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BAC Library Construction and Characterization of Suyuan 7235, a Cotton Germplasm with High Fiber Strength

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Abstract: The bacterial artificial chromosome (BAC) library of Suyuan 7235, a cotton germplasm with high fiber strength has been constructed following the partial digestion of genomic DNA with HindIII. The pIndigoBAC-5 (*Hind*III-cloning ready) cloning vector was used for the library. The BAC library included 30336 clones. Analysis of 96 recombinants showed that the insert DNA size ranged from 50 kb to 140 kb, averaged 120 kb with less than 2.1% of empty clones. As much as 89.6% clones inserts are over 100 kb. As a genomic resource, the library has potential use in further research of fiber strength gene analysis and gene cloning, as well as SSR primer development.

Key words: cotton (*Gossypium hirsutum*); fiber quality; bacterial artificial chromosome (BAC) library

Along with the development of molecular biology, identification and cloning of elite fiber quality genes become the hotspots in cotton biotechnology research. Bacterial artificial chromosome (BAC) library with large insert DNA fragments is an important genomic resource for map-based cloning[1], physical mapping[2-4], genome sequencing[5] and SSR primer development[6]. Today, BAC libraries have been constructed in many plants, such as wheat, barley, soybean, sorghum, potato, *Arabidopsis thaliana*, sugarcane, pearl millet, citrus, coffee tree, and chickpea. These BAC libraries accelerate the research of plant genomics[7].

Suyuan 7235, as an important basic material for fiber quality improvement[8], is a cotton germplasm bred from wild species, *Gossypium anomalum*, with high fiber strength. Molecular linkage map and QTL mapping of fiber strength have been reported using the variety as parent[9]. Therefore, the BAC library construction of this variety is very significant for identification and cloning of elite fiber quality genes.

1 Materials and Methods

1.1 Materials

Suyuan 7235 was obtained from the Cotton Research Institute of Chinese Academy of Agricultural Sciences (CRI, CAAS). Competent cell DH10B (Invitrogen, USA) and cloning vector pIndigoBAC-5 (*HindIII*-cloning ready, Epicentre Technologies, USA) were used for the cloning.

1.2 Methods

1.2.1 *Preparation of high-molecular-weight (HMW) genomic DNA.* Megabase-sized DNA was prepared from 25 g fresh yellow cotyledons of seedlings grown for less than 1 week in a growth chamber. The cotyledons were grinded to powder in liquid nitrogen for 1 h. The powder was transferred to 250 mL ice-cold 1×HB wash buffer (with TritonX-100 and 0.15% β- mercaptoethanol) and swirled on ice for 15 min. Then the homogenate was filtered into several 50 mL centrifuge tubes through two layers of

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cheesecloth and two layers of miracloth and spinned the tubes in a centrifuge at 4℃, 4500 r • min^{-1} for 20 min. The supernatants were decanted, then 1 mL ice-cold 1×HB (without TritonX-100, with 0.15% β- mercaptoethanol) was added to each pellet, and gently re-suspended the nuclei with a small paintbrush. In each tube, 1× HB (without TritonX-100, with 0.15% β-mercaptoethanol) was added to 50 mL and centrifuged at 4℃, 4500 r • min^{-1} for 15 min (the above step was repeated for 3 times). The supernatants were decanted, then ice-cold 1 × HB (without TritonX-100 and 0.15% β- mercaptoethanol) was added to each pellet and centrifuged at 4℃, 4500 r • min^{-1} for 15 min. The nuclei acid was re-suspended and adjusted with 1× HB (without TritonX-100 and β-mercaptoethanol) to be clear.

The tube containing nuclei was placed in the 45°C water bath for 5 min and mixed with an equal volume of the pre-warmed LMP agarose solution. Then nuclei/agarose mixture was placed into the wells of the pre-chilled plug mold. The plug mold was solidified on ice for 30 min. The plugs were incubated in the lysis buffer with 0.2 g • L^{-1} proteinase K at 50°C for 18 h, then the lysis buffer was changed, and the plugs were incubated at 50°C for 21 h. Final washes of the plugs were carried out in $T_{10}E_{10}$ (10 mmol • L^{-1} Tris-HCl, pH 8.0; 10 mmol • L^{-1} EDTA pH 8.0) containing 1 mmol • L^{-1} PMSF on ice for 1 h and with $T_{10}E_1$ (10 mmol • L^{-1} Tris-HCl, pH 8.0; 1 mmol • L^{-1} EDTA pH 8.0) for 4 times on ice for 1 h each. The washed plugs were stored in $T_{10}E_1$ at 4°C. Samples of 1/2, 1/4, and 1/8 plug pieces were performed with PFGE (BioRad CHEF DRII, USA) in 0.5 × TBE buffer under conditions of 6.0 V • cm^{-1}, initial pulse 50 s, final pulse 50 s, pump 80, 11°C, 18 h in order to examine the DNA qualities and concentrations of the plugs. Based on relative staining intensity of the samples compared to lambda standards, a mean DNA amount per plug could be estimated.

