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RESEARCH PAPER

Construction of Vector of Gene Targeting to Multiple Loci for Leghorn Chicken Based on BAC with *Cre/lox* P System

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Abstract: Based on the sequence of BAC (Bacterial Artificial Chromosome) along with the *Cre/lox* P system, the gene-targeting vectors to multiple loci of the repetitive internal transcribed spacers between rDNA genes in Leghorn chicken were constructed. The main material of gene targeting to multiple loci in Leghorn chicken would be obtained.

First, the plasmid of pYLSV-TDN with *TK*, HRDS2, and *Neo* genes was constructed. The *TK*-HRDS2-*Neo* DNA fragment obtained from the plasmid of pYLSV-TDN was digested by *Not* I/*Hin*d III and inserted into the upstream of the l*ox* P site of BAC plasmid for obtaining the selective vector of BAC-TDN. The expression vector of pYLVS-GID with *EGFP*, *hIFN* genes, and HRDS1 was then obtained. The plasmid of BAC-TDN-VS-GID was obtained by cotransformation of the selective vector of BAC-TDN and the expression vector of pYLVS-GID to *E. coli* NS3529 through the action of *Cre/lox* P system. The gene-targeting vector of BAC-TDN-GID to multiple loci of the ITS region in Leghorn chicken was obtained by cleaving the sequence of pYLVS with the homing endonuclease of I-*Sce* I and ligating with the linker of LS. The insertion and the insert direction of DNA fragments were identified by restriction digestion or PCR and sequencing in each clone.

The significance of the technique of gene-targeting vector to multiple loci is shown as follows. First, the targeting loci were increased to 100–300. Second, the problems of unstable expression of inserted genes were partially solved. Third, the need for safety against toxicity integration was resolved. Fourth, the forbidden zone of gene integrating on the repetitive DNA sequences was broken through.

Key Words: gene targeting; multiple loci; vector; BAC; *Cre/lox* P system; Leghorn chicken; repetitive sequence

By introducing foreign gene into the cock in 1993, Sang first obtained a hen with human interferon protein in its vitelline[1]. Leghorn chicken has faster breeding cycle and high egg yield. The transgenic product of the egg has tremendous potential value, so transgenic Leghorn chicken can be a perfect kind of oviduct bioreactor[2]. Wang Xiaotong, Sun Huaichang, and Jamie Love *et al* successfully obtained the transgenic chicken into whose genome the reporter gene of *Lac* Z and the human growth hormone gene at random was inserted by microinjecting the foreign gene into the chick embryo[3,4]. Besides the chick embryo microinjection, there were other transgenic chicken techniques such as the ballistic transfection, the retrovirus vector, and the sperm vector for the primordial embryo cell and the methods for the stem cells

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such as primordial germ cells (PGC)[3].

However, the methods of production of transgenes at random considerably blocked the efficient integration, the stable heredity, and the expression of the foreign gene in transgenic animals. The gene-targeting technique wherein the foreign gene is inserted into a selected locus is an ideal measure to overcome the problems mentioned above. Although in the gene-targeting techniques, there were some problems such as low efficiency and the need to ensure safety, .the technique of gene targeting of multiple loci of repetitive sequences set up by the authors will resolve these problems to some extent[5].

Based on the sequences of BAC (Bacterial Artificial Chromosome) with *Cre/lox* P system, the gene-targeting vectors to multiple locus of the repetitive internal transcribed spacers between rDNA genes in Leghorn chicken carrying *EGFP* (enhanced green fluorescence protein) gene and *hIFN* (human interferon) genes were constructed. The main material of gene targeting to multiple loci in Leghorn chicken would be obtained.

1 Materials and methods

1.1 Materials

The materials used were as follows: Plasmid of p*CTKNeo* (kept at the Molecular Biology Laboratory of Foshan University, plasmid of p*EGFP* (kept at the Biochemistry and Molecular Biology Laboratory of Jinan University, plasmid of BAC747N, pYLSV, and pYLVS and the *E. coli* strain of NS3529 (donated by Professor Liu YG, Southern China Agriculture University[6], recombinant plasmid of pCDNA3-*hIFN* (donated by Laboratory of Infectious Disease of Xiangya Hospital, gel extraction kit, LA PCR Taq polymerase, GC buffer, restriction enzymes (from TaKaRa) and the homing endonuclease of I-*Sce* I (from New England Biolabs).

