

Establishment and biological characteristics of Qingyuan partridge chicken fibroblast line

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ABSTRACT The aim of this work was to seek a different approach to conserving important domestic animals in imminent danger. A Qingyuan partridge chicken embryonic fibroblast line, containing 336 cryovials with 8×10^6 cells each, was successfully established from 60 Qingyuan partridge chicken embryos using explant culture and cryopreservation techniques. The cells were morphologically consistent with fibroblasts. The growth curve was a typical "S" shape having a detention phase, a logarithmic phase, and a plateau phase. The population doubling time was approximately 72 h. Tests for bacteria, fungi, viruses, and *Mycoplasma* were all negative. Isoenzyme polymorphism indicated that the genetic characteristics of the cell line were stable in vitro. Karyotyping analysis suggested that the chromo-

some number of a normal cell was $2n = 78$ and 93.4% of the entire population was diploid. The transfection efficiencies of 6 fluorescent proteins (pEGFP-C1, pEGFP-N3, pDsRed-N1, pEYFP-N1, pECFP-N1, and pECFP-mito) optimal at 48 h were from 15.1 to 39.8%. The cell line met all of the criteria from the American Type Culture Collection. Not only has the germline of this important chicken breed been preserved at the cell level, but also valuable material has been provided for genome, postgenome, and somacloning research. Moreover, the establishment of this technical platform may provide both technical and theoretical support for storing the genetic resources of other animals and poultry at the cell level.

Key words: Qingyuan partridge chicken, fibroblast line, biological characteristic, cryopreservation

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INTRODUCTION

Worldwide spread of high-yield domestic breeds has been undermining genetic diversity gradually (Woelders et al., 2006), especially in the field of livestock and poultry (Ma and Wu, 2001). In the later half the 20th century, 740 livestock and poultry breeds had become extinct, 531 were endangered, 1,092 were seriously endangered, and only 2,395 breeds were relatively safe (Pattison et al., 2007). Ma and Wu (2001) reported that among the 231 livestock and poultry breeds in China, 99 were threatened at different degrees. It would be a tremendous loss for the theoretical research of genomic resources and life sciences if these breeds had not been conserved in any form before their complete extinguishment (Guan and Ma, 2003).

The conservation of endangered breeds as live animals, semen, embryos, or DNA were all strategies for governments and breeding organizations to maintain genetic diversity, which could subsequently be used for breeding and production in agriculture (Falge et al., 1996; Wu, 1999). In addition to the methods outlined above, establishment of somatic cell banks using cryopreservation techniques was a novel and effective approach to conserve and maintain the diversity of domestic animals (Guan and Ma, 2003).

Qingyuan partridge chicken originated from Guangdong, China. As one of the broiler-type breed, these chickens have a typical body conformation of 1 cuneate, 2 slim, and 3 spotty. One cuneate denotes that the hen's body is shaped like a wedge. Two slim denotes that the head and feet are slim. Three spotty denotes that the hen's back feather mainly have 3 kinds of colors, yellow, coffee, and brown. Qingyuan partridge chicken was listed as one of the 78 nationally protected domestic animals by the Chinese government in 2000.

In this study, a fibroblast line of the nationally protected Qingyuan partridge chicken was established for

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the study of their biological characteristics. The aim is to preserve the breed resources at cell level and in the meantime to provide valuable material for reviving endangered breeds by cloning and for other genomic research.

MATERIALS AND METHODS

Cell Culture

Eight-day-old embryos were isolated from Qingyuan partridge chicken eggs then rinsed and cropped into 1-mm³ pieces, which were subsequently seeded in a flask and cultured at 37°C for 4 to 5 h in an incubator with 5% CO₂. Modified Eagle's medium (MEM) containing 10% fetal bovine serum (FBS) was added into inverted flasks. The culture was observed every 24 h for cells migrated from the tissue pieces. Cells at the logarithmic phase were harvested. The cell sheet was rinsed twice with PBS to remove all traces of trypsin inhibitors in the serum and 0.05% trypsin solution was added. The flasks were inverted and incubated for 3 min at 37°C before being turned over. Subsequently, the flasks were shaken gently to detach cells from their walls and the medium was added to the cell suspension to stop the trypsinization. The cells were split into new flasks under the ratio 1:2 or 1:3 and incubated at 37°C with 5% CO₂ (Hay, 1992).

Cryopreservation and Recovery

After 3 to 5 passages, the cells were harvested according to the subculture protocol described above and then centrifuged at 168 × *g* for 8 min to form a pellet. The supernatant was removed and the cell pellet was resuspended in a freezing media containing 10% dimethylsulfoxide, 50% FBS, and 40% MEM to reach a final cell density of 8 × 10⁶ viable cells/mL. The cell suspension was aliquoted into sterile cryovials labeled with breed, sex, passage number, and the date: kept at 4°C for 30 min; put into a programmed cryopreservation system for 12 h; and finally transferred to liquid nitrogen for long-term storage.

