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Preliminary Study of BAC Library Construction in Black Tiger Shrimp, *Penaeus monodon*

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ABSTRACT

Availability of shrimp genome information is necessary for shrimp genetic studies and large-insert DNA clones, bacterial artificial chromosome (BACs) serve as valuable tools for obtaining genomic sequences. The construction of a BAC library was achieved from this preliminary study of *P. monodon*. High molecular weight (HMW) genomic DNA was isolated from abdominal muscle and the resulting hemocytes were of high quality and sufficient quantity for a BAC library construction. This BAC library was constructed from hemocyte HMW genomic DNA fragments ranging between 100 and 300 kb ligated to a pBAC-lac vector. The average insert size of the BAC clones was calculated as 100 kb ranging from 40 - 150 kb with over 50 % of the clones containing inserts larger than 100 kb. This BAC library contained approximately 60,000 BAC clones representing a 3-fold coverage of the haploid shrimp genome size. Furthermore, the consistency of BAC clones was also proven as no apparent rearrangements were observed before and after 100 generations in serial growth. From the above result, it is essential to improve the insert size and clone numbers of the BAC library to be a powerful tool in the future.

Keywords: BAC, *Penaeus monodon*, genome

INTRODUCTION

The black tiger shrimp has been one of the most important export products for Thailand with an annual income of more than 50 billion baht. The culture areas are mainly located nearby the coast along the Gulf of Thailand and Andaman Sea. Since diseases, especially the yellow-head virus and white spot syndrome virus (WSSV) have become more widespread, the number of culture areas has dramatically decreased.

Genome mapping and isolation of quantitative traits loci (QTLs) and economical traits loci (ETLs) are of importance for breeding, e.g. growth rate, stress tolerance, disease resistance, etc. Physical genome mapping includes methods where the position of and distances between DNA markers are physically determined, in contrast to genetic mapping of genomes, in which distances are determined statistically based on segregation of polymorphic markers in family material and expressed as recombination fractions. Physical mapping complements genetic mapping by enabling the assignment of linkage groups to chromosomes [1]. In addition, high resolution physical mapping in terms of chromosome walking, cloning and sequencing must be used in order to localize important traits, e.g. QTL. Physical mapping, including known genes as markers, makes it possible to employ non-polymorphic markers and use genomic information gathered from other species.

Large insert libraries have made valuable contributions to genome analysis and molecular genetics. The most commonly used system for constructing large insert libraries is the bacterial artificial chromosome (BAC) system [2]. The BAC vector is an F plasmid-based vector that is maintained as a single-copy plasmid in a recombination-deficient *Escherichia coli* host to promote sequence stability [3]. Although the insert size found in most BAC libraries is much smaller than the size found in some yeast artificial chromosome (YAC) libraries, the BAC system offers some important advantages over the YAC system. In contrast to YACs, BAC inserted DNA is easy to isolate, manipulate and clone instability and chimeras, a particular problem in YAC libraries [4-6] which has been reported to be low [7] or absent [3,8] in BAC libraries. Such large insert BAC libraries are similar to shotgun libraries in genome coverage, whose cloned DNA fragments are randomly derived from the different regions of a genome, and thus, would have truly high genome coverage. The number of clones from such complementary libraries equivalent to 6 - 8X haploid genomes would be sufficient for different genome research purposes, including global genome physical mapping and sequencing [9].

The genome of shrimps was estimated as large as 70 % of the human genome [10]. In order to understand shrimp genome information, *Penaeus monodon* has also been investigated by several groups of researchers including, marker development [11-14], gene identification [15-18], and genetic mapping [19,20]. However, high-density genetic maps of *P. monodon* have not been obtained. In order to facilitate shrimp genome research in the near future, it is necessary to develop the BAC library in *P. monodon* for further genetic structure identification, physical mapping and genome sequencing.

MATERIALS AND METHODS

High-Molecular Weight (HMW) Genomic DNA Preparation

HMW *P. monodon* DNA was isolated from hemocyte and abdominal muscle by the method modified from Zhang [21]. The quality and quantity of HMW DNA was then investigated by cutting with *Hin*dIII and then separated on 1 % agarose PFGE.

