Construction and Characterization of a Bacterial Artificial Chromosome Library from the Huoyan Goose

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Abstract: Huoyan goose is a famous Chinese local breed for its egg productivity. In this study, a bacterial artificial chromosome library of the Huoyan goose was successfully established using the Hind III site of the vector pBeloBAC11, comprising of 115, 200 clones arrayed in 543, 384 well microplates , with an average insert size of 102 kb and the content of the library was 11.4 genome equivalents, which yielded a theoretical probability of 99.93% for isolating a particular DNA sequence. BAC clones of the library were stable in the bacterial host for at least 100 passages.
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1. Introduction

Bacterial artificial chromosome (BAC) was one of the favorite vectors for cloning genomic DNA due to its large capacity, high clone stability and low DNA chimerism (Shizuya, 1992), bacterial artificial chromosome library, which could be also used in the study of comparative genomics (Jung, 2006), map position cloning (Quiniou, 2007), physical mapping (Tao, 2001; Zhebentyayeva, 2008) and large-scale genome sequencing, Furthermore, single chromosome segregation technique has made the construction of specific chromosome become true (Gingrich, 1996).

The Huoyan goose was listed as one of the 78 nationally protected domestic animals by the Chinese government in the year of 2000. It was used for both meat and egg production, and was celebrated for its egg productivity during the winter. It has a genome size of approximate 1200 Mb/C. The establishment and characterization of Chinese Huoyan goose BAC library , our aim is not only to preserve this nationally protected breed resource at genomic level, but also supply valuable materials for positional cloning or genomic sequencing studies. Moreover, following the completion of whole genome sequencing of human and several model species, there was an ever-increasing demand for BAC libraries from species that were phylogenetic and/or biomedical importance.

2. Material and Methods

The Wing vein blood cells of Huoyan goose were embedded in agarose microbeads to prepare high molecular weight (HMW) genomic DNA (Ouyang, 2010). Quality of the prepared DNA was evaluated by pulse field gel electrophoresis (PFGE).

Genomic DNA was partially digested by three restriction enzymes Hind Ⅲ, EcoR Ⅰ and BamH Ⅰ to determine the optimal partial digestion condition. The number of restriction enzyme units ranged from 15 to 70 units. Partially digested genomic DNA was subjected to size selection by two rounds of PFGE.

Size-selected genomic DNA was ligated to 30 ng prepared pBeloBAC11 in a molar ratio of 5:1. Ligation product was used to transform 20 μl E. coli Electro MAX DH10BTM cell suspension (Invitrogen) by electroporation. White colonies were picked with a Genetix robot (Genetix, New Milton, UK) in 384-well plates (Corning) containing LB freezing medium and incubated overnight at 37℃. The whole library was stored at −80°C.

Each of 400 randomly selected clones was incubated overnight in LB medium followed by an alkaline lysis procedure. The DNA pellet was dissolved in 50 μl TE and digested with 0.2 U Not I (New England Biolabs), followed by PFGE and photographed.

Clones were randomly picked from the library and incubated, primary cultures were considered as generation 0, Then the cultures were diluted 106 fold in 25 ml LB and incubated overnight. The process was repeated for 5 consecutive days, representing 100 generations. Samples were taken for analysis by Hind III digestion.

3. Results

An important aspect in large insert library construction is to gain HMW genomic DNA. Pulsed-field gel electrophoresis showed that the Huoyan

goose genomic DNA was not degraded during preparation (Figure 1a).

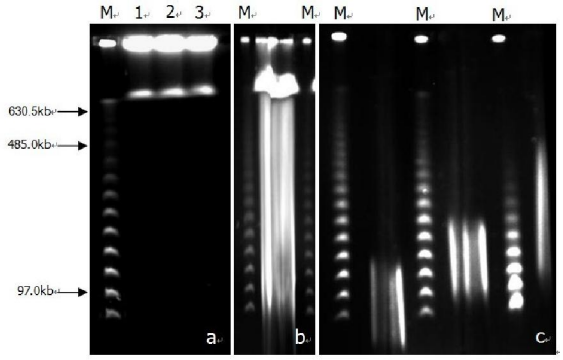

Figure 1. The preparation of HMW DNA from Huoyan goose detected by PFGE
a, HMW genomic DNA, lanes 1-3 were DNA samples; b, PFGE result of recovered fragment by first recovery after partial digested genomic DNA; c, Sencond recovery after partial digested genomic DNA. M was Lambda Ladder PFG Marker.

It was necessary to determine the optimal conditions to generate the maximum concentration of DNA within the desired size range (Nilmalgoda, 2003). In this study, HMW genomic DNA was optimally digested with Hind III spreaded at 100-500 kb, then they were excised from the gel slices, the piece was cut into three pieces with sizes 100-200 kb, 200-300 kb and 300-500 kb, they were subjected to a second PFGE, excised, recovered, and dialyzed (Figure 1 b and c).

The recovered HMW DNA was quantified with λDNA as control and its concentration was larger than 6.25 ng/μl. Desalting of the ligation reactions before transformation was found to improve transformation frequency two-fold, and thus was used frequently. In this study, 10% PEG8000 was used for concentration and we also got higher transformation efficiency (Osoegawa, 1998). The ligation solution should be used as soon as possible, or the large fragment DNA would degrade and aggregate.

