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Construction of Genomic DNA Library of Beijing Fatty Chick InλphageEMBL3 and Screen of Chick Heart Type Fatty Acid Binding Protein Gene

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Abstract: A genomic DNA library of Beijing Fatty chick was constructed in phage EMBL3 with a titer of 3.85×10^5. To testify the utility of this library we screened recombinant phages containing chick heart type fatty acid binding protein gene by Southern hybridization. In order to simplify the process of screen we determined sub-library which contained target gene firstly by PCR with specific primers. Several positive recombinants were detected. We selected one randomly to be digested with *Sal* I. Results showed inserted exogenous DNA was about 15Kb.

Key words: DNA library, Beijing fatty chick, fatty acid binding protein gene, screen

Introduction

Beijing Fatty Chick is one of Chinese traditional chick breeds. In Qing dynasty Beijing Fatty chick was reared only for consumption of the royal courts. Nowadays this cherish chick breed is reared only in the conservative farm in Constitute of Animal Science, Chinese Academy of Agricultural Sciences. Beijing Fatty Chick is famous for its excellent meat quality. Results of researches relevant to its good traits have shown that it has low abdominal fat and high intramuscular fat (IMF) content compared with broils and many other Chinese local chick breeds (Chen *et al*., 2006; Chai *et al*., 2005) Since IMF is directly connected with meat quality such as juicy and flavor, genes involved in the metabolism of triglyceride have been selected to be studied as candidate genes contributed to its outstanding performance. Adipocyte type and heart type fatty acid binding protein (H-FABP) genes, which belong to one family, are two of them (Ye *et al*., 2002, 2003, 2007).

In this study, we constructed a genomic DNA library of Beijing fatty chick in phage EMBL3 with average inserted fragments of 9-20Kb to promote the conservation of this unique chick breed and facilitate clone of more candidate genes. We also testify the practical application of this library by screening chick H-FABP gene from it.

Materials and Methods

Construction of Genomic DNA Library

Extract of genomic DNA: Blood from a healthy female Beijing Fatty chick was obtained from the conservative farm in Constitute of Animal Science, Chinese Academy of Agricultural Sciences. 30 μL blood was added into 470 μL extract buffer containing 10mM Tris-Cl (pH8.0), 100 mM EDTA (pH8.0) and 20 μg mL^{-1} pancreatic RNase. Proteinase K and SDS were added further to final concentration of 200 μg μL^{-1} and 0.5% respectively. After thoroughly mixed, digestion was performed in 56°C overnight. Phenol and trichloromethane were used to remove protein contents and ice-cooled alcohol to precipitate DNA.

Partly digestion of genomic DNA to 9-20Kb: *Sau3A*I (Promega) was used to digest genomic DNA. Dilution buffer contained 0.1 mg mL^{-1} actyl-BSA. Enzyme solution was diluted to 3 Units per μL. To select the optimum reaction condition 45 μL genomic DNA solution, which contained about 1 μg genomic DNA, was mixed with 5 μL *Sau3A*Isolution of ten different dilution proportions (Table 1). Mixture was incubated in 37°C for 30min. Reaction products were detected by electrophoresis in 0.3% Agarose gel. Tube, in which most products centered in the size of 9-20Kb, contained the most optimum reaction proportion of enzyme and genomic DNA.

Recovery of DNA fragments by electrophoresis and dialysis: Genomic DNA was digested in large scale under optimum reaction condition. Products were separated in 0.3% Agarose gel by electrophoresis and stained with ethidium bromide. Agarose gels contained 9-20Kb fragments were cut by a knife under UV and put in a dialytic bag filled with 1×TAE buffer. Get rid of air and extra 1×TAE buffer carefully and secure gels were immerged in as little TAE buffer as possible. Put dialytic bag in electric field. Electrophoresis was performed under 4-5V/cm overnight. When DNA had transferred from gel to the wall of dialytic bag, we inverted the direction of electric field for 1min. TAE buffer in dialytic bag was collected and dialytic bag was washed with 1×TAE buffer for once. TAE buffer containing DNA of 9-20Kb was collected together and centrifuged under 12000g for 30 seconds to remove gel scales. Then they were extracted by n-Butyl alcohol for several times to