Vector Preparation

The highly-purified pBAC-lac vector was kindly provided by Mr. Takashi Koyama, Laboratory of Genome Science, Graduate School of Marine Science and Technology, Tokyo University of Marine Science and Technology. Vector DNA was digested with *Hin*dIII and dephosphorylated by a calf intestinal alkaline phosphatase (CIAP) enzyme. The dephosphorylated pBAC-lac/*Hin*dIII was dissolved in deionized water and stored at 4 °C in aliquots until used.

Serial Partial Digestion

The plugs containing HMW *P. monodon* DNA were used to determine the unit of restriction enzyme suitable for size selection by serial partial digestion [21] briefly as: each plug was firstly cut into 8 slices by a glass slide cover. The plug slices were incubated in digestion buffer I (1X digestion buffer, 2 mM spermidine, 1 mM DTT) on ice for 30 min 2 times. The plug slices were transferred into 6 1.5 ml tubes, 8 slices per tube. Then 300 µl of digestion butter II (1X digestion buffer, 2 mM spermidine, 1 mM DTT, 0.1 mg/ml BSA) was added per tube. The *Hin*dIII restriction enzyme was then added to each tube at serial amounts including 0, 2.0, 4.0, 8.0 and 16.0 units. The reactions were incubated on ice for 60 min and then immediately incubated in a water bath at 37 °C for 30 min. The reactions were stopped by immediately transferring the tubes onto ice and adding 1/10 V 0.5 M EDTA (pH 8.0). The digested plugs were analyzed by pulsed-field gel electrophoresis under the following conditions: 12.5 °C (cooler settings), 80 (pump settings), 120° angle, 6 V/cm, initial pulse time 50 s and final pulse time 50 s for 18 h. The results were detected by ethidium bromide staining.

The concentration of the enzyme under which most of the partially-restricted fragments fell in the range 100 - 300 kb on the gel was selected for large-scale partial digestion for BAC cloning.

The First Size Selection

A total of 12 plugs (12 reactions) were used for large-scale partial digestion with the optimum enzyme concentration. The plug slices were loaded into the sample well along with a well for lamda ladder marker. The DNA samples and marker in the wells were then sealed with the molten agarose. Size-selection of the partial digestion was run by pulsed-field gel electrophoresis under the following conditions: 12.5 °C (cooler settings), 80 (pump settings), 120° angle, 6 V/cm, initial pulse time 90 s and final pulse time 90 s for 18 h. The gel zone containing the DNA fragments ranging from 100 - 300

kb was excised by a glass slide cover and divided into 2 sections horizontally: 100 - 200 kb (fraction I), 200 - 300 kb (fraction II). Each gel section was electroeluted with the selected DNA placed in a dialysis tube.

The Second Size Selection

The 2 sections of eluted DNA from the first size selection were run by pulsed-field gel electrophoresis on 1 % agarose gel under the conditions: 12.5 °C (cooler settings), 80 (pump settings), 120° angle, 4 V/cm, initial pulse time 5 s and final pulse time 5 s for 10 h. The compressed DNA zone on the gel was excised with a glass slide cover and used to perform the electroelution of the DNA. The DNA eluted from the gel slice was directly dialyzed in the same tube against ice-cold 0.5X TE 3 times by 1 h each at 4 °C.

BAC Ligation and Transformation

Two samples of *Hin*dIII-digested shrimp DNA (fraction I, II) were quantified by 1 % agarose gel electrophoresis compared to lambda DNA as a concentration standard. The size-selected DNA fragments were mixed with pBAC-lac/*Hin*dIII vectors at a vector of: DNA as 1:6 (1 ng vector: 6 ng DNA) and ligated in 1X T4 DNA ligase buffer with 4 Weiss units T4 ligase (MBI Fermentas) in 200 µl at 16 ^{o}C for 12 h. The reactions were then desalted by spotting onto the dialysis filter (0.025 µm) floating on 0.2X TE buffer in a Petri dish for 1 h on ice.