At recovery, the vials were taken from the liquid nitrogen and thawed in a 42°C water bath and then transferred into a flask with 10 mL of MEM containing 10% FBS. The cells were cultured at 37°C with 5% CO₂. The medium was renewed 24 h later (Hay, 1992; Stacey and Masters, 2008).

Epithelial Cell Detection

For testing epithelial cell contamination, cryopreserved cells were thawed and plated on 6-well plates. The cells were harvested and total RNA was extracted. We designed PCR primer pairs (primer A: CTTCCGCAAATACTGTGGACAA and primer B: GCAATCTGGGCTTGTAGGC) for amplifying 1 coding region of

Homo sapiens keratin 18, which was expressed in epithelial cells. Complementary DNA synthesis from total RNA extracts of cells and the PCR were carried out. In the control group, total RNA of chicken liver was extracted and then subjected to reverse transcription PCR.

Viability Assay

Viabilities before cryopreservation and after recovery were detected using the trypan blue exclusion test. Cells were seeded in 6-well plates and 1,000 cells were counted for calculation (Strober, 2001).

Growth Kinetics

Cells were plated on 24-well microplates with a density of 4 × 10⁴ cells/well and were cultured for 7 d. Cells were counted every 24 h until the plateau phase. Mean values were used to plot a growth curve and to calculate the population doubling time (Kozak, 1992).

Microorganism Detection

Cells were cultured in antibiotic-free medium. Bacterial, fungal, and yeast contamination was observed within 3 d as described by Stacey (1997). Under normal culture conditions, cells were selected randomly for cytopathogenic examination using phase-contrast microscopy following Hay's hemadsorption protocol (Hay, 1992). *Mycoplasma* contamination was detected by a modified Hoechst 33258 (Invitrogen Corp., Carlsbad, CA) staining procedure as described by Battaglia et al. (1980).

Chromosome and Karyotype Analysis

Cells were cultured in media containing colcemid for 6 h at 37°C and were then harvested. Cell chromosomes were prepared using a slight modification of the method of Frederic (1961). A Qingyuan partridge chicken cell karyotype was prepared according to the method of Masabanda et al. (2004) and chromosomes were counted. Ten photographs of representative chromosome sets at metaphase were selected. The long and short arms of the 10 pairs of macrochromosomes were measured. The chromosomal parameters of relative length, centromere index, and kinetochore type were calculated as described by Lavania and Sharma (1981).

Isoenzyme Polymorphism

The isoenzyme patterns of lactate dehydrogenase (LDH) and malate dehydrogenase (MDH) from Qingyuan partridge chicken embryonic (QPCE) fibroblasts were analyzed using modified PAGE as described by Zeng and Chen (1997). Cells were harvested and pro-

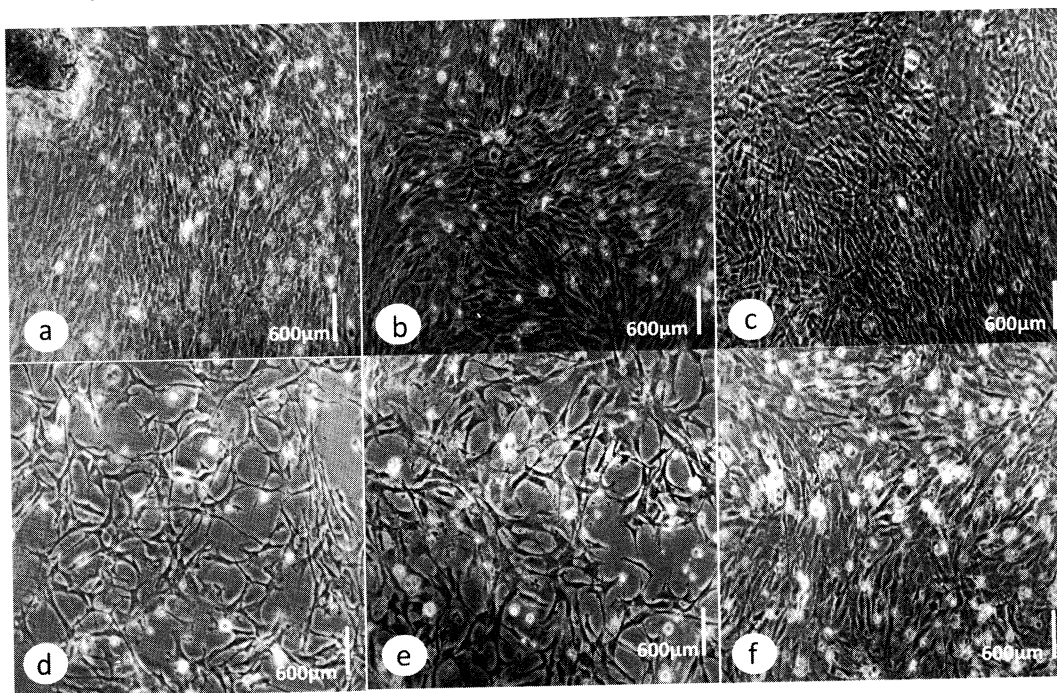


Figure 1. Morphology of Qingyuan partridge chicken embryonic fibroblasts. a) Primary cultured cells from Qingyuan partridge chicken embryo, b) fibroblasts after 1 passage, c) fibroblasts before cryopreservation, d) fibroblasts after thawing and cultured at 12 h, e) fibroblasts after thawing and cultured at 24 h, f) fibroblasts after thawing and cultured at 48 h. Scale bar = 600 μm . Color version available in the online PDF.

