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A BAC-based physical map of the channel catfish genome

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Abstract

Catfish is the major aquaculture species in the United States. To enhance its genome studies involving genetic linkage and comparative mapping, a bacterial artificial chromosome (BAC) contig-based physical map of the channel catfish (*Ictalurus punctatus*) genome was generated using four-color fluorescence-based fingerprints. Fingerprints of 34,580 BAC clones (5.6× genome coverage) were generated for the FPC assembly of the BAC contigs. A total of 3307 contigs were assembled using a cutoff value of 1×10^{-20}. Each contig contains an average of 9.25 clones with an average size of 292 kb. The combined contig size for all contigs was 0.965 Gb, approximately the genome size of the channel catfish. The reliability of the contig assembly was assessed by both hybridization of gene probes to BAC clones contained in the fingerprinted assembly and validation of randomly selected contigs using overgo probes designed from BAC end sequences. The presented physical map should greatly enhance genome research in the catfish, particularly aiding in the identification of genomic regions containing genes underlying important performance traits.

Keywords: BAC; Contig; Physical map; Catfish; Genome; Fingerprint

The channel catfish (*Ictalurus punctatus*) is the major aquaculture species in the United States, accounting for over 60% of all U.S. aquaculture production. In 2006, its production in the United States reached almost 700 million pounds. Additionally, the channel catfish is a well-developed research model for comparative immunology and toxicology. Rapid progress in catfish genomics has been made in the past several years. Large numbers of molecular markers have been developed and evaluated for linkage mapping [1,2], framework genetic linkage maps have been constructed [3,4], and genome repeat structure has been characterized [2,5]. More than 55,000 ESTs have been generated [6–10], and an ongoing large-scale EST project by the Joint Genome Institute of the Department of Energy will significantly further expand the EST resources in both channel catfish and blue catfish [11]. Microarrays have been used to study genome-wide expression in catfish [12–15]. Two bacterial artificial chromosome (BAC) libraries, CCBL1 and CHORI-212, have been constructed using different restriction endonucleases and subsequently characterized [16,17]. More than 20,000 BAC end sequences from the channel catfish CHORI-212 library have been generated and characterized [2]. Of the two BAC libraries, CCBL1 was constructed using DNA from a homozygous gynogenetic female. Gynogens were produced from eggs induced to develop using UV-irradiated sperm; the diploid state was restored by hydrostatic pressure shock that induced the retention of the second polar body. The other BAC library, CHORI-212, was constructed using DNA from a diploid male catfish of which the genomic DNA contains all autosomes and sex chromosomes and the natural level of polymorphism [16]. The two libraries were also constructed using different restriction endonucleases, *Hin*dIII for CCBL1 and *Eco*RI for CHORI-212. BAC contigs have been developed recently from CCBL1 [18]. In this work, the objective was to

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construct a BAC contig-based physical map using the CHORI-212 BAC library.

A BAC-based physical map is important for the understanding of genome structure and organization and for position-based cloning of economically important genes. A well-characterized physical map can often be an important foundation for whole genome sequencing. A BAC-based physical map would also allow exploitation of existing genomic information from map-rich species using comparative mapping. The channel catfish's evolutionary position allows comparison of its genome information with that of the model species zebrafish, accelerating catfish genome research while facilitating zebrafish genome annotation. Because of their importance to genome research, physical maps have recently been constructed in aquaculture finfish species including Nile tilapia (*Oreochromis niloticus*) and Atlantic salmon (*Salmo salar*) [19,20]. Here we report the construction of a BAC contig-based physical map of the channel catfish genome.

Results

BAC fingerprinting

A total of 40,416 BAC clones was processed from the channel catfish BAC library CHORI-212, and 34,580 (85.6% success) fingerprints were validated and used in the final FPC assembly. The valid fingerprints represent approximately 5.6-fold coverage of the catfish genome, while the total number of processed clones represents approximately 6.5-fold coverage of the catfish genome (Table 1). Each BAC clone contains, on average, 95.2 restriction fragments, with 60 to 120 bands in most of the samples (Fig. 1).

Using a tolerance of 4 and a cutoff stringency of 1×10^{-20} (see below), we tested FPC assembly of 5000, 10,000, 15,000, 20,000, 25,000, and 30,000 BAC clones to assess if the number of fingerprinted BAC clones was sufficient to cover the entire genome. When the assembled contig numbers were plotted against the clone numbers (Fig. 2), the contig number reached a plateau when the total clone number reached 25,000, suggesting that the number of fingerprinted BAC clones was sufficient to cover the catfish genome.

Table 1
Statistics of the BAC contig assembly of the catfish genome

Total number of BAC clones fingerprinted	40,416	6.5× genome equivalent
Valid fingerprints for FPC assembly	34,580	5.6× genome coverage
Total number of contigs assembled	3,307	
Clones contained in the 3307 contigs	30,582	
Average BAC clones per contig	9.25	
Average estimated size per contig	292 kb	
Number of Q-contigs	517	
Number of Q-clones	1,494	4.3%
Number of singletons	3,998	
Average insert size of the BAC library	161 kb	
Average number of bands per fingerprinted BAC clone	95.2	
Average size each band represents	1.6912 kb	
Total number of bands included in the contigs	570,766	18.7 bands per BAC clone in the consensus map
Total physical length of assembled contigs	965,279 kb	~1× genome size

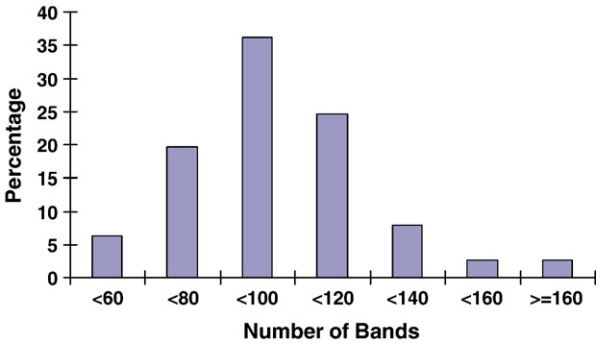

Fig. 1. Distribution of band numbers in the catfish BAC fingerprints.

