

## Species identification of meat products using the cytochrome b gene

Hsing-Mei Hsieh,<sup>1</sup> Ph.D.; Chin-Cheng Tsai,<sup>2</sup> M.S.; Li-Chin Tsai,<sup>1</sup> M.S.; Hsiao-Ling Chiang,<sup>2</sup> M.S.; Nu-En Huang,<sup>2</sup> Ph.D.; Rocky Tai-Ping Shih,<sup>3</sup> M.D.; Adrian Linacre,<sup>4</sup> Ph.D.; James Chun-I Lee,<sup>5,\*</sup> Ph.D.

<sup>1</sup> Department of Forensic Science, Central Police University, 56 Shu Jen Road, Taoyuan 333, Taiwan ROC.

<sup>2</sup> Criminal Investigation Bureau, National Police Administration, Taiwan ROC.

<sup>3</sup> DADE Private Medical Examiner's Office, 17010 Dew Drop Lane, Houston, TX 77095-1249, USA.

<sup>4</sup> Forensic Science Unit, Department of Pure & Applied Chemistry, University of Strathclyde, Glasgow G1 1XW, UK.

<sup>5</sup> Department of Forensic Medicine, College of Medicine, National Taiwan University, No.1 Jen-Ai Road Section 1, Taipei 10051, Taiwan ROC.

Received: March 9, 2005 /Accepted: April 06, 2005

### ABSTRACT

The partial sequence (402 bp) of cytochrome b gene has been proved to be of value in the identification of animal species from unknown samples. In this study, the analysis of 402 bp sequences was used to confirm the materials of 87 processed meat products. There were 11 samples that did not conform to the original indicated materials. The inconsistent ratio was about 12.6 %. There were 9 and 10 haplotypes of 32 beef samples and 55 pork samples respectively. The genetic distances between intraspecies ranged 0.00 to 1.77 (pig), and 0.00 to 2.29 (cow). The genetic distances between interspecies (pig and cow) ranged 19.11 to 21.90. In this study, we have illustrated the value of a simple DNA test to identify the animal species used in processed meats and investigate the situation about the fraudulent misdescription of food contents on product labels in Taiwan markets. This system could also be used in the speciation of other biological products.

**Keywords:** cytochrome b gene, meat products, genetic distances

### Introduction

Consumers rarely have a problem identifying fresh meat when bought at markets or in shops. The particular color and shape of beef can be distinguished from pork or poultry when the meat is fresh. Processed meat, such as sausage, jerky, and canned foods, poses more of a problem as the product cannot be identified by bare eyes. In such cases where the meat is thought to be unrepresentative of the advertised product, a dispute between the consumer and the seller may arise.

In this study, a DNA test was used to screen random samples of processed meat products bought from supermarkets in Taiwan. Some techniques for DNA testing have been developed to identify the species in meat

products, such as CAPS (cleavable amplified polymorphic sequences) [1], Southern blotting by using species-specific satellite DNA as probes [2], RAPD (random amplification of polymorphic DNA) fingerprinting [3, 4], multiplex PCR [5, 6], quantitative intra-short interspersed element PCR [7] and sequencing the specific gene such as the mitochondrial 12S rRNA [8]. Processed meats are likely to contain degraded DNA, therefore mitochondrial DNA (mtDNA) was considered more suitable than nuclear DNA for this analysis. The cytochrome b gene on the mitochondria has been used successfully in species identification [9, 10, 11] and in taxonomic and phylogenetic studies [12, 13, 14]. However, the complete sequencing of the cytochrome b gene is time-consuming and laborious due to its size (1140 bp). Additionally

\* Corresponding author, e-mail: jimlee@ntumc.org

this large fragment is frequently not amplified from powdered samples or processed products, which may be of a highly degraded nature. The partial sequence (402 bp) of cytochrome b gene was evaluated and proved to be usable for animal identification recently [11]. In this study, the analysis of 402 bp sequences was used to confirm the identity of meat substances from 87 processed meat products. Genetic distances between intraspecies or inter-species were evaluated by GCG computer programs.