tein extraction solution was added after the cell density was adjusted to 5×10^7 cells/mL. These samples were centrifuged, and after the supernatant was removed, they were stored in aliquots at -80°C . An equal volume of 40% sucrose solution and 2.5 μL of loading buffer was added to the samples and mixed before performing the PAGE. The mobility of different patterns was represented by the relative mobility fronts, which were calculated as the ratio of the migration distance of the isozyme band to that of the bromochlorophenol blue.

Expression of 6 Fluorescent Proteins in QPCE Fibroblasts

Cells were plated on 24-well microplates and transfected with the plasmid DNA of 6 fluorescent proteins (pEGFP-N3, pEGFP-C1, pDsRed-N1, pEYFP-N1, pECFP-N1, and pECFP-mito) with Lipofectamine 2000 (Invitrogen Corp.), according to the method of Kain et al. (1995). The medium was renewed 6 h after transfection, and the cells were observed 24 h, 48 h, 72 h, 2 wk, and 1 mo after transfection using a Nikon TE-2000-E inverted confocal microscope (Nikon Corporation, Tokyo, Japan) with excitation wavelengths of 405, 488, and 543 nm to determine the transfection efficiency and the morphology of positive cells. For each experimental group, images were captured from 10 visual fields to determine the total and positive cell counts in each field for the calculation of transfection efficiencies (Guan et al., 2006).

RESULTS

Cell Morphology

Epithelial-like and fibroblast-like cells migrated from tissue pieces 3 to 6 d after explanting (Figure 1a). As time passed, cells continued to proliferate and were subcultured when they were 90% confluent (Figure 1b). After subculture, the fibroblasts proliferated rapidly and gradually outgrew and excluded their epithelial counterparts. Purified fibroblasts were then obtained after 1 to 2 passages. The cells were cryopreserved after 3 passages (Figure 1c). Fibroblasts were whirlpool-migrating, frame-like, or radiating shapes with turgor

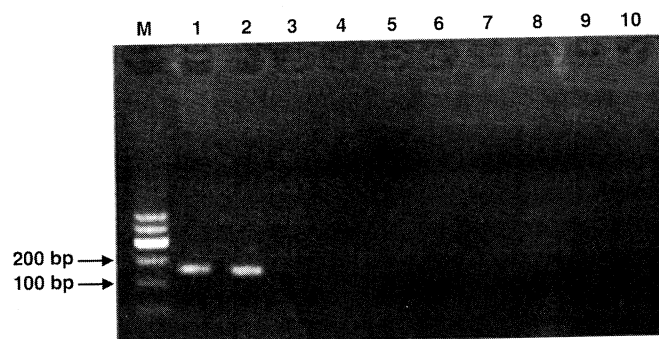


Figure 2. Polymerase chain reaction test for epithelial cell contamination. Lanes 1 and 2 = positive control; lanes 3 to 10 = PCR products from different Qingyuan partridge chicken embryonic (QPCE) fibroblast samples; M = DNA marker I. Result indicated that there was no epithelial cell contamination in the QPCE fibroblasts line.

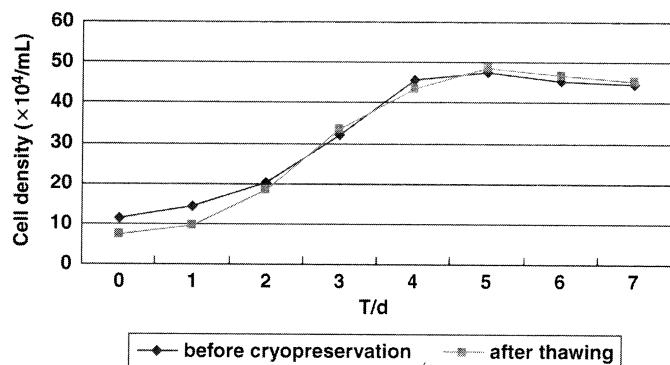


Figure 3. The growth curve of the Qingyuan partridge chicken embryonic fibroblast cell line. The growth curve was a typical “S” shape. Lag of around 24 h was observed after cells were seeded. Then, cells proliferated and entered the logarithmic phase. The population doubling time was approximately 72 h. From d 5, cells reached the plateau phase. T/d = times per day. Color version available in the online PDF.

vitalis cytoplasm. In addition, they were viable and grew well after they were thawed (Figure 1d, e, and f). There was no epithelial cell contamination when the cells were subjected to keratin 18 PCR testing (Figure 2). In the control group, there was a coding region section of keratin 18 about 260 bp in size (Figure 2).