Table 1: Genomic DNA digested by *Sau3A* I of different dilutions

Tube			Dilution buffer	Dilution proportion	Enzyme Unit/μg DNA
1	10 μL Enzyme solution	+	140 μL	1:15	1
2	μL 1:15 diluted Enzyme solution	+	90 μL	1:150	0.1
3	μL 1:150 diluted Enzyme solution	+	10 μL	1:300	0.05
4	μL 1:150 diluted Enzyme solution	+	30 μL	1:600	0.025
5	μL 1:150 diluted Enzyme solution	+	50 μL	1:900	0.017
6	μL 1:150 diluted Enzyme solution	+	70 μL	1:1200	0.0125
7	μL 1:150 diluted Enzyme solution	+	90 μL	1:1500	0.01
8	μL 1:150 diluted Enzyme solution	+	110 μL	1:1800	0.0083
9	μL 1:150 diluted Enzyme solution	+	190 μL	1:3000	0.005
10	μL 1:150 diluted Enzyme solution	+	290 μL	1:4500	0.0033

Table 2: Ligations of vectors arms and insert DNA fragments under different proportions

	Positive control	A	B	C	Negative control
Vector arms (digested by *Bam* H I, 0.5 μg μL^{-1})	2 μL	2 μL	2 μL	2 μL	2 μL
Inserted fragments (9-20Kb, 0.2-0.3 μg μL^{-1})	2 μL pTI11 (digested by *Bam*H I)	3 μL	2 μL	1 μL	
10×Buffer	1 μL	1 μL	1 μL	1 μL	1 μL
T_4DNA Ligase (3U μL^{-1})	1 μL	1 μL	1 μL	1 μL	1 μL
Distilled water	4 μL	3 μL	4 μL	5 μL	6 μL
Total volume	10 μL	10 μL	10 μL	10 μL	10 μL

reduce in bulk until final volume reached 400 μL. Phenol, trichloromethane and isoamyl alcohol (24:23:1) were used to remove protein and alcohol was used to precipitate DNA. Recovery DNA was diluted in 20 μL TE (pH8.0). Length of products was detected by electrophoresis in 0.4% Agarose gel to detect its purity and concentration.

Ligation of DNA fragments and vector arms from λ phage EMBL3: Protruded fragments digested by *Sau3A*I can be ligated by T4 DNA Ligase with vector arms digested by *BamH*I. We fixed the concentration of vector arms (Promega) and changed concentration of inserted DNA fragments to determine the optimum condition of ligation (Table 2). Ligation was performed in 4°C overnight. Products were detected by electrophoresis in 0.4% Agarose.

Package *in vitro* and determination of titer: In the function of package proteins, ligation products of genomic DNA and vector arms would be packaged to form recombined EMBL3 phages. All the processes were conducted according to the direction of protocol. *Ecoli*. KW251 was used as host strain. After recombinant phages were diluted to different dilution, titer was determined according to the number of Phage Forming Unit (pfu).

Screen of Chick *H-FABP* gene from the library

Primary screen by PCR to determine sub - library: Primer P_1 was designed (using Software Primer5.0), with upstream sequence 5'-AGCACCTTCAAGAACACAGAGA-3' and downstream sequence 5'-GACCAGCTTGCCTCCATCTA-3', to amplify the second intron of chick *H-FABP* gene. Target fragment was 2054bp. Another primer P_2 with upstream sequence 5'-TAGCATAGTTTCCACCACCC-3' and down stream sequence 5'-CATTGCAGCATACTTCTG TCTT-3' was designed to amplify 221bp fragment within the product of P_1.

Host strain *Ecoli*. KW251 from one clone was cultured to reach exponential growth stage in LB medium. 100ul host strain was mixed with recombinant phage that contained 10^4pfu and incubated in 37°C for 20min. Then LB medium was added to a final volume of 9.5ml. When mixed thoroughly, 100ul per well was distributed to a 96-well plate. The last well that contained host strain only was negative control. Plate was incubated in 37°C for 5-6 hours until phage reached lysis stage.

