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# Species authentication of commercial beef jerky based on PCR-RFLP analysis of the mitochondrial 12S rRNA gene

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**Abstract**

In this study, we determined species-specific variations by analyzing the mitochondrial 12S rRNA gene sequence variation (~440 bp) in 17 newly obtained sequences and 90 published cattle, yak, buffalo, goat, and pig sequences, which represent 62 breeds and 17 geographic regions. Based on the defined species-specific variations, two endonucleases, *Alu* I and *Bfa* I, were selected for species authentication using raw meat/tissue samples and the PCR-RFLP method. Goat and pig were identified using the *Alu* I enzyme, while cattle, yak, and buffalo were identified by digestion with *Bfa* I. Our approach had relatively high detection sensitivity of cattle DNA in mixed cattle and yak products, with the lowest detectable threshold equaling 20% of cattle DNA in a mixed cattle/yak sample. This method was successfully used to type commercial beef jerky products, which were produced by different companies utilizing various processing technologies. Our results show that several yak jerky products might be implicated in commercial fraud by using cattle meat instead of yak meat.

*Keywords*: 12S rRNA gene; PCR-RFLP; meat species identification; beef jerky; commercial fraud

## Introduction

In 2008, the total meat production in China approximated 72.8 million tons, of which 46.2 million was pork, 6.1 million was beef, and 3.8 million was mutton (Li et al., 2009). The demand for meat is projected to escalate steadily over the next decade as a result of increasing consumption. The commercial fraud of jerky products, particularly for yak jerky which is much more expensive than cattle jerky, may cause many problems. For the sake of public health, commercial protection, and religious customs, it is imperative to develop an applicable method to authenticate meat origin. Although many reliable and powerful detection methods, including protein-based (Ayaz et al., 2006) and DNA-based approaches (Ballin et al., 2009), have been developed in the past decades, their application in commercial jerky products can be further improved.

The target genes and DNA fragments used as markers for identifying meat species mainly come from the mitochondrial genome, including the cytochrome *b* gene (Murugaiah et al., 2009), the 16S rRNA gene (Mitani et al., 2009), the 12S rRNA gene (Girish et al., 2005; Fajardo et al., 2009; Rojas et al., 2009; Yin et al., 2009; Wang et al., 2010), and the mtDNA control region (Bellagamba et al., 2001; Dooley et al., 2004). The approaches, such as

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PCR-restriction fragment length polymorphism (RFLP) (Bellagamba et al., 2001; Murugaiah et al., 2009), DNA sequencing (Mitani et al., 2009), species-specific primers amplification (Rojas et al., 2009), and real-time PCR assays (Dooley et al., 2004), have been widely used for meat product identification. Among them, the PCR-RFLP was regarded to be one of the most efficient methods in terms of the cost, detection power, and applicability to large scale screening.

Previous reports using the PCR-RFLP method selected restriction sites based on only a few sequences; thus, they could have overlooked the potential genetic variations among the breeds and/or geographical populations of the same species. For instance, we found that divergent haplogroups differed from each other by up to 30 variations in domestic yak mtDNA control region sequences (~891 bp) (Lai et al., 2007). It is conceivable that the intra-species variation would lead to a false negative result during the meat identification process *via* gain or loss of restriction sites. Prado and colleagues (2007) reported the simultaneous co-amplification of two 359 bp cytochrome *b* gene fragments, belonging to the divergent *Bos indicus* and *Bos taurus* lineages, which affected the identification of cattle in the PCR-RFLP food authentication assay. With the accumulation of more livestock mtDNA sequences in public data sets, such as GenBank, it is now feasible to conduct an initial analysis based on the available data, and avoid such problems.

In this study, we first attempted to establish a reliable and rapid assay for authenticating cattle (*Bos taurus*), yak (*Bos grunniens*), buffalo (*Bubalus bubalis*), goat (*Capra hircus*), and pig (*Sus scrofa*) meat products according to the RFLP profile of the mitochondrial 12S rRNA gene. The selection of the appropriate restriction enzyme was based on the intra-species variation that was defined by the large-scale data gathered in this study and from GenBank. The optimized assay was utilized to authenticate commercial beef jerky.