1.2.2 *Determination of the optimal enzyme concentration of partial digestion and size selection.* The optimal enzyme concentration was determined with partial digestion of plug DNA with 0, 0.4, 1, 2, 3, 4, 6, 8, 10, 18 Units of HindⅢ. Large scale restriction digestion at the optimal enzyme concentration was processed for six plugs. Two size selection method was taken to obtain the optimal size fragments. After first size selection of PFGE, 100～200 kb, 200～300 kb and 300～400 kb of DNA were cut from the gel based on 50 kb lambda ladder marker. Initial size selection condition was: 1% agarose gel, 11℃, initial pulse 50 s, final pulse 50 s, 18 h run time, 6 V • cm^{-1}, angel 120°, pump 80. The gel slices were transferred to perform the second PFGE and run at 1% agarose gel, 11℃, initial pulse 3 s, final pulse 5 s, 16 h, V • cm^{-1}, angel 120°, pump 80. The gel slices were excised, and DNAs were removed from the agarose and electroeluted for 2 h using the BioRad Electro-Eluter system. DNA concentrations were examined on 0.8% agarose gel. If the concentrations were very low, DNAs were concentrated with dialyzing on 10% PEG8000 for 90 min.

1.2.3 *Ligation and transformation.* The ligation was performed with the molar ratios of 5:1, 10:1 and 15:1 of vector DNA and HMW DNA. The ligates were dialyzed on the ddH_2O for 2 h, and then concentrated using 30% PEG8000 for 30 min. Two microlitres of ligates was transformed to 20 μL electrocompetent DH10B TM cells using the Cell-porator Electroporation System (Gibco BRL Company). The Cell-porator settings were 350 V, 330 μF capacitance, low ohms impedance, fast charge rate, and 4 kΩ resistance. After the electroporation, the cells were immediately transferred to 1 mL of SOC medium and cultured with 225 r • min^{-1} shaking at 37 °C for 1 h. Recombinant colonies were selected on LB agar plates containing 500 mg • L^{-1} IPTG and 50 mg • L^{-1} X-gal with 12.5 mg • L^{-1} chloramphenicol at 37 °C for 16～24 h.

1.2.4 *Isolation and analysis of insert DNA.* To

estimate the insert sizes, the alkaline lysis method was used for preparing the plasmid DNA from random clones of the BAC library. Plasmid DNA samples were digested with NotI, and then loaded on 1% agarose gel. PFGE was performed under conditions of 11°C, 6.0 V • cm^{-1}, 5～15 s pulse, angle 120°, pump 70 and 16 h. After the electrophoresis, the gel was stained for 30 min in 0.5 mg • L^{-1} ethidium and distained for 30 min in ddH_2O. The average insert size and empty clone of the BAC library were estimated by comparison with a Lambda ladder PFG marker.

2 Results

2.1 Quality and concentration of DNA plugs

In the study, diethyldithiocarbamic acid (DIECA) and polyvinylpyrolidone (PVP40) were added to 1× HB wash buffer (with TritonX-100 and 0.15% β-mercaptoethanol) in order to inhibit the high levels of phenolic compounds in the seedling tissue. PFGE results showed that DNA plugs had good quality, high concentration and low degradation (Figure 1).

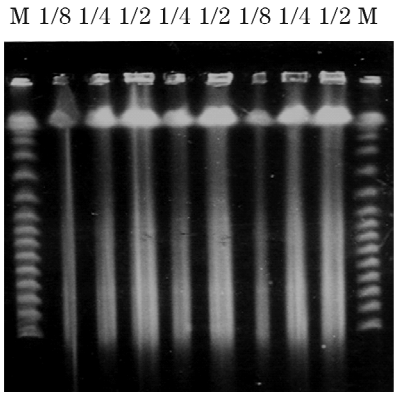


Fig. 1 PFGE products of DNA plugs

2.2 Determination of the optimal enzyme concentration

Partial digestion was carried out in the 30 min by changing the concentration of restriction enzyme *Hind*III. PFGE results showed that 3～4 Units of *Hind*III were optimal, and digested DNA concentrated on 100～400 kb (Figure 2).

