

# Cloning, homological analysis and construction of Eg95 Xinjiang strain DNA vaccine

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**OBJECTIVES:** To study the structure specificity of *Echinococcus granulosus* 95 (Eg95) gene and the open reading frame (ORF) of the full-length cDNA sequence in Xinjiang, northwestern China and construct Eg95 Xinjiang strain DNA vaccine.

**METHODS:** Primers of Eg95 were designed on the basis of the sequence of Eg95 antigen cDNA. Genomic DNA was extracted from *E. granulosus* protoscoleces (sheep) in Xinjiang. The Eg95 gene and full-length Eg95 cDNA were amplified by PCR from the genomic DNA and protoscolex cDNA library of *E. granulosus* in Xinjiang, respectively. The Eg95 gene was cloned into pUCm-T plasmid and the Eg95 cDNA into eukaryotic expression plasmid pcDNA3 for the construction of full-length ORF DNA vaccine pcDNA3-Eg95/XJ. Both Eg95 gene and Eg95 cDNA were sequenced and analyzed by DNAMAN and NCBI/Blast program.

**RESULTS:** DNA sequence analysis of Eg95 Xinjiang strain (Eg95/XJ) cDNA fragment indicated that the coding region of the full-length of Eg95/XJ was 471bp and that encoding a peptide with 156aa and the genomic DNA size was 1191bp. Homological comparison showed that the ORF of Eg95/XJ cDNA was identical to the cDNA sequence of Eg95 reported in the reading frame, but the genomic DNA was a new sequence, named Eg95/XJ and the multiple nucleotide differences, which were represented in Eg95/XJ gene in comparison with those of the New Zealand strain, occurred predominantly in the non-coding regions of the gene. The pcDNA3-Eg95/XJ positive clone was the exact recombinant plasmid and could be used as a DNA vaccine.

**CONCLUSION:** pcDNA3-Eg95/XJ Xinjiang strain DNA vaccine is successfully constructed.

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**Key words:** *Echinococcus granulosus*; Eg95/XJ gene; Eg95/XJ cDNA; homological analysis

## Introduction

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*Echinococcus granulosus* (*E. granulosus*) is an etiological agent of cystic echinococcosis (CE), which causes zoonotic infection and serious public problem particularly in regions where pastoral activities are prominent. Although a wide variety of animals can serve as intermediate hosts, sheep are the most important one worldwide.<sup>[1,2]</sup> Use of the *Echinococcus granulosus* 95 (Eg95) antigen of *E. granulosus* as a vaccine has been reported to provide high levels of protection against challenging infection with *E. granulosus* in sheep. Such success adds to the potential of the vaccine to be developed for direct use in humans.<sup>[3,4]</sup> But there is substantial genetic variability within the *E. granu-*

losus species<sup>[5]</sup> and antigenic variability in the Eg95 protein has the potential to limit the effectiveness of the Eg95-based vaccine in different *E. granulosus* isolates.<sup>[6]</sup>

DNA vaccines represent a new approach to the development of subunit vaccine. A number of studies have shown that DNA vaccination may induce antibody and cell-mediated responses to a variety of bacterial, viral and parasitic antigens. DNA vaccines should generate strong antibody and cell-mediated responses efficiently.<sup>[7]</sup> In this study, we sought to present the genomic structure and the cDNA sequence of Eg95 Xinjiang strain and to construct a recombinant plasmid of the Eg95 Xinjiang strain DNA for a vaccine development.

## Methods

### Primer design and synthesis

According to the published Eg95 cDNA sequences (GeneBank accession number X90928), the forward primer and reverse primer were designed using DNAMAN biosoftware and synthesized by Sangon Co., Shanghai, China. The forward primer, CGGAATTCATGGCATTCCAGTTATGTCTC, incorporated a *EcoR* I restriction site (*italics*) immediately upstream of the transcription initiation codon (**bold**), while the reverse primer, GCCTCGAGTCAAGTAAGACAAC, incorporated a *Xho* I site (*italics*) and a stop codon (**bold**).