## Materials and methods

### *Sample sources and DNA extraction*

The meat samples of 87 processed products, numbered from A1 to A87, were collected from markets in Taiwan. These samples were labeled as the products of pork (44 samples) or beef (43 samples). DNA was extracted from 25 mg of each sample with QIAamp DNA Mini Kit (QIAGEN) and quantified with ultra-violet detection by spectrophotometer.

### *PCR amplifications and sequencing of PCR products*

The universal primers for PCR amplification were L14724 and H15149, as described by Kocher et al. [9] and Irwin et al. [10]. Numbering of these primers was according to the human mtDNA sequences [15]. The sequences of L14724 and H15149 were 5'-CGAAGCTTGATATGAAAACCATCGTTG-3' and 5'-AAACTGCAGCCCCTCAGAATGATATTTGTCCTCA-3' respectively. PCR amplification was performed in a 50  $\mu$ l of reaction mixture, which contained 10 ng genomic DNA, reaction buffer (10 mM Tris-HCl, pH 8.3, 2.5 mM MgCl<sub>2</sub>, 50 mM KCl, 0.1% (w/v) gelatin), 2.5 unit of VioTaq DNA polymerase (Viogene, USA) and 0.15  $\mu$ M each of primers. Amplification was conducted in a 480 Perkin-Elmer thermal cycler with the following conditions: initial denaturation at 95°C for 10 min followed by 35 cycles of 95°C for 45 sec, 50°C for 45 sec and 72°C for 90 sec, then 72°C for 10 min for further extension. Cycle sequencing of PCR products was also conducted in a 480 Perkin-Elmer thermal cycler with the following conditions: 25 cycles of 95°C for 45 sec, 50°C for 15 sec and 72°C for 4 min. Sequencing was performed using the primers L14724

or H15149 and the BigDye™ Terminator Kit (ABI PRISM™ BigDye™ Terminator Cycle Sequencing Ready Reaction Kit). The cycle sequencing products were separated by 5% denatured Long Ranger™ gel (FMC BioProducts, Rockland, Maine, USA) and detected using a PE Applied Biosystems 373A DNA sequencer.

### *Analysis of genetic distances*

Sequences were aligned using the PileUp program of GCG computer package (Wisconsin Package Version 10.3, Accelrys Inc, San Diego, CA), and the genetic distances were generated by Kimura's 2-parameter model of Genetic distances program.

## Results and discussion

The size of PCR products, using the primers L14724 and H15149, was 486 bp. This size was confirmed by DNA sequence analysis. Of the 486 bp, the cytochrome b gene comprised 402 bp. All of the sequences were compared with the sequences registered in GenBank and EMBL by GCG computer package. The most similar species and similarity of each sample are shown in Table 1. In 87 samples, there were 11 samples (A14, A15, A18, A32, A45, A50, A57, A63, A64, A65 and A78) which did not conform to the original indicated material. The ratio of inconsistent samples to whole samples was therefore approximately 12.6%. All the inconsistent samples were advertised as being beef but were found to be more closely related to pork, and the similarities of all these products with pig were higher than 99.5%. The sequence similarities of other consistent samples with the original indicated materials were all higher than 98%, and the sequence diversity (variations per 100 bases) was less than 3%. Our previous study showed that the percentage range of sequence diversity in the same species was from 0.25 to 2.74% (less than 3%), and that between the different species was from 5.97 to 34.83% [11].

There were 9 haplotypes found within the 32 beef samples and 10 haplotypes found within the 55 pork samples. The sequences of all these haplotypes were aligned by the PileUp program of the GCG computer package, and the consensus sequences were deduced by Pretty program.

