Construction and characterization of a bacterial artificial chromosome library for mandarin fish
Siniperca chuatsi (Basilewsky)

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Siniperca chuatsi (Basilewsky), a demersal piscivore, is an endemic freshwater fish species in China. For the purpose of genomics research, we have constructed the first bacterial artificial chromosome (BAC) library for S. chuatsi. The BAC library comprised a total of 84,480 clones with an average insert size of 124.6 kb and less than 2.5% empty clones, corresponding to a 10.5-fold coverage of the S. chuatsi genome. The probability of isolating genes of interest was more than 99%. To validate the library, we screened 220 superpools and found that 1–19 were positive for six SSR markers, while none was positive for two mitochondrial gene markers. Therefore, the S. chuatsi BAC library will provide useful genomics resources and tools for cloning, functional genomics research and identification of economically important genes in this species.

Key words: bacterial artificial chromosome library, Siniperca chuatsi (Basilewsky), genome, PCR screening

A bacterial artificial chromosome (BAC) library is essential for aspects of genomics research such as whole genome assembly. BAC libraries are also particularly useful not only for the construction of BAC fingerprint-based physical maps, but also for integration of physical and linkage maps, and as source material for position-based candidate gene cloning (Li et al., 2011). Many BAC libraries have been constructed in aquaculture species including Haplochromis chilotes (Watanabe et al., 2003), Salmo salar (Thorsen et al., 2005), Cyprinus carpio L. (Li et al., 2011) and Dicentrarchus labrax (Whitaker et al., 2006), and some of them have contributed to genome sequencing projects.

The mandarin fish Siniperca chuatsi (Basilewsky), a demersal piscivore, is an endemic freshwater fish species in China and in the River Amur along the Russian border, and is especially abundant in the Yangtze River drainage in China (Liang, 1996). The global aquaculture production of S. chuatsi was reported to be almost 284,780 tonnes in 2013 (http://www.fao.org/fishery/culturedspecies/Siniperca_chuatsi/en). Because of its large size, rapid growth and gastronomic popularity, the mandarin fish can bring significant economic benefits in China. It also has a very particular food preference, only accepting live prey fish and refusing dead prey fish or artificial diets (Liang et al., 1998). Consequently, developing genomics research for S. chuatsi is essential because of its economically important traits and biological characteristics such as the particular food preference.

However, there are no reports of construction of a S. chuatsi BAC library. Here we describe for the first time the construction and characterization of a publicly available BAC library of S. chuatsi. The BAC library will be an important tool for genome sequencing of S. chuatsi and will be useful for providing a better understanding of the mechanism of its particular food preference. Currently, the development of diverse genomic resources for this species is in progress. As part of this effort, we constructed a publicly available BAC library using blood from a female mandarin fish, which was obtained from Luhu mandarin fish farm (Wuhan, Hubei Province, China).

Mandarin fish blood was collected using sterile syringes and stored in 5-ml vacuum blood tubes with EDTA anticoagulant at 4 °C. The blood was then mixed with an equal volume of low-melting agarose and shaped by plug molds. High molecular weight (HMW) genomic DNA was extracted in situ. Partial digestions and two size selections were as described by Luo and Wing (2003) with modifications. A series of eight pilot partial digestions were performed at 37 °C for 15 min using different con-
centrations (0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5 U/μl) of Hind III to maximize the yield of DNA fragments in the range of 100–200 kb. According to the result of these partial digestions, we selected 3.5 U/μl for large-scale DNA digestion (Fig. 1). After digestion, the DNA was treated with two rounds of electrophoresis to remove the smaller fragments. HMW DNA was ligated into pHZAUBAC1 vector (Shi et al., 2011) and transformed into DH10B competent cells. A total of 84,480 BAC clones were chosen and arrayed in 220 × 384-well plates in LB medium containing 12.5 μg/ml chloramphenicol. To estimate the average size of the insert DNA, 40 clones were randomly selected from the BAC library. The plasmid DNA was then extracted and digested with NotI and examined on a pulsed-field gel (Fig. 2). The insert sizes ranged from 97 kb to 145.5 kb, with an average of 124.6 kb, and less than 2.5% of the clones were empty (Table 1). For simple and effective PCR screening of BAC clones with specific DNA sequences and for evaluating the BAC library, 220 superpools were constructed. Each superpool contained all of the 384 BAC clones from one original plate and was stored at –80 °C in a 50-ml centrifuge tube.