A 5 µl ligation reaction was transformed to 30 µl competent cells of *Escherichia coli* strain DH10B by electroporation using a GENE Transfer Equipment GTE-10 (SHIMADZU) with a power of 15 kV/cm. The transformed cells were transferred into a 1 ml SOC medium and incubated at 37 °C with stirring at 250 rpm for 1 h, then plated on an LB solid medium containing 12.5 µg/ml chloramphenicol, 50 µg/ml X-gal, and 25 µg/ml IPTG and incubated at 37 °C for 24 h.

Characterization of the BAC Library

The total white and blue colonies obtained from each transformation were examined. In order to determine the insert size of the BAC clones, a total of 20 white colonies were cultured in an LB medium with 12.5 µg/ml chloramphenicol for 16 - 20 h at 37 °C with shaking at 250 rpm. A standard alkaline lysis method was used to isolate the BAC DNA. Inserts were excised with *Not* I and resolved by pulsed-field gel electrophoresis under the following conditions: 12.5 °C (cooler settings), 80 (pump settings), 120° angle, 6 V/cm, initial pulse time 5 s and final pulse time 15 s for 16 h. The results were detected by ethidium bromide staining.

Insert Stability of BAC Clone

To examine the stability of *P. monodon* inserted DNA in the vector pBAC-lac, 3 selected BAC clones were each inoculated into 10 ml of LB medium containing 12.5 µg/ml chloramphenicol and cultured at 37 °C with 250 rpm shaking overnight. Five µl

of the overnight culture was inoculated in another 10 ml of LB medium containing 12.5 μg/ml of chloramphenicol and incubated at 37 °C overnight. Serial cultures were made continuously for 5 days (approximately 100 generations). BAC DNAs were isolated from day 1 and day 5 cultures, digested by *Hae*III and analyzed by electrophoresis on 1.5 % agarose gel [22].

RESULTS

High-Molecular Weight (HMW) Genomic DNA Isolation

Examining HMW DNA using 1 % pulsed-field gel electrophoresis, plugs containing HMW genomic DNA from both abdominal muscle and hemocytes of *P. monodon* showed that it was of good quality indicated by the fact that little degraded DNA fragments were found at lower size ranges compared to the 1,000 kb DNA fragment of λ PFG (**Figure1**; 0 unit enzyme in hemocyte's HMW DNA and abdominal muscle's HMW DNA). The plugs also contained sufficient quantity for BAC library construction estimated using lambda DNA as a standard through 0.8 % agarose gel electrophoresis as a 2 μg/plug (data not shown). Based on the staining intensity of the DNA samples compared to each other, the amount of DNA per plug in the hemocytes was higher than abdominal muscle.

BAC Library Construction

For this study the HMW genomic DNA from the hemocytes was used to construct the BAC library because it seemed to have a larger amount of DNA. When partial digestion of the HMW was performed using varying units of *Hin*dIII, fragment sizes of 100 - 300 kb were found to be suitable when a digestion reaction was incubated with 4 units of *Hin*dIII (**Figure 1**).

Large-scale DNA fragments isolation was achieved from an optimum enzyme concentration of 4 units of *Hin*dIII with a total of 12 digestion reactions (12 plugs, approximately 20 μg of HMW DNA). Two regions of agarose gel containing the desired DNA fragment sizes between 100 - 300 kb were cut and electroeluted for the first size selection. The gel zone containing DNA fragments 100 - 200 kb was assigned as fraction I and 200 - 300 kb as fraction II. After electroelution, DNA fragments were then fractionated by second size selection, electroeluted and dialyzed in a 0.5X TE buffer.

The dialyzed DNA fragments were quantified by 1 % agarose gel electrophoresis. The total DNA fragments obtained from fraction I and II were 1,000 ng and 2,400 ng, respectively. DNA fragments were then ligated to the dephosphorylated pBAC-lac/*Hin*dIII vector with a ratio of insert to vector of 6 ng to 1 ng. The ligation reactions were prepared for fractions I and II to give a total volume of 400 μl and 800 μl, respectively and incubated at 16 °C for 12 h.