	1	50
Consensus	ATGACCAACA TCCGAAAATC ACACCCACTA ATAAAAATTA TCAACAACGC	
A7	.....	.....
A19	.....	.....
Pig1	.....	.....
A9	.....	.....
A10	.....	.....
A62	.....	.....
A77	.....	.....
A6	.....	.....
A23	.....	.....
A41	.....	.....
Pig2	.....	.....
A60	.....	.....
A4	....t...t .t...g.. c..... .g .a...t..	
A74	....t...t .t...g.. c..... .g .a...t..	
A1	....t...t .t...g.. c..... .g .a...t..	
A59	....t...t .t...g.. c..... .g .a...t..	
Cow2	....t...t .t...g.. c..... .g .a...t..	
A2	....t...t .t...g.. c..... .g .a...t..	
A34	....t...t .t...g.. c..... .g .a...t..	
A27	....t...t .t...g.. c..... .g .a...t..	
A87	....t...t .t...g.. c..... .g .a...t..	
Cow1	....t...t .t...g.. c..... .g .a...t..	
A30	....t...t .t...g.. c..... .g .a...t..	

	101	150
Consensus	GTTCCCTCTT AGGCATCTGC CTAATCTTGC AAATCCTAAC AGGCCTGTTC	
A7	.....	.....
A19	.....	.....
Pig1	.....	.....
A9	.....	.....
A10	.....	.....
A62	.....	.....
A77	.....	.....
A6	.....	.....
A23	.....	.....
A41	.....	.....
Pig2	.....	.....
A60	.....	.....
A4	.....c .g .a..... .c .a..... .c..... .a...	
A74	.....c .g .a..... .c .a..... .c..... .a...	
A1	.....c .g .a..... .c .a..... .c..... .a...	
A59	.....c .g .a..... .c .a..... .c..... .a...	
Cow2	.....c .g .a..... .c .a..... .c..... .a...	
A2	.....c .g .a..... .c .a..... .c..... .a...	
A34	.....c .g .a..... .c .a..... .c..... .a...	
A27	.....c .g .a..... .c .a..... .c..... .a...	
A87	.....c .g .a..... .c .a..... .c..... .a...	
Cow1	.....c .g .a..... .c .a..... .c..... .a...	
A30	.....c .g .a..... .c .a..... .c..... .a...	

	51	100
Consensus	ATTCATTGAC CTCCCAGCCC CCTCAAACAT TTCATCATGA TGAACCTTCG	
A7	.....	.....
A19	.....	.....
Pig1	.....	.....
A9	.....	.....
A10	.....	.....
A62	.....	.....
A77	.....	.....
A6	.....	.....
A23	.....	.....
A41	.....	.....
Pig2	.....	.....
A60	.....	.....
A4	.....c... .t..... .a..... .t.....	
A74	.....c... .t..... .a..... .t.....	
A1	.....c... .t..... .a..... .t.....	
A59	.....c... .t..... .a..... .t.....	
Cow2	.....c... .t..... .a..... .t.....	
A2	.....c... .t..... .a..... .t.....	
A34	.....c... .t..... .a..... .t.....	
A27	.....c... .t..... .a..... .g .t.....	
A87	.....c... .t..... .a..... .g .t.....	
Cow1	.....c... .t..... .a .g..... .g .t.....	
A30	..... .t..... .a..... .g .t.....	

	151	200
Consensus	TTAGCAATAC ATTACACATC AGACACAACA ACAGC.TTCT CATCAGTTAC	
A7	.....	.....
A19	.....	.....
Pig1	.....	.....
A9	.....	.....
A10	.....	.....
A62	.....	.....
A77	.....	.....
A6	.....	.....
A23	.....	.....
A41	.....	.....
Pig2	.....	.....
A60	.....	.....
A4	c..... .c..... c..... .a..... .c .t.....	
A74	c..... .c..... c..... .a..... .c .t.....	
A1	c..... .c..... c..... .a..... .c .t.....	
A59	c..... .c..... c..... .a..... .c .t.....	
Cow2	c..... .c..... c..... .a..... .c .t.....	
A2	c..... .c..... c..... .a..... .c .t.....	
A34	c..... .c..... c..... .a..... .c .t.....	
A27	c..... .c..... c..... .a..... .c .t.....	
A87	c..... .c..... c..... .a..... .c .t.....	
Cow1	c..... .c..... c..... .a..... .c .t.....	
A30	c..... .c..... c..... .a..... .c .t.....	