Determination of tolerance

The tolerance level dictates how closely two restriction fragments must match to be considered the same fragment across gel runs. The tolerance was determined by identifying identical fragments in many different fingerprints and computing the standard deviation of their sizes in different fingerprints. For this purpose, vector fragments from BAC vector pTARBAC2.1 were identified from 300 randomly picked fingerprints and the standard deviation of each vector fragment was computed. The standard deviations of the three vector fragments (59.1, 157.3, and 369.8 bp) were 0.099, 0.081, and 0.085 bp, respectively, with an average of 0.088 bp. Since the three vector fragments did not cover the whole range of fragment sizes (50–500 bp),

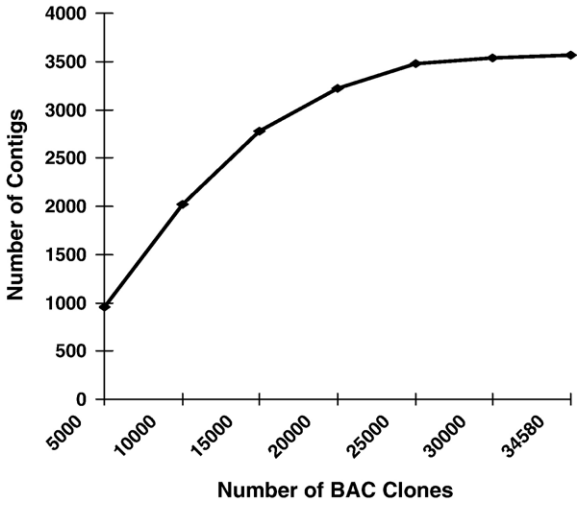

Fig. 2. The relationship of the number of fingerprinted BAC clones and the number of BAC contigs assembled using a cutoff value of 1×10^{-20} and a tolerance of 0.4.

we also computed the standard deviations of selected GS500-LIZ internal size standard fragments (Applied Biosystems, Foster City, CA, USA), 100, 160, 340, and 490 bp. The standard deviations lay between 0.06 and 0.13, with an overall average of 0.087 bp. Thus, the tolerance value was estimated at 0.36 according to the size deviation with 95% confidence interval. The tolerance value used in FPC assembly was set at 4 since all the fragment sizes were multiplied by 10, and decimals were not allowed in the FPC program.

Determination of cutoff values for the contig assembly

The cutoff value is the threshold of the Sulston score, the probability that fingerprint bands match by coincidence. Lowering the cutoff value (e.g., 1×10^{-12} to 1×10^{-15}) would increase the stringency and therefore increase the likelihood that reported overlapping BAC clones are truly overlapping. However, setting an appropriate stringency of Sulston score is always challenging; too low a cutoff would lead to splitting of true contigs into multiple contigs or singletons, whereas too high a cutoff would lead to chimeric contigs. During our assembly of the catfish physical map, a series of cutoff values ranging from 1×10^{-12} to 1×10^{-40} was tested. The resulting numbers of contigs, Q-contigs (questionable contigs), singletons, and Q-clones were considered. At high stringencies (1×10^{-25} to 1×10^{-40}), the number of singletons increased drastically (Fig. 3), causing many contigs to collapse. As expected, a lower number of contigs were assembled using lower stringencies: only 1798 and 2460 contigs resulted using cutoff values of 1×10^{-12} and 1×10^{-15}, respectively; but a large number of clones were in the category of Q-clones, 31.0 and 13.7%, respectively. Clearly, these stringencies (1×10^{-12}, 1×10^{-15}) were too low, as almost 1/3 of the contigs contain Q-clones, and the percentage of Q-clones was too high. It was noted from the plot of the numbers of contigs, singletons, and Q-clones versus the assembly stringencies that these values cross over each other at approximately 1×10^{-17}, at which the number of contigs, the number of singletons, and the number of Q-clones were all reasonably low (Fig. 3). This provided a starting point for determining the proper cutoff value. At the stringencies of 1×10^{-18} and 1×10^{-20}, all indicators were similar, suggesting these

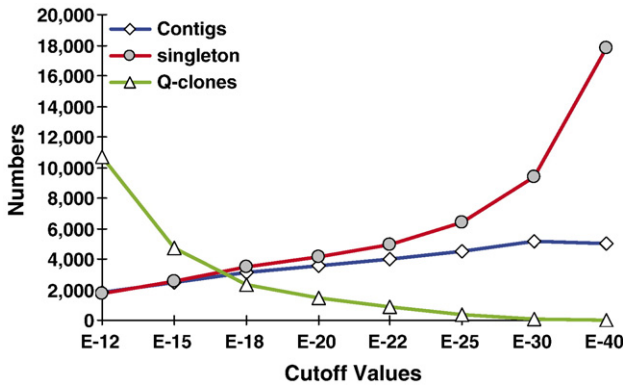

Fig. 3. Plot of the numbers of contigs, singletons, and Q-clones over the stringencies used for the assemblies.