Partially digested DNA was ligated to vector DNA, 9 ligations and 89 transformations were performed to complete the construction of the Huoyan goose BAC library. In total, 115, 200 clones were picked, deposited in 543 384-well microplates and stored at -80℃.

The percentage of non-insert clones was 0.8% among the 400 selected clones. Inserts varied from 48 to 150 kb with an average size of 102kb, corresponding to 11.4 genome equivalents, yielding a probability of 99.93% of isolating a particular DNA sequence.

The stability of the BAC library was analyzed by continuously incubating 5 random clones for 5 days. The result demonstrated that the restriction patterns of the 5 days were identical and the clones of the library were stable in the bacterial host for at least 100 generations (Figure 2).

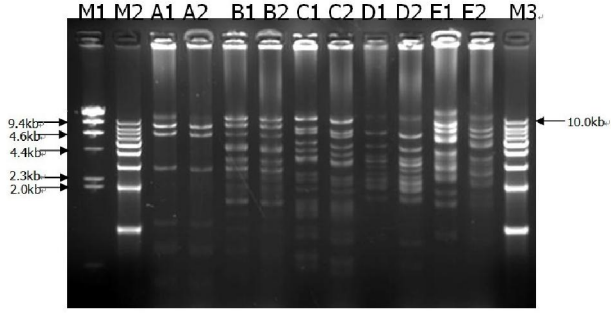

Figure 2. The stability detection of BAC library. M:1kb Marker; A1, A2, B1, B2, C1, C2, D1, D2, E1, E2 was digested by Hind Ⅲ.

4. Discussions

An important aspect in large insert library construction is to gain HMW genomic DNA. During the preliminary preparation of goose mega-base-size DNA, a series of varied restriction digestions were performed using three restriction enzymes Hind Ⅲ, EcoR Ⅰ and BamH Ⅰ. This was necessary to determine the optimal conditions to generate the maximum concentration of DNA within the desired size range (100-500 kb). In this study, high molecular weight genomic DNA (HMW) DNA was partially digested by three restrictive enzymes Hind Ⅲ, EcoR Ⅰ and BamH Ⅰ. The results showed that HMW DNA was optimally digested with Hind III spreaded at 100-500 kb, so Hind III was the best. The gradient digestion showed that 40 U/μl Hind III was suitable.

Frijters used two enzymes in constructing a Lettuce BAC library to reduce chances of cloning bias owing to non-uniform distribution of restriction sites (Frijters, 1997). Similarly, Nilmalgoda constructed a hexaploid wheat library using the enzymes Hind III and BamH I (Nilmalgoda, 2003). Size distributions of randomly selected Hind III and BamH I clones were illustrated. Although the average insert size of the BamH I clones was slightly smaller than that of the Hind III clones, there had no significant difference. In our experiments, it showed that Hind Ⅲ was the best for preparation of goose mega-base-size DNA. The most critical factor in achieving appropriate digestion was time which

should not extend the digestion time longer than 40 min.

Pulsed-field gel electrophoresis was carried out using a double size selection strategy (Woo et al., 1994) in order to fractionate the goose HMW DNA. Previously studies have shown that two rounds of size selection eliminated small DNA fragments co-migrating with the selected range in the first pulsed-field fractionation (Cai, 1995). This strategy resulted in an increase in insert sizes and a more adqulis insert size distribution. However, the second sizes election obviously decreased the ligation and transformation efficiency (Wang, 2001). Similar losses after repeated size selection were also reported by Choi (Choi, 2000) and Nilmalgoda (Nilmalgoda, 2003). Nevertheless, O'Sullivan reported that single-step size selection was used to construct BAC library with good results (O'Sullivan, 2001). In our experiments, we have observed the insert size loss also, and insufficient DNA was obtained after the second pulsed-field fractionation to allow efficient ligation. Therefore, one-round purified DNA fragments were used to construct 80% of the Huoyan goose library, and double size selection procedure was used to remove the small DNA fragments usually trapped in the coils of the HMW DNAs.

The general parameters for assessing the quality of large insert libraries are the average insert size, the stability and integrity of inserts, the level of contamination by organelle DNA, the genome representation, and the potential of the library to provide a clone of interest (Moullet, 1999). In our study, the average insert size of the Huoyan Goose library was estimated as 102kb, which was a promising quality for the use of this library in large-scale sequencing and gene-screening projects. Size of insertion element in libraries varies with different investigation purposes. For large scale sequencing, the larger the insert size is, the better. If we want to clone a modicus gene, smaller insert size is enough. Likewise, the content of the libraries differs for different study purposes. When used for gene cloning and sequencing, higher genomic coverage was required (Zhang, 2000).

The BAC library we constructed from a female Huoyan goose consisted 115, 200 clones with an average insert size of 102 kb. Excluding the 0.6% empty clones, the coverage of this library was 11.4 genome equivalents. It was as demonstrated that the Huoyan goose genetic materials could be conserved by constructing and storing their BAC libraries at -80 ℃ for a long term storage.

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