201 250

Consensus .CACATCTGT CGAGACGTAA ATTACGGATG AGTTATTCCG TACCTACATG

A7 a.....t.....t.....

A19 a.....t.....t.....

Pig1 a.....t.....t.....

A9 a.....t.....t.....

A10 a.....t.....t.....

A62 a.....t.....t.....

A77 a.....t.....t.....

A6 a.....t.....t.....

A23 a.....t.....t.....

A41 a.....t.....t.....

Pig2 a.....t.....t.....

A60 a.....t.....t.....

A4 c..t.....c.....g..c.....c.....a.c.c.c.a..a.....c.

A74 c..t.....c.....g..c.....c.....a.c.c.c.a..a.....c.

A1 c..t.....c.....g..c.....c.....a.c.c.c.a..a.....c.

A59 c..t.....c.....g..c.....c.....a.c.c.c.a..a.....c.

Cow2 c..t.....c.....g..c.....c.....a.c.c.c.a..a.....c.

A2 c..t.....c.....g..c.....c.....a.c.c.c.a..a.....c.

A34 c..t.....c.....g..c.....c.....a.c.c.c.a..a.....c.

A27 c..t.....c.....g..c.....c.....a.c.c.c.a..a.....c.

A87 c..t.....c.....g..c.....c.....a.c.c.c.a..a.....c.

Cow1 c..t.....c.....g..c.....c.....a.c.c.c.a..a.....c.

A30 c..t.....c.....g..c.....c.....a.c.c.c.a..a.....c.

301 350

Consensus GGCCTATATT ACGGATCCTA TATATTCCTA GAAACATGAA ACATTGGAGT

A7 ..t.....c.....t.....

A19 ..t.....c.....t.....

Pig1 ..t.....c.....t.....

A9 ..t.....c.....t.....

A10 ..t.....c.....t.....

A62 ..t.....c.....t.....

A77 ..t.....c.....t.....

A6 ..t.....c.....t.....

A23 ..t.....c.....t.....

A41 ..t.....c.....t.....

Pig2 ..t.....c.....t.....

A60 ..t.....c.....t.....

A4 ..t.....c.....g..t..c.ct..t.....t.....

A74 ..t.....c.....g..t..c.ct..t.....t.....

A1 ..t.....c.....g..t..c.ct..t.....t.....

A59 ..t.....c.....g..t..c.ct..t.....t.....

Cow2 ..t.....c.....g..t..c.ct..t.....t.....

A2 ..t.....c.....g..t..c.ct..t.....t.....

A34 ..t.....c.....g..t..c.ct..t.....t.....

A27 ..t.....c.....t..g..t..c.ct..t.....t..c.....

A87 ..t.....c.....t..g..t..c.ct..t.....t..c.....

Cow1 ..t.....c.....t..g..t..c.ct..t.....t..c.....

A30 ..t.....c.....t..g..t..c.ct..t.....t..c.....

251 300

Consensus CAAACGGAGC ATCCATGTT. TTTATTTGCC TATTATCCA CGTAGGCCGA

A7 .....a.....c.....

A19 .....c.....

Pig1 .....a.....c.....

A9 .....a.....c.....

A10 .....a.....c.....

A62 .....c.....

A77 .....c.....

A6 .....c.....

A23 .....c.....

A41 .....c.....

Pig2 .....c.....

A60 .....c.....

A4 .....t..a.....t.....c.....t.....at..g.....a...