Cell Viability

A viable cell has clear cytoplasm, whereas a non-viable cell exhibits a blue cytoplasm when dyed with trypan blue (Strober, 2001). The mean viability before cryopreservation was 96.8% and it was 92.6% after recovery. The results indicated that cells were viable, and the culture conditions were appropriate and cells were not significantly affected by cryopreservation.

Growth Kinetics

The growth curve of the QPCE fibroblast line before cryopreservation and after recovery displayed a typical

“S” shape (Figure 3). A lag phase of approximately 24 h was observed after the cells were plated, which corresponded to the recovery period against trypsin damage. Afterward, the cells proliferated rapidly and entered the exponential growth phase until the stationary phase after about 5 d. From d 5, growth plateaued and the population began to degenerate. The population doubling time calculated from the curve was about 72 h.

Microbial Analysis

Tests for contamination from bacteria, fungi, and yeasts were negative and no other microorganisms were observed in the media. The existence of viruses was disproved by the hemadsorption test. Under a fluorescence microscope after Hoechst 33258 staining, fibroblast nuclei appeared as blue ellipses, indicating that the newly established fibroblast line was *Mycoplasma*-negative (Figure 4a). To the contrary, particles of fluorescent mycoplasmal DNA and small filamentous surface fragments were present in the positive control (Figure 4b).

Chromosome and Karyotype Analysis

The chromosome number of Qingyuan partridge chicken was $2n = 78$, consisting of 10 pairs of macrochromosomes and 29 pairs of microchromosomes. The sex chromosome type was ZZ (male)/ZW (female) (Figure 5). Chromosome 1 and sex chromosome Z were metacentric; chromosomes 2, 4, 9, and sex chromosome W were submetacentric; and chromosomes 3, 6, 7, 8, and 10 were subtelocentric (Table 1). In size, chromosome 1 was the largest, chromosome 4 was close to chromosome 3, chromosome Z was approximately as large as chromosome 5, and chromosome W was of similar size to chromosome 9. Chromosomes were counted at first and third passages and 93.4% of the cells analyzed were diploid ($2n = 78$), implying that the cell line was reproducibly diploid in vitro.

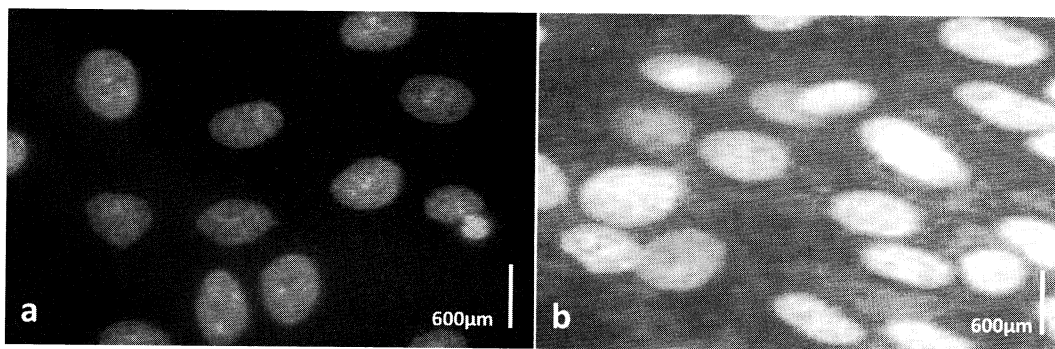


Figure 4. *Mycoplasma* contamination for the Qingyuan partridge chicken embryonic fibroblast cell line. a) Cells after thawing were stained with Hoechst 33258 (Invitrogen Corp., Carlsbad, CA); under a fluorescence microscope, fibroblast nuclei appeared as blue ellipses, showing that the established cell line was *Mycoplasma*-negative; b) positive control of *Mycoplasma* contamination stained with Hoechst 33258. Scale bar = 600 μ m. Color version available in the online PDF.

Table 1. Chromosome parameters of Qingyuan partridge chicken (male)¹

Chromosome number	Chromosome parameter		
	Relative length (%)	Centromere index (%)	Centromere morphology ²
1	24.52 ± 0.014	43.75 ± 0.001	M
2	18.39 ± 0.095	33.33 ± 0.004	SM
3	11.49 ± 0.052		ST
4	10.34 ± 0.012	25.93 ± 0.012	SM
Z	9.96 ± 0.021	50.12 ± 0.045	M
6	8.43 ± 0.009		ST
7	6.13 ± 0.078		ST
8	3.06 ± 0.062		ST
9	3.12 ± 0.023	50.78 ± 0.006	M
10	2.34 ± 0.012		ST

¹Mean ± SE.²M = metacentric chromosome; SM = submetacentric chromosome; ST = subtelocentric chromosome.

Isoenzyme Polymorphism

Lactate dehydrogenase and MDH isoenzyme patterns were obtained from QPCE fibroblasts and were compared with those of other 4 breeds. Five bands, LDH1, LDH2, LDH3, LDH4, and LDH5, were apparently arranged from anode to cathode (Figure 6). The patterns of MDH exhibited 3 bands (Figure 6). One band of mitochondrial MDH was found near the cathode and 2 bands of cytosolic MDH were located near the anode. The relative mobility fronts of LDH (Table 2) and MDH (Table 3) were calculated. Different species and breeds have distinctive isoenzyme patterns and each band has a different relative mobility. Those results indicated that the genetic characteristics of QPCE fibroblasts were stable *in vitro*.