To validate the genome coverage and value of our BAC library, we selected six primer pairs for PCR screening of the 220 BAC superpools. Two hundred and twenty PCR reactions were necessary for screening the superpools. All six primer pairs could amplify positive clones, and the results are shown in Table 2. We also used two primer pairs designed for two mitochondrial genes (Chen et al., 2012). There were no positive clones in any of the 220 superpools for these two primer amplifications.

Table 1. Summary statistics of mandarin fish BAC library

| Total clones | 84,480 |
|--------------|--------|
| Total 384-well plates | 220  |
| BAC vector | pHZAUBAC1 |
| Competent cells | DH10B competent cells |
| DNA source | Blood |
| Average insert size | 124.6 kb* |
| Haploid genome equivalent | ~10.5 × |
| Insert-empty BACs | < 2.5%* |

* These values are based on an examination of a random sample of 40 clones.

Fig. 1. Determination of the optimum enzyme dosage for HMW DNA. M, λ ladder PFG marker; 1–8 indicate 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5 U/μl of HindIII, respectively.

Fig. 2. Insert sizes of 40 randomly selected BAC clones. M, MidRange I PFG marker (New England Biolabs).
Table 2. Results of screening the BAC library with SSR and mitochondrial gene primers

| Target name | Primers 5’-3’ | Size (bp) | Number of hits from superpool |
|-------------|---------------|----------|-------------------------------|
| TSC71       | F:ATTGCGTGAGGTTTATATCTT G:CTTTTTTCTCCTGAGGCTTAT  | 249      | 19                            |
|             | R:GTGACACGCCCTCTCATAGAC C:GGCAGTGGTTTTCTATGCTATCT  | 264      | 8                             |
| TSC87       | F:ACTGTGGAAGCTAGAAACCA G:GGTCTCTTTGGAAGCGGAGGCT  | 250      | 18                            |
|             | R:ACTATGTGGCTATTCCACTG C:ACGGTATCAGCTATGCTTATG  | 220      | 17                            |
| TSC290      | F:ACATATAAGGCTAGACGAC G:GGTAGCTTTGGAAGCCAGGCT  | 1100     | 12                            |
|             | R:ATGTTACGACTTGCCTCCC C:GTGCTGGTCTTCCTGTTGT   | 1000     | 1                             |
| TSC316      | F:GTAGAAAGGATTCGGTGACT G:CTAGAGCAGGTCATGTCAA  | 538      | 0                             |
|             | R:CTAGAGGACGTCACTGTCAAA C:TGCTTTGGCGTAGACGAG    |          |                               |

Here, we reported the successful construction of the first BAC library for Siniperca chuatsi. In brief, the library consisted of 84,480 clones arrayed in 220 384-well plates, with an average insert size of 124.6 kb (result from 40 random clones) and less than 2.5% empty clones. Six SSR markers were used for screening 220 superpools, and positive superpools were obtained for all six markers. According to the screening result, no mitochondrial DNA was present in the BAC library. The BAC library was shown to have 10.5-fold coverage of the haploid mandarin fish genome based on the estimated genome size of 800 Mb (Cui et al., 1991). Therefore, the probability of identifying any gene of interest in this library was estimated to be greater than 99% according to the formula

\[ N = \frac{\ln(1 - p)}{\ln(1 - f / G)} \] (Clarke and Carbon, 1976).

This BAC library will be a significant genomics resource for studying the classification, evolution, and relationships among species of Siniperca. It can be used as a foundation for further integration of the physical map and molecular genetics mapping.

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