## Materials and methods

### *Sample collection and extraction of genomic DNA*

Seventeen raw meat or ear tissue samples were collected from five farm animals: cattle ($n$ = 7), yak ($n$ = 2), buffalo ($n$ = 4), goat ($n$ = 2), and pig ($n$ = 2). Before sampling, all animals were morphologically confirmed. The samples were stored at –20ºC until further processing. Eleven commercial beef jerky products from different companies, all labeled as yak meat and flavored in particular ways (spicy, roasted, satay, peppery, or braised), were purchased from the local supermarkets or retail markets in Kunming, Yunnan ($n$ = 9) and Xining, Qinghai ($n$ = 2). Genomic DNA from the raw meat or ear tissue was extracted by using the standard phenol/chloroform method. To effectively extract the largely degraded genomic DNA from the processed samples, we combined the guanidine hydrochloride lysis and phenol/chloroform method together. In brief, jerky samples were rinsed in distilled water overnight and were stored at –20ºC. We incubated 10 mg of each jerky sample in the presence of proteinase K (400 ng/mL) and guanidine hydrochloride (5 mol/L) solution in a total volume of 1 mL for 10 h at 55ºC. The supernatant was collected after centrifugation at 12,000 g for 15 min, followed by the standard phenol/chloroform extraction.

### *PCR amplification and sequencing*

A pair of universal primers (Forward: 5′-CAA ACT GGG ATT AGA TAC CCC ACT AT-3′; Reverse: 5′-GAG GGT GAC GGG CGG TGT GT-3′) were used to amplify a fragment (~440 bp) in the mitochondrial 12S rRNA gene (Kocher et al., 1989; Girish et al., 2005). PCR amplification was performed in a 50 μL reaction mixture containing 100 ng of DNA, 10 mmol/L Tris-HCl (pH 8.3), 2.5 mmol/L $MgCl_2$, 50 mmol/L KCl, 10 pmol/L of each primer, and 1 unit of *Taq* polymerase (TaKaRa, Dalian, China). The amplification conditions consisted of 33 cycles of 50 s at 94ºC, 50 s at 62ºC, and 60 s at 72ºC.

PCR products were purified on spin columns (Watson BioTechnologies, Shanghai, China) and directly sequenced in both directions using the BigDye Terminator sequencing kit (Applied Biosystems, Foster City, California, USA). Sequencing was performed on a 3700 DNA sequencer (Applied Biosystems) according to the manufacturer's instructions. The 17 sequences have been deposited in GenBank under the accession Nos. GQ926965–GQ926966, GQ926969–GQ926974, GU066734–GU066738, and GU119953–GU119956.

### *Restriction fragment length polymorphism*

The 17 newly sequenced samples were edited using the

DNAstar program (DNAS Inc, Madison, WI, USA) and were aligned with 90 reported mitochondrial 12S rRNA gene sequences of cattle, yak, buffalo, goat, and pig available in GenBank (Table 1 and Supplemental Table 1). These sequences were selected to cover nearly all divergent mtDNA lineages (e.g., *Bos indicus* and *Bos taurus* types of cattle) and geographical populations/breeds (e.g., Asian breeds and European breeds of domestic pig). For each species, we obtained the consensus sequence of the newly generated sequences and the reported sequences. The intra-species variations, whether occurring as single variants or parsimonious variants, were taken into consideration. The species-specific variations were identified by aligning the consensus sequences of the farm animals and were exported by using MEGA 4.0 (Tamura et al., 2007).

Based on the species-specific variations, two restriction enzymes, *Alu* I and *Bfa* I (Fermentas, Shanghai, China), were chosen for RFLP analyses. The predicted restriction sites and fragment length polymorphisms are listed in Table 1 and Fig. 1. The PCR products of 12S rRNA gene were digested with *Alu* I and *Bfa* I at 37ºC for 6 h in a total volume of 10 μL, which contained 1 μL of PCR product, 3 U of restriction enzymes, and 1 μL of digestion buffer. The digested products were visualized by electrophoresis on a 10% polyacrylamide gel and stained with ethidium bromide. Electrophoresis was run at 15 V/cm for 50 min at room temperature.

### *Sensitivity test*

To determine the sensitivity of our method in detecting mixed meat products, we prepared a serial mixture of cattle and yak genomic DNA with the following weight/weight ratios: 1:9, 2:8, 3:7, 4:6, and 5:5. A total of 10 ng of mixed DNA was amplified using the above described amplification conditions and thermal cycles. PCR products were digested with *Bfa* I following the same procedure.