Expression of 6 Fluorescent Protein Genes in QPCE Fibroblasts

Six fluorescent proteins, pEGFP-C1, pEGFP-N3, pDsRed-N1, pEYFP-N1, pECFP-N1, and pECFP-mito, were highly expressed in QPCE fibroblasts (Figure 7). Positive cells were observed at 24 h, 48 h, 72 h, 2 wk, and 1 mo after transfection. The green, yellow, and cyan fluorescence could be observed throughout the cytoplasm and nuclei of control cells except in cryptomere vesicle, whereas red fluorescence was mostly shown in cytoplasm with a perforated pattern (Figure 8). The strongest fluorescent intensities and the highest transfection efficiencies of the 6 fluorescent proteins appeared at 48 h after transfection between 15.1 and 39.8% (Figure 9). The expression of the red fluorescent



Figure 5. Chromosome at metaphase and karyotype of Qingyuan partridge chicken (male). Cells were grown in medium containing colcemid for 6 h at 37°C, then cell chromosomes were prepared: the karyotype of Qingyuan partridge chicken consisted of 10 pairs of macrochromosomes and 29 pairs of microchromosomes. The sex chromosome type was ZZ (male)/ZW (female).

Table 2. Relative mobility fronts of lactate dehydrogenase (LDH) in different species and breeds

Breed	LDH relative migration distance (%)				
	LDH1	LDH2	LDH3	LDH4	LDH5
Beijing duck	82.85	74.29	68.57	62.86	51.43
Qingyuan partridge chicken	85.71	73.86	65.71	54.29	45.71
Silkie bantam	80.19	71.43	62.86	54.88	46.45
Wencheng chicken	81.54	68.57	61.58	53.96	42.86
White Leghorn chicken	79.32	65.71	54.29	42.86	34.29
White ear lobe chicken	77.14	69.11	62.12	51.43	43.12

protein (pDsRed-N1) was maximal, and the cyan fluorescent proteins (pECFP-N1 and pECFP-mito) were significantly lower in content than those of the green, yellow, and red fluorescent proteins ($P < 0.01$). Most positive cells shrunk and detached 72 h after transfection. The number of cells expressing fluorescent proteins decreased and the fluorescence intensity gradually faded and vanished 7 d after transfection, although some cells still expressed fluorescent proteins after 2 wk and even after 1 mo. By screening G418 resistance and monoclonal culture for 1 mo, we obtained 6 positive cell strains that expressed enhanced green fluorescent protein stably (Figure 8g, h, and i). These results suggested that the expression of exogenous genes has no obvious effects on the growth and proliferation of the QPCE fibroblast.

DISCUSSION

With the development of science and technology, the roles of fibroblast lines will become increasingly prominent, and there will be currently unforeseen applications. Somatic cells, such as diploid fibroblasts, can be used for nuclear donors, and they can be subjected to genetic manipulation in vitro (Campbell et al., 1996; Polejaeva et al., 2000; Yin et al., 2005; Oh et al., 2008; Thongphakdee et al., 2008). Chicken fibroblasts can also be used for the preparation of live vaccine and can provide feeder layers for stem cell culture (Hornemann et al., 2003; Liu et al., 2009). In the present study, using a characteristic Chinese chicken breed to initiate the establishment of cell lines and the study of their biological characteristics, our aim is to cryopreserve this nationally protected genomic resource for the purposes

of reviving endangered breeds by nuclear transfer. The QPCE fibroblasts were transfected with fluorescent exogenous genes and some were replicated, transcribed, translated, and modified, which will provide valuable materials for transgenic research.

The morphological observation indicated that the primary cells usually coexist with epithelial cells, which may grow either in groups or scattered (Grout et al., 1990). Epithelial cells and fibroblasts have different tolerances to trypsin, so we can collect fibroblasts at appropriate trypsinization times. The fibroblasts were first detached from the flask while the epithelial cells remained adherent when being subcultured (Hay, 1992; Hodges, 2006; Morris, 2007), and a purified QPCE fibroblast line could be obtained by 1 to 2 passages.

Normal cells possessed stable chromosome numbers, shape, and structure. Therefore, karyotype analysis constitutes an effective method for distinguishing normal cells from variants, which could also be used for the identification of the sex and origin (Stacey, 1997; de Oliveira et al., 2005). Chicken chromosomes were categorized into 3 groups: a) macrochromosomes 1 to 5 and Z, b) medium-sized chromosomes 6 to 10, and c) 19 microchromosomes (de Oliveira et al., 2005). The genetic nature of the cells may be affected by in vitro culture after many passages; therefore, a minimal passage number is needed to protect them (Wu et al., 2008). The QPCE fibroblasts were cryopreserved within 3 passages. As difficult as the chromosomes of birds were to distinguish, 50 spreads of the first and third passages were sampled. The numbers of hypodiploid, diploid, and hyperdiploid chromosomes were counted, and the diploid rate was 93.4%, indicating that the cell line was genetically stable. By passage 5, the diploid accounted for approximately 91.6% of the population.

