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***Sang L., Chen D.J., Sun S.K., Chen Y.F., Xie X.P.***

SEQUENCE ANALYSIS OF cDNA ENCODING FUJIAN YELLOW  
RABBIT INHIBIN BETA-A SUBUNIT PRECURSOR PROTEIN.

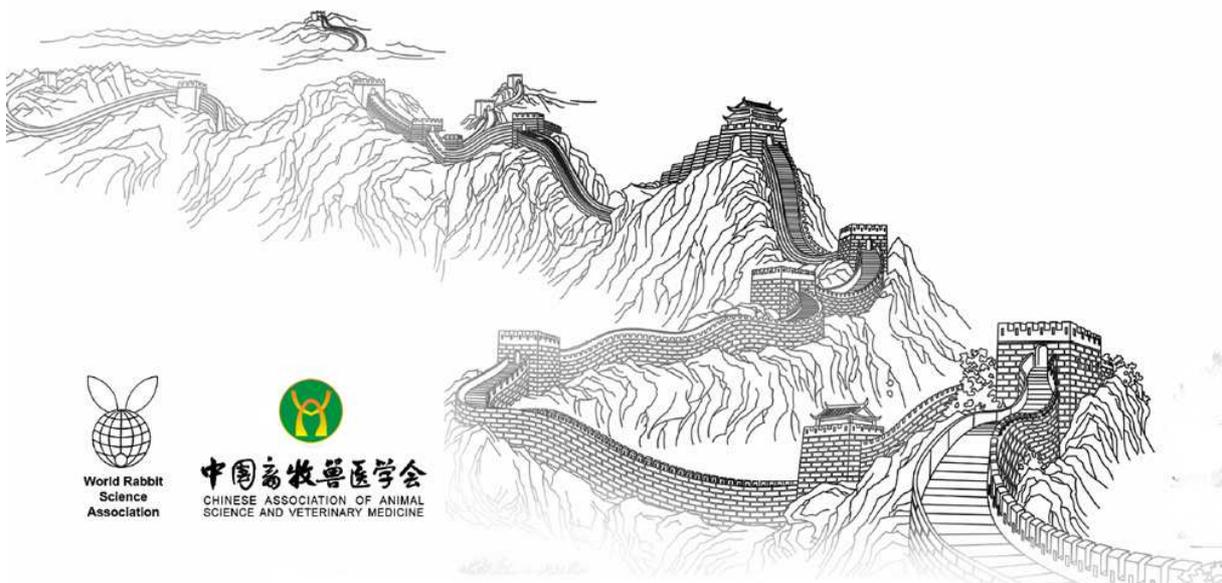
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## SEQUENCE ANALYSIS OF CDNA ENCODING FUJIAN YELLOW RABBIT INHIBIN BA SUBUNIT PRECURSOR PROTEIN

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### ABSTRACT

The total RNA obtained from the ovary of Fujian yellow rabbit was used to amplify the cDNA for the precursor of Fujian yellow rabbit inhibin  $\beta$ A subunit by RT-PCR, and the opening reading frame of 1275 bps was acquired. The fragments amplified were ligated with T-Vector pMD™20 vector and transformed into the competent *E. coli* Competent cells JM109. The positive clones were verified, sequenced and submitted to GenBank, (accession number : KC831577). Based on the ORF sequence, the upstream and downstream sequences were isolated by 5'- and 3'- RACE, respectively. The full cDNA (2339bp) consists of a 5'-terminal untranslated region (UTR) (280bp), a 3'-terminal UTR (784bp) with a polyadenylation signal sequence (AATAA), a poly (A) tail, and an ORF(1275bp). The sequence result was compared with homologous sequences from mammalian animals including human, cow, sheep, and mouse. Sequence alignment showed that ranks of their similarities were above 95%, and meanwhile, the results of genetic evolution analysis of the gene showed a fairly high degree of homology between rabbits and primates.

**Key words:** INHBA, RACE, Fujian Yellow Rabbit, Sequence analysis

### INTRODUCTION

Fujian Yellow rabbit is a valuable local breed under natural selection for a long time, which was mainly distributed in Fuzhou city of Fujian Province. In 2006, it was identified and named Fujian Yellow Rabbit and list in the Catalogue for protection and conservation of livestock and poultry Genetic Resources in China by the National Commission on livestock and Poultry Genetic Resources. Fujian Yellow rabbit has a well-proportioned body, beautiful features, a pair of short and thick ears, black eyes, and is fully coated with the yellow fur. It also presents many excellent characteristics of wide adaptability, high disease resistance, juicy and delicious meat. It is perceived to be good for the health in China, and consumed as a kind of medicinal food by the people. Over the years a loss of genetic characteristics of this rabbit was observed due to the lack of a suitable selection scheme.. From the early of last century, Fujian Academy of Agricultural Sciences has carried out a series of research on their body figure, growth development, reproductive performance, and genetic diversity to protect, conserve, utilize, and develop the rabbit meat breeds resources. After years of hard work, the traits of the somatotype, fur color, and ear shape of the Fujian Yellow rabbits tend to be homogeneity, and the production performance of yellow rabbits has been improved by selection.