## Results

### *PCR amplification and sequence analysis of the 12S rRNA gene*

We successfully performed PCR amplification using universal primers on all five farm animals. The amplicon of the mitochondrial 12S rRNA gene had the expected size of 440 bp. The sequence authenticity was further verified by direct sequencing of the PCR products in both directions.

In total, we identified 3 single variable sites, 3 insertion/deletion sites, and 59 parsimony informative sites among the 17 newly obtained sequences and 90 reported sequences, which altogether represent 62 breeds from 17 regions (Table 1 and Supplemental Table 1). The consensus sequence of each species was constructed using the IUPAC coding system and aligned using the cattle DNA as the reference sequence (Fig. 1).

### *RFLP analyses*

The PCR products of the 12S rRNA gene were subjected to *Alu* I and/or *Bfa* I digestion. The five animals showed species-specific restriction patterns (Fig. 2). *Alu* I digestion of pig PCR products resulted in two fragments (162 bp and 278 bp), which were distinguished from the two fragments (200 bp and 240 bp) observed for goat PCR products. Cattle and yak had the same digestion pattern (91 bp and 349 bp) using *Alu* I, so they were indistinguishable. The buffalo PCR product could not be digested by *Alu* I. After digestion by *Bfa* I, the cattle and yak PCR products were separated. Cattle had three bands (48 bp, 82 bp, and 310 bp) and yak had two bands (130 bp and 310 bp). The *Bfa* I restriction enzyme generated the same digestion pattern (130 bp and 310 bp) for goat and yak PCR products, so they could not be distinguished. Three fragments were observed for both pig (30 bp, 100 bp, and 310 bp) and buffalo (35 bp, 130 bp, and 275 bp) PCR products after *Bfa* I digestion. The small bands (30 bp, 35 bp, and 48 bp) observed in corresponding species using *Bfa* I digestion were elucidated using a 10% polyacrylamide gel.

Table 1
RFLP analysis of the mitochondrial 12S rRNA gene of five farm animals

| Species | Number of samples (Breeds/Countries) [a] | Restriction enzymes and fragments (bp) | |
|---|---|---|---|
| | | *Alu* I | *Bfa* I |
| Cattle | 50 (21/13) | 91, 349 | 48, 82, 310 |
| Yak | 8 (5/2) | 91, 349 | 130, 310 |
| Buffalo | 14 (10/5) | 440 | 35, 130, 275 |
| Goat | 10 (8/5) | 200, 240 | 130, 310 |
| Pig | 25 (18/8) | 162, 278 | 30, 100, 310 |
| Total | 107 (62/17) | — | — |

[a] The numbers in brackets refer to the samples that were classified by breed and/or geographical origin.

```
 Cattle  CAAACTGGGA TTAGATACCC CACTATGCTT AGCCCTAAAC ACAGATAATT ACATAAACAA AATTATTCGC CAGA   74
    Yak  .......... .......... .......... .......... .......... .......... .......T.. ....
Buffalo  .......... .......... .......... .......... ...A...... .T..T..... .......... ....
   Goat  .......... .......... .......... .......... ...A...... ...G...... .......... ....
    Pig  .......... .......... ........C. .......... C..A...G.. ......-... ..C....... ....

 Cattle  GTACTACTAG CAACAGCTTA AAACTCAAAG GACTTGGCGG TGCTTTATAT CCTTCTAGAG GAGCCTGTTC TATA  148
    Yak  .......C.. .......... .......... .......... .......... ..C....... .......... .G..
Buffalo  .......CG. ...Y..C... .......... .......... .......... ..CY...... .......... ....
   Goat  .......CG. ......CCG. .......... .......... .........C .......... .......... ....
    Pig  ........C. ....T.C... .......... .......... .....C.C.. ..AC...... .......... ....

 Cattle  ATCGATAAAC CCCGATAAAC CTCACCAATT CTTGCTAATA CAGTCTATAT ACCGCCATCT TCAGCAAACC CTAA  222
    Yak  .......... .......... .......G.. .......... .......... .......... .......... ....
Buffalo  .......... .......G.. .......... .........G ..S....... .......... .....R.... ....
   Goat  .......... .......... .........C .......... ......R... .......... .......... ....
    Pig  .......... .......R.. ..T.....CC .....C...T ...C...... .......... .......... ....

 Cattle  AAAGGAAAAA AAGTAAGCGT AATTATGATA CATAAAAACG TTAGGTCAAG GTGTAACCTA TGAAATGGRA AGAA  296
    Yak  .......... ........RC ...Y...... .......... .......... .......... ..R.....A. ....
Buffalo  .....T.C.. .........C ...C.CA.YG .......... .......... .......... ........G. ....
   Goat  .......C.. ........TC ...C.CA.C. ......G... .......... ........C. ..G.....G. ....
    Pig  ......RC.. T.......AC ...C..ARC. .......... .......... ......G.T. ..GGT...A. ....

 Cattle  ATGGGCTACA TTCTCTACAC YAAGAGAATC AA-GCACGAA AGTTATTATG AAACCARTAA CCAAAGGAGG ATTT  370
    Yak  .......... .......... T.......Y. ..T....... .......... ...TT.G... .......... ....
Buffalo  .......... ..T....... C....AY.C. C.ACACGA.. GT.AT.ATGA ..RTT.A... .......... ....
   Goat  .......... ..T.....CT T....A...T ..--T..... ..CC...... ...TT.A.G. .......... ....
    Pig  .......... ..T......T A.GA.T.YM. .CCAY..... ....T..... ....T.AA.. .......... ....

 Cattle  AGCAGTAAAC TAAGAATAGA GTGCTTAGTT GAATTAGGCC ATGAAGCACG CACACACCGC CCGTCACCCT C    441
    Yak  .......... .....G.... .......... .......... .......... .......... .......... .
Buffalo  .......... .......... .......... ...C...... .......... .......... .......... .
   Goat  ..T....... .......... .......... .......... .......... .........S .......... .
    Pig  .........T C......... ......GA.. ....A..... .......... .......... .......... .
```