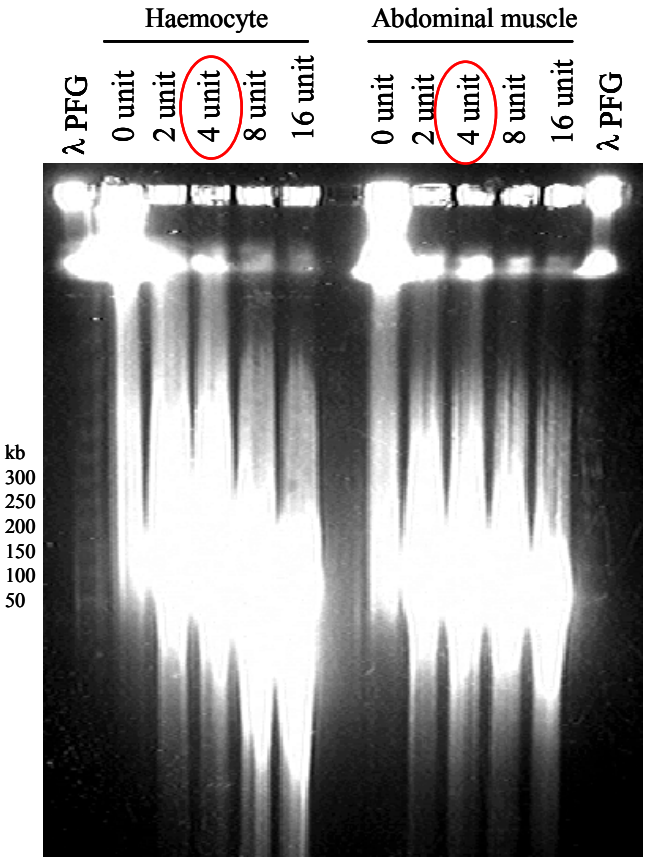

Figure 1 Serial partial digestion of *P. monodon* HMW DNA by *Hin*dIII (0, 2, 4, 8, 16 units). Two tissues were used to isolate the HMW genomic DNA; hemocyte and abdominal muscle.

The number of white colonies obtained from the transformation of a 5 µl ligation reaction for fractions I and II was around 400 and 180 colonies, respectively and also contained an equal number of blue colonies. To estimate the insert size, we randomly took 20 BACs from each library. After digestion with *Not*I and separation of the fragments by PFGE, we calculated the average insert size of each library (**Figure 2**). The average insert size of the BAC clones generated from fractions I and II was similar to the calculated 100 kb with sizes ranging from 40 to 150 kb with over 50 % of the clones containing insert sizes larger than 100 kb (**Figure 2**). BAC clones were picked and kept in a freezing medium at −80 °C for further analyses. This BAC library contained approximately 60,000 BAC clones. According to a haploid genome size of approximately 2,100 Mb for *P. monodon* [10], this BAC library represents a 3-fold coverage of the haploid genome size.

