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Construction of a BAC library for *Haplochromis chilotes*, a cichlid fish from Lake Victoria

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Cichlid fishes in Lake Victoria are model organisms for studying rapid radiation and speciation. On the way to examine the molecular basis of how these cichlid fishes achieved such a remarkable morphological diversification, we constructed a bacterial artificial chromosome (BAC) library derived from a cichlid species, *Haplochromis chilotes*, from Lake Victoria. The library includes 157,056 clones with the average insert size of 128 kb, corresponding to a 10-fold coverage of the *H. chilotes* genome. Given that the cichlid fishes endemic to Lake Victoria are closely related to one another phylogenetically and their genomes are nearly identical, this BAC library can be utilized to isolate genes from the more than 200 Haplochromine cichlid species in Lake Victoria.

Key words: cichlid, BAC library, Lake Victoria

BAC libraries (Shizuya et al., 1992) are very useful for analyzing genome structure in almost all organisms. To date, many BAC libraries have been constructed, and some have been utilized for genome sequencing projects. Regarding a cichlid BAC library, Katagiri et al. (2001) constructed a series of four BAC libraries using Nile tilapia (*Oreochromis niloticus*). These BACs cover 2–64x of the genome, and have been utilized in the cichlid genome project. Nile tilapia is recognized as an important commercial food source and is presently cultivated in more than 65 countries. From an evolutionary viewpoint, the tilapia constitutes an ancient lineage of East African cichlids, but displays less morphological diversity than the Haplochromine cichlids (Fryer and Iles, 1972; Greenwood, 1984; Stiassny, 1991). In East Africa, there are three Great Lakes, namely Lakes Tanganyika, Malawi and Victoria within which reside >1000 morphologically diverse endemic cichlid species that have adapted to these ecosystems over 10 My of evolution. In particular, the Haplochromine cichlid species in Lake Victoria are regarded as model organisms of adaptive radiation and rapid speciation (Barel et al., 1991; Johnson et al., 1996).

We constructed a BAC library derived from *Haplochromis chilotes* of Lake Victoria. Genomic DNA was prepared from 10^9 reticulocytes isolated from two *H. chilotes* specimens. After anesthetizing the fish with 3-aminobenzoic acid ethyl ester (MS-222, Sigma, USA), arterial blood was collected and mixed with an equal volume of buffer (10 mM Tris-HCl [pH 8.0], 0.5 M EDTA). The reticulocytes were washed twice with phosphate-buffered saline and then treated with lysis buffer (10 mM Tris-HCl pH 8.0, 50 mM NaCl, 1% lithium dodesyl sulfate, 100mM EDTA) (Amemiya et al., 1996) for 2 hr at 37°C in 1.0% agarose gel plugs, each plug containing 5×10^7 reticulocytes, then stored in 20% NDS solution consists of 0.2% N-laurylsarcosine, 2 mM Tris-HCl (pH 9.0), 0.14 M EDTA. After exchanging the NDS buffer with TE buffer (10 mM Tris-HCl [pH 8.0], 0.1 mM EDTA), the genomic DNA was then partially digested with *Sac* I and 150-250 kb DNA fragments were isolated using pulse field gel electrophoresis. The DNA fragments eluted from the gel were ligated into pKS145 (Fujiyama et al., 2002) and an aliquot of the ligation reaction was used to transform *E. coli* DH10B. Using Flexys robot (Genomic Solutions, USA) and 3D:Biomek FX robot (BECKMAN COULTER, USA), a total of 157,056 BAC clones were chosen and arrayed in 409 × 384-well microtiter plates in LB medium containing 10% glycerol and 25 μmg/ml ampicillin. The plates were incubated overnight at 37°C and then stored at –80°C. The BAC library was constructed so as to be

