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# Gene cloning, homology comparison and analysis of the main functional structure domains of beta estrogen receptor in Jining Gray goat

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ABSTRACT

To clarify the molecular evolution and characteristic of beta estrogen receptor (ERβ) gene in Jining Gray goat in China, the entire ERβ gene from Jining Gray goat ovary was amplified, identified and sequenced, and the gene sequences were compared with those of other animals. Functional structural domains and variations in DNA binding domains (DBD) and ligand binding domains (LBD) between Jining Gray goat and Boer goat were analyzed. The results indicate that the ERβ gene in Jining Gray goat includes a 1584 bp sequence with a complete open-reading-frame (ORF), encoding a 527 amino acid (aa) receptor protein. Compared to other species, the nucleotide homology is 73.9–98.9% and the amino acid homology is 79.5–98.5%. The main antigenic structural domains lie from the 97th aa to the 286th aa and from the 403rd aa to the 527th aa. The hydrophilicity and the surface probability of the structural domains are distributed throughout a range of amino acids. There are two different amino acids in the DBD and three different amino acids in the LBD between Jining Gray and Boer goats, resulting in dramatically different spatial structures for ERβ protein. These differences may explain the different biological activities of ERβ between the two goat species. This study firstly acquired the whole ERβ gene sequence of Jining Gray goat with a complete open reading frame, and analyzed its gene evolutionary relationship and predicted its mainly functional structural domains, which may very help for further understanding the genome evolution and gene diversity of goat ERβ.



## 1. Introduction

Jining Gray goats are widely distributed in the Jining and Heze regions in Shandong Province of China and are noted for the wavy patterns of their kid-pelts, which are a traditional commodity in international markets and a significant display of sexual precocity, year-round estrus, and high prolificacy. It has been reported that Jining Gray goats reach sexually mature at 60–90 days after birth, produce 2–3 pregnancies per year, and have a lamb rate of 204–326% per pregnancy. Jining Gray goats were selected as one of important state-protected breeds in China [Tu, 1989]. The Boer goat is recognized as a meat-type goat, with an average sexual maturity at 6 months and a lambing rate of 162–164% [Greyling, 2000]. Yunling Black goat, another famous goat breed in the Southwest of China, is also a meat-type goat species and has a reproduction performance similar to that of the Boer goat (1.0–1.5 pregnancies per year, sexually mature at approximately 6 months of age, and a lambing rate of 104–189%) [Tu, 1989]. The genetic mechanism underlying the breeding performance of these three different goat species is a very interesting and significant subject of study.

Estrogen receptor β (ERβ) belongs to the steroid/thyroid hormone receptor superfamily and regulates gene transcription by binding to specific response elements. ERβ plays an important role in follicular maturation [Emmen et al., 2005] and is one of the key receptors that determine high prolificacy or reproductive function in mammals [Gibson and Saunders, 2012]. The ERβ genes of some animal species, such as rat, human, bovine, mouse, sheep, swine, and chicken, have been cloned since ERβ was first reported in mice in 1996 [Kuiper et al., 1996]. Previously, published goat ERβ whole gene sequences only included Africa Boer goat and Chinese Yunling Black goat (GenBank #EU847291 and #EU847286); the ERβ whole gene sequence of Jining Gray goat has not been reported. The gene sequence homology and differences in the functional protein domains have not been compared and analyzed, limiting the

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**Table 1**
PCR primers were used in present study.

| Primer names | Primer sequences (5′–3′) | Accession no. | Product size |
|---|---|---|---|
| ERβ1-forward | CCGGAATTCATGGATGTCAAAAACTCACC | NM_001009737.1 | 640 bp |
| ERβ1-reverse | CCCAAGCTTACTTCACCATTCCGACCT | | |
| ERβ2-forward | CGCGGATCCCGACTGCGGAAGTGCTATGAG | NM_001009737.1 | 981 bp |
| ERβ2-reverse | CCCAAGCTTTCACTGAGCCTGGGGTTTCTG | | |

*Note*: the characters with underline mean the sites of restriction enzyme digestion (GAATTC: EcoRI; AAGCTT: HindIII).

understanding of the ERβ gene and protein diversity in goats. The objectives of this study were to first obtain the gene sequence of goat ERβ, analyze its homology with other animals and make predictions about the main functional protein domains, and then to analyze the amino acid (aa) variations in DBD and LBD between Jining Gray and Boer goats and discuss the biological significance of these variations.

## 2. Materials and methods

### 2.1. Sample collection and RNA extraction

Ovary samples were collected from six different Jining Gray goats (Jining Gray goats breeding and conservation base, Jining region, Shandong Province, PR China). Samples were homogenized in Trizol Reagent (Invitrogen, CA, USA) and total RNA isolation was completed following the manufacturer's protocol. The extracted RNAs were treated with 1.2% formaldehyde, analyzed with 1% agarose gel, quantitatively measured using an ultraviolet spectrophotometer (Shimadzu, Japan), and then stored at −80 °C.