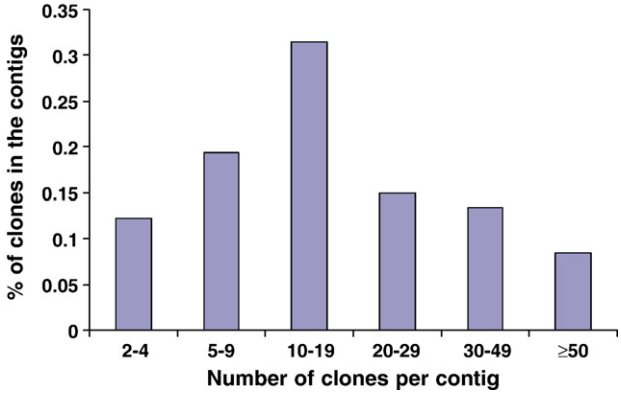

Fig. 4. Distribution of BAC clones in contigs of various sizes.

cutoff values provide relatively stable assemblies. However, the number of Q-contigs was significantly more with 1×10^{-18} than with 1×10^{-20} (Fig. 3). Higher levels of stringency, such as 1×10^{-22} and 1×10^{-25}, would increase the reliability of the assembly, but also increase the chances of splitting true contigs. Further experiments using overgo hybridizations (see below) demonstrated that the cutoff values of 1×10^{-22} and 1×10^{-25} were too stringent, leading to the breaking of many *bona fide* contigs. We therefore chose 1×10^{-20} as the cutoff value for the assembly of the physical map.

Contig assembly

Contigs were assembled from the fingerprint data using the computer program FPC version 8.5 (http://www.agcol.arizona.edu/software/fpc/). A total of 3307 contigs were assembled with the valid fingerprints of 34,580 BAC clones using FPC with a cutoff value of 1×10^{-20} and a tolerance level of 4 (see below), followed by end-to-end merging and end-to-single merging at progressively lower stringencies. A total of 30,582 clones were placed into the 3307 contigs, leaving the remaining 3998 BAC clones as singletons. The assembly can be accessed through a Web-based physical map viewer, WebFPC, at http://titan.biotec.uiuc.edu/WebAGCoL/AU02-20/WebFPC/.

The contig size (clones per contig) distribution is shown in Fig. 4. The top half of the contigs (1654 contigs) contained 83% (25,398 clones) of the total assembled clones. The top 580 contigs (17.5%) contained 50% of BACs in the contigs. The largest contig contained 364 BAC clones, while the smallest contig contained 2 BAC clones. The contigs contained an average of 9.25 clones each and had an average estimated length of 292 kb per contig.

There were a total of 570,766 consensus bands distributed in the 3307 contigs, representing approximately 0.96 Gb (965,279 kb) linear length of DNA according to the average band size of 1.69 kb (the BACs have an average insert size of 161 kb and produced an average of 95.2 bands per BAC, and therefore, each band represents an average segment size of 1.69 kb), equivalent to the genome size of the channel catfish [21]. On average, each BAC in the contigs contributed 18.7 unique consensus bands to the assembly, or approximately 31.6 kb to the linear length of the contig assembly.

Table 2
Distribution of Q-clones in contigs assembled using a cutoff value of 1.00×10^{-20}

Number of contigs	Q-clones/contig	Percentage of all contigs
2790	0	84.4
301	1	9.1
96	2	2.9
44	3	1.3
19	4	0.6
16	5	0.5
41	>5	1.2

Note that 84.4% of the contigs are free of Q-clones, and most Q-clones are involved in a small number of contigs.

Q-contigs and dQ-process

There were a total of 1494 questionable clones (4.3%) distributed in 517 Q-contigs. However, the distribution of the Q-clones was uneven. The vast majority of contigs (84.4%) were free of Q-clones; 301 contigs had only 1 Q-clone each; 96 and 44 contigs had 2 and 3 Q-clones, respectively, while the vast majority of Q-clones were placed into a small number of contigs (Table 2). This indicated that it is likely that some highly repetitive elements could have been involved in the contigs containing many Q-clones, and they need to be assembled at a higher stringency. The dQer in the FPC program was used to eliminate the Q-clones in the Q-contigs with more than 5 Q-clones. The dQer automatically reran the assembly algorithm with lower cutoffs of 1×10^{-21}, 1×10^{-22}, and 1×10^{-23}; split Q-contigs; and then assembled the generated contigs and singletons with existing contigs. Finally, dQer generated 523 Q-contigs, in which 15 contigs still have more than 5 Q-clones. The final contig and singleton numbers were 3366 and 4104, respectively. There were only 55 contigs impacted in the dQer reassembly, which suggested high stability of the contig assembly.

Assessment of the physical map reliability

Several approaches were used to assess the quality of the contig assembly. First, we checked if the BAC clones containing known genes were actually assembled into the same contigs. Overgo probes were designed for known genes using available cDNA sequences. Overgo hybridization was used to screen the high-density CHORI-212 channel catfish BAC library filters. All the positive clones were identified. The positive clones of each gene were tabulated to test the validity of contigs assem-

