livestock species and different breeds, there are fewer band differences on the LDH isozymogram. These domestic animals had their own unique bands with a different relative mobility. These results show that there was no cross-contamination of the TSF19 fibroblasts from different cell lines established in our laboratory during the same time frame. The order of TSF19 LDH activity from low to high was LDH3 < LDH2 < LDH1 < LDH4 < LDH5.

MDH patterns are shown in Fig. 2*b* for Texel sheep,

Fig. 1. Cell morphology, growth curve, and karyotype of TSF19 cells. (*a*) Primary cells (×100). The cells had a typical long spindle shape with slower growth. (*b*) Subcultured cells (×100). The cells were subcultured until they reached 90% confluence. After passage, growth accelerated and plateaued after 3–4 d. (*c*) Cells prior to cryopreservation (×100). The cells were healthy and in a mitotic growth phase. (*d*) Cells in recovery after thawing (×100). The cells were cultured for 48 h after thawing. (*e*) Growth curve of TSF19 cells. The curve had a typical "S" shape, with cell density on the left axis. The growth curve included a latency phase, exponential growth phase, and stationary phase. The population doubling time calculated from the growth curve was approximately 24.9 h. (*f*) Metaphase chromosomes (left) and karyotype (right) of TSF19 cells (male) (×1000). The Texel sheep 2*n* chromosomes number is 54 in which three pairs are metacentric chromosomes and 23 pairs are telocentric chromosomes. The X chromosome is the longest acrocentric chromosome and the Y chromosome is the shortest submetacentric chromosome.

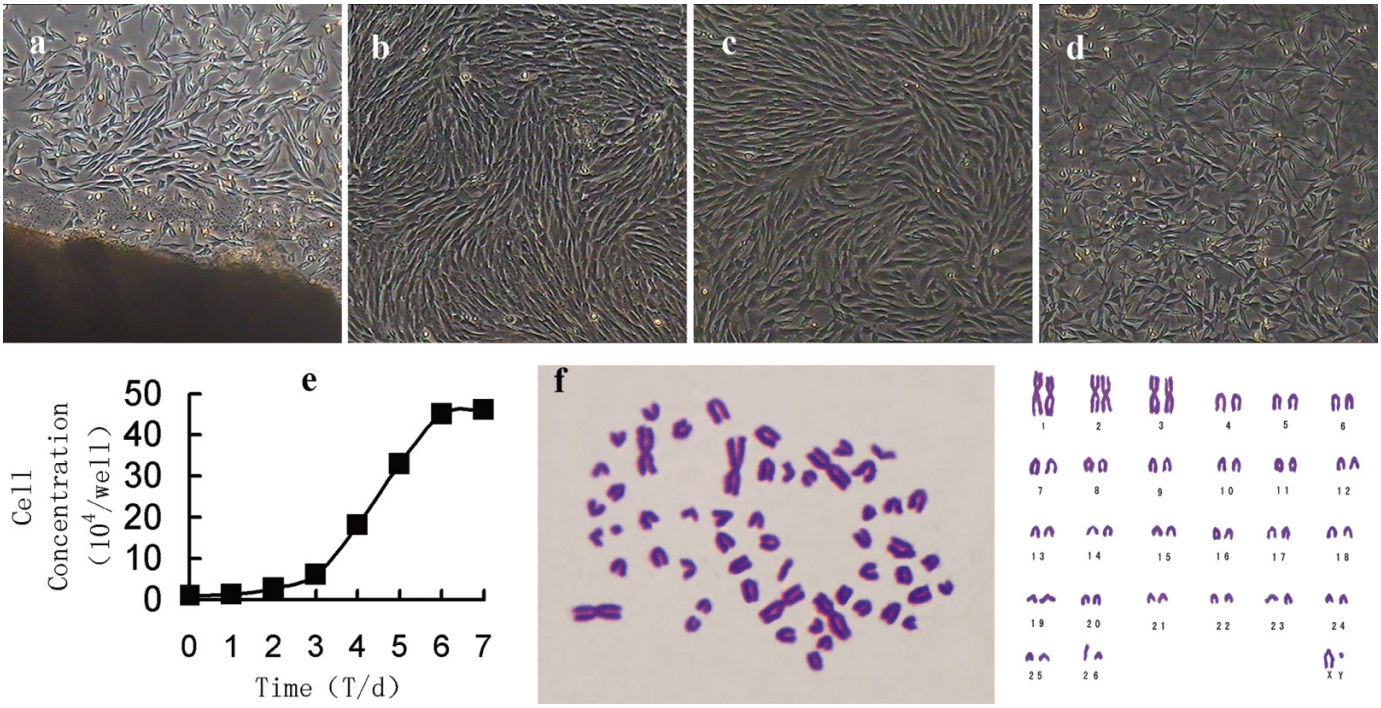

Table 1. Chromosome number in TSF19 cells.