A74 .....g..t..a.....t.....c.....t.....at..g.....a...

A1 .....t..a.....t.....c.....t.....at..g.....a...

A59 .....t..a.....t.....c.....t.....at..g.....a...

Cow2 .....t..a.....t.....c.....t.....at..g.....a...

A2 .....t..a.....t.....c.....t.....at..g.....a...

A34 .....t..a.....t.....c.....t.....at..g.....a...

A27 .....t..a.....t.....c.....t.....at..g.....a...

A87 .....t..a.....t.....c.....t.....at..g.....a...

Cow1 .....t..a.....t.....c.....t.....at..g.....a...

A30 .....t..a.....t.....c.....t.....at..g.....a...

351 400

Consensus AGTCCTACTA TTTACCGTTA TAGCAACAGC CTTCATAGG. TACGTCCTGC

A7 .....c.....

A19 .....c.....

Pig1 .....c.....

A9 .....c.....

A10 .....c.....

A62 .....c.....

A77 .....c.....

A6 .....c.....

A23 .....c.....

A41 .....c.....

Pig2 .....c.....

A60 .....c.....

A4 .....a.....t..g..c.c.a.a.a.....c.....a..t.....a.....a.

A74 .....a.....t..g..c.c.a.a.a.....c.....a..t.....a.....a.

A1 .....a.....t..g..c.c.a.a.a.....c.....a..t.....a.....a.

A59 .....a.....t..g..c.c.a.a.a.....c.....a..t.....a.....a.

Cow2 .....a.....t..g..c.c.a.a.a.....c.....a..t.....a.....a.

A2 .....a.....c..g..c.c.a.a.a.....c.....a..t.....a.....a.

A34 .....a.....t..g..c.c.a.a.a.....c.....a..t.....a.....a.

A27 .....a.....t..g..c.c.a.a.a.....c.....a.....a.....a.

A87 .....a.....t..g..c.c.a.a.a.....c.....a.....a.....a.

Cow1 .....a.....t..g..c.c.a.a.a.....c.....a.....a.....a.

A30 .....a.....t.....c.c.a.a.a.....c.....a.....a.....a.

401	
Consensus	CC
A7	..
A19	..
Pig1	..
A9	..
A10	..
A62	..
A77	..
A6	..
A23	..
A41	..
Pig2	..
A60	..
A4	.a
A74	.a
A1	.a
A59	.a
Cow2	.a
A2	.a
A34	.a
A27	.a
A87	.a
Cow1	.a
A30	.a

**Fig.1** The sequences of all the haplotypes in this study were aligned by the PileUp program of GCG computer package, and the consensus sequences were deduced by the Pretty program. A dot '.' indicates the same base as the consensus sequence. Pig1, Pig2, Cow1 and Cow2 were the respective sequences extracted from GenBank (AF034253, AF304200, D34635 and J01394 respectively). There are four nucleotide differences between the sequences of Pig1 and Pig2, and five between Cow1 and Cow2 in 402 bp.

The genetic distances between intraspecies or inter-species were generated by Kimura's 2-parameter model. The distances are shown in Table 2. Number 1 to 12 represented the sequences of pig and pork samples, they were A7, A19, Pig1, A9, A10, A62, A77, A6, A23, A41, Pig2 and A60 respectively. Number 13 to 23 represented the sequences of cow and beef samples, they were A4, A74, A1, A59, Cow2, A2, A34, A27, A87, Cow1 and A30 respectively. The genetic distances between intraspecies, ranged from 0.00 to 1.77 (pig), and 0.00 to 2.29 (cow). The genetic distances between interspecies (pig and cow) ranged 19.11 to 21.90. The value represented the evolutionary distance between two sequences. Our previous study showed that the genetic distance between the different species was from 6.33 to 40.59 [11].