The inhibin beta A subunit joins the alpha subunit to form a pituitary FSH secretion inhibitor. Inhibin has been shown to regulate gonadal stromal cell proliferation negatively and to have tumor-suppressor activity. Furthermore, the beta A subunit forms a homodimer, activin A, and also joins with a beta B subunit to form a heterodimer, activin AB, both of which stimulate FSH secretion (Oshima et al., 2014). Thus, the inhibin beta A gene might be a candidate gene for reproduction performance of rabbit.

### MATERIALS AND METHODS

#### Experiment animals

Animal care and handling were in accordance with the policy on the Care and Use of Animals of the Ethical Committee, Fujian Academy of Agricultural Sciences. Female Fujian Yellow rabbits (2 to 2.5 kg and more than 6 months old) purchased from Yuhuashan Rabbit Breeding Farm of Lianjiang (Fuzhou,

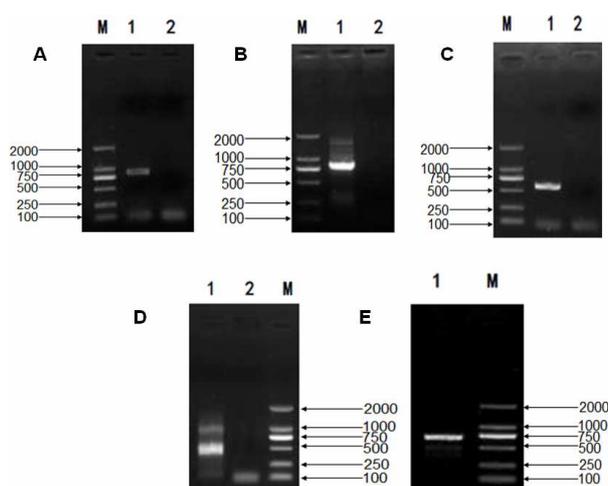
China) were raised with a free access to food and water at all time, and maintained on a 12L/12D (light/dark) cycle and in an air-conditioned room.

### RNA extraction and quality determination

Total RNA was extracted from female Fujian Yellow rabbit ovary tissues by TRIzol (Invitrogen, Carlsbad, CA, USA) according to the standard protocol. The RNA samples were treated with DNase I (TaKaRa, Japan) for 4 h. RNA was quantified by measuring the absorbance at 260 nm using a Nanodrop spectrophotometer (Thermo Fisher Scientific Inc., San Jose, CA, USA). The ovary RNA was assessed by the ratio of the absorbance at 260 and 280 nm. The integrity of the RNA samples was examined with an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA).

### Rapid amplification of 5' and 3' cDNA ends (RACE)

Based on the conserved sequence of *INHBA* genes from closely related species, one pair of primers, F1(5'-ATGCCCTTGCTCTGG-3' bp) and R1(5'-CTATGAGCAGCCACAC-3'), were designed to amplified an *INHBA* cDNA fragment (1275bp) from rabbit. Cycling parameters for PCR amplification were one cycle of 94°C for 3 min followed by 35 cycles of 94°C for 30 sec, 50°C for 30 sec, 72°C for 30 sec and a final extension step at 72°C for 10 min. The PCR products were gel-purified and sequenced (Fig 1).



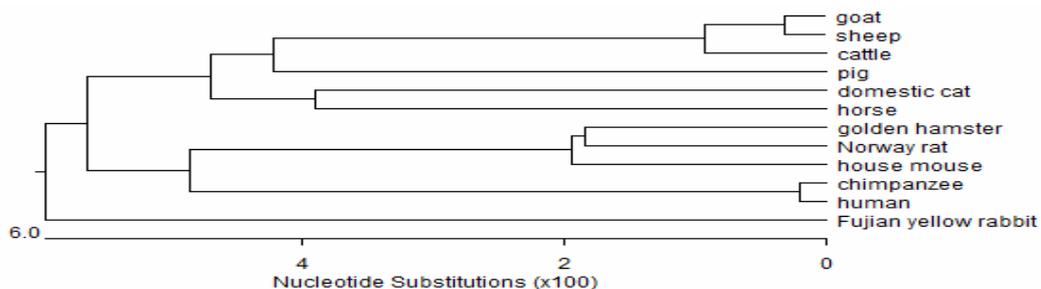
**Figure 1:** *INHBA* of Fujian Yellow rabbit by PCR. A: Core sequence of *INHBA*. The first lane is the PCR product, and the second lane is a negative control; B: first 3'RACE of *INHBA*. The first lane is the PCR product, and the second lane is a negative control; C: Core sequence of downstream region of *INHBA*. The first lane is the PCR product, and the second lane is a negative control; D: second 3'RACE of *INHBA*. The first lane is the PCR product, and the second lane is a negative control; E: 5'RACE of *INHBA*.