2.3 Two size selections of digested DNA

Large scale digestion was performed using 3.5 Units of *Hind*III. In the first size selection, 100～400 kb fragments were excised (Figure 3A). Then the main bands of 100～400 kb were cut into 100～200 kb, 20～300 kb and 300～400 kb for the second size selection. Due to 100～200 kb was a smear, only 200～300 kb and 300～400 kb of fragments were reclaimed and used for electroelution (Figure 3B).

2.4 Ligation and transformation

Transformation efficiency was influenced by the molar ratio of vector and HMW DNA. In the study, the molar ratio of 5 : 1, 10 : 1 and 15 : 1 of vector and HMW DNA were used. The transformation results showed that 10 : 1 was suitable

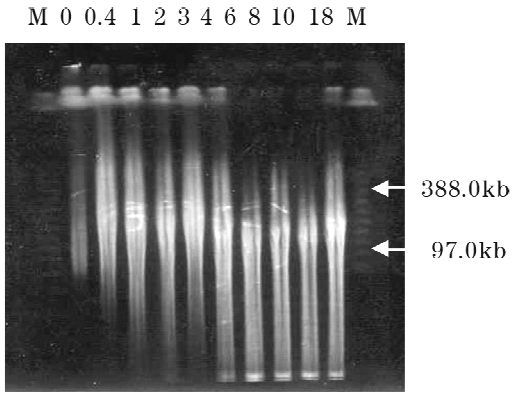
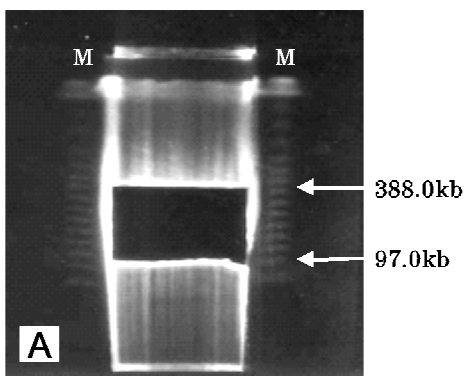


Fig. 2 PFGE products of different *Hind*III concentrations



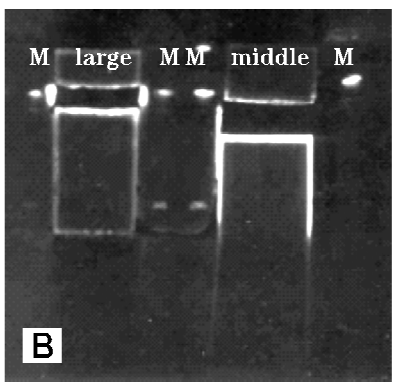


Fig. 3 PFGE products of 1st (A) and 2nd (B) selection for large DNA fragments

and had a high transformation efficiency. 1200 clones were obtained at each transformation, and recombination efficiency was high. According to the ratio, mass transformation was done, and 30336 clones of the BAC library were obtained.

2.5 Isolation and analysis of insert DNA

Ninety-six BAC clones were checked randomly and extracted according to alkali lysis method. PFGE results showed that insert DNA ranged from 50 kb to 140 kb, averaged 120 kb with less than 2.1% of empty clones (Figure 4).

The distribution of insert DNA from 96 clones was analyzed. The clones of less than 60 kb accounted for 1.0%. The clones with 60～80 kb, 80～100 kb, 100～120 kb and 120～140 kb of the insert DNA accounted for 2.1%, 7.3%, 72.9% and 15.6%, respectively. One clone had over 140 kb insert fragment. As much as 89.6% clones had inserts over 100kb (Figure 5).