Fig. 1. Alignment of modified consensus sequences of the mitochondrial 12S rRNA gene fragments for five farm animals. The intra-species polymorphic sites identified in each species (cattle, $n$ = 50; yak, $n$ = 8; buffalo, $n$ = 14; goat, $n$ = 10; and pig, $n$ = 25) are marked by rectangles and are presented by IUPAC codes, e.g., "Y" means the nucleotides of C or T. The restriction sites of endonucleases *Alu* I (5′-AG^CT-3′) and *Bfa* I (5′-C^TAG-3′) are underlined. Dots (·) denote the identity of the reference sequence. Short dashed lines (–) represent the deletion of a base pair relative to the reference sequence.

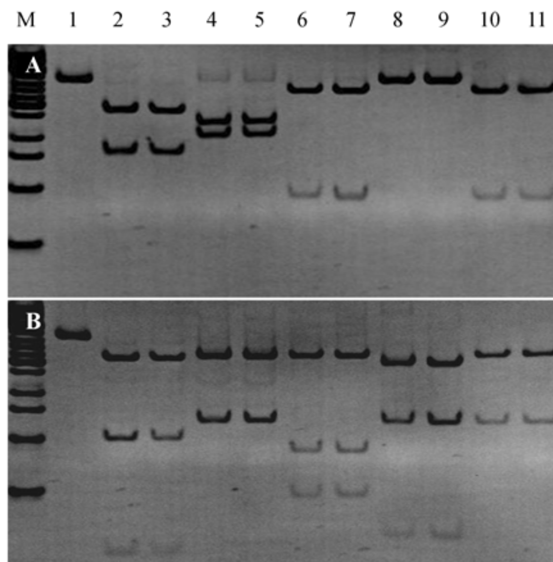


Fig. 2. Electropherograms of mitochondrial 12S rRNA gene fragments of five farm animals digested by using *Alu* I (**A**) and *Bfa* I (**B**). Lane M, 50 bp DNA ladder; lane 1, undigested PCR product; lanes 2–3, pig; lanes 4–5, goat; lanes 6–7, cattle; lanes 8–9, buffalo; lanes 10–11, yak.

To test the sensitivity of our method, we created arbitrary mixtures of cattle and yak genomic DNA with different ratios (1:9; 2:8; 3:7; 4:6 and 5:5). We could identify cattle DNA in yak DNA after *Bfa* I digestion except for in the 1:9 ratio condition. The resulting gel showed four bands of 48 bp, 82 bp, 130 bp and 310 bp (Fig. 3). Because the reaction buffers for *Alu* I and *Bfa* I were the same, we attempted to digest the PCR products using one reaction with both enzymes simultaneously. However, the resulting digestion pattern presented a similar profile (data not shown) and could not clearly allow for the distinction of species.