PCR was performed in a reaction volume of 12ul containing 1ul bacterial lysis from each well as template, 0.5μM of each primer, 1×PCR buffer [10mM Tris-HCl (pH9.0) and 1.5mM $MgCl_2$], 0.2mM dNTPs and 2 units of *Taq* DNA polymerase (Takara). The first cycle was 5min at 95°C followed by 34 cycles of 30s at 94°C, 30s at 62°C and 2min (P_1) or 30s (P_2) at 72°C, ending with a 10min extension phase at 72°C. PCR products were detected by Agarose gel or 0.8% polyacrylamide gel electrophoresis (PAGE). Wells which had been successfully amplified to show target fragments were determined to be where the sub-library that contained chick *H-FABP* gene located.

Southern hybridization: Probe was prepared by using random primer of 6bp. PCR products amplified by P_1 from chick genomic DNA was used as template DNA. dUTP was labeled by DIG and antibody to DIG was connected with Alkaline Phosphatase (AP). When substrate, CSPD (Roche), was dephosphorated by AP, luminescence from intermittent products could be

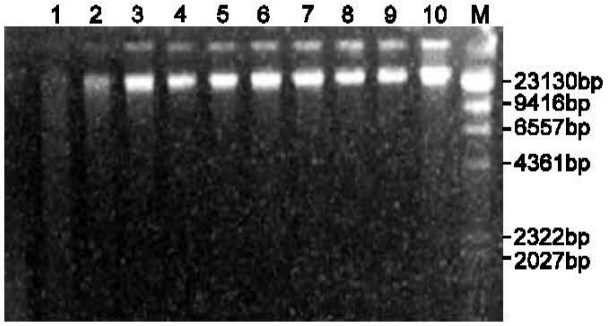

Fig. 1: Genomic DNA digested by different dilutions of *Sau3A*I (0.3%Agarose), Lane1-10: Tubes with different dilution of *Sau3A*I. Lane M: λDNA/*Hind*III

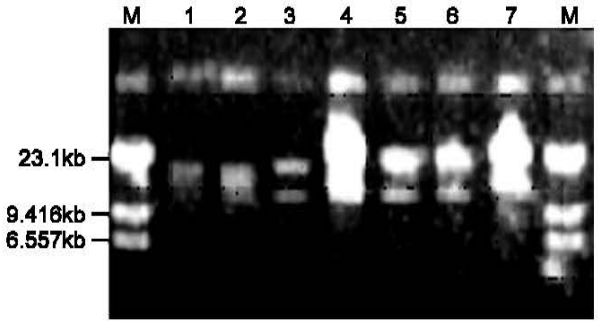

Fig. 2: Ligation of vector and exogenous DNA under different proportions (0.4%Agarose), Lane 1 and 2: DNA fragments prepared by electrodialysis after digested with *Sau*3AI, Lane 3: negative control. Lane 4, 5, 6: Proportion of vector arms to exogenous DNA was 2:1, 1:1 and 1:1.5 respectively. Lane 7: positive control. Lane M: λDNA/*Hind*III

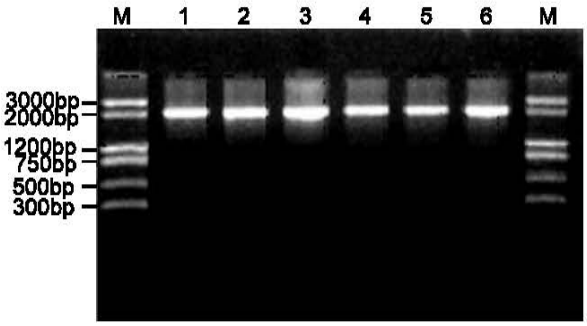

Fig. 3: PCR products of *H-FABP* gene intron2 from genomic DNA, Lane1-6:Individual PCR products

captured by X film. Processes of southern hybridization were conducted according to the protocol.

Detect the length of inserted fragment in positive recombinant phage: We selected randomly one positive recombinant phage to be incubated with *E coli*. KW251 until lysis stage. Recombinant phage DNA were prepared and digested by *Sal* I. Products were separated in 0.7% Agarose by plectrophoresis.

Results

Construction of Genomic DNA Library

Preparation of genomic DNA: We selected blood as the source of genomic DNA because red cell of chick has nuclear. Integrity of genomic DNA was of great importance to the construction of genomic DNA library. In our study we detected DNA preparation by pulsed field gel electrophoresis. Only DNA preparation more than 150kb were used to be further digested with *Sau3A* I.