The 3' and 5' ends were obtained by rapid amplification of cDNA ends (RACE) approaches using 3'-Full RACE Core Set with PrimeScript™ RTase and 5'-Full RACE Kit with TAP (TaKaRa, Japan) following the manufacturer's instructions. Primers for 3'-RACE and 5'-RACE were listed in Table 1. The PCR products were ligated into T-Vector pMD™20 vector (TaKaRa, Japan) and transformed into the competent *E. coli* Competent cells JM109. Positive clones with the expected-size inserts were determined by PCR on clones and DNA sequencing.

**Table 1:** Primers for 3'-RACE and 5'-RACE

Name	Primer sequence(5'→3')	Annealing temperature (°C)
Specific reverse primer for 5'-RACE PCR	Outer: CTCCTGGCTGTGCCTGACTCG	60
	Inner: AGAGGCGGATGGTGACTTTGGT	60
Specific forward for first 3'-RACE PCR	Outer: CATCTTCCCGTCTCCAGCAGC	60
	Inner: AGCGAACTCTTGCTGTCGGAAA	60
Homology sequence of 3'-downstream region	Forward: TGCCAACCTCAAATCGTGCTGT	60
	Reverse: CTGGGTAATTGGGTAGGAAAGT	
Specific forward for second 3'-RACE PCR	Outer: GAGATGAAGCAGTGAAGGAGACAG	60
	Inner: CCAGTTGTAAATGGGGTATTGTCC	60





**Figure 3:** Homology analysis of *INHBA* on nucleotides in kinds of mammals

### CONCLUSIONS

The full cDNA (2339bp) of *INHBA* gene consists of a 5'-terminal untranslated region (UTR) (280bp), a 3'-terminal UTR (784bp) with a polyadenylation signal sequence (AATAA), a poly (A) tail, and an ORF(1275bp). The sequence result was compared with homologous sequences from mammalian animals including human, cow, sheep, and mouse. Sequence alignment showed that ranks of their similarities were above 95%, and meanwhile, the results of genetic evolution analysis of the gene showed a fairly high degree of homology between rabbits and primates.

### ACKNOWLEDGRMRNTS

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# Sequence analysis of cDNA encoding Fujian Yellow rabbit Inhibin $\beta$ A subunit precursor protein



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## The Message

Obtain the sequence of cDNA encoding Fujian Yellow rabbit Inhibin  $\beta$  A subunit precursor protein by methods of RT-PCR and RACE.

Obtain the result of the sequence cDNA encoding Fujian Yellow rabbit Inhibin  $\beta$  A subunit precursor protein. Multiple sequences alignment and phylogenetic analysis were performed using MEGA3.1.

## Introduction

Fujian Yellow rabbit is a valuable local breed under natural selection for a long time, which was mainly distributed in Fuzhou city of Fujian Province. It has a well-proportioned body, beautiful features, a pair of short and thick ears, black eyes, and is fully coated with the yellow fur. It also presents many excellent characteristics of wide adaptability, high disease resistance, juicy and delicious meat.

The inhibin beta A subunit joins the alpha subunit to form a pituitary FSH secretion inhibitor. Inhibin has been shown to regulate gonadal stromal cell proliferation negatively and to have tumor-suppressor activity. Furthermore, the beta A subunit forms a homodimer, activin A, and also joins with a beta B subunit to form a heterodimer, activin AB, both of which stimulate FSH secretion (Oshima et al., 2014). Thus, the inhibin beta A gene might be a candidate gene for the improvement of rabbit production.



## Methods

### Experiment animals

Female Fujian Yellow rabbits (2 to 2.5 kg and more than 6 months old) were purchased from Yuhuashan Rabbit Breeding Farm of Lianjiang (Fuzhou, China).

### RNA extraction and RT

Total RNA was extracted from female Fujian Yellow rabbit ovary tissues by TRIzol (Invitrogen, Carlsbad, CA, USA) according to the standard protocol. Based on the conserved sequence of INHBA genes from closely related species, one pair of primers, F1 (5'-ATGCCCTTGCTCTGG-3') and R1 (5'-CTATGAGCAGCCACAC-3'), were designed to amplify an INHBA cDNA fragment (1275bp) from rabbit.