The fraudulent misdescription of food contents on

product labels is a widespread problem, particularly with high added-value products. It could become a criminal problem especially when the materials were from the conservation animals. Some of the techniques and molecular markers have been successfully applied for detection of food contents. At least, these items include the speciation of meats, fish and fruit in processed food products [16]. We have illustrated the value of a simple DNA test to identify the animal species used in processed meats and investigate the situation about the fraudulent misdescription of food contents on product labels in Taiwan markets. The information from this test could support the law enforcement to decrease the fraudulent misdescription situation. This system could also be used in the speciation of other biological products.

## References

1. R. Meyer, U. Candrian, J. Luthy, Detection of pork in heated meat products by the polymerase chain reaction, *J. AOAC Int.* 77 (1994) 617-22.
2. D.J. Hunt et al., Identification of the species of origin of raw and cooked meat products using oligonucleotide probes, *Food Chem.* 60 (1997) 437-42.
3. I. Martinez, I.M. Yman, Species identification in meat products by RAPD analysis, *Food Res. Intern.* 31 (1998) 459-66.
4. R. Saez, Y. Sanz, F. Toldra, PCR-based fingerprinting techniques for rapid detection of animal species in meat products, *Meat Sci.* 66 (2004) 659-65.
5. T. Matsunaga, K. Chikuni, R. Tanabe, S. Muroya, K. Shibata, J. Yamada, Y. Shinmura, A quick and simple method for the identification of meat species and meat products by PCR assay, *Meat Sci.* 51 (1999) 143-8.
6. A. Dalmaso, E. Fontanella, P. Piatti, T. Civera, S. Rosati, M.T. Bottero, A multiplex PCR assay for the identification of animal species in feedstuffs, *Mol. Cell. Probes* 18 (2004) 81-7.
7. J.A. Walker, D.A. Hughes, B.A. Anders, J. Shewale, S.K. Sinha, M.A. Batzer, Quantitative intra-short interspersed element PCR for species-specific DNA identification, *Anal. Biochem.* 316 (2003) 259-69.
8. P.S. Girish, A.S.R. Anjaneyulu, K.N. Viswas, M. Anand, N. Rajkumar, B.M. Shivakumar, S. Bhaskar, Sequence analysis of mitochondrial 12S rRNA gene can identify meat species, *Meat Sci.* 66 (2004) 551-6.
9. T.D. Kocher, W.K. Thomas, A. Meyer, S.V. Edwards,

- S. Paabo, F.X. Villablanca, A.C. Wilson, Dynamics of mitochondrial DNA evolution in mammals: amplification and sequencing with conserved primers, *Proc. Natl. Acad. Sci. USA* 86 (1989) 6196-200.
10. D.M. Irwin, T.D. Kocher, A.C. Wilson, Evolution of the cytochrome b gene of mammals, *J. Mol. Evol.* 32 (1991) 128-44.
  11. H.M. Hsieh, H.L. Chiang, L.C. Tsai, S.Y. Lai, N.E. Huang, A. Linacre, J.C.I. Lee, Cytochrome b gene for species identification of the conservation animals, *Forensic Sci. Int.* 122 (2001) 7-18.
  12. R. Kuwayama, T. Ozawa, Phylogenetic relationships among European red deer, wapiti, and sika deer inferred from mitochondrial DNA sequences, *Mol. Phylogenet. Evol.* 15 (2000) 115-23.
  13. R.O. Polziehn, C. Strobeck, A phylogenetic comparison of red deer and wapiti using mitochondrial DNA, *Mol. Phylogenet. Evol.* 15 (2002) 342-56.
  14. X.J. Shen, S. Ito, M. Mizutani, Y. Yamamoto, Phylogenetic analysis in chicken breeds inferred from complete cytochrome b gene information, *Biochem. Genet.* 40 (2002) 129-41.
  15. S. Anderson, A.T. Bankier, B.G. Barrell, M.H.L. de Bruijn, A.R. Coulson, J. Drouin, I.C. Eperon, D.P. Nierlich, B.A. Roe, F. Sanger, P.H. Schreier, A.J.H. Smith, R. Staden, I.G. Young, Sequence and organization of the human mitochondrial genome, *Nature* 290 (1981) 457-65.
  16. M. Woolfe, S. Primrose, Food forensics: using DNA technology to combat misdescription and fraud, *Trends Biotechnol.* 22 (2004) 222-6.