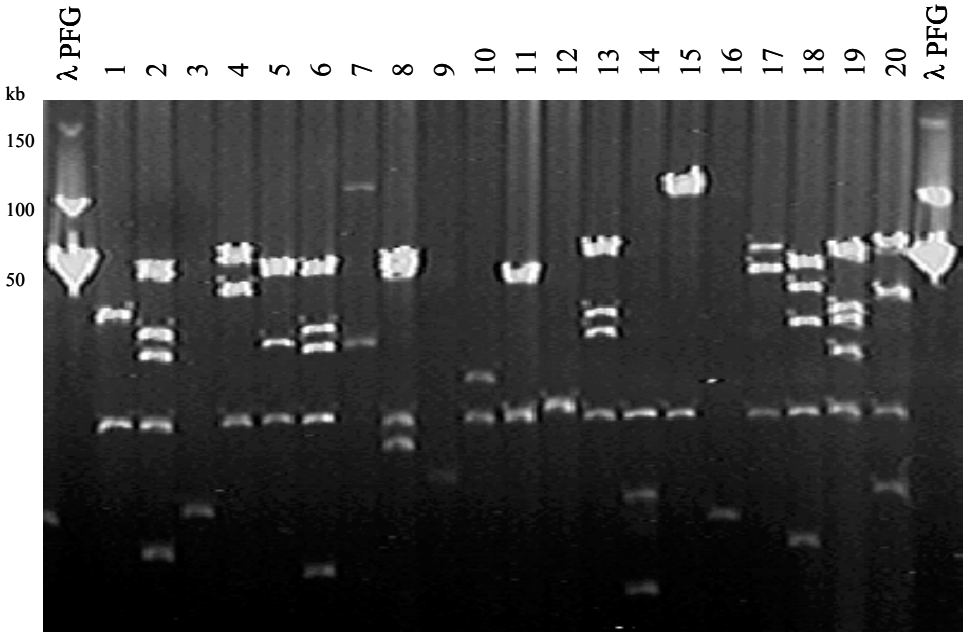

Figure 2 Determination of the BAC clones of *P. monodon* insert size (1 - 20). BAC clones were isolated, cut with *Not*I and electrophored though 1 % agarose gel electrophoresis. Ethidium bromide staining was used to visualize the DNA fragments (λPFG = standard marker).

To analyze the stability of the *P. monodon* BAC clones, the selected clones were grown for 6 days in a liquid medium, and BAC DNA was isolated from the cultures after each day of growth and subjected to *Hae*III fingerprinting (**Figure 3**). Identical restriction patterns were observed between the DNAs of all 3 clones isolated from the day 1 and day 5 cultures, indicating that the clones of the libraries are stable in the bacterial host for at least 100 generations.

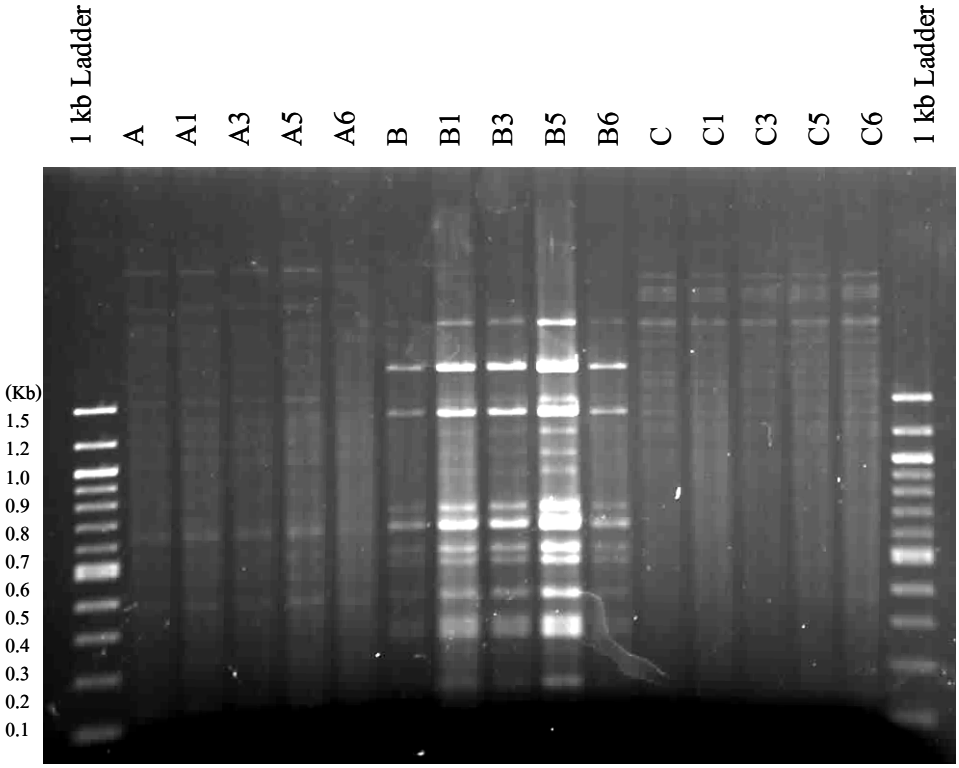

Figure 3 Stability test of BAC clones. *Hae*III fingerprints produced from BAC DNA extracted from 3 BAC clones (A, B, C) after 1, 3, 5 and 6 days of growth (lane A, B, C = control). One Kb ladder was used as a size standard.

