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Construction, characterization and chromosomal mapping of bacterial artificial chromosome (BAC) library of Yunnan snub-nosed monkey (*Rhinopithecus bieti*)

Huai-liang Xu[1,3,4], Ya-ping Qian[2], Wen-hui Nie[1], Jian-xiang Chi[1,3], Feng-tang Yang[1] & Bing Su[1,2]*
[1]*Key Laboratory of Cellular and Molecular Evolution, Kunming Institute of Zoology, The Chinese Academy of Sciences, Kunming, China; E-mail: sub@mail.kiz.ac.cn;*
[2]*Center for Genome Information, Department of Environmental Health, University of Cincinnati, OH, USA; Tel: (+1)513-558-6678; Fax: (+1)513-558-4397; E-mail: bing.su@uc.edu;*
[3]*Graduate School of the Chinese Academy of Sciences, Beijing, China;* [4]*College of Animal Science and Technology, Sichuan Agricultural University, Yaan, China*
**Correspondence*

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Abstract

We constructed a high redundancy bacterial artificial chromosome library of a seriously endangered Old World Monkey, the Yunnan snub-nosed monkey (*Rhinopithecus bieti*) from China. This library contains a total of 136 320 BAC clones. The average insert size of BAC clones was estimated to be 148 kb. The percentage of small inserts (50–100 kb) is 2.74%, and only 2.67% non-recombinant clones were observed. Assuming a similar genome size with closely related primate species, the Yunnan snub-nosed monkey BAC library has at least six times the genome coverage. By end sequencing of randomly selected BAC clones, we generated 201 sequence tags for the library. A total of 139 end-sequenced BAC clones were mapped onto the chromosomes of Yunnan snub-nosed monkey by fluorescence *in-situ* hybridization, demonstrating a high degree of synteny conservation between humans and Yunnan snub-nosed monkeys. Blast search against human genome showed a good correlation between the number of hit clones and the size of the chromosomes, an indication of unbiased chromosomal distribution of the BAC library. This library and the mapped BAC clones will serve as a valuable resource in comparative genomics studies and large-scale genome sequencing of nonhuman primates. The DNA sequence data reported in this paper were deposited in GenBank and assigned the accession number CG891489-CG891703.

Introduction

Comparison between mammalian genomes provides insight into the common features of biologic mechanisms and helps to identify experimental models for studying complex processes and unique details of gene structure and function (Collins *et al.* 1998). It also has been considered as a powerful approach complementary to experimentation that helps to identify functional elements and evolutionary constraints within the genomes (Sidow 2002). With the completion of whole genome sequencing of human and a series of model species (Lander *et al.* 2001, Waterston

et al. 2002, Larkin *et al.* 2003), there has been an ever-increasing need for large insert DNA libraries of other species that are phylogenetically informative and biomedically significant. Currently, the bacterial artificial chromosome (BAC) library is the common choice of resource for research. It provides an easy access to stable material for DNA manipulation such as exon trapping, cDNA selection, direct sequencing, microsatellite marker isolation, fluorescence *in-situ* hybridization (FISH) and physical mapping (Beck 2001, Martins-Wess 2002, Li 1999).

In particular, sharing a close behavioral and genetic kinship with humans, nonhuman primates are the most suitable biomedical models for comparative genomic studies. However, most of the primate species are seriously endangered and may face extinction (Mittermeier *et al.* 1999). Hence, construction of nonhuman primate libraries would provide the most practical and cost-effective means to interrogate the genetic bases for primates traits relevant to evolution and human disease, and also resources which can be utilized persistently (Eichler and de Jong, 2002). To date, a number of nonhuman primates libraries have been constructed (Pieter de Jong's laboratory at Children's Hospital Oakland Research Institute, ⟨http://www.chori.org/bacpac⟩; Qian *et al.* 2003), and more BAC libraries are certainly necessary to cover the diverse evolutionary lineages in primates.

The Yunnan snub-nosed monkey (*Rhinopithecus bieti*) diverged from the human lineage about 25 million years ago (Goodman 1998, 1999), and is one of the rarest and most endangered primates in the world (Long *et al.* 1994). Morphological study suggests it occupies an intermediate position between the Old World monkeys and the lesser apes (Peng *et al.* 1985, 1988), and is, therefore, a keystone species in understanding primate evolution. Unfortunately, the low genetic diversity, small population size and fragmented distribution all act to increase the endangered status of this rare species with less than 2000 individuals in the wild (Long *et al.* 1994, Lan *et al.* 1995, Su & Shi 1995).