Table 1 The results of the most similar species and similarity of 87 samples searched in GenBank and EMBL databases. Samples in bold indicate inconsistent results observed

Sample no.	Original indicated sample name	The most similar species	Similarity	Sample no.	Original indicated sample name	The most similar species	Similarity
A1	Beef	Beef	100.0 %	A28	Pork	Pork	100.0 %
A2	Beef	Beef	99.8 %	A29	Beef	Beef	100.0 %
A3	Beef	Beef	100.0 %	A30	Beef	Beef	99.0 %
A4	Beef	Beef	99.8 %	A31	Pork	Pork	100.0 %
A5	Beef	Beef	100.0 %	<b>A32</b>	Beef	Pork	100.0 %
A6	Pork	Pork	99.0 %	A33	Beef	Beef	100.0 %
A7	Pork	Pork	100.0 %	A34	Beef	Beef	99.5 %
A8	Beef	Beef	100.0 %	A35	Pork	Pork	100.0 %
A9	Pork	Pork	99.8 %	A36	Pork	Pork	99.0 %
A10	Pork	Pork	99.5 %	A37	Beef	Beef	100.0 %
A11	Beef	Beef	100.0 %	A38	Beef	Beef	100.0 %
A12	Pork	Pork	99.8 %	A39	Pork	Pork	100.0 %
A13	Beef	Beef	100.0 %	A40	Pork	Pork	99.0 %
<b>A14</b>	Beef	Pork	100.0 %	A41	Pork	Pork	99.3 %
<b>A15</b>	Beef	Pork	99.5 %	A42	Pork	Pork	100.0 %
A16	Pork	Pork	100.0 %	A43	Beef	Beef	100.0 %
A17	Beef	Beef	100.0 %	A44	Beef	Beef	99.8 %
<b>A18</b>	Beef	Pork	100.0 %	<b>A45</b>	Beef	Pork	100.0 %
A19	Pork	Pork	100.0 %	A46	Pork	Pork	100.0 %
A20	Pork	Pork	100.0 %	A47	Pork	Pork	99.8 %
A21	Pork	Pork	100.0 %	A48	Beef	Beef	100.0 %
A22	Pork	Pork	100.0 %	A49	Pork	Pork	99.8 %
A23	Pork	Pork	99.5 %	<b>A50</b>	Beef	Pork	100.0 %
A24	Pork	Pork	99.8 %	A51	Beef	Beef	99.8 %
A25	Beef	Beef	100.0 %	A52	Pork	Pork	100.0 %
A26	Pork	Pork	100.0 %	A53	Pork	Pork	100.0 %
A27	Beef	Beef	99.8 %	A54	Beef	Beef	99.8 %

Table 1 The results of the most similar species and similarity of 87 samples searched in GenBank and EMBL databases. Samples in bold indicate inconsistent results observed (continued)