DISCUSSION

A BAC library is an essential tool for physical mapping and genome sequencing. A BAC library was constructed for *P. monodon* in this study. Construction of a BAC library is time-consuming; so numerous improvements have been developed to simplify the procedure. In BAC library construction, the preparation of a vector is the key factor for success. A lot of time is spent in obtaining suitable quantities of high quality vector DNA. The preparation of insert DNA is another key factor in the construction of a BAC library. In BAC library construction, large inserts are necessary for a large genome. To obtain a large insert, a 2-step size selection is often used to fractionate partly digested HMW DNA in the construction of the BAC library. However, the second size selection obviously decreased the ligation and transformation efficiency and the average insert size of BAC clones was much lower than the selected sizes [23].

The appearance of nonrecombinant background clones (blue colony) in a BAC library depends not only on the quality of the vector preparation, but also on the ligation conditions [24]. The pBAC-lac vector used for this study was digested with *Hin*dIII, and

treated with alkaline phosphatase, which would result in the undigested vector (open circular or supercoiled plasmid). The ratio of insert to vector was 1:10, a ratio that is useful for reducing the number of noninsert clones [24]. For this study we performed ligation with the amount of insert to vector at 6 ng insert to 1 ng vector, equivalent to a 1:1 molar ratio that caused low numbers of white colonies and high numbers of blue colonies. This information would be useful for the next trials to optimize varying molar ratios as previously reported. However, it is recommended that if the percentage of false positive clones is > 10 %, it is possible that the vector DNA was damaged during dephosphorylation. An extremely high percentage of blue clones (> 40 %) suggest possible problems during vector preparation, ligation, and/or transformation [25].

The insert sizes of the BAC clones in this *P. monodon* library indicate a bimodal distribution, where a cluster of clones were centered around 100 kb. Small insert clones (< 50 kb) were likely derived from small DNA fragments trapped by the large fragments during size selection as previously suggested [8,26,27]. BAC clones with insert sizes in excess of 100 kb are desired if the library is to be used for assembly of contigs spanning hundreds of kilobases.

The total size of the *P. monodon* genome is estimated to be about 2,100 Mb [10]. From the size distribution (average 100 kb), it was calculated that about 20,000 BAC clones in the library correspond to 1 coverage of the *P. monodon* genome. In this study, a total of 60,000 BAC clones were assumed as 3x the genome equivalent for *P. monodon*.

Concerning the instability of the insert, the DNA fingerprint pattern was found to be unaltered during 6-days of growth, suggesting that the BAC clones were relatively stable upon prolonged growth. We were also unable to detect any major rearrangements for primary transformants when digested with *Not*I. In contrast to the study by Song and co-workers [28], we were unable to demonstrate any major instability problems for wheat BAC clones.

The BAC library reported herein will serve as a useful resource for modification or a preliminary method for BAC construction for *P. monodon* and will be partially subjected to use for physical mapping, positional cloning, and sequencing in the future.

CONCLUSIONS

This preliminary study has modified the optimized methodology for BAC library construction in *P. monodon*. This BAC library contained approximately 60,000 clones with an average insert size of 100 kb, covering a 3-fold genome coverage of the shrimp genome. Physical mapping is further aimed to analyze these BAC clones.

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REFERENCES

[1] H Ellegren, BP Chowdhary, M Johansson, L Marklund, M Fredholm, I Gustavsson and L Andersson. A primary linkage map of the porcine genome reveals a low rate of genetic recombination. *Genetics* 1994; **137**, 1089-100.

[2] W Vanhouten and SA Mackenzie. Construction and characterization of a common bean bacterial artificial chromosome library. *Plant Mol. Biol.* 1999; **40**, 977-83.

[3] H Shizuya, B Birren, UJ Kim, V Mancino, T Slepak, Y Tachiiri and M Simon. Cloning and stable maintenance of 300-kilobase-pair fragments of human DNA in *Escherichia coli* using an F-factor-based vector. *Proc. Natl. Acad. Sci. USA*. 1992; **89**, 8794-97.

[4] ED Green, HC Riethman and JE Dutchik. Detection and characterization of chimaeric yeast artificial chromosome clones. *Genomics* 1991; **11**, 658-69.