### *Authentication of beef jerky products*

To test the practical application of the methodology in this study, we performed PCR-RFLP analysis for 11 commercial jerky products from different companies in Yunnan and Qinghai provinces. The 11 jerky flavors (all labeled as yak meat) represented the different processing technolo-

gies prevalent in the Chinese jerky industry. We extracted genomic DNA from the jerky samples despite the obvious DNA degradation (Fig. 4A). All 11 samples yielded amplification by PCR and two bands (91 bp and 349 bp) were observed when using the *Alu* I enzyme (data not shown). The *Bfa* I digestion generated similar profiles for 9 of the 11 samples, which matched the expected restriction profile of cattle. The remaining two jerky products presented bands (130 bp and 310 bp) that correlated to yak (Fig. 4B).

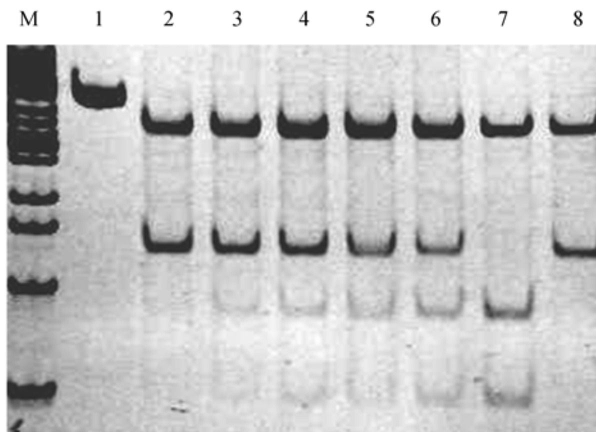


Fig. 3. Electropherograms of mitochondrial 12S rRNA gene fragments of mixed cattle and yak DNA digested with *Bfa* I. Lane M, 50 bp DNA ladder; lane 1, undigested PCR product; lanes 2–6 refer to a mixture of cattle and yak DNA in ratios of 1:9, 2:8; 3:7; 4:6, and 5:5; lanes 7–8 are positive controls: pure cattle and yak samples, respectively.

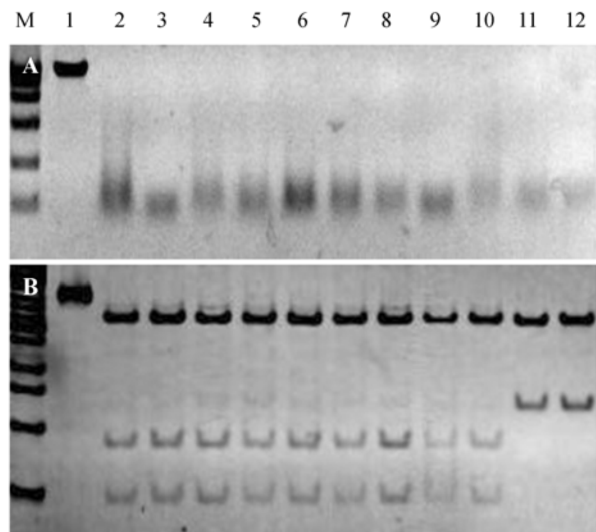


Fig. 4. Electropherograms of genomic DNA and *Bfa* I digested mitochondrial 12S rRNA gene fragments of 11 commercial jerky products. Lane M, DNA ladder; lane 1 refers to genomic DNA from fresh muscle tissue (**A**) or undigested PCR product (**B**); lanes 2–12, genomic DNA from 11 commercial jerky products (**A**) and digested PCR product of each sample (**B**).

## Discussion

Techniques for authenticating the origin of meat products have advanced recently (Ballin et al., 2009). The DNA-based methods are rooted in the assumption that there are always species-specific genetic differences among the studied species (Hajibabaei et al., 2007; Frézal and Leblois, 2008). However, in most published reports, only a few sequences were analyzed to deduce the so-called species-specific DNA sequence variations (Murugaiah et al., 2009). With increasing sample sizes and more geographically distant populations/breeds, some of the proposed genetic markers might show intra-species variations and would not be reliable in identifying the species. This problem is most obvious when attempting to identify mtDNA sequence variations for domestic animals containing multiple matrilineal components (Lai et al., 2006, 2007; Liu et al., 2009).