Table 3. Relative mobility fronts of malate dehydrogenase (MDH) in different species and breeds

Breed	MDH relative migration distance (%)		
	Mitochondrial MDH	Cytosolic MDH1	Cytosolic MDH2
White ear lobe chicken	31.43	60.12	68.57
Silkie bantam	28.57	61.86	67.14
White Leghorn chicken	22.86	65.71	71.43
Wencheng chicken	25.71	62.85	69.89
Qingyuan partridge chicken	20.36	59.23	70.23
Beijing duck	30.63	57.14	65.71

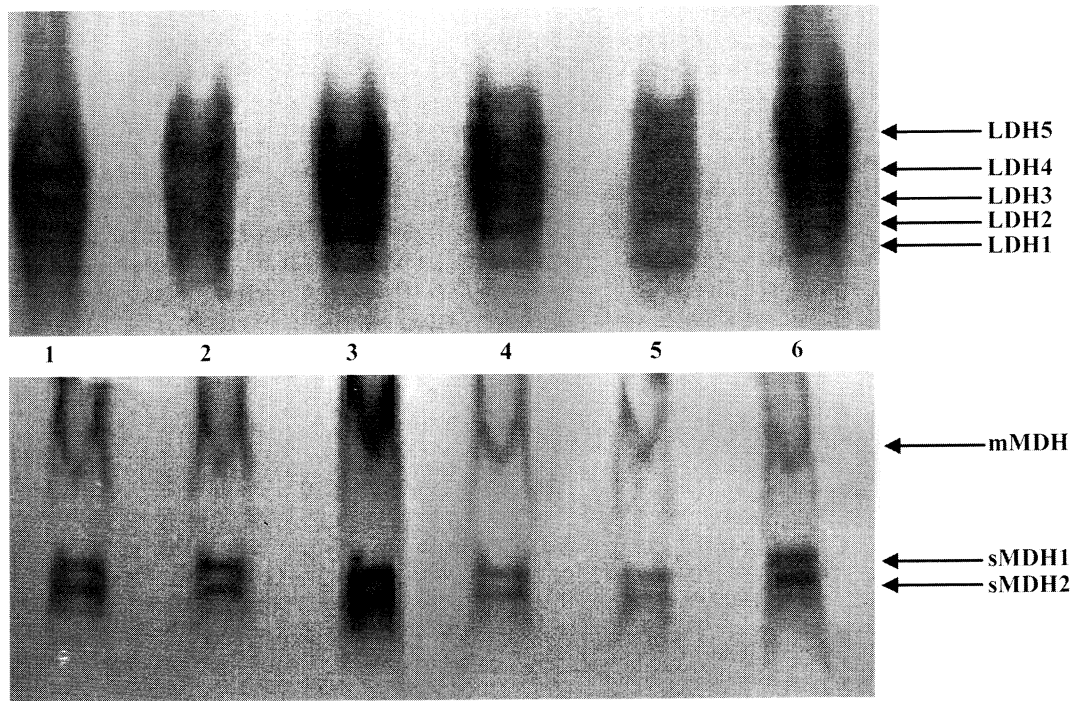


Figure 6. Lactate dehydrogenase (LDH) and malate dehydrogenase (MDH) isoenzymes for different species or breeds. Lactate dehydrogenase isoenzymes (upper panel) for Beijing duck, Qingyuan partridge chicken, Silkie bantam, Wencheng chicken, White Leghorn chicken, and white ear lobe chicken corresponding to lanes 1 to 6, respectively; MDH isoenzymes (lower panel) for white ear lobe chicken, Silkie bantam, White Leghorn chicken, Wencheng chicken, Qingyuan partridge chicken, and Beijing duck corresponding to lanes 1 to 6, respectively.

The Qingyuan partridge chicken karyotyping results are in accordance with the results from Frederic (1961), Zheng and He (2003), Masabanda et al. (2004), Xu et al. (2004), and Liu et al. (2006). There existed a low

rate of abnormal chromosomes, presumably as a result of chromosome loss or overlap during spread or chromosomal damage during in vitro culture and passage (Hay, 1992).