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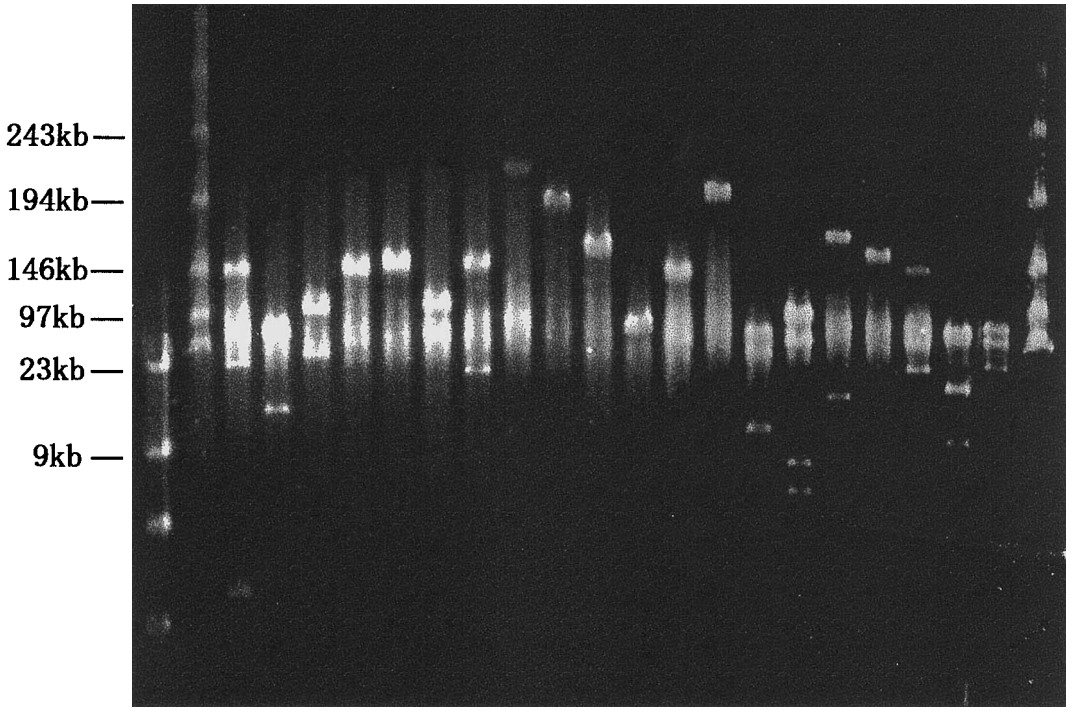

Fig. 1. Analysis of the average size of the BAC library inserts. The insert sizes of 20 randomly selected BAC clones were analyzed by pulse field gel electrophoresis. After digestion with *Not* I, an aliquot of each digest was applied to the gel. Each lane contains an insert with a vector fragment (7.4 kb). Electrophoresis was performed by CHEF-DR III (Bio-Rad) in 0.5x TBE buffer at 160 V for 15 hr at 16°C.

amenable to the dimension pooling system for PCR screening. To estimate the size of the average DNA insert in the BAC library, 80 randomly selected clones were digested with *Not* I and analyzed by pulse field gel electrophoresis. Only 20 clones are shown in Fig. 1. The average insert size was 128 kb, corresponding to 10x coverage of the *H. chilotes* genome The genome size is assumed by that of *Oreochromis niloticus* (Majumdar and McAndrew, 1986). As a pilot screening experiment, we obtained independent seven BAC clones of the *lws* (a long wavelength-sensitive) opsin gene from this library (data not shown). The number of the clones isolated confirmed that this BAC library covers almost 10x genome, since the *lws* gene was shown to be a single copy gene in cichlid from Lake Victoria (Terai et al, 2002c).

Recent studies from our laboratory (Takahashi et al., 2001a, 2001b, 2002; Terai et al., 2002a, 2002b) and others (Mayer et al., 1990; Sultmann et al., 1995; Nagl et al., 1998, 1999, 2000; Booton et al., 1999) have elucidated the close phylogenetic relationships among East African cichlid fishes. Remarkably, the genomes of species in Lake Victoria are nearly identical (Nagl et al., 1998, 1999; Terai et al., submitted). In fact, the DNA sequences of some housekeeping genes among several Haplochromine species are identical (Watanabe and Okada, unpublished data). Although nucleotide variations are present in a cichlid genome, most of them distribute as inter-specific polymorphisms among Haplochrominie cichlids in Lake Victoria. This is also an indication that the genomes of cichlids in Lake Victoria are essentially the same (Nagl et al., 1998, 1999; Terai et al., submitted). Thus, the BAC library constructed from *H. chilotes* may also prove to be useful for studies of the more than 200 Haplochromine cichlid species in Lake Victoria, although we must bear in mind that a very few genes responsible for speciation of *Haplochromis chilotes* are distinct from those of other species.

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