In the Old World monkeys, genome-wide comparative chromosome maps between human and three species of leaf monkeys have been established by cross-species chromosome painting with human chromosome-specific probes (Bigoni *et al.* 1997a, Nie *et al.* 1998). These comparative maps showed a high degree of conservation of chromosomal syntenies, and only a few interchromosomal rearrangements (involving human chromosomes 1, 2, 6, 14, 15, 16, 19, 21 and 22) differentiate the karyotypes of the leaf monkeys and humans. This observation suggests that human 14 and 15 homologues as well as 21 and 22 homologues have been involved in Robertsonian translocation during the evolution of Asian colobine monkeys (Bigoni *et al.* 1997a, 1997b, Nie *et al.* 1998, Wu *et al.* manuscript in preparation). The leaf monkeys are closely related to the snub-nosed monkeys with the same diploid chromosome number ($2n = 44$) like most of the Asian colobine monkeys (Bigoni *et al.* 2003). In this study, we constructed a high-redundancy BAC library of the Yunnan snub-nosed monkey using pBACe3.6 vector (Frengen *et al.* 1999). With insert size testing, BAC clone end sequencing, we showed that this library has an average insert size of 148 kb, and at least six times the genome coverage with unbiased chromosomal distribution. Using FISH, we mapped the chromosomal locations of 139 BAC clones. Our results demonstrated a high degree of synteny conservation between humans and Yunnan snub-nosed monkeys.

Materials and methods

A. BAC library construction

(1) Preparation of high-molecular-weight (HMW) DNA

The lymphoblast cell line (KCB-96009) of a male Yunnan snub-nosed monkey with a normal $2n = 44$ karyotype was obtained from Kunming Cell Bank and the cell concentration was adjusted to 5×10^8 cells/ml. An equal volume of prewarmed 1% InCert agarose (BioWhittaker Molecular Applications) was added. The agarose/cell mixture was briefly stirred and transferred into a disposable plug mold (Bio-Rad) placed on ice. The plugs were then incubated twice (3 h and overnight with change of the buffer) at 50°C in L buffer (0.1 mol/L EDTA pH 8.0, 0.01 mol/L Tris-Cl pH 7.6, 0.02 mol/L NaCl and 1% *N*-lauroylsarcosine) containing 0.1 mg/ml of

proteinase K. The plugs were washed five successive times at 4°C in TE buffer (pH 7.6, 10 mmol/L Tris-Cl, 1 mmol/L EDTA) and incubated for 30 min at 50°C in TE buffer containing 40 μg/ml PMSF (phenylmethylsulfonyl fluoride from Sigma, 20 mg/ml in isopropanol stored at –20°C), followed by three successive 30-min dialyses against TE (pH 7.6). The DNA plugs were stored at 4°C in 20% NDS (2 mmol/L Tris, 6.8 mmol/L *N*-lauroylsarcosine and 127 mmol/L EDTA pH 9.0).

(2) Preparation of insert DNA

Five agarose plugs were equilibrated twice in sterile 0.5 × TE (pH 8.0) for 1 h at 4°C and then in the EcoRI digestion buffer (2 mmol/L $MgCl_2$) for 30 min. The optimal amount of enzyme combination was determined by digesting the DNAs with 1 U EcoR I and varying amounts of EcoR I methylase. Partial digestion of each plug was performed with 10 μl of EcoRI restriction endonuclease (0.1 U/μl) and 1 μl of EcoRI methylase (25 U/μl) containing 420 μl EcoR I digestion buffer, 1.25 μl SAM (final concentration: 80 μmol/L) and 5 μl 100 × BSA (final concentration: 100 ng/ml) at 37°C overnight. Reactions were stopped by adding 20% NDS. The partial digested DNAs were size-selected twice by pulsed-field gel electrophoresis (PFGE) on 1% agarose gel (Fisher Biotech) using the CHEF-DRIII system (Bio-Rad). The agarose gel containing 100–250 kb DNAs was cut out and sliced into two equal parts.A second PFGE was then performed to remove small DNA fragments trapped within the gel slices using the same conditions.