### 2.2. Primers and amplification and sequencing of Jining Gray goat ERβ

According to the published ERβ whole gene length from other animals (approximately 1600 bp), the whole gene of Jining Gray goat could not have been cloned using only one pair of primers. As shown in Table 1, two pairs of continuous primers (ERβ1 and ERβ2) were designed according to the ERβ gene sequences in other species available in GenBank (NM_001009737.1) using Primer 5.0 software and were synthesized by Shanghai Biological Engineering Technology Services Limited Co. The first-strand of cDNA was synthesized according to the PrimeScript® RT Reagent Kit protocol. The RT-PCR product was stored at −20 °C.

The cDNA was then used as a template for the subsequent PCR reactions according to the High Fidelity Taq enzyme protocol. PCR was performed by mixing 2 μL of cDNA, 2 μL of dNTPs (2.5 mM each), 1 μL (20 pM) each of forward and reverse primers, 0.25 μL (5 U/μL) of High Fidelity Taq enzyme (TAKARA BIO Inc., Dalian, China), 5 μL of 10× PCR buffer ($Mg^{2+}$ plus) and 36.75 μL of sterilized and double distilled water in a 50 μL volume. Touchdown PCR reactions of ERβ1 gene were performed using a Mastercycler_5333 (Eppendorf AG, Hamburg, Germany) under the following conditions: at 94 °C for 3 min (initial denaturation), 94 °C for 30 s, 65 °C for 30 s (decrease by 0.5 °C per cycle); 30 cycles of at 72 °C for 50 s, 94 °C for 30 s, 45 °C for 30 s; and 15 cycles of 72 °C for 50 s, 72 °C for 10 min and 4 °C for 30 min. Touchdown PCR reactions of ERβ2 gene were performed under following conditions: 94 °C for 3 min (initial denaturation), 94 °C for 30 s, 65 °C for 30 s (decrease by 0.5 °C per cycle); 30 cycles with 72 °C for 75 s, 94 °C for 30 s, 50 °C for 30 s; and 15 cycles with 72 °C for 75 s, 72 °C for 10 min and 4 °C for 30 min. One fifth of each PCR reaction was electrophoresed on a 1.0% agarose gel, stained with ethidium bromide, and visualized under UV light.

The PCR products of ERβ1 and ERβ2 were extracted with Agarose Gel DNA Fragment Recovery Kit Ver. 2.0 protocols (TaKaRa, Dalian, China) and inserted into pMD18-T Simple Vectors (TaKaRa, Dalian, China) according to the provided protocols. The recombinant plasmids were transformed into competent *E. coli* Top10 cells. Positive cells were cloned at 37 °C for 8 h, and the positive recombinant plasmids were extracted with a Plasmid Extraction Kit (OMEGA) and digested with double enzymes: EcoR I/Hind III and BamH I/Hind III (TaKaRa, Dalian, China). After preliminary identification using PCR, the positive plasmids were named pMD18-T-ERβ1 and pMD18-T-ERβ2 and sequenced using two-way sequencing method by Beijing Liuhe Huada Gene Co. Ltd. (Beijing, China).

### 2.3. Bioinformatics analysis of Jining Gray goat ERβ

#### 2.3.1. Homologous analysis of the ERβ nucleotide and amino acid sequences

The nucleotide and amino acid sequences of Jining Gray goat ERβ were compared with those published for other species, including *Capra hircus* breed Boer (EU847291.1), *Capra hircus* breed Yunling Black (EU847286.1), *Ovis aries* (NM_001009737.1), *Bos taurus* (NM_174051.3), *Sus scrofa* (AF267736.1), *Homo sapiens* (AF060555.1), *Mus musculus* (BC145329.1), and *Gallus gallus* (NM_204794.1), using DNAstar Lasergen7.0 software. The amino acid sequence alignments of Jining Gray goat ERβ and other species were constructed using software Clustal X (1.83). The neighbor-joining phylogenetic tree of ERβ among these animal species was constructed by MEGA (4.0) software (http://www.megasoftware.net), and the bootstrap values to support the nodes of the tree were based on 1000 iterations of the heuristic search [Sullivan and Joyce, 2005; Zhang, 1999]. The evolutionary kinship was clarified according to the results of the comparison.

#### 2.3.2. Prediction and analysis of the main functional domains of Jining Gray goat ERβ

The main antigenic domains (MAD) of ERβ in Jining Gray goat were predicted using Jameson-Wolf methods [Jameson and Wolf, 1988]. The hydrophilic and transmembrane domains of the ERβ protein were predicted using Plot-Kyte–Doolittle online methods (http://www.expasy.Org/tools/protscale.html and http://www.cbs.dtu.dk/services/TMHMM-2.0/) [Kyte and Doolittle, 1982]. The surface probability domains were predicted with seven amino acids residues as a group scheme according to the Plot-Emini principle [Emini et al., 1985]. The specific amino acid residues in the structure were calculated using Garnier–Robson methods [Garnier et al., 1978]. The secondary structures of the ERβ protein were predicted by Chou–Fasman from amino acid residues in the crystal structure [Zhang et al., 2010]. The protein backbone flexibility was predicted with Karplus–Schultz methods [Karplus and Schulz, 1985]. The three-dimensional structures of the mature ERβ peptides in Jining Gray goat and Boer goat were predicted using the Swiss-model web server (http://swissmodel.expasy.org/) according to the human ERβ three-dimensional structure obtained using X-ray diffraction analysis as the model [Peitsch, 1996].