Sample no.	Original indicated sample name	The most similar species	Similarity	Sample no.	Original indicated sample name	The most similar species	Similarity
A55	Pork	Pork	100.0 %	A72	Pork	Pork	100.0 %
A56	Pork	Pork	99.8 %	A73	Beef	Beef	100.0 %
<b>A57</b>	Beef	Pork	100.0 %	A74	Beef	Beef	99.5 %
A58	Beef	Beef	100.0 %	A75	Beef	Beef	100.0 %
A59	Beef	Beef	100.0 %	A76	Pork	Pork	100.0 %
A60	Pork	Pork	99.8 %	A77	Pork	Pork	98.8 %
A61	Beef	Beef	100.0 %	<b>A78</b>	Beef	Pork	100.0 %
A62	Pork	Pork	99.5 %	A79	Beef	Beef	100.0 %
<b>A63</b>	Beef	Pork	100.0 %	A80	Pork	Pork	100.0 %
<b>A64</b>	Beef	Pork	100.0 %	A81	Pork	Pork	100.0 %
<b>A65</b>	Beef	Pork	100.0 %	A82	Beef	Beef	100.0 %
A66	Pork	Pork	100.0 %	A83	Beef	Beef	99.8 %
A67	Pork	Pork	100.0 %	A84	Pork	Pork	100.0 %
A68	Pork	Pork	100.0 %	A85	Pork	Pork	100.0 %
A69	Pork	Pork	100.0 %	A86	Pork	Pork	100.0 %
A70	Pork	Pork	100.0 %	A87	Beef	Beef	99.5 %
A71	Pork	Pork	100.0 %				

Table2 Genetic distances of samples in this study

Sampl	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1	0.00	0.50	0.00	0.25	0.25	1.01	1.01	1.01	1.01	0.75	1.01	1.52	21.16	21.51	20.84	21.19	20.84	20.84	21.19	21.55	21.19	21.90	20.49
2		0.00	0.50	0.75	0.75	1.01	0.75	1.01	1.01	0.50	1.01	1.52	21.16	21.51	20.84	21.19	20.84	20.84	21.19	21.55	21.19	21.90	20.49
3			0.00	0.25	0.25	1.01	1.01	1.01	1.01	0.75	1.01	1.52	21.16	21.51	20.84	21.19	20.84	20.84	21.19	21.55	21.19	21.90	20.49
4				0.00	0.50	1.26	1.26	1.26	1.26	1.01	1.26	1.77	21.16	21.51	20.84	21.19	20.84	20.84	21.19	21.55	21.19	21.90	20.49
5					0.00	1.26	1.26	1.26	1.26	1.01	1.26	1.77	20.49	20.84	20.17	0.52	20.17	20.17	20.52	20.87	20.52	21.23	19.82
6						0.00	0.75	0.75	0.75	0.75	0.75	1.26	20.45	20.80	20.14	20.49	20.14	20.14	20.49	20.84	20.49	21.19	19.79
7							0.00	1.01	1.01	1.26	1.01	1.52	19.79	20.14	19.48	19.82	19.48	19.82	20.17	19.82	20.52	19.13	
8								0.00	0.25	0.50	0.00	0.50	19.76	20.11	19.45	20.14	19.45	19.45	19.79	20.14	19.79	20.49	19.11
9									0.00	0.25	0.25	0.75	20.11	20.45	19.79	19.79	19.79	19.79	20.14	20.49	20.14	20.84	19.45
10										0.00	0.50	1.01	20.45	20.80	20.14	20.14	20.14	20.14	20.49	20.84	20.49	21.19	19.79
11											0.00	0.50	19.76	20.11	19.45	20.14	19.45	19.45	19.79	20.14	19.79	20.49	19.11
12												0.00	20.45	20.80	20.14	20.84	20.14	20.14	20.49	20.84	20.49	21.19	19.79
13													0.00	0.25	0.25	0.75	0.25	0.50	0.50	1.26	1.51	1.51	2.03
14														0.00	0.50	1.00	0.50	0.75	0.75	1.51	1.77	1.77	2.29
15															0.00	0.50	0.00	0.25	0.25	1.01	1.26	1.26	1.77
16																0.00	0.50	0.75	0.75	1.52	1.77	1.77	2.29
17																	0.00	0.25	0.25	1.01	1.26	1.26	1.77
18																		0.00	0.50	1.26	1.52	1.52	2.03
19																			0.00	1.26	1.52	1.52	2.03
20																				0.00	0.25	0.25	0.75
21																					0.00	0.50	0.50
22																						0.00	1.01
23																							0.00

Note Representative of each number was showed in the text.