(3) Recovery of high-molecular-weight EcoRI DNA fragments

The DNA size-selected agarose slices were recovered by electroelution. After electroelution, the DNA solution was dialyzed against 0.5 × TE (pH 8.0) at 4°C overnight. DNA concentration was estimated by gel electrophoresis with λ DNA of known concentration.

(4) Ligation and transformation

Using a 1:10 molar ratio of insert over vector molecules, the electroeluted HMW DNA fragments were incubated with the pBACe3.6 vectors in a 50-μl total volume containing 0.5 unit of T4 DNA ligase (Invitrogen) at 15°C overnight. The ligation mixture was dialyzed using microdialysis filters (0.025-μm, Millipore) in sterile 0.5 × TE (pH 8.0) buffer for 1.5 h. Two microliters of ligation product was used to transform 20 μl of ElectroMAX DH10B competent *E. coli* cells (Invitrogen). Electroporation was carried out using a Cell-Porator *E. coli* Electroporation System (Gibco BRL). After eletroporation, the cells were incubated in 1 ml S.O.C. medium at 37°C for 1 h. The cells were then inoculated on LB medium containing 12.5 μg/ml chloramphenicol and incubated at 37°C overnight.

B. BAC library characterization

(1) Size estimation of BAC clones

A total of 225 BAC clones were randomly picked from the Yunnan snub-nosed monkey library. These clones were inoculated in 2 ml LB medium containing 12.5 μg/ml chloramphenicol at 37°C for 16 h. The insert DNAs were extracted using a modified alkaline-lysis method (Osoegawa *et al.* 2002). The insert DNAs were digested with NotI (New England Biolabs) and then subjected to PFGE. The molecular weights of the BAC inserts were calculated using the image analysis program TotalLab 1D gel analysis (http://www.totallab.com/home.asp) with the Low-Range size marker (New England BioLabs).

(2) BAC end sequencing

A total of 206 BAC clones were randomly picked from the library. DNA extractions were performed using plasmid BAC mini-prep kit (Oligo-Chem). A 20-mer T7 primer: 5′ TAATACGACTCACTATAGGG 3′ was used for 5′ end sequencing. Fifteen clones were also sequenced at the 3′ ends using the SP6 primer: 5′ ATTTAGGTGACACTATAGAAGGATC 3′. The ABI 3100 Genetic Analyzer (Applied Biosystem) was used.

C. Chromosomal mapping using FISH

The metaphase preparation and G-banding followed the procedures described in Nie *et al.* (1998).

A total of 139 BAC clones of the end-sequenced clones were mapped using FISH. Mini-preps of BAC DNAs were conducted using the standard alkaline lysis method (Sambrook *et al.* 2001). The DNA samples were subject to further purification by phenol/chloroform extraction and isopropanol precipitation. A 1–2 μg aliquot of DNA was used to prepare probes labeled with biotin-14-dCTP by nick-translation in 25 μl mixtures containing 2.5 μl 10 × nick-translation buffer, 1.9 μl ATG (0.5 mmol/L dATP, dTTP, GTP), 1 μl DNase I (1 mU), 1.8 μl biotin-14-d CTP (0.4 mmol/L), 2 μl DNA polymerase/DNase I (0.5 U DNA polymerase I – 0.4 mU DNase I/μl) and 0.5 μl DNA polymerase I (10 U/μl) (Invitrogen). The mixtures were incubated for 1–1.5 h at 16°C and stopped by adding 1/10 volume of 0.5 mol/L EDTA (pH 8.0) and incubated for 10 min at 65°C. About 2 μl (about 100 ng) of labeled BAC DNA and 1 μg of human Cot-1 DNA were mixed with 12 μl hybridization buffer (50% deionized formamide, 10% dextran sulfate, 2 × SSC, 0.5 mol/L phosphate buffer, pH 7.3 and 1 × Denhardt's solution). The probes were denatured for 10 min at 70°C, directly transferred to 37°C, and allowed to pre-anneal for at least 30 min. The metaphase slides were treated with 0.01% pepsin in 10 mmol/L HCl for 7 min at room temperature and stopped in 2 × SSC solution, and then subject to dehydration in an ethanol series (70%, 90% and 100%) for 2 min. The slides were incubated for 1–3 h at 65°C before hybridization. The slides were denatured in 70% formamide-2 × SSC for 1–1.5 min at 65°C, quenched in ice-cold 70% ethanol, and dehydrated in an ethanol series. After air-drying, slides were hybridized to probes at 37°C for 15–18 h. Following hybridization, the coverslips were removed and the slides were washed in 50% formamide-2 × SSC twice and 2 × SSC twice for 5 min at 43°C. The hybridization was checked with Cy3-avidin (1:1000 dilution), and then were stained in 2 × SSC containing 0.6 μg/μl DAPI (4′,6-diamidino-2-phenylindole). The fluorescent images were captured using the Genus system (Applied Imaging) with a Cohu CCD camera mounted on a Zeiss microscope (Axioplan 2). The chromosomes of the Yunnan snub-nosed monkey were identified based on the DAPI-bandings which are similar to G-bandings following the nomenclature described by Wu *et al.* (manuscript in preparation).