	Chromosome number				
Generation	Hypodiploid	Diploid	Hyperdiploid	Total cell score	Percentage of 2*n*
2	0	49	1	50	98
3	1	48	1	50	96
4	2	45	3	50	90
6	2	43	5	50	86
8	2	41	7	50	82
10	3	41	6	50	82
13	3	38	9	50	76

white grey goat, Angus, large Yorkshire, and Mongolian horse marginal ear tissue fibroblasts. All five domestic animals had two bands, and there was a single m-MDH band near the cathode and two s-MDH bands near the anode. Similar activity was seen for both m-MDH and s-MDH1, while s-MDH2 had weaker activity. These domestic animals had their own unique bands with a different relative mobility. These results show that there was no cross-contamination between different breeds.

Expression results for fluorescent protein genes in TSF19

Under a defined wavelength of excitation light (pEGFP-N3, 488 nm; pECFP-N1, 405 nm; pDsRed1-N1, 543 nm; pEYFP-N1, 495 nm), the expression of pEGFP-N3, pECFP-N1, pDsRed1-N1, and pEYFP-N1 was observed at 24, 48, and 72 h using laser confocal microscopy and recorded (Fig. 3). The results showed that the transfection efficiencies of pECFP-N1 were significantly lower than those of the pEGFP-N3, pDsRed1-N1, and pEYFP-N1 ($p < 0.01$), with the transfection efficiency of pEYFP-N1 being maximal. The expression efficiencies of the four fluorescent protein genes at 24, 48, and 72 h after transfection were all between 21.8% and 46.5% (Table 2). The numbers of fluorescent cells decreased at 1 week after transfection. By screening

Fig. 2. LDH zymotype and MDH zymotype of TSF19 cells. Domestic animals have their own unique bands with a different relative mobility. Results showed that there was no cross-contamination between different breeds. SDS–PAGE electrophoresis of (*a*) LDH and (*b*) MDH. Species: lane 1, Texel sheep; lane 2, white grey goat; lane 3, Angus; lane 4, large Yorkshire; lane 5, Mongolian horse. Lanes 2–5 were control species. On Fig. 2*a* are LDH5, LDH4, LDH3, LDH2, and LDH1. On Fig. 2*b* are mMDH, sMDH1, and sMDH2, with sMDH2 showing weaker activity.