Table 3
Assembly of BAC clones positive for selected gene probes

Gene	Positive clones[a]	Contigs	Clones in the contig	No. in the same contig
BPI	**005B2E02, 013A1F03, 017A1B07, 030B1E12, 077A1G10, 104A2A02, 107B1H12**, 126B1D02, 174B2D01	212	005B2E02, 013A1F03, 017A1B07, 030B1E12, 077A1G10, 104A2A02, 107B1H12	100%
LEAP-2	*007B1D12, 009A2G03*, **023A1G01, 027B1B07, 033B1E02, 035A2F08, 063B2E04, 063B2F04**, *066B2D04*, **076A2D04, 080A1B04, 087B2B06, 101B1C12**, 113B2C06, 113A2D02, 134A1A05, 138A2G04, 154A2A05, 160A2H10, 171A2F06, 172B2A09, 178B1E12, 178A1H11	1162 2525	023A1G01, 033B1E02, 063B2E04, 063B2F04, 076A2D04, 087B2B06, 101B1C12 027B1B07, 035A2F08, 080A1B04	Split into 2 contigs
IL-1β	*017B1E01*, **028A2A02, 039B1H01, 039B1H11, 042B2H09, 051A1A07, 072A1A09**, *089B1F09, 093A1B01, 094A1H03,* **102B2C12**, 175B2F11, 187A1H06	699	028A2A02, 039B1H01, 039B1H11, 042B2H09, 051A1A07, 072A1A09, 102B2C12	100%
Hepcidin	**010B2C11, 047B1B09, 049B2G01, 090B2H07, 095A1G05, 102B1F01**, 132A2D08, 176A2C06, 188B2A07	948 1788	010B2C11, 049B2G01, 095A1G05, 102B1F01 047B1B09, 090B2H07	Split into 2 contigs
TLR20	**005A1G05, 047A1G09, 050B1C09, 070A1H02, 093A2B06, 105B2D06**, 136A2B09	1754	005A1G05, 050B1C09, 070A1H02, 105B2D06	4 of the 6 clones in this contig
TLR21	**006A2D09, 009B1A01**, *025A1E09,* **075A1G05, 086A1H05**, *092A2D05,* **104A1C08, 108B1B01**, 160B2F10, 161B1F08, 164A1E11, 176B2H06, 192B2H03	1703	006A2D09, 009B1A01, 086A1H05, 104A1C08, 108B1B01	5 of the 6 clones in this contig
CXCL8-like	**001B1A07**, *025A2D04*, **053B2F01, 056B1C06, 066A2A05**, 189A1D12	1734	001B1A07, 053B2F01, 056B1C06, 066A2A05	100%
CXCL10	**010B1E05**, *016A2F06*, **024A1D07, 036B2D06, 037A1C06**, *050A1C12*, **056B1C06, 060A1A04, 063A2E12, 107A1B01**, 176B1G02, 180B2B03, 182A1B12	972 1734	024A1D07, 060A1A04, 063A2E12, 107A1B01 036B2D06, 037A1C06, 056B1C06	Split into 2 contigs
CXCL12	**019B2H05, 024A1G02, 031A2H09, 034B1C09**, *037A2C01*, **038B1B07, 052A1G07, 059B1E12, 068B2C11, 081B1F10, 090A1G01, 096B1D08**, *099B1B07*, **111A2D11**, 130A1E05, 135B1C01, 136A1B04, 139A2G09, 142A2D12, 158B2E11, 160B1B06, 170B2G05, 184A1F01, 185B2H12, 189B2E12, 191B1G09	766	019B2H05, 024A1G02, 031A2H09, 034B1C09, 038B1B07, 052A1G07, 059B1E12, 068B2C11, 081B1F10, 090A1G01, 096B1D08, 111A2D11	100%
CXCL14	**010A2E01, 020B1C02, 026B1B01, 035A2F03, 039B1G09, 040B2C05, 053A1G05, 060A1F01, 079B2H12**, 118B1H10, 123A2E12, 143A1D10, 144B1E02, 164B2C01	981	010A2E01, 020B1C02, 026B1B01, 035A2F03, 039B1G09, 040B2C05, 053A1G05, 060A1F01	8 of the 9 clones in this contig

[a] Hybridization was conducted using the high-density filters containing all BAC clones in the CHORI 212 library, whereas only a portion of the CHORI BAC library was fingerprinted for the construction of the physical map. Boldface indicates the successfully fingerprinted clones. Italic indicates clones for which the fingerprints did not pass the quality cutoff. Clones in normal font were not fingerprinted but were positive to the overgo probes.

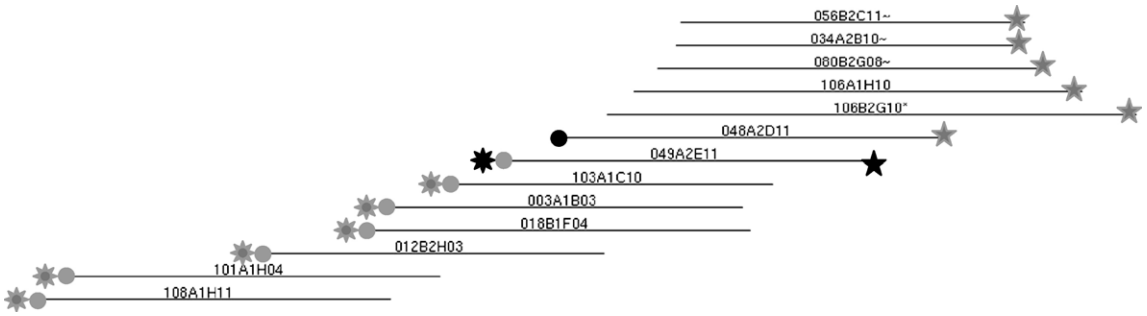

Fig. 5. An example of contig validation using overgo hybridization. Three probes were used (black symbols). Using these probes allowed detection of all positive clones hybridizing to each probe (with same-shaped symbols, but gray filling). The three probes collectively hybridized to all clones, with many hybridizing to multiple clones, allowing confirmation of the contig.

bled using FPC with a cutoff of 1×10^{-20} and tolerance of 4 (Table 3). In general, two types of situations were observed. In the first case, all the fingerprinted positive clones fell within a single contig, confirming the reliability of the assembly; these included the genes for bactericidal permeability-increasing protein (BPI), interleukin-1β (IL-1β), and chemokines CXCL8 and CXCL12. In the second case, the fingerprinted positive clones were split into two contigs or a major contig plus singletons, suggesting that the stringency of the assembly was too high, these genes are duplicated in the catfish genome, or the fingerprinting of some of those clones was suboptimal. These included genes for liver-expressed antimicrobial peptide 2 (LEAP-2), hepcidin, CXCL10, CXCL14, TLR20, and TLR21.