### Rapid amplification of 5' and 3' cDNA ends (RACE)

The 3' and 5' ends were obtained by rapid amplification of cDNA ends (RACE) approaches using 3'-Full RACE Core Set with PrimeScript<sup>TM</sup> RTase and 5'-Full RACE Kit with TAP (TaKaRa, Japan) following the manufacturer's instructions. Primers for 3'-RACE and 5'-RACE were listed in Table 1. The PCR products were ligated into T-Vector pMDTM20 vector (TaKaRa, Japan) and transformed into the competent E. coli Competent cells JM109. Positive clones with the expected-size inserts were determined by PCR on clones and DNA sequencing.

## Methods

Table 1: Primers for 3'-RACE and 5'-RACE

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Homology sequence of 3'-downstream region	Forward: TGCCAACTCAAATGTGCTGT Reverse: CTGGGTAATTGGGTAGGAAAGT	60
Specific forward for second 3'-RACE PCR	Outer: GAGATGAAGCAGGAGGAGACAG Inner: CCAGTGTAAATGGGATGTGTC	60

### Sequence analysis

cDNA sequences of INHBA from Fujian Yellow rabbit were blasted to obtain known homologous sequences of INHBA genes from other species. Multiple sequences alignment and phylogenetic analysis were performed using MEGA3.1.

## Results

### Product of RT-PCR and RACE

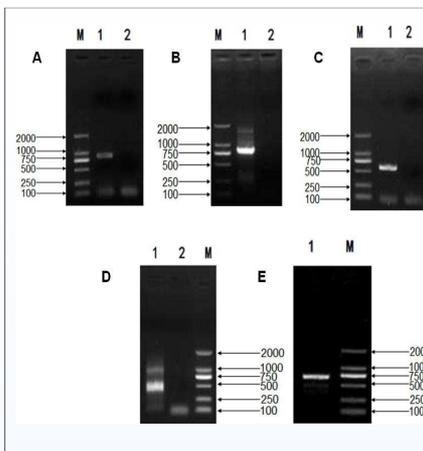


Figure 1: INHBA of Fujian Yellow rabbit by PCR. A: Core sequence of INHBA. The first lane is the PCR product, and the second lane is a negative control; B: first 3'RACE of INHBA. The first lane is the PCR product, and the second lane is a negative control; C: Core sequence of downstream region of INHBA. The first lane is the PCR product, and the second lane is a negative control; D: second 3'RACE of INHBA. The first lane is the PCR product, and the second lane is a negative control; E: 5'RACE of INHBA.

### Sequence analysis

The length of rabbit INHBA open reading frame was 1275 bp, encoding a protein with 424 amino acids residues. The 5'UTR is located 280 bp upstream of the putative start codon (ATG) and 3'UTR (784bp) is followed by a poly (A) tail. The full-length of nucleotide sequence and the deduced amino acid sequence are shown in Figure 2. The structure analysis indicated that there were two conserved domains of propeptide superfamily and TGF $\beta$  superfamily on INHBA protein.

## Results

Multiple sequence alignment showed that nucleotide sequence of Fujian Yellow rabbit INHBA shared high similarity with other INHBA sequence from goat, sheep, cattle, pig, domestic cat, horse, golden hamster, Norway rat, house mouse, chimpanzee, and human. Sequence alignment showed that ranks of their similarities were above 95%, and meanwhile, the results of genetic evolution analysis of the gene showed a fairly high degree of homology between rabbits and primates.

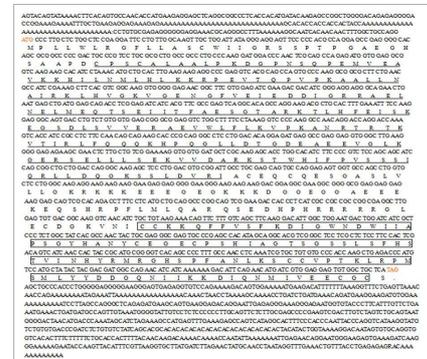


Figure 2: Sequence of INHBA from Fujian Yellow rabbit and its amino acid sequence. There are two classic conserved domains on the INHBA protein:  $\Delta$  represent TGF $\beta$  propeptide superfamily; A represent TGF $\beta$  superfamily.

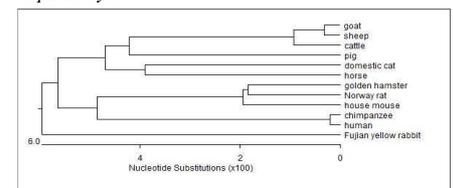


Figure 3: Homology analysis of INHBA on nucleotides in kinds of mammals

## Conclusion

The full cDNA (2339bp) of INHBA gene consists of a 5'-terminal untranslated region (UTR) (280bp), a 3'-terminal UTR (784bp) with a polyadenylation signal sequence (AATAA), a poly (A) tail, and an ORF(1275bp).

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