Results and discussion

Library description

To generate enough BAC clones for sufficient genome coverage, genomic DNAs were prepared and partially digested with EcoRI, size fractionized, and then ligated with pBACe3.6 vectors (Frengen 1999). A total of 136 320 BAC clones were produced and arrayed into 355 384-well microtiter plates.

Insert size testing

To estimate the average insert size of the library, a total of 225 clones were randomly selected and tested through pulse field gel electrophoresis (PFGE) (Figure 1 and Figure 2). By subtracting the vector size (8.7 kb), the insert size was calculated using the *TotalLab 1D gel* analysis program. Our result showed that the average insert size is 148 kb. Six non-insert clones were observed in the 225 clones tested (2.67%).The insert size distribution of the BAC clones is shown in Figure 2. Most of the clones in the library fall in the range of 120 kb to 170 kb (80.4%). The ratio of small inserts (<100 kb) is only 2.74% (6/219), indicating a high ratio of large insert clones in this library. According to the published data on haploid C values (CV) of the primate genomes (http://www.genomesize.com/mammals.htm), assuming a similar genome size with the average size in subfamily Colobinae, the Yunnan snub-nosed monkey BAC library has at least six times coverage of its genome.

BAC clone end-sequencing and blast search analysis

In order to generate sequence tags and evaluate the chromosomal distribution of the BAC clones, we sequenced a total of 206 randomly picked BAC clones at the 5′ ends using the T7 primer.There were five clones showing no inserts (2.43%), consistent with the test using PFGE. The BAC end sequences were subjected to blast search against the human genome and dbEST databases. A summary of the blast search result is shown in Table 1. The blast search result