Second, map reliability was assessed by checking randomly selected contigs to determine if all the BAC clones truly belong to the contigs. If all the BAC clones truly belong to the contig, then use of a few probes should allow hybridization of all clones in the contig, thereby validating the contig. As shown in Fig. 5, use of three probes allowed validation of contig 1046, as the three probes collectively hybridized to all BAC clones. Similarly, a total of five randomly selected contigs were all validated this way (Table 4), providing strong evidence for the high reliability of the physical map.

Third, after initial assembly at the cutoff of 1×10^{-20}, trial assemblies were conducted at higher stringencies such as 1×10^{-22} and 1×10^{-25}. As the stringency was increased, some contigs were split into two or more contigs. Based on the position of the breaking points, overgo probes were designed from BAC end sequences from the clone covering the breaking points. In all 10 instances examined, the overgo probes hybridized to both splitting contigs, confirming that the contigs split at the cutoff value of 1×10^{-22} or 1×10^{-25} were indeed *bona fide* contigs, and they were split simply because the overlapping regions were not long enough and the stringency was too high (Table 5). This set of hybridization experiments not only provided evidence for the high reliability of the contig assembly, but also provided a strong experimental basis for the selection of assembly cutoff values.

Table 4
Assessment of map reliability using overgo probes designed from BAC end sequences

Contig at 1×10^{-20}	Number of BACs	Number of probes	Total number of positive clones	Contig assembly completely validated
1046	13	3	22	Yes
1558	11	2	12	Yes
673	16	2	18	Yes
586	13	4	29	Yes
284	17	2	21	Yes

A number of probes were designed from BAC end sequences such that all clones in the contigs were positive (also see Fig. 3). Note that some clones are positive to multiple probes, leading to the larger number of positives than the total number of clones in the contig; thereby collaterality was established.

Discussion

This work produced a high-quality BAC-based physical map of the diploid catfish genome with 3307 contigs spanning approximately 0.965 Gb, equivalent to the size of the catfish genome. The generation of this physical map filled a critical gap in catfish genome research. This BAC-based physical map should provide a material and information basis for comparative mapping [22,23] and large-scale analysis of the catfish genome using existing genome sequence information from several model fish species. The availability of the contig information and BAC-end-associated polymorphic markers also provides the opportunity for integration of the physical map with genetic linkage maps. Polymorphic microsatellite markers are being generated from the BAC end sequences, mapping of which to the genetic linkage map would allow integration of the genetic and physical maps. With the physical map, chromosomal regional markers can be developed from targeted genomic regions for fine mapping of candidate genes associated with performance traits important to aquaculture, laying grounds for eventual positional cloning of economically important genes [24]. This physical map will also allow generation of a minimal tiling path in preparation for whole genome sequencing.

A plot analysis of the fingerprinted clones versus the number of contigs (Fig. 2) indicated that the fingerprinted BACs (5.6× genome coverage) should provide a reasonable coverage of the whole genome of catfish. Although the calculated consensus band (CB) map distance was the same size as the catfish genome, the actual physical map length could be longer, as the 3998 singletons were not included in the map length. In addition, there would be gaps among the contigs as well. However, such gaps and map distances represented by non-contig singletons could be offset by undetected overlaps among the

Table 5
Validation of contigs through overgo hybridizations and collateral inferring

Contigs 1×10^{-20}	Contigs 1×10^{-22}	Contigs 1×10^{-25}	Probe location	Positive contigs
Contig 1246 (12)	Contig 1405 (12)	Contig 1477 (8)	Singleton	Contig 1477
		Contig 3765 (3)		Contig 3765
		1 singleton		Singleton
Contig 135 (42)	Contig 138 (42)	Contig 148 (19)	Contig 492	Contig 148
		Contig 492 (22)		Contig 492
		1 singleton		
Contig 199 (51)	Contig 261 (30)	Contig 3413 (4)	Contig 271	Contig 3413
	Contig 192 (17)	Contig 271 (30)		Contig 271
	Contig 3000 (4)	Contig 175 (17)		
Contig 276 (53)	Contig 275 (41)	Contig 322 (41)	Contig 322	Contig 974
	Contig 793 (12)	Contig 974 (12)		Contig 322
Contig 121 (41)	Contig 118 (36)	Contig 2470 (4)	Contig 107	Contig 2470
	Contig 2079 (4)	Contig 107 (33)		Contig 107
	1 singleton	Contig 3373 (3)		
		1 singleton		
Contig 1046 (13)	Contig 1088 (13)	Contig 1104 (7)	Contig 1104	Contig 2624
		Contig 2624 (6)		Contig 1104
Contig 1997 (10)	Contig 1435 (10)	Contig 2972 (7)	Contig 2972	Contig 4477
		Contig 4477 (3)		Contig 2972
Contig 958 (21)	Contig 2616 (3)	Contig 1116 (10)	Contig 1116	Contig 2909
	Contig 980 (16)	Contig 2064 (6)		Contig 2064
	Contig 3724 (2)	Contig 2909 (3)		Contig 1116
		Contig 4363 (2)		
Contig 366 (24)	Contig 364 (24)	Contig 1915 (4)	Contig 1915	Contig 339
		Contig 339 (20)		Contig 1915
Contig 579 (21)	Contig 591 (14)	Contig 642 (14)	Contig 642	Contig 2853
	Contig 2340 (7)	Contig 2853 (3)		Contig 3277
		Contig 3277 (4)		Contig 642