Determination of optimum reaction condition to produce 9-20Kb DNA fragments: From Fig. 1 we could see most genomic DNA in lane 1 and lane 2 were degraded to small fragments by high concentration of *Sau3A* I. Genomic DNA in lane 3 had the largest quantity of fragments about 9-20Kb with only small parts of products shorter than 9Kb. From lane 4 to lane 10, genomic DNA was digested incompletely. We concluded the most suitable proportion between restriction enzyme and genomic DNA was 0.05U *Sau3A* I/μg DNA.

Recovery of DNA fragments and ligation with vector arms: 9-20Kb fragments produced by partly digestion of genomic DNA by *Sau3A*I were successfully recovered by electrodialysis (lane 1 and 2 in Fig. 2). *Sau3A*I and *Bam*H I have the same cohesive ends. Vector arms digested by *Bam*H I and DNA fragments digested by *Sau3A*I could be ligated together by T4 Ligase. Since ligation efficiency in lane 4 was almost the same as the positive control, we concluded the optimum proportion of vector arms and inverted fragments was 2:1.

Titer of genomic DNA library: The genomic DNA library from Beijing Fatty chick we conducted had a titer of 3.85×10^5.

Screen of Chick *H-FABP* gene from the library

Results of P_1 and P_2 amplification by PCR: Target fragments could be specifically amplified by P_1 when genomic DNA was used as template (Fig. 3). However, when phage lysis was used as template, products amplified by P_1 could only been weakly seen by PAGE. We presumed length of the fragment (2054bp) was too long to affect the efficiency of amplification. Another primer P_2, with product of 221bp and located in the middle of P_1 product was used to determine where sub-library located. Wells in which 221bp target fragment could be successfully amplified were deemed as where positive clones located (Fig. 4).

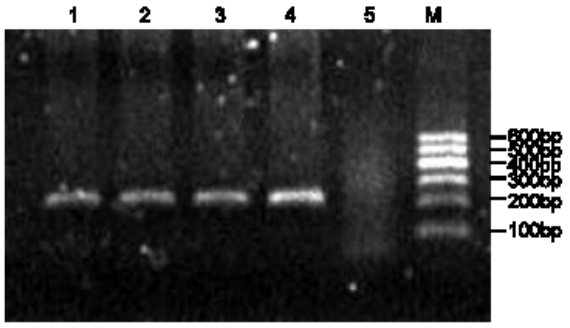

Fig. 4: PCR products of P_2 from different wells where sub-library located. (1% Agarose), Lane 1-4: Individual wells. Lane 5: Negative control. Lane M: 100bp ladder

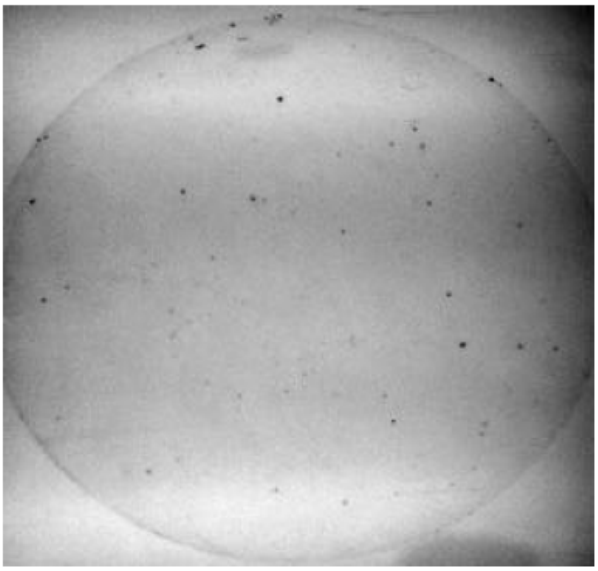

Fig. 5: Result of Southern hybridization

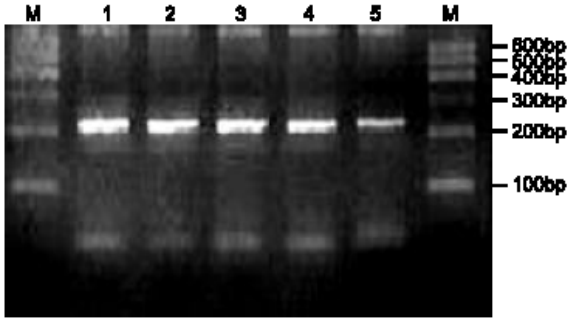

Fig. 6: Results of PCR amplified by P_2, Lane 1-5: Different positive clone. Lane M: 100bp ladder