[5] R Dunford, L Vilageliu and G Moore. Stabilization of a yeast artificial chromosome containing plant DNA using a recombination-deficient host. *Plant Mol. Biol.* 1993; **21**, 1187-89.

[6] Y Umehara, A Inagaki and H Tanoue. Construction and characterization of a rice YAC library for physical mapping. *Mol. Breeding.* 1995; **1**, 79-89.

[7] E Bent, S Johnson and I Bancroft. BAC representation of two low-copy regions of the genome of *Arabidopsis thaliana*. *Plant J.* 1998; **13**, 849-55.

[8] SS Woo, J Jiang, BS Gill, AH Paterson and RA Wing. Construction and characterization of a bacterial artificial chromosome library of *Sorghum bicolor*. *Nucleic Acids Res.* 1994; **22**, 4922-31.

[9] HB Zhang and C Wu. BAC as tools for genome sequencing. *Plant Physiol. Biochem.* 2001; **39**, 195-209.

[10] S Chow, WJ Dougherty and PA Sanfer. Meitotic chromosome complements and nuclear DNA contents of four species of shrimps of the genus *Penaeus*. *J. Crustacean Biol.* 1990; **10**, 29-36.

[11] A Tassanakajorn, A Tiptawonnukul, P Supunkul, V Rimphanitchayakit, D Cook, P Jarayabhand, S Klinbunga and V Boonsaeng. Isolation and characterization of microsatellite markers in the black tiger prawn *Penaeus monodon*. *Mol. Mar. Biol. Biotechnol.* 1998; **7**, 55-61.

[12] Z Xu, AK Dhar, J Wyrzykowski and A Alcivar-Warren. Identification of abundant and informative microsatellites from shrimp (*Penaeus monodon*). *Anim. Genet.* 1999; **30**, 150-6.

[13] S Pongsomboon, V Whan, SS Moore and A Tassanakajorn. Characterization of tri- and tetranucleotide microsatellites in the black tiger prawn, *Penaeus monodon*. *ScienceAsia* 2000; **26**, 1-8.

[14] S Wuthisuthimethavee, P Lumobol, A Vanavichit and S Tragoonrung. Development of microsatellite markers in the black tiger shrimp (*Penaeus monodon* Fabricius). *Aquaculture* 2003; **224**, 39-50.

[15] SA Lehnert, KJ Wilson, K Byrne and SS Moore. Tissue-specific expressed tags from the black tiger shrimp *Penaeus monodon*. *Mar. Biotechnol.* 1999; **1**, 465-76.