Only one overgo probe was used to cover the break points of randomly selected contigs when one single contig assembled at a lower stringency (1×10^{-20}) was split into more than one contig at a higher stringency (1×10^{-22} or 1×10^{-25}). Note that the contigs were assigned different numbers in each assembly. Numbers in parentheses are the numbers of clones in the contigs. For instance, Contig 1246 (12) was one contig containing 12 BACs in the assembly using a cutoff value of 1×10^{-20}; this contig remained as a single contig in the assembly using a cutoff value of 1×10^{-22} (though with a different contig number, Contig 1405 now); however, this contig was split into two contigs plus a singleton in the assembly using a cutoff value of 1×10^{-25}. An overgo probe designed from the singleton BAC clone hybridized to some clones contained within Contigs 1477 and 3765 as well as to itself, providing evidence that the contig was split because of the high assembly stringency.

contigs. The CB map estimation is very similar to that estimated from a gynogen catfish [18] for which the physical map size was estimated to be 0.93 Gb. The number of consensus bands used for the assembly was also very similar (516,956 in Ref. [18] and 570,766 here).

The contig numbers initially increased with the number of clones being fingerprinted. However, the number of contigs reached a plateau around 25,000 clones. Due to budget limitations, fingerprinting of additional BAC clones was not possible at this time. Additional fingerprinting in the future could potentially fill some gaps, allowing contigs to be merged, thereby reducing the total number of contigs. However, the use of a complementary BAC library using a different restriction enzyme may be more effective in gap filling as some genomic regions would have been left out during library construction using restriction digestion.

One key issue for the assembly of a physical map is the selection of a proper cutoff value. With agarose gels, a Sulston cutoff value of 3×10^{-12} was used for the human genome (3×10^{9} bp) for automated assembly [25], and a less stringent score of 1×10^{-9} was used for the smaller *Arabidopsis* genome [26]. For the construction of the tilapia BAC contigs, a cutoff value of 10^{-8} was used [19], and for the Atlantic salmon Ng et al. used a cutoff value of 10^{-16} for initial contig assembly, then the contigs were merged with a cutoff value of 10^{-10} [20]. A more stringent cutoff value is required for the assembly of fingerprints produced using high-information-content fingerprinting (HICF) [27] compared to the assemblies of fingerprints produced by agarose gels. However, the use of too stringent a cutoff value could lead to a split of many *bona fide* contigs. In a recent assembly of BAC contigs using HICF, a cutoff value of 1×10^{-40} was used for the contig assembly [18]. However, the BAC library, CCBL1, was constructed using DNA from a gynogenetic female whose genome was homozygous, whereby sequence polymorphism was minimal, if any. It is obvious that the level of sequence polymorphism, reflected in the DNA from a single diploid organism as sequence differences between the two sets of homologous chromosomes, could greatly affect the choice of cutoff values. The greater the sequence divergence, the less stringent the cutoff value should be. In addition, the genome size should be considered for the selection of the cutoff value. In this regard, the catfish genome is approximately 1/3 the size of the Atlantic salmon genome and is similar to the size of the tilapia genome. With all existing information, we believe that the assembly using 1×10^{-20} should provide a reasonably conservative assembly that can easily be updated when more genome data of the channel catfish become available. Our overgo hybridization experiments strongly support the use of

1×10^{-20} as the proper cutoff value for the assembly of the catfish physical map. At this cutoff, the percentage of Q-clones was reasonably low (4.3%) (for comparison, 7.3% Q-clones in Ref. [18]). Obviously, at various cutoff values, some true contigs would have been split, while a low level of false contigs might exist, as ultimate validation would need verification of all contigs using an approach different from the fingerprinting approach used in this study. Systematic integration of the physical and linkage maps by mapping microsatellites from BAC end sequences to linkage map should also validate the contig assembly, and we are attempting to acquire funding toward this goal in the near future.

The quality of the physical map was assessed by both hybridizations of selected genes and validation of randomly selected contigs using overgo hybridizations with probes designed from BAC end sequences. The vast majority of contigs (84.4%) were free of Q-clones. Even in the contigs with Q-clones, these may not be a result of fingerprinting analysis. They could represent truly questionable clones caused by several means. First, teleost fish genomes are well known for their whole-genome duplication events [28–30]. In addition to the whole-genome duplications, teleost fish also exhibit a high level of tandem and segmental gene duplications [31–33]. Such genome duplications would certainly add complexity to physical genome analysis, including the possibility of producing Q-clones. Second, the use of four sets of restriction endonucleases, while providing great advantages, also increased the sensitivity for the detection of polymorphism. Some Q-clones could truly represent polymorphic genomic regions derived from homologous chromosomes of the diploid catfish.