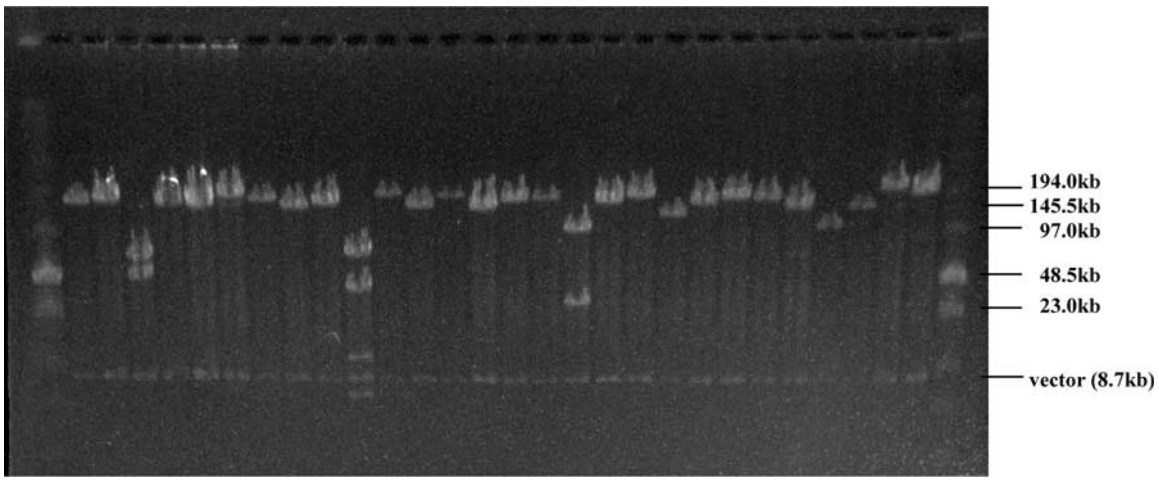

Figure 1. The electromorph of PFGE for insert size testing. There are 28 BAC clones tested. The low-range molecular weight marker was used as size standard in the two flanking lanes. The vector band is 8.7 kb.

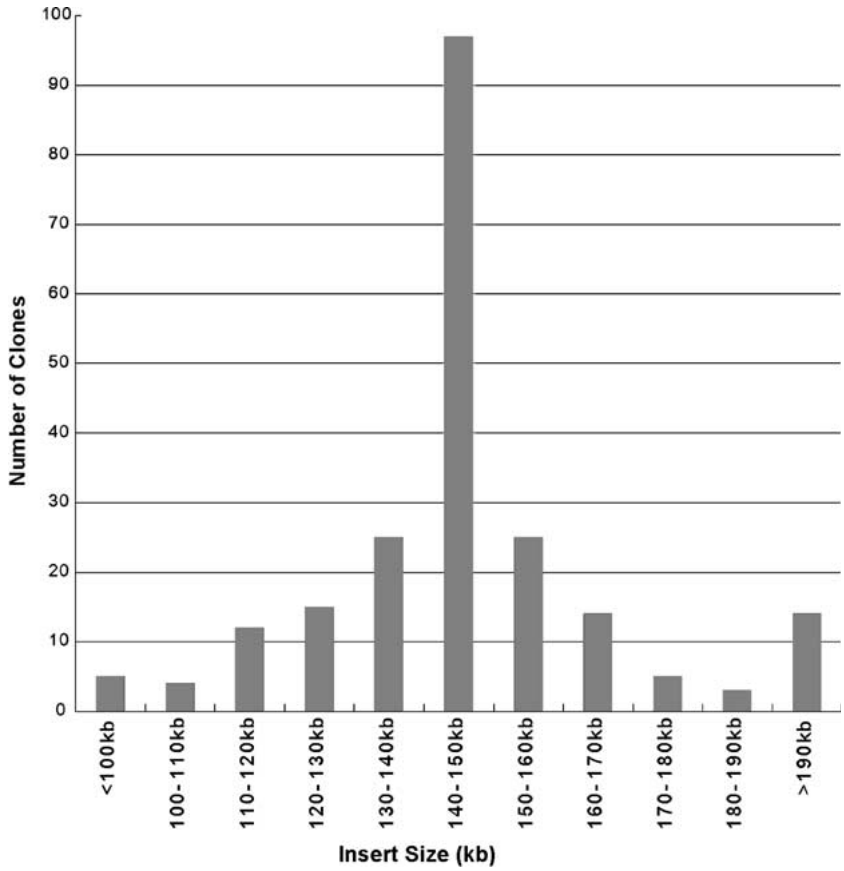

Figure 2. The insert size distribution of the Yunnan snub-nosed monkey BAC library. Insert sizes were determined for 225 BAC clones. The horizontal axis refers to the size ranges in kb while the vertical axis indicates the number of clones.

Table 1. Summary of the blast search result of the 201 BAC end sequences.

	Repet	Uniq	Unknown	EST
No. of hits	95	102	4	24
Percentage (%)	47.3	50.7	2.0	11.9

Note: Repet, repetitive sequences, Uniq, unique sequences, Unknown, no sequence matches in the human genome, EST, sequences with hits on human ESTs (score $\geqslant$ 300). The average trimmed read length of the 201 BAC-end sequences is 484 bp, and the average sequence similarity between human and Yunnan snub-nosed monkey is 92.2%.