[16] P Supungul, S Klinbunga, R Pichyangkura, S Jitrapakdee, I Hirono, T Aoki and A Tassanakajorn. Identification of immune-related genes in hemocytes of black tiger shrimp (*Penaeus monodon*). *Mar. Biotechnol.* 2002; **4**, 487-94.
[17] J Tong, SA Lehnert, K Byrne, HS Kwan and KH Chu. Development of polymorphic EST markers in *Penaeus monodon*: applications in penaeid genetics. *Aquaculture* 2002; **208**, 69-79.
[18] S Wuthisuthimethavee, P Lumobol, A Vanavichit and S Tragoonrung. EST-based identification of genes expressed in the branchiae of black tiger shrimp (*Penaeus monodon* Fabricius). *ScienceAsia* 2005; **31**, 137-44.
[19] K Wilson, Y Li, W Whan, S Lehnert, K Byrne, S Moore, S Pongsomboon, A Tassanakajorn, G Rosenberg, E Ballment, Z Fayazi, J Swan, M Kenway and J Benzie. Genetic mapping of the black tiger shrimp *Penaeus monodon* with amplified fragment length polymorphism. *Aquaculture* 2002; **204**, 297-309.
[20] S Wuthisuthimethavee, P Lumobol, T Toojinda, S Tragoonrung and A Vanavichit. SSLP-based genetic linkage map construction of *Penaeus monodon* Fabricius. *ScienceAsia* 2005; **31**, 87-93.
[21] HB Zhang. *Construction and manipulation of large-insert bacterial clone libraries*, Manual. GENEfinder Genomic Resources, Texas A&M University, USA, 2000, p. 107.
[22] Q Tao and HB Zhang. Cloning and stable maintenance of DNA fragments over 300 kb in *Escherichia coli* with conventional plasmid-based vectors. *Nucleic Acids Res.* 1998; **26**, 4901-09.
[23] DM O'Sullivan, PJ Ripoll, M Rodgers and KJ Edwards. A maize BAC library from the European flint line F2. *Theor. Appl. Genet.* 2001; **103**, 425-32.
[24] K Osoegawa, PY Woon, B Zhao, E Frengen, M Tateno, JJ Catanese and PJ de Jong. An improved approach for construction of bacterial artificial chromosome libraries. *Genomics* 1998; **52**, 1-8.
[25] DG Peterson, JP Tomkins, DA Frisch, RA Wing and AH Paterson. Construction of plant Bacterial Artificial Chromosome (BAC) libraries: An illustrated guide. *J. Agr. Genomics* 2000; 5, 1-100.
[26] O Moullet, HB Zhang and ES Lagudah. Construction and characterization of a large DNA insert library from the D genome of wheat. *Theor. Appl. Genet.* 1999; **99**, 305-13.
[27] SD Nilmalgoda, S Cloutier and AZ Walichnowski. Construction and characterization of a bacterial artificial chromosome (BAC) library of hexaploid wheat (*Triticum aestivum* L.) and validation of genome coverage using locus-specific primers. *Genome* 2003; **46**, 870-8.
[28] J Song, F Dong, JW Lilly, RM Stupar and J Jiang. Instability of bacterial artificial chromosome (BAC) clones containing tandemly repeated DNA sequences. *Genome* 2001; **44**, 463-9.

บทคัดย่อ

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การศึกษาเบื้องต้นในการสร้าง Bacterial Artificial Chromosome (BAC) ในกุ้งกุลาดำ

Bacterial Artificial Chromosome (BAC) เป็นการโคลนจีโนมิคดีเอ็นเอขนาดใหญ่ สามารถนำไปใช้ในการค้นหาและศึกษายีนต่างๆในสิ่งมีชีวิต สำหรับในกุ้งกุลาดำได้ศึกษาเบื้องต้นในการสร้างห้องสมุดดีเอ็นเอ BAC โดยเริ่มจากการสกัดดีเอ็นเอคุณภาพสูง การตัดดีเอ็นเอให้มีขนาดอยู่ในช่วงระหว่าง 100 ถึง 300 กิโลเบส การเชื่อมต่อชิ้นดีเอ็นเอกับเวคเตอร์ pBAC-lac การถ่ายฝากเข้าสู่แบคทีเรีย และการทดสอบโคลน พบว่าดีเอ็นเอคุณภาพสูงสามารถสกัดได้จากเนื้อเยื่อส่วนกล้ามเนื้อลำตัวและเม็ดเลือดกุ้งกุลาดำโดยมีคุณภาพไม่แตกต่างกัน และจากการสร้างห้องสมุดดีเอ็นเอ BAC ของกุ้งกุลาดำพบว่าได้โคลนที่มีค่าเฉลี่ยของชิ้นดีเอ็นยาวประมาณ 100 กิโลเบส จำนวนประมาณ 60,000 โคลน โดยคิดเป็นประมาณ 3 เท่าของจีโนมกุ้งกุลาดำ นอกจากนี้ดีเอ็นเอกุ้งกุลาดำในโคลนแต่ละโคลนยังมีความเสถียรหลังจากมีการเพิ่มจำนวน 100 ชั่วอายุ อย่างไรก็ตามห้องสมุดดีเอ็นเอ BAC นี้ก็สามารถนำไปใช้ประโยชน์ในการค้นหายีนได้แต่ควรมีการพัฒนาวิธีการต่อไปเพื่อให้ได้ขนาดของชิ้นดีเอ็นเอที่ใหญ่ขึ้นและมีจำนวนโคลนที่มากขึ้นซึ่งจะสามารถใช้เป็นห้องสมุดดีเอ็นเอ BAC อ้างอิงสำหรับการหาลำดับเบสจีโนมกุ้งกุลาดำต่อไป

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