In this study, we attempted to design an efficient species identification method that avoids the aforementioned limitations. We analyzed a total of 107 sequences, including samples from 62 breeds and 17 regions (Supplemental Table 1). We detected 21 intra-species polymorphisms in the 440 bp fragment of the 12S rRNA gene (Fig. 1) and filtered them during the selection of appropriate restriction enzymes. By using *Alu* I and *Bfa* I, we accurately identified the DNA source from five species of farm animals (Fig. 2). In a previous report that traced meat origins (Chen et al., 2008), *Bsp*H I endonuclease was used to identify the sequence of "5′-T^CATGA-3′" in yak species. An audit for the sequence revealed that this restriction site, which was located in region 245–250 in our sequences (Fig. 1), contained an intra-species variation at the second nucleotide position of the enzyme recognition site and would cause problems during the digestion. This finding supports the notion that improper selection of restriction endonucleases has the potential to incorrectly identify meat species. Sufficient analysis of the available sequences may help to avoid this problem.

DNA-based authentication methods are well-known for their application in processed meat products. Previous reports always simulated the conditions of processed meat samples (Girish et al., 2007). In this study, we purchased 11 commercial yak jerky products of different flavors produced by prevalent processing methods in the industry. Undoubtedly, such a sampling would be more representative and suitable than any simulated processed samples.

However, there are many difficulties in DNA extraction and PCR amplification when one deals with processed samples, especially for commercial jerky products. We were able to successfully extract genomic DNA from all the jerky samples by using the modified protocol of combining guanidine hydrochloride lysis with the phenol/chloroform method despite the obvious DNA degradation. The high efficiency of DNA extraction by our modified protocol could also be demonstrated by the successful amplification of a PCR fragment up to 440 bp (Fig. 4), which was not easily obtained by regular methods from heat-processed samples.

Unexpectedly, 9 out of 11 beef jerky products analyzed in this study showed a digestion profile unique to cattle species, suggesting commercial fraud of using cattle meat instead of yak meat. Short supply and high price of yak meat may account for the high frequency of fake yak jerky. Although only 11 jerky samples were investigated, we think this may suggest a common fraudulent practice in the meat jerky industry. Systematic screening and standardization of jerky production are necessary to protect consumer interest and the authenticity of yak jerky products.

Many analytical approaches are used to quantify the proportion of mixed meat products, but detection sensitivities are variable (Ballin et al., 2009). Using the PCR-RFLP method, Girish et al. (2005) failed to detect mixed meat samples. We were able to successfully detect cattle DNA in yak DNA at ratios of 2:8 to 5:5 in one reaction in this study. However, the sensitivity of our method is lower than the multiplex PCR-based approach designed recently by Yin and colleagues (2009). To achieve a higher sensitivity, other approaches such as real-time PCR assay should be designed to precisely quantify the proportion of mixed cattle and yak meat.

In short, we developed an efficient PCR-RFLP method for authenticating meat products from five farm animals on the basis of the mitochondrial 12S rRNA gene sequence variations. We avoided intra-species variation by analyzing the available sequences for related species and selecting appropriate restriction enzymes. We showed that our approach can effectively authenticate meat species used in commercial jerky products.

## Acknowledgments

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## Supplemental data

Supplemental Table 1 associated with this article can be found in the online version at www.jgenetgenomics.org.

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Supplemental Table 1
Mitochondrial 12S rRNA sequences of five farm animals analyzed in this study