1.2 Construction of the selective vector of BAC-TDN

1.2.1 Construction of the plasmid of pYLSV-TDN: HRDS1 (homogenous recombination direct sequence, HRDS) DNA fragment ending in ITS1 (internal transcribed spacer, ITS) was amplified from the rDNA gene family of Leghorn chicken genomic DNA by LA Taq PCR[7,8]. HRDS1 DNA fragment, which was digested with *Bam*H I/*Sal* I, was inserted into the plasmid of pYLSV for obtaining the plasmid of pYLSV-HRDS1. The *TK* (thymidine kinase gene) and *Neo* gene DNA fragment amplified from the plasmid of pCTKNeo and digested with *Sal* I/*Hin*d III and *Xho* I/*Bam*H I, respectively, were inserted into the upstream and downstream, respectively, of HRDS1 of the plasmid of pYLSV-HRDS1. The plasmid of pYLSV-TDN (pYLSV-*TK*-HRDS1-*Neo*) was constructed through identification using enzyme digestion and DNA sequencing.

1.2.2 Construction of the selective vector of BAC-TDN: The *TK*-HRDS1-*Neo* DNA fragments of pYLSV-TDN digested with *Hin*d III/*Not* I were separated on 0.8 % agarose/TAE gel and purified from the gel with the gel extraction kit according to the manufacturer's instruction, and then inserted into the plasmid of BAC747. The selective vector of BAC-TDN was constructed through identification using PCR and DNA sequencing.

1.3 Construction of the expression vector of pYLVS-GID

For deleting the multiple cloning site between pCMV (CMV promoter) and *EGFP* CDS (coding domain sequence) in the plasmid of p*EGFP*, the pCMV and *EGFP* CDS DNA fragment were amplified beyond the multiple cloning sites and digested with *Sal* I/*Pst* I and *Pst* I/*Hin*d III, respectively, following which it was inserted into the plasmid of pUC18. The plasmid of pUC-pCMV-*GFP* was constructed following identification using PCR.

pCMV-*GFP* was amplified from the plasmid of pUC-pCMV-*GFP*, whereafter digested with *Hin*d III/*Sal* I and inserted into the plasmid of pYLVS for constructing the plasmid of pYLVS-*GFP*. HRDS2 DNA fragment was amplified beginning with ITS1, from the rDNA gene family of Leghorn chicken genomic DNA. HRDS2 DNA fragment digested with *Nh*e I/*Cl*a I was inserted into the plasmid of pYLVS-*GFP* for obtaining the plasmid of pYLVS-*GFP*-HRDS2. The *hIFN* gene DNA fragment was amplified from the plasmid of pCDNA3-*hIFN* and digested with *Sal* I/*Nh*e I, and then inserted into the plasmid of pYLVS-*GFP*-HRDS2. Finally, the expression vector of pYLVS-GID (pYLVS-*GFP*-*hIFN*-HRDS2) was constructed through identification using PCR, enzyme digestion, and DNA sequencing.

1.4 Construction of the vector of BAC-TDN-GID

The plasmid of BAC-TDN-VS-GID was obtained by the cotransformation of the selective vector of BAC-TDN and the expression vector of pYLVS-GID to *E. coli* NS3529 through the action of *Cre/lox* P system. To screen positive colonies, two kinds of antibiotics, namely, chloramphenicol and kanamycin were used. The plasmid of BAC-TDN-VS-GID was isolated from the mixture of the plasmids of BAC-TDN-VS-GID, BAC-TDN and pYLVS-GID by gathering all positive colonies and extracting the mixture of the plasmids for transformation to *E. coli* DH10B.

The gene-targeting vector of BAC-TDN-GID to multiple loci of Leghorn chicken was then obtained by cleaving the sequence of pYLVS with the homing endonuclease of I-*Sce* I and ligating with the linker of LS (Fig. 1). The insertion and the insert direction of DNA fragments were identified by restriction digestion or PCR and DNA sequencing of each clone.

2 Results

2.1 Identification of the plasmid of pYLSV-TDN

The double enzyme digestion products of pYLSV-TDN

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