A large number of Q-clones generally result from one or several false positive overlaps. Once a clone in the contig overlaps with another clone in another contig, the whole contig will be brought to that contig. However, in this case, FPC may not assign the appropriate linear order to each clone on the CB map. So the clones coming from different contigs stack on top of each other. The dQer can automatically increase the cutoff stringencies and split the Q-contigs. However, there are still many contigs harboring Q-clones. Most possibly, the Q-contigs could be caused by duplicated genome regions or repetitive elements in the genome. Repetitive sequences occupy a significant fraction of the catfish genome [2,5,34]. In previous analysis of repeat sequences from 11.4 million base pairs of the channel catfish BAC end sequences, approximately 11% sequences were masked by RepeatMasker software using zebrafish and *Takifugu* repeat databases [2]. Some of the Q-clones could have been attributed to such repetitive sequences.

Materials and methods

BAC library and BAC fingerprinting

The CHORI-212 BAC library contains a total of 72,067 recombinant clones with average insert size of 161 kb, representing approximate 10.6× coverage of channel catfish genome [16].

BAC clones were inoculated into 2.2-ml 96-well culturing blocks with each well containing 1.5 ml 2×YT medium and 12.5 μg/ml chloramphenicol from 384-well stocking plates using a 96-pin replicator (V&P Scientific, San Diego, CA, USA). Each 384-well plate of BAC clones was inoculated into four 96-well culturing blocks. To ensure clone tracking, the BAC clones were always taken using the 96-pin replicator with the first pin of the replicator aligned with position A01 of the 384-well plates as the set "A" samples (which takes A01, A03, A05,…, C01, C03, C05, …, and O01, O03, O05, …, followed by the first pin of the replicator aligned with position A02, B01, and B02 as the "B" set, "C" set, and "D" set of samples. The four sets of samples were later decoded to their original 384-well locations. The 96-well culture blocks were covered with air-permeable seals (Excel Scientific, Wrightwood, CA, USA) and incubated at 37°C for 24 h on a HiGro shaker incubator (Gene Machines, San Carlos, CA, USA) at 450 rpm. The blocks were centrifuged at 2500*g* for 10 min in an Eppendorf 5810 bench-top centrifuge to precipitate the bacteria. The culture supernatant was decanted, and the blocks were inverted and tapped gently on paper towels to remove remaining liquid. BAC DNA was isolated using Qiagen REAL Prep 96 plasmid kit (Valencia, CA, USA) according to the manufacturer's instructions. BAC DNA was collected in 96-well plates and stored at −20°C before use.

For fingerprinting, the four fluorescence-labeled restriction fragments were first created using the SnapShot kit from Applied Biosystems (Foster City, CA, USA) as previously described [35]. BAC DNA was digested with *Bam*HI, *Eco*RI, *Xba*I, *Xho*I, and *Hae*III restriction endonucleases (New England Biolabs, Ipswich, MA,USA) simultaneously at 37°C for 4 h. The 6-bp cutter restriction endonucleases *Eco*RI (G|AATTC), *Xba*I (T|CTAGA), *Bam*HI (G|GATCC), and *Xho*I (C|TCGAG) generate 5′-protruding ends allowing differentially fluorescence-labeled A, C, G, and T to be incorporated at the 3′ ends of fingerprints, while the 4-bp cutter *Hae*III cleaves the fragments to small segments, making them suitable for analysis using an automated sequencer. All the procedures for restriction digestion reactions and labeling reactions followed the protocols of the SnapShot kit provided by the manufacturer. The labeled BAC fragments were precipitated and analyzed with the ABI GS500-LIZ internal size standard (Applied Biosystems) on an ABI 3730XL DNA analyzer (Applied Biosystems) at the W.M. Keck Center at the University of Illinois.

Data processing

The fragment sizes in each BAC fingerprint profile were collected by the ABI Data Collection program. The data off the ABI 3730XL genetic analyzer were processed using the computer software package GenoProfiler (http://wheat.pw.usda.gov/PhysicalMapping/) and FPminer (http://www.bioinforsoft.com). Briefly, the fragment size calling was conducted using an automatic algorithm in FPminer. Several quality checks were applied to the fingerprints: the empty well was removed, fingerprints with fewer than 25 fragments or more than 250 fragments were removed, the background fragments were identified and removed using the FPminer embed algorithm, and the off-scale fragments with peak height greater than 6000 were removed. The data were then transferred to Genoprofiler to remove the vector fragments and frequent fragments. Only the fragments between 50 and 500 bp were used for contig assembly in FPC assembly.

BAC contig assembly

The program FPC version 8.5 was used to assemble the BAC fingerprint data to contigs. The size tolerance value was determined by the mean size deviation of the vector fragments and the size standard fragments in the GS500-LIZ internal size standard (Applied Biosystems). The 250-bp fragment was not used since this fragment migrates abnormally under denaturing conditions (Applied Biosystems, personal communication, January 2007).

Library screening and assessment of physical map quality

Overgo hybridization was conducted to screen the BAC library to assess the physical map quality. Two assessment approaches were conducted. First, the overgo probes designed from genes were hybridized to high-density filters of a channel catfish BAC library, which were purchased from BACPAC Resources at Children's Hospital of Oakland Research Institute (BACPAC Resources, CHORI, Oakland, CA, USA). All the primer sequences for the probes are listed in Table 6. Second, overgo probes designed from BAC end sequences were used to hybridize to the BAC clones in the selected contigs. DNA (200 ng) of each BAC clone from the same contig was treated for dot-blot analysis [36]