showed that there are 102 (50.7%) unique sequences and 95 (47.3%) repetitive sequences, and 4 (2%) sequences without obvious homologies in the human genome. This observation is lower than the ratio of repetitive sequences (58%) observed in the human genome (Zhao *et al.* 2000), which is probably due to lack of EcoRI recognition sites in some of the repetitive regions in the Yunnan snub-nosed monkey. Based on the analysis of the 102 unique sequences, the sequence similarity between humans and Yunnan snub-nosed monkeys was estimated as 92.2% on average (Table 1).The blast search result also indicated a fairly good correlation between the number of hit clones and the size of the human chromosomes (Figure 3), hence, an implication of unbiased chromosomal distribution of the BAC clones of the library. There are 24 (11.9%) end sequences showing hits in the dbEST database (score $\geq$ 300, according to Zhao *et al.* 2000). In addition, we also sequenced 15 of the 201 clones at the 3′ ends using the SP6 primer, the blast results indicated the same chromosome hits as the 5′ end sequences. The average read length of the BAC ends after trimming the vector sequences is 484 bp and the total length of all the BAC end reads is 104.8 kb. The 201 sequence tags were deposited in GenBank under accession Nos CG891489-891703.

FISH analysis

Mini-prepared DNAs from the BAC clones were used in the FISH experiments to evaluate the chimerism of the library and to map the chromosomal locations of the clones. A total 139

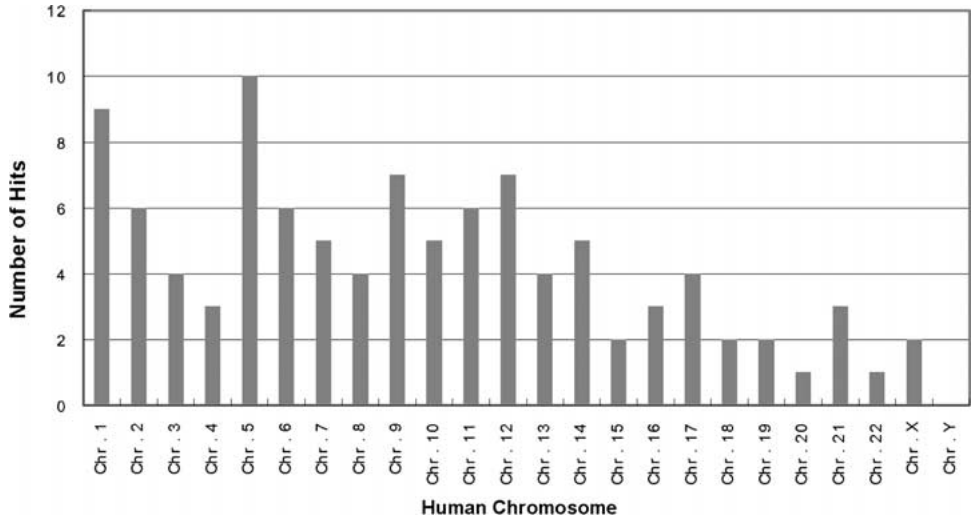

Figure 3. Human chromosomal distribution of the 100 unique BAC end sequences of the Yunnan snub-nosed monkey. The data was generated by blast search against the human genome database.

end-sequenced BAC clones were mapped using FISH onto metaphase chromosomes of the Yunnan snub-nosed monkey. The FISH examples were shown in Figure 5 and the chromosomal assignment of all BAC clones were summarized on a G-banded karyotype of the Yunnan snub-nosed monkey (Figure 6 and Table 2). Among the 139 BAC clones tested, 10 clones had hybridization signals at the centromeric regions of all the chromosomes except for the Y chromosome (Figure 5a), an implication of repetitive sequences in those BAC clones. All the other 129 BAC clones had unique chromosomal locations and no chimeric hybridization signals were observed. We were able to confidently determine the approximate chromosomal locations of 100 BAC clones on well-spread chromosomes (Table 2 and Figure 6). The chromosomal distribution of the mapped clones is illustrated in Figure 4, demonstrating a good correlation between the number of clones and the size of the chromosomes of the Yunnan snub-nosed monkey, which is consistent with the blast search result against human genome (Figure 3). Based on the chromosomal homology between human and Yunnan snub-nosed monkey established by chromosome painting (Wu *et al.* 2003, manuscript in preparation), most of the BAC clones hitting individual human chromosomes were hybridized onto the corresponding monkey chromosomes, indicating conserved chromosomal syntenies between human and Yunnan snub-nosed monkey. There are five clones (243A5, 183E2, 243A3, 330D1 and 330N3) hitting sequences on multiple human chromosomes. This is probably caused by segment duplications that frequently occurred in the human genome (Johnson *et al.* 2001). Interestingly, the clones hitting human chromosome 14/15 were mapped onto chromosome 5 of the Yunnan snub-nosed monkey, and the clones hitting human chromosome 21/22 were mapped onto chromosome 15 of the Yunnan snub-nosed monkey. This observation is consistent with the proposed HAS 14/15, 21/22 Robertsonian translocation by chromosome painting studies (Wienberg *et al.* 1990, 1992, Bigoni *et al.* 1997a, 1997b, Nie *et al.* 1998, Wu *et al.* 2003, manuscript in preparation). Our result also suggested that human chromosome 1 has four syntenic regions in the Yunnan snub-nosed monkey genome, which was confirmed by the chromosome