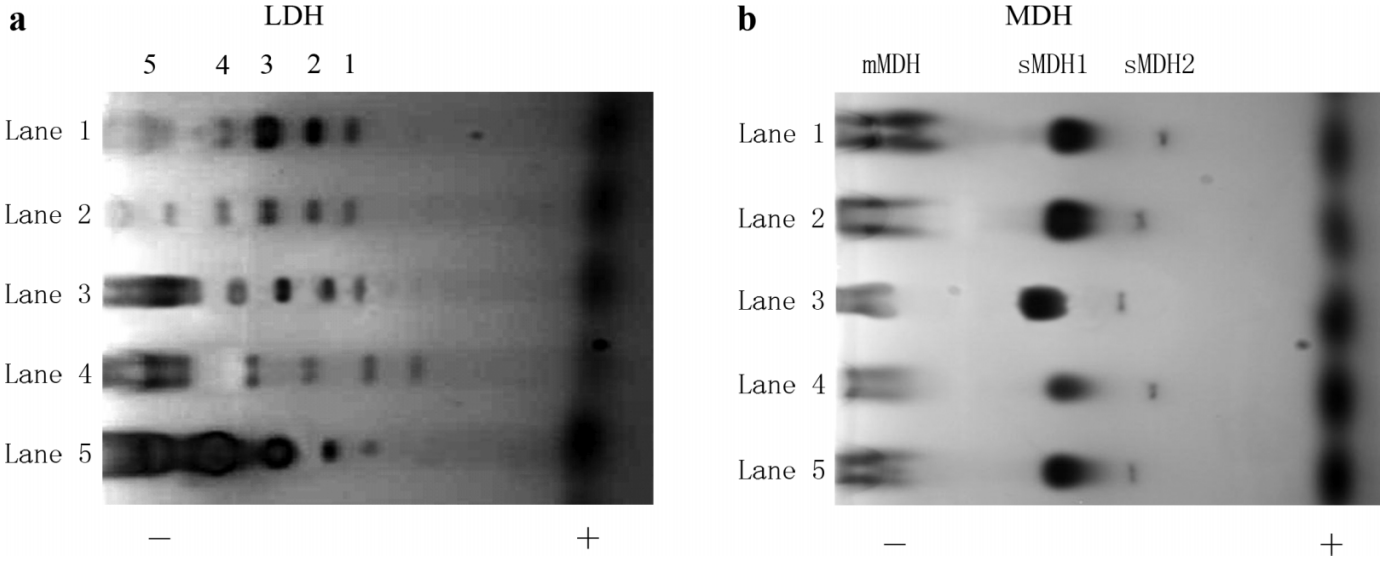

Fig. 3. Efficient transfection of TSF19 cells with pEGFP-N3, pECFP-N1, pDsRed1-N1, and pEYFP-N1 using Lipofectamine 2000 (×100). (*a*) Transfection of 48 h cells resulted in 42.7% pEGFP-N3-positive cells. (*b*) Transfection of 48 h cells resulted in 21.8% pECFP-N1-positive cells. (*c*) Transfection of 48 h cells resulted in 40.9% pDsRed1-N1-positive cells. (*d*) Transfection of 48 h cells resulted in 46.5% pEYFP-N1-positive cells.

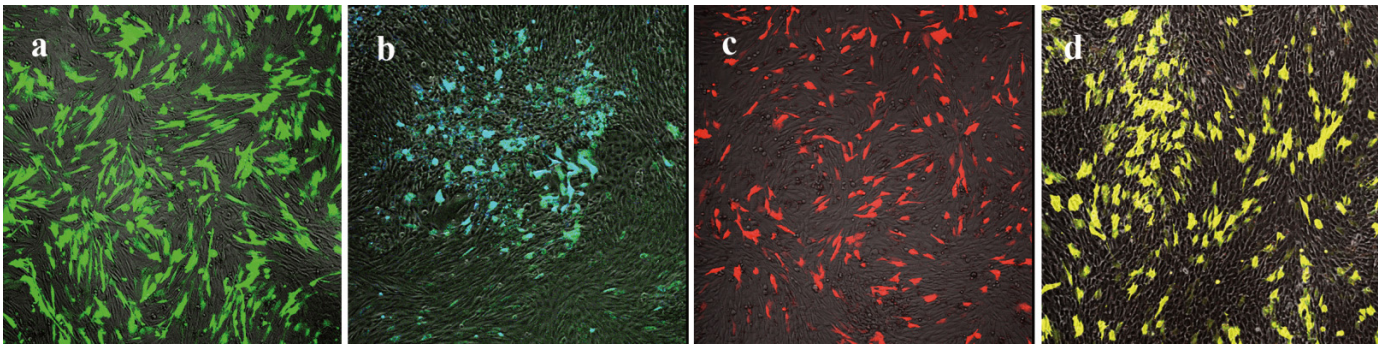

Table 2. Transfection efficiency for four fluorescent proteins.

Transfection time (h)	pEGFP-N3 (%)	pECFP-N1 (%)	pDsRed1-N1 (%)	pEYFP-N1 (%)
24	33.4	15.1	32.4	34.9
48	42.7	21.8	40.9	46.5
72	40.6	20.4	38.3	44.3

for G418 resistance and monoclonal culture for 1 month, we obtained three positive cell strains that stably expressed pEGFP-N3, pDsRed1-N1, and pEYFP-N1. RT-PCR and Western blotting confirmed that pEGFP-N3, pECFP-N1, pEYFP-N1, and pDsRed1-N1 were integrated into the Texel sheep fibroblast genome and could thus access the normal cellular protein expression machinery (data not shown).

Discussion

Establishment of TSF19

All of our analyses of these cells indicate that this newly established TSF19 is genetically stable and grows rapidly. After freezing and thawing, the cell survival rate decreased to some extent, possibly owing to cell injury that occurred

during the process of refrigeration and recovery. To ensure the motility rate of the cells recovered at late stages, the cells were frozen within four generations when their concentration exceeded 3×10^6 cells/mL. Because excessive passaging and trypsin digestion may adversely affect the biological characteristics of these cells, in particular the cell hereditary characteristics, all cells used to preserve a species should undergo a minimum number of passages.

Our morphological observations indicated that there were both epithelial and fibroblast cells present during the primary and early passages of the explanted tissue. Different tolerances to trypsin were evident, as the fibroblasts detached first when treated with trypsin and adhered more rapidly after passaging, while most epithelial cells were not adherent or stably adherent and detached with only mechanical agitation. Using these procedures for selection, purified fibroblasts were obtained after two or three passages.

Mycoplasma detection

A pure cell culture can easily become contaminated with bacteria or fungi. Air, equipment, serum, tissue samples, and handling errors can all be sources of such contamination. Repeated bacterial, oomycete, and mycete contamination, which introduce turbidity into the media, could be observed with the naked eye. Viral infections could be identified under the microscope, but mycoplasmas were not found. Mycoplasmas are difficult to remove and can coexist with cells for long periods of time. They are therefore more difficult to detect than bacteria, oomycetes, mycetes, or viruses. The methods of mycoplasma detection included direct solid agar microbiological culture, indirect DNA fluorescent staining, and new DNA stylet hybridization. Because mycoplasma DNA fluorescent staining is simple and rapid, it is a method commonly utilized by cell culture collection institutions.