| Livestock | Geographic regions | Breeds / Populations [a] | GenBank accession No. | References [b] |
|---|---|---|---|---|
| Cattle | America | Charolais | AY676861 | Unpublished |
| | | Simmental | AY676855 | Unpublished |
| | England | Angus | AY676857; AY676859; AY676862; AY676865; AY676866; AY676871; AY676873 | Unpublished |
| | Poland | Ukrainian grey | GQ129208 | Unpublished |
| | China | Unknown | EU851893 | Wang et al., 2010 |
| | | Tongjiang | GU066734 - GU066738 | This study |
| | | Wenshan | GQ926965; GQ926966 | This study |
| | France | Limousin | AY676856 | Unpublished |
| | Iran | Iranian | EU177860; EU177870 | Achilli et al., 2008 |
| | Italy | Chianina | EU177816; EU177819; EU177828; EU177841; EU177853 | |
| | | Rendena | EU177861 | |
| | Japan | Japanese Black | AB074962; AB074964; AB074967 | Mannen et al., 2003 |
| | Korea | Beef cattle | DQ124396; DQ124397 | Unpublished |
| | | Korean cattle | DQ124371; DQ124374; DQ124375; DQ124382; DQ124385 | Unpublished |
| | | Korean Holstein-Friesian | DQ124404; DQ124406; DQ124407; DQ124409; DQ124418 | Unpublished |
| | Mongolia | Mongolia cattle | FJ971088 | Achilli et al., 2009 |
| | South Asia | Zwergzebu | AF492350 | Hiendleder et al., 2008 |
| | India | Haryana | DQ867006 | Unpublished |
| | | Karnataka | AJ490501 | Girish et al., 2004 |
| | | Nellore | AY126697; NC_005971 | Unpublished |
| | Iraq | Iraqi | EU177868; EU177869 | Achilli et al., 2008 |
| Yak | India | Unknown | EU521723 | Unpublished |
| | | Unknown | AF091686 | Hassanin and Douzery, 1999 |
| | China | Maiwa | AY684273 | Gu et al., 2007 |
| | | Unknown | EF494177; EF494178; EF494179 | Unpublished |
| | | Jiulong | GQ926973; GQ926974 | This study |
| Buffalo | India | Murrah | DQ867005 | Unpublished |
| | | | EU760478 | Unpublished |
| | | Unknown | AF547270 | Unpublished |
| | | Unknown | EU908276 | Unpublished |
| | | Unknown | AJ457159 | Girish et al., 2004 |
| | | Unknown | AJ490502 | Girish et al., 2004 |
| | Italy | Unknown | AY488491 | Parma et al., 2004 |
| | Russia | Unknown | AF231028 | Kuznetsov et al., 2001 |
| | Spain | Unknown | AJ846850 | Lopez-Calleja et al., 2005 |
| | China | Haikou | AY702618 | Unpublished |
| | | Binhu | GU119953 - GU119956 | This study |
| Goat | Italy | Unknown | NC_005044 | Parma et al., 2003 |
| | China | Unknown | EU851902; EU851903 | Wang et al., 2010 |
| | | Nanjiang | GQ926969; GQ926970 | This study |
| | India | Black Bengal | DQ867007 | Unpublished |

| | | | | |
|---|---|---|---|---|
| | | Barbari | AJ490504 | Girish et al., 2004 |
| | Spain | Unknown | AJ630113 | Rodríguez et al., 2004 |
| | | Unknown | AJ885199 | Fajardo et al., 2006 |
| | America | Unknown | M55541 | Kraus and Miyamoto, 1991 |
| Pig | Sweden | Unknown | AJ002189 | Ursing and Arnason, 1998 |
| | America | Duroc | AF486858 | Yang et al., 2003 |
| | China | Aba pig | EF545578 | Wu et al., 2007 |
| | | Jinhua | AF486863 | Yang et al., 2003 |
| | | Meishan | AF304200 | Kijas and Andersson, 2001 |
| | | Ningxiang | AF486857 | Yang et al., 2003 |
| | | Rongchang | AF486860 | |
| | | Yunnan Saba | EF545567 | Wu et al., 2007 |
| | | Wannanhua | AF486873 | Yang et al., 2003 |
| | | Wuzhishan | AF486867 | |
| | | Xiang pig | AF486859 | |
| | | Yunnan | GQ926971; GQ926972 | This study |
| | Denmark | Landrace | AF304202 | Kijas and Andersson, 2001 |
| | | | AF486866 | Yang et al., 2003 |
| | England | Hampshire | AY574046 | Unpublished |
| | | Large White | AF486874 | Yang et al., 2003 |
| | | | AP003428 | Unpublished |
| | Japan | Ohmini pig | AB298688 | Unpublished |
| | Korea | Jeju native black | AY334492 | Unpublished |
| | Spain | Iberian | FJ236991; FJ236992; FJ236993 | Unpublished |
| | | Duroc | FJ236996; FJ236997 | Unpublished |
| Total | 17 | 62 | 107 | — |

[a] These sequences deposited in GenBank by the same group but without any breed or population information were regarded as samples from the same breed and/or population. [b] The unpublished sequences were only deposited in GenBank database.

## Supplemental references

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