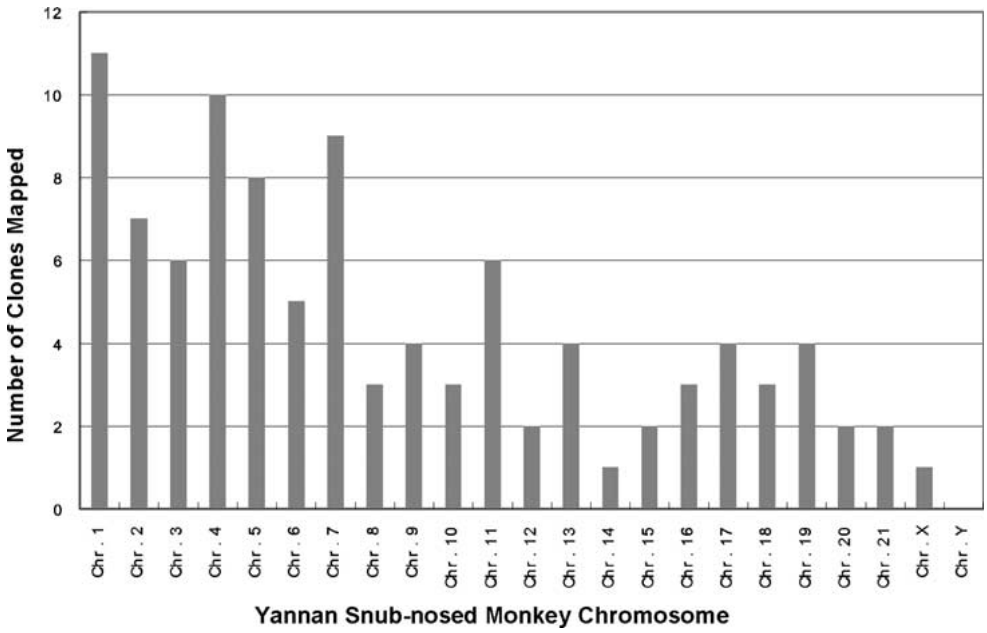

Figure 4. Chromosomal distribution of the 100 BAC clones mapped onto the chromosomes of Yunnan snub-nosed monkey using FISH.

painting study (Wu *et al.* 2003, manuscript in preparation). Seventeen clones with a single hit on both human and monkey chromosomes were found to be located at different arms of the homologous chromosomes (Table 2), which implies that these chromosomes have probably undergone intra-chromosomal rearrangements during evolution.

In summary, we have constructed a Yunnan snub-nosed monkey BAC library containing 136 320 clones. Based on the estimated insert size, the number of clones and the putative genome size, this library should represent at least 6-fold coverage of the *R. bieti* genome. We have demonstrated that this library has no obvious chromosome-coverage bias. As a preliminary effort, we have generated 201 BAC end sequences and 139 of them were mapped onto the chromosomes of the Yunnan snub-nosed monkey. Therefore, the Yunnan snub-nosed monkey BAC library would serve as a valuable resource for cloning, mapping and comparative genomics studies.