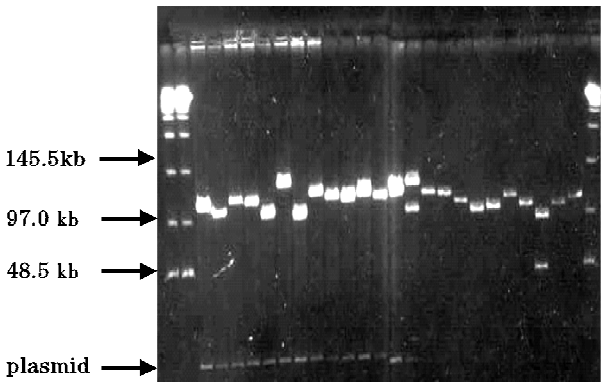


Fig. 4 Insert size of Suyuan7235 BAC recombinants

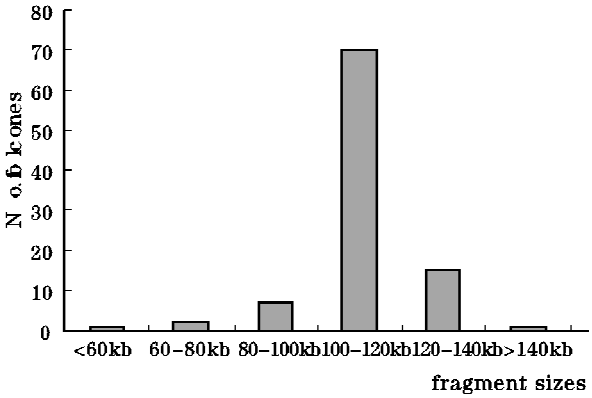


Fig. 5 Insert size distribution of clones in the BAC library

3 Discussion

According to 2250 Mb of the haploid genome of *Gossypiun hirsutum*, the library of Suyuan 7235 had the 1.6 haploid genome coverage. The probability of cloning particular gene was 80%. The BAC library of Suyuan 7235 provided important and available genomic resources for cotton genome research. Based on this BAC library, gene cloning, development of PCR-based marker, functional and comparable genomics of cotton can be carried out. At present, this BAC library was exploited by other team for cotton genomic research. In chickpea, BAC library was constructed, and 223 SSR primers had been exploited by screening the BAC library using 8 oligonucletides of (GA)10, (GAA)7, (AT)10, (TAA)7, (TGA)7, (CA)10, (CAA)7 and (CCA)7[6]. This research provided an important tool for molecular breeding and genomics research. In mungbean, two PCR-based markers closely linked to a major locus related to bruchid resistance were developed based on the BAC library[10].

References:

[1] GIRAUDAT J, Hauge B, Valon C, et al. Isolation of the *Arabidopsis ABI3* gene by positional cloning [J]. Plant Cell, 1992, 4: 1251-1261.

[2] MOZO T, Dewar K, Dunn P, et al. A complete BAC-based physical map of the *Arabidopsis thaliana* genome [J]. Nat Genet, 1999, 22: 271-275.

[3] WU C, Sun S, Nimmakayala P, et al. A BAC- and BIBAC-based physical map of the soybean genome [J]. Genome Research, 2004,14(2):319-326.

[4] WU C, Nimmakayala P, Santos F A, et al. Construction and characterization of a soybean bacterial artificial chromosome library and use of multiple complementary libraries for genome physical mapping [J]. Theor Appl Genet, 2004, 108:1432-2242.

[5] OSOEGAWA K, Mammoser A G, Wu Chen-yan, et al. A bacterial artificial chromosome library for sequencing the complete human genome[J]. Genome Research, 2001,11(3): 483-496.

[6] LICHTENZVEIG J, Scheuring C, Dodge J, et al. Construction of BAC and BIBAC libraries and their applications for generation of SSR markers for genome analysis of chickpea, *Cicer arietinum* L. [J]. Theor Appl Genet, 2004, 110:492-510.

[7] WANG Xing-fen, Zheng Yong-min, Zhang Gui-yin, et al. The optimized method for construction of bacterial artificial chromosome (BAC) library in cotton [J]. Cotton Science, 2004,16(3):170-174 (in Chinese).

[8] PANG Chao-you, Du Xiong-ming, Ma Zhi-ying. Progress of enhancement and utilization of upland cotton elite germplasms with wild cotton genes[J]. Cotton Science, 2005,17(3):171-177 (in Chinese).

[9] ZHANG Tian-zhen, Yuan You-lu, John Yu, et al. Molecular tagging of a major QTL for fiber strength in upland cotton and its marker-assisted selection [J]. Theor Appl Genet, 2004, 106(2):262-268-2242.

[10] MIYAGI M, Humphry M, Ma Z Y, et al. Construction of bacterial artificial chromosome libraries and their application in developing PCR-based markers closely linked to a major locus conditioning bruchid resistance in mungbean(*Vigna Radiata* L. Wilczek) [J]. Theor Appl Genet, 2004,110(1):151-156. ●