Screen of positive clones from sub-library by Southern hybridization: Wells in which sub-library located were used to screen positive clones containing chick *H-FABP* gene by Southern hybridization (Fig. 5). We selected randomly several positive clones detected by Southern hybridization and used phage lysis from single clone as template to be amplified by P_2 (Fig. 6). Target fragments could be amplified specifically.

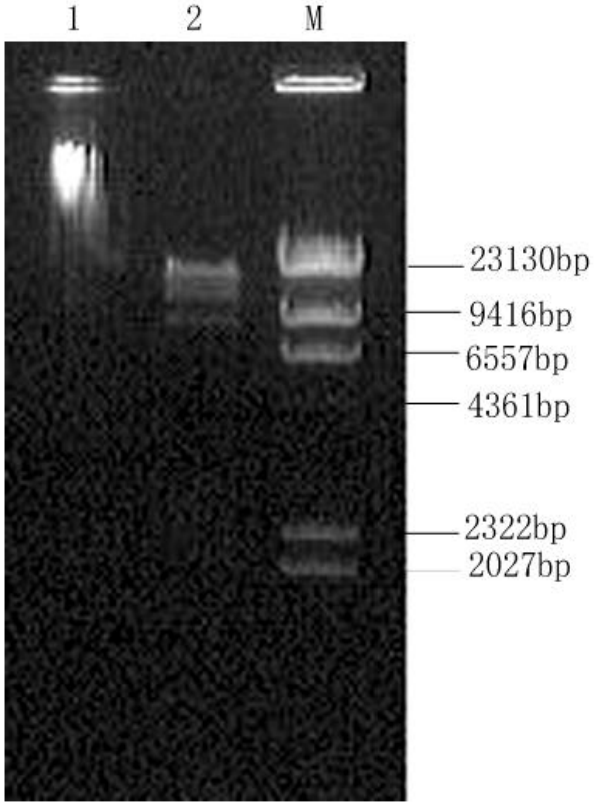

Fig. 7: Identification of the length of inserted DNA, Lane1: Recombinant DNA. Lane2: Recombinant DNA digested by *Sal* I

Identification of inserted fragments: After digested by *Sal* I, positive recombinant phage DNA could produce integrate exogenous fragment. Three fragments were produced: 20.5Kb and 9Kb were two vector arms from phage and 15Kb fragment was inserted DNA fragment.

Discussion

Compared with cDNA library, DNA library contains more information of organisms' genome. It is useful in the screen of target genes to obtain their whole sequences including introns and exons. In this study we constructed a genomic DNA library from Beijing Fatty chick in phage EMBL3 with a titer of 3.85×10^5. In theory each gene has the chance to be cloned from a integrate library. To testify the practicality of this library we tried to screen chick *H-FABP* gene from it. Several positive recombinant phages

were successfully detected. We concluded that determination of sub-library where target gene located before Southern hybridization could minimize the scope and promote the process of screen greatly. Our results showed that this DNA library could represent the integrity of genomic DNA and could be used to screen interested gene in the future. Up till now, this is the first report about the construction of genomic library of Beijing Fatty Chick. This work makes Beijing fatty chick, a national important genetic resource preserved in DNA level. It also provides future researches an ideal experimental material for related genetic studies. H-FABP gene is a candidate gene, which influences the content of IMF of chick. We have already found a *Hha*I and a *Msp* I polymorphic site by RFLP analysis in the second intron of H-FABP gene. Their relationship with fatness related traits such as content of IMF, body weight, percentage of abdominal fat weight to body weight were studied in Beijing fatty chick and Dwarf chick. We found out that specific performance was significantly related to different genotypes in tested populations (Ye *et al*., 2003, 2007). Clone and sequence of chick *H-FABP* gene will help us reveal extra genetic variations and find useful genetic polymorphisms that contribute to the excellent performance of Beijing fatty chick.

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