### Amplification of the full-length Eg95 Xinjiang strain cDNA and construction of DNA vaccine pcDNA3-Eg95/XJ

DNA from the protoscoleces cDNA library of *E. granulosus* Xinjiang strain (sheep) was used as a template in PCR reaction with the primers specific for the Eg95 sequence. Standard cycling conditions for PCR were used with Pfu polymerase (Sangon, Shanghai, China): 95 °C, 6 min; 94 °C 30 s, 55 °C 30 s and 72 °C 2 min, for 30 cycles; and 72 °C 10 min. Eukaryotic expression plasmid pcDNA3 (Invitrogen, USA) was used to construct the DNA vaccine (Fig. 1). The PCR product was purified by the standard method and double digested by restriction enzyme *EcoR* I/*Xho* I (Takara, Da-

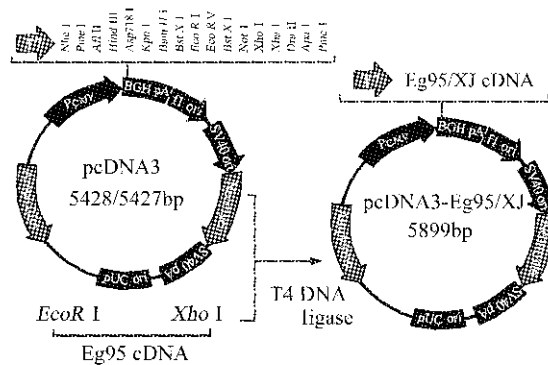


Fig. 1. Construction of pcDNA3-Eg95/XJ DNA vaccine.

lian, China). The digested PCR product was recovered using DNA recovery kit (Sangon, Shanghai, China) and was ligated to an *EcoR* I/*Xho* I digested pcDNA3 backbone to construct the pcDNA3-Eg95/XJ DNA vaccine. *E. coli* DH5 $\alpha$  was transformed with the resultant recombinants pcDNA3-Eg95/XJ and the positive bacteria colonies were screened by ampicillin. The recombination pcDNA3-Eg95/XJ was identified by *EcoR* I/*Xho* I digestion. Agrose gel electrophoresis demonstrated if the pcDNA3-Eg95/XJ was constructed successfully and the recombinants were sequenced.

### Isolation of the genomic DNA and construction of recombinant pUCm-Eg95/XJ

*E. granulosus* specimens used in this investigation were the protoscoleces of the Xinjiang sheep isolate which was obtained from naturally infected sheep. Genomic DNA was extracted from parasites using trizol reagent (Life Technologies, USA). PCR condition was the same as described above. The PCR products were cloned into pUCm-T vector (Sangon, Shanghai, China) and the positive clones were screened by blue/white screening with X-gal (Promega, USA) and IPTG (Promega, USA).

### Sequencing and analysis

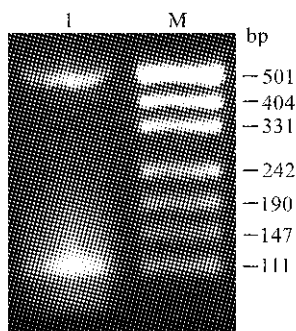
All DNA recombinants pcDNA3-Eg95/XJ and pUCm-Eg95/XJ- were verified by sequencing (Sangon, Shanghai, China). The sequence reports were analyzed using DNAMAN and BLAST biosoftware.

The large-scale production of pcDNA3-Eg95 DNA vaccine was accomplished using a standard method.