Table 6
All primers, probes, and their sequences used in this study

Probe	Probe target	Overgo primer Ova	Overgo primer Ovb
AU50480	Hepcidin	CTGCTGCAGGTTCTAATAACGGAC	TGAAAACTTGCATGTGGTCCGTTA
AU50493	LEAP-2	AGGAGATCAGAGGTCACTCAAGAG	TGTCATACGGGCCATTCTCTTGAG
AU50531	BPI	TATCAGCCTTCACCCTGAACTCAG	TTGTACACGAATCCGGCTGAGTTC
AU50591	CXCL12	TTGCTGAACCAGCACTTAACCTGC	GAGGCAAGCAAGGTTTGCAGGTTA
AU50592	CXCL14	CAAATGCAGATGCACCAGGAAAGG	GTATCGTATCTTGGGGCCTTTCCT
AU50620	CXCL10	GAGAATCTTCAGAGCATCGAGTGT	CCTCTTGCTCTTGAACACACTCGA
AU50621	CXCL8	CAGTAACTGCCTTCTGCTGCTTTG	AAGGCAAACACTGTGGCAAAGCAG
AU50814	IL-1β	AATATTCAGTCCACGGAGTTCACC	TGAAAAGCTCCTGGTCGGTGAACT
AU50860	TLR20	TGGGACTGGTGTTCTTCATGCTGG	TGATGGAGCAGCACTACCAGCATG
AU50861	TLR21	GCTTGTTACACTTCGCCTGGACAA	ATCAGACAGAAGGTTGTTGTCCAG
AU51030	Contig 121	CAGTATTGGTAGCTAGCCATTTTC	ACGACAGTAGCTTTGTGAAAATGG
AU51056	Contig 135	GACTGGCTTTGAAACGTGGGAAGC	GGACTGGCTTTTGTCTGCTTCCCA
AU51063	Contig 199	CGCGAGTTTTGTCTTTGAGTCATC	CCAGATCATGCTCATGGATGACTC
AU51079	Contig 276	TTTATATTGTAGGTGTTACCTAGG	GTCAACTTCCAGTTGCCCTAGGTA
AU51083	Contig 1246	GTGGGACGAACAGATTTTAAGTTT	AACGAACTGCCACCTTAAACTTAA
AU51111	Contig 366	ATTACCTTGTATATTGCAAATGGG	GGAATGACCAAAAAAACCCATTTG
AU51135	Contig 958	CCAACCTTGCCTTATGCTTTTTTC	GAAAGAAAGAAAGAAAGAAAAAAG
AU51141	Contig 284	GTGTGCCGATCTAAACGTATCAGG	CAGTAATGATGCTCTACCTGATAC
AU51142	Contig 284	TTGTTTTCCTTGGCCAACCTGTTC	CTAACAACCAGACATGGAACAGGT
AU51144	Contig 579	CAGTGTATTAGGGTGGAAGTGGTG	GTAATGAAGACCCTGCCACCACTT
AU51146	Contig 586	ACCACATGAAAAGTGCTCTATAAA	CTACTGATGAACTTCTTTTATAGA
AU51147	Contig 586	TCGGTTGGAAACCTGACAAAAATG	AGCATTTCGACCCTCTCATTTTTG
AU51148	Contig 586	CTCCATGTAAGTTCAGACACACCG	TGTAAACACCGCAGTCCGGTGTGT
AU51149	Contig 586	GAGAAGCACAGTCAATAAACGCTG	GCCATGCCATTATGAGCAGCGTTT
AU51150	Contig 673	ACACAAAATTGATTTTCATGCAAG	AGCTCACGAAAGTATGCTTGCATG
AU51151	Contig 673	GGCGTGGATCACAGATGCTATATG	CGCGTCGCGTGATATACATATAGC
AU51152	Contig 1046	AAAATCATGTGGAAATCAATGATC	AACCATTATGCACATGGATCATTG
AU51153	Contig 1046	AACACGATTGAATCATTTCACTTG	GAGAAATCTAGCTGAGCAAGTGAA
AU51154	Contig 1046	TTACTAAAACATATATCAATATTC	AATCAGTCGGTCTCAGGAATATTG
AU51164	Contig 1558	ACCCAATGTACTGTAAAATTTGTG	TCACTCTTAGACTCTACACAAATT
AU51165	Contig 1558	TAGACGAGCTCGTAGTTGAGAGAG	CTCTCAAGAACTTAGTCTCTCTCA
AU51168	Contig 1997	TTTGCACCTTGGGGTTGAAGTAGC	CAGCCACAGTCCTGCCGCTACTTC

Ova and Ovb are a set of two primers for overgo probes.

and spotted on a piece of nylon membrane using a pipette and crosslinked to the membrane using UV radiation and a Stratalinker 2400 (Stratagene, La Jolla, CA, USA). Overgo hybridization was conducted as we previously described [37]. Briefly, overgo primers were selected following a BLAST search against GenBank to screen out repeated sequences and then purchased from Sigma Genosys (Woodlands, TX, USA). Two hundred nanograms of each overgo primer was labeled with 40 μl of a freshly prepared master mix composed of 14.0 mM Tris–HCl (pH 7.5), 5 mM $MgCl_2$, 0.02 mM dGTP, 0.02 mM dTTP, 20 μCi [α-^{33}P]dCTP, 20 μCi [α-^{33}P]dATP (3000 Ci/mmol; Amersham, Piscataway, NJ, USA), and 5 units of Klenow enzyme (Invitrogen). Labeling reactions were carried out at room temperature for 2 h. After removal of unincorporated nucleotides using a Sephadex G50 spin column, probes were denatured at 95°C for 10 min and added to the hybridization tubes containing high-density BAC filters. Hybridization was performed at 54°C for 18 h in hybridization solution (50 ml of 1% BSA, 1 mM EDTA at pH 8.0, 7% SDS, 0.5 mM sodium phosphate, pH 7.2). BAC filters were washed with 2× SSC at room temperature for 15 min and exposed to X-ray film at −80°C for 2 days.

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