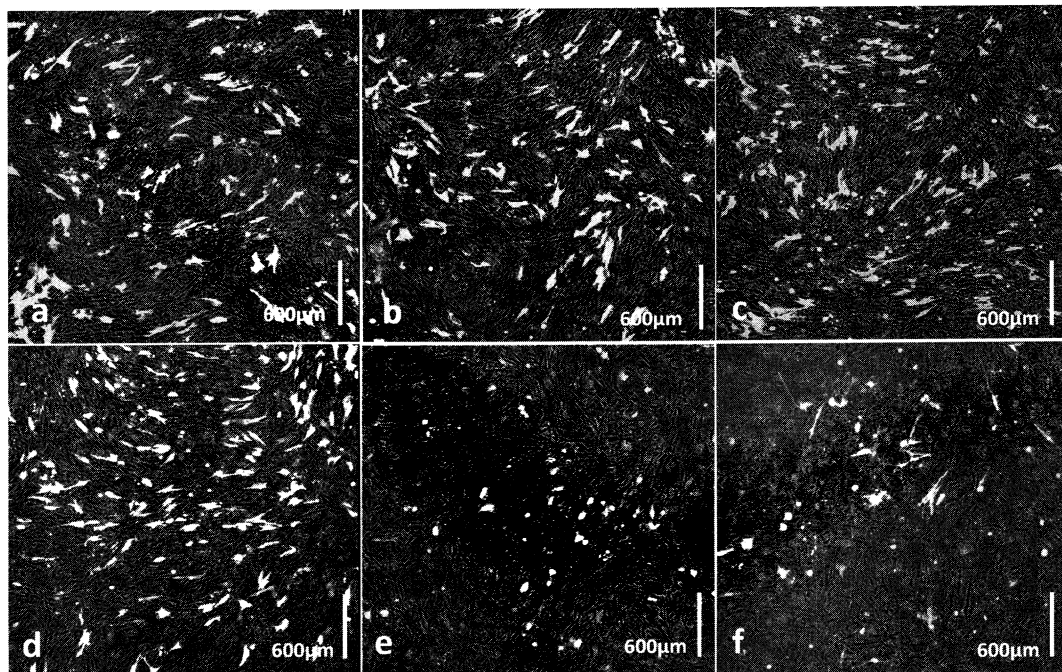


Figure 7. Comparative figures of 6 fluorescent proteins at 48 h after transfection in Qingyuan partridge chicken embryonic fibroblasts using a Nikon TE-2000-E inverted microscope (Nikon, Tokyo, Japan) with excitation wavelengths of 405, 488, and 543 nm to determine the transfection efficiency. Panels a, b, c, d, e, and f were the transfection results of pEGFP-C1, pEGFP-N3, pDsRed1-N1, pEYFP-N1, pECFP-N1, and pECFP-mito, respectively. Scale bar = 600 μm. Color version available in the online PDF.

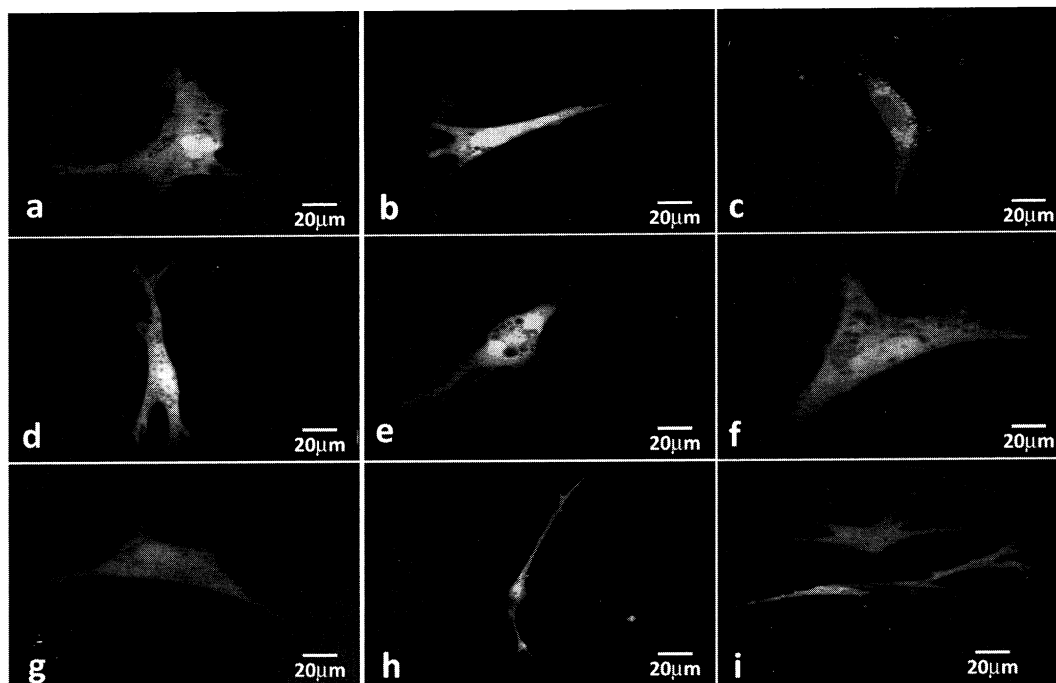


Figure 8. The expression and distribution of 6 fluorescent proteins in Qingyuan partridge chicken embryonic fibroblasts. a) The transfection result of pEGFP-C1 at 24 h after transfection; b) the transfection result of pEGFP-N3 at 48 h after transfection; c) the transfection result of pDsRed1-N1 at 72 h after transfection; d) the transfection result of pEYFP-N1 at 48 h after transfection; e and f) the transfection results of pECFP-N1 and pECFP-mito at 72 h after transfection; g, h, and i) the positive cell strains that expressed enhanced green fluorescent protein stably by screening G418 resistance and monoclonic culture for 1 mo. Scale bar = 20 μ m. Color version available in the online PDF.