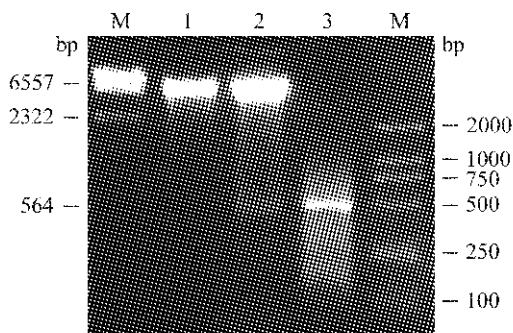
**Results**

**Construction of DNA vaccine pcDNA3-Eg95/XJ**

The size of 0.5kb Eg95 fragment containing the whole ORF was amplified from the *E. granulosus* protoscolex cDNA library of Xinjiang strain by using the specific Eg95 primers (Fig. 2). The DNA vaccine pcDNA3-Eg95 was constructed with the Eg95 cDNA fragment inserted into the cloning site of eukaryotic expressed vector pcDNA3. The recombinants were selected by *EcoR* I/*Xho* I restriction enzyme digestion and agarose gel electrophoresis



**Fig. 2.** Amplification of full-length Eg95 antigen cDNA. M: pUC19 DNA marker; Lane 1: amplified Eg95 cDNA from *E. granulosus* cDNA library.



**Fig. 3.** The restriction endonuclease analysis of pcDNA3-Eg95. M:  $\lambda$ -HindIII/DL2000 DNA marker; Lane 1: *EcoR* I digested result; Lane 2: *EcoR* I/*Xho* I double digested result; Lane 3: full-length Eg95 cDNA PCR fragment.

(Fig. 3). The sequences showed that the cloned cDNA segments in selected recombinants-pcDNA3-Eg95 were 471bp and encode 156 amino acid. The pcDNA3-Eg95 recombinant was constructed successfully and could be used as a DNA vaccine.

**Amplified the genomic DNA and construction of pUCm-Eg95/XJ**

The Eg95 DNA fragment was amplified from the genomic DNA of protoscolex of *E. granulosus* Xinjiang strain and was inserted into the pUCm-T vector. The white clones were picked out and sequenced completely. The fragment was 1191bp.

**Homological analysis**

Homological comparison showed the full-length ORF of Eg95/XJ cDNA was identical to the cDNA sequence of Eg95 reported by Lightowlers MW et al<sup>[3]</sup> (GeneBank accession No. X90928) in the open reading frame. But the genomic sequence report indicated a new sequence of *E. granulosus*, named Eg95/XJ (Genebank accession number AF-465599) and multiple nucleotide differences in the Eg95/XJ gene in comparison with those of the New Zealand strain, which occur predominantly in the non-coding regions of the gene.

**Discussion**

Cystic echinococcosis, caused by infection with larval stage of *E. granulosus*, affects both humans and domestic animals and it is recognized as one of the worldwide major zoonoses.<sup>[1,2]</sup> In some highly endemic regions of Xinjiang, Qinghai, Gansu, Ningxia in China, more than 5%–10% people have been infected by this parasite.<sup>[8]</sup> Use of vaccine has been considered as the most effective way to control this sort of transmission-infected diseases.<sup>[3,9]</sup> The Eg95 protein was regarded as a vaccine candidate to prevent from hydatid transmission for use in the parasite's natural animal intermediate hosts.<sup>[3,4,10]</sup> This study found that the genomic DNA sequence of Eg95/XJ was different from that of the New Zealand strain, but the open reading frame of Eg95 antigen was identical between the Xinjiang

strain and the other strains. These results show that the Eg95 antigen gene could be conserved and the degree of the conservation in different strains suggests that the Eg95 protein performs a function that may be vital in the parasite's biology. DNA vaccines represent a novel means of expressing antigens in vivo for the generation of both humoral and cellular immune responses and could be considered to have potential advantages because of easier construction, ability to induce long-lasting immune responses, high temperature stability, and low production cost. Ease and speed of production could be important, particularly for those vaccine antigens that differ from one epidemic to another; as such, DNA vaccination might provide several important advantages over current vaccines.<sup>[11-13]</sup> The pcDNA3 vector can express the insert gene fragment in eukaryotic cell efficiently and the constructed Eg95 Xinjiang strain DNA vaccine may provide a potential way to control echinococcus infection in China.

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### Competing interest

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

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