Chromosomal morphology analysis

In the present experiments, TSF19 was verified to be a stable diplont cell line. There are some minute differences among autosomes, which may be due to differences in digestion time or the chromosome film handling process. These problems obscure the individual chromosome banding pattern and accurate differentiation becomes difficult. This problem may be linked with concrete culture and the processing of the chromosome slide. If the colchicine is too concentrated, the chromosome contracts excessively and a monomer may be scattered. If the centrifugal speed is excessively high or the centrifugation time is too long, the cell aggregate is difficult to disrupt, the chromosomes accumulate, mechanical scatter is insufficient, and the cell fragments accumulate. Excessive disruption can prematurely break cells, resulting in chromosome loss. If the hypotonic treatment is insufficient, the chromosomes may clump together. Excessive hypotonic treatment can rupture all of the cells, possibly resulting in chromosome loss. Contamination or an unclean glass slide and insufficient slide cooling can also affect a chromosome spread. Thus, in addition to macroscopic observation error, numerous factors may result in perceived changes in the number of chromosomes.

We found that the karyotype, chromosome number, and morphology of Texel sheep were very similar to those of Tan sheep and Hu sheep. From these data, the origin of Texel sheep may be the same as that of Tan sheep and Hu sheep. However, the relationships between these breeds remain a topic for future investigations. These different breeds of sheep may have a common ancestor, with natural and artificial selection leading to the formation of a different breed.

Isoenzyme analysis

LDH and MDH have been broadly utilized for the study of aerobic and nonaerobic metabolism based on their participation in cell glycolysis and the citric acid cycle, respectively. MDH is a dimer composed of cytosolic MDH (s-MDH) and mitochondrial MDH (m-MDH) alleles. Isoenzymes harbor polymorphisms across species, races, and individual or diverse tissues, and intercellular cross-contamination can be detected by isoenzyme analysis when 10% of the cells are affected.

In the organization of aerobic metabolism, such as in the heart, the dominant position in LDH is LDH1 (LDH-B), whereas in the organization of nonaerobic metabolism, such as in skeletal muscle, the dominant position is LDH5 (LDH-A) (Hammond et al. 1976). M subunits and H subunits have a certain proportion in the same organization of different animals owing to the specificity of in vivo functions. The energy of goats and milked caprine self-metabolism was provided by H subunit catalysis of lactose to pyruvic acid and M subunit catalysis of pyruvic acid to lactose. Therefore, the contents of H subunits are maximal and M subunits are higher. The LDH activity of the Lubeibai goat (Li et al. 1998), green goat (Zhang et al. 1993), and goat (Lin et al. 1985) is LDH1 > LDH3 > LDH2 > LDH5 > LDH4. The woolers have no use for this enzyme in energy consumption; thus, the content of M subunits in serum is very low (Tang and Luo 1997). The LDH zymogram of cultured TSF19 cells was LDH3 < LDH2 < LDH1 < LDH4 < LDH5 and the cellular content of H subunits and M subunits was lower than detected in goat species, which is in accord with previous publications. These results further demonstrate that the specificity of LDH isodynamic enzymes has close connection with physiological functions, the metabolic type, and economical usage.

Expression of fluorescent protein genes

DNA concentration, lipofectine concentration, DNA incubation time and lipofectine combination, and the presence of serum can all affect cell transfection efficiency (Escriou et al. 2001; Rui et al. 2006). In the present study, the transfection efficiency for pECFP-N1 was significantly lower than for pEGFP-N3, pDsRed1-N1, and pEYFP-N1. It is possible that the ratio of plasmid DNA (micrograms) to Lipofectamine 2000 (microlitres) (1:3) was not appropriate for pECFP-N1. RT-PCR and Western blotting confirmed that the four fluorescent protein genes were integrated into the Texel sheep fibroblast genome and could thus access the normal protein expression machinery. Under optimal conditions, transfection had no significant effects ($p > 0.05$) on apoptosis rate, cell shape, growth, or reduplication status compared with untransfected control cells.

The fluorescent protein positive cell strains that were developed will provide abundant donor cells for transgenic an-

imal cloning. The recombinant plasmids were constructed by integrating express functional genes with fluorescent protein genes that can be observed under a fluorescence microscope. Thus, these fibroblasts can be widely used as tools for investigating the functions of exogenous genes. This new resource will be important for future identification of breed-specific genetic markers and for nuclear transplantation and transgenic cloning investigations.

Conclusions

We have established a new fibroblast cell line from Texel sheep. This TSF19 cell line is genetically stable and rapidly proliferating, suggesting that it may be useful in the conservation of this unique breed. It also represents an effective new experimental resource for further genetic studies on the Texel sheep.

Acknowledgments

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