Isoenzyme polymorphism was commonly selected to confirm the species origin of the cells, distinguish between normal and tumor cells, and act as a biochemical index of animal classification (Steube et al., 1995). American Type Culture Collection considered the bio-

chemical analysis of isoenzyme polymorphism to be a routine method for detecting intercellular contamination (Hay, 1992). In almost all vertebrate tissues examined, the enzyme LDH has been shown to consist of 5 distinct isoenzymes (Coffin and Hall, 1974). Us-

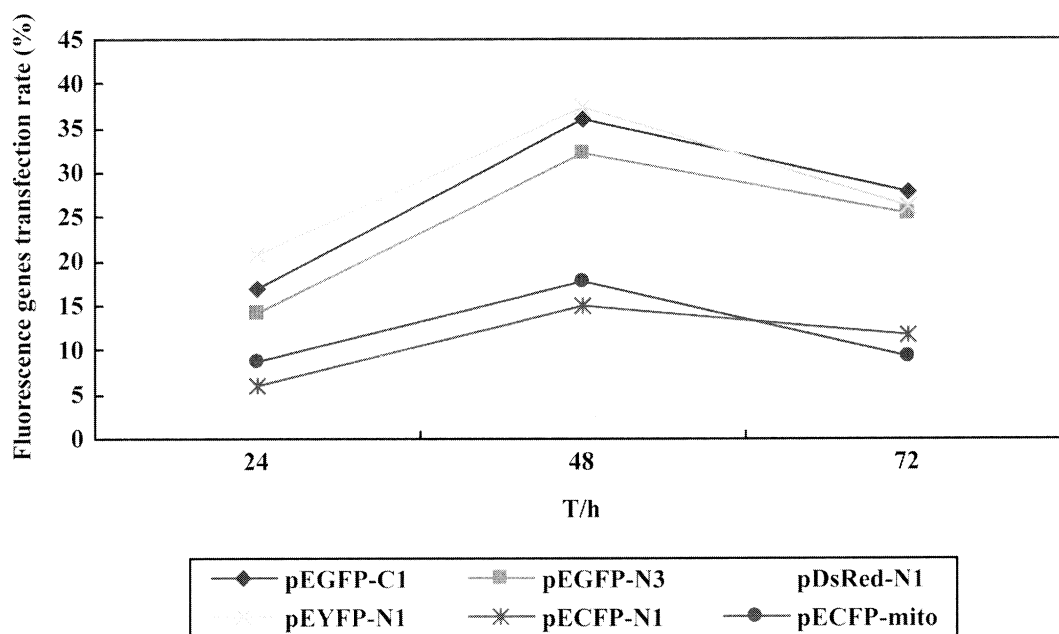


Figure 9. Transfection efficiencies of 6 fluorescence genes in the Qingyuan partridge chicken embryonic fibroblasts. The strongest fluorescence intensity and the highest transfection efficiency of the 6 fluorescent proteins appeared 48 h after transfection between 39.8 and 15.1%. The transfer efficiency of the red fluorescent protein (pDsRed1-N1) was maximal, whereas the cyan fluorescent proteins (pECFP-N1 and pECFP-mito) were significantly lower than those of the green, yellow, and red fluorescent proteins ($P < 0.01$). T/h = times per hours. Color version available in the online PDF.

ing isoelectric focusing at a pH range of 3.0 to 9.0, a good and clear separation of all 5 LDH isoenzymes in chicken organs was achieved by Heinova et al. (1999). Mitochondrial MDH and cytosolic MDH were both obtained during early and middle development (1 to 16 d) of chicken embryos and newly hatched chick (Ma and Qiu, 1995). In this study, we obtained isoenzymes for LDH and MDH and improved the American Type Culture Collection starch gel electrophoresis method. The QPCE fibroblast isoenzyme bands of LDH and MDH in QPCE cells were clear and the isoenzyme activities in vitro were similar to those in the original tissues. The results indicated that the genetic characteristics of QPCE fibroblasts were stable. It was applicable to preserve Qingyuan partridge chicken genetic materials by freezing their fibroblasts in liquid nitrogen for long-term storage.

Fluorescent proteins were widely applied as probes for intracellular dynamics and useful tools in super-resolution microscopy (Shaner et al., 2007). They were proven to be excellent tools for live-cell imaging studies (Muller-Taubenberger et al., 2006). In this study, the QPCE fibroblasts were transfected with plasmid fluorescent protein coding DNA and a significant fluorescent signal was observed, suggesting gene expression and in vitro protein localization in the cells were monitored. Under optimal conditions, transfection had no significant effects ($P > 0.05$) on cell morphology, growth, apoptosis rate, or reduplication status compared with untransfected control. The numbers of fluorescent cells decreased at 1 wk after transfection, but a few scattered cells remained positive after 1 mo, indicating that the exogenous genes in the fibroblasts were replicated, transcribed, translated, and modified. The fluorescent protein-positive cell strains observed here will provide donor cells for transgenic animal cloning. Thus, the QPCE 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.

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