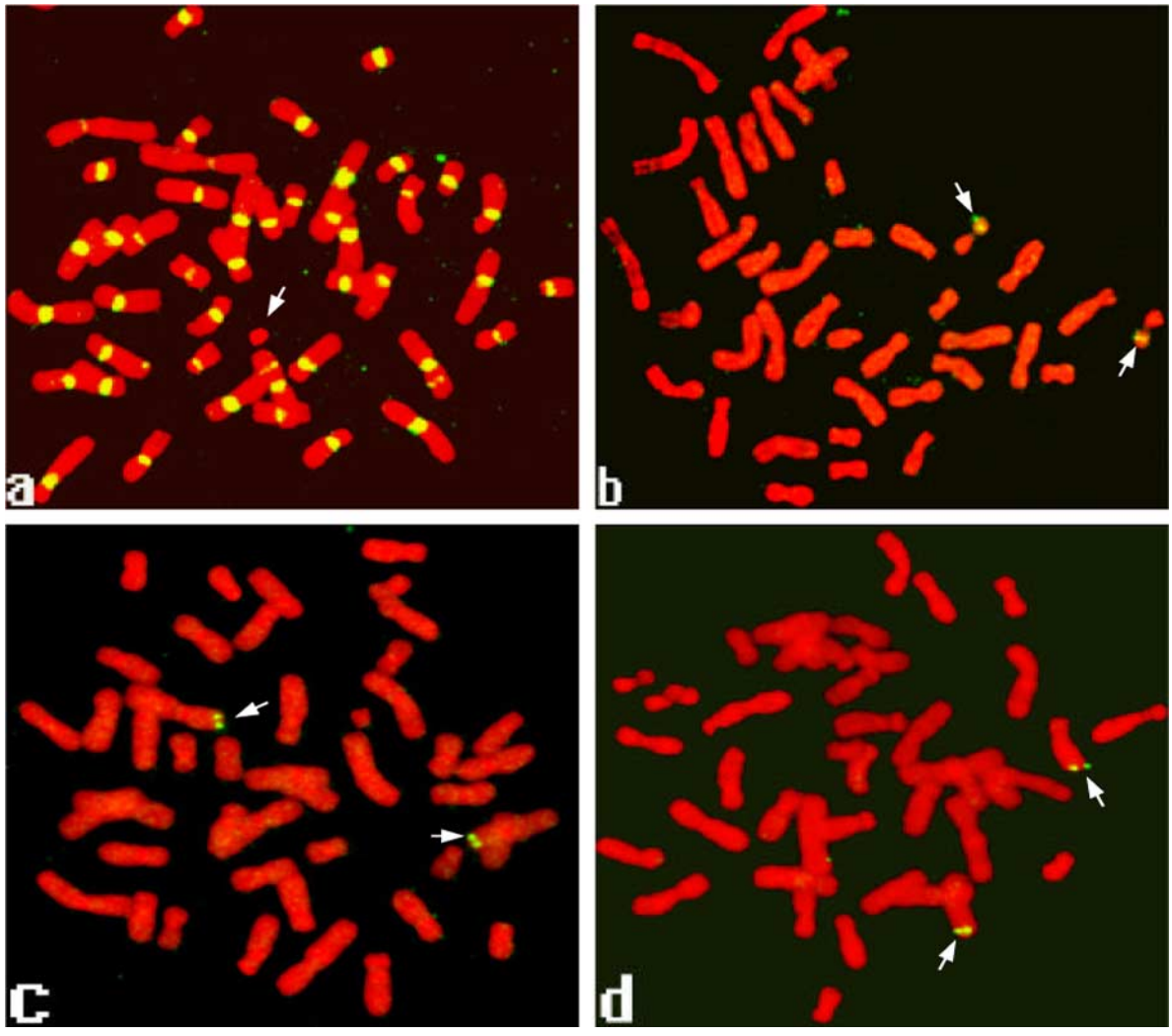

Figure 5. The examples of FISH mapping results of four BAC clones. (a) BAC clone_243M5 with signals on the centromeric regions of all chromosomes except for the Y chromosome (arrowed). (b) BAC clone_243J2 hybridized onto the middle region of the long arm of chromosome 15. (c) BAC clone_243N6 hybridized onto the distal regions of the short arm of chromosome 2 (d) BAC clone_243J6 hybridized onto the distal regions of the long arms of chromosome 7. No chimerism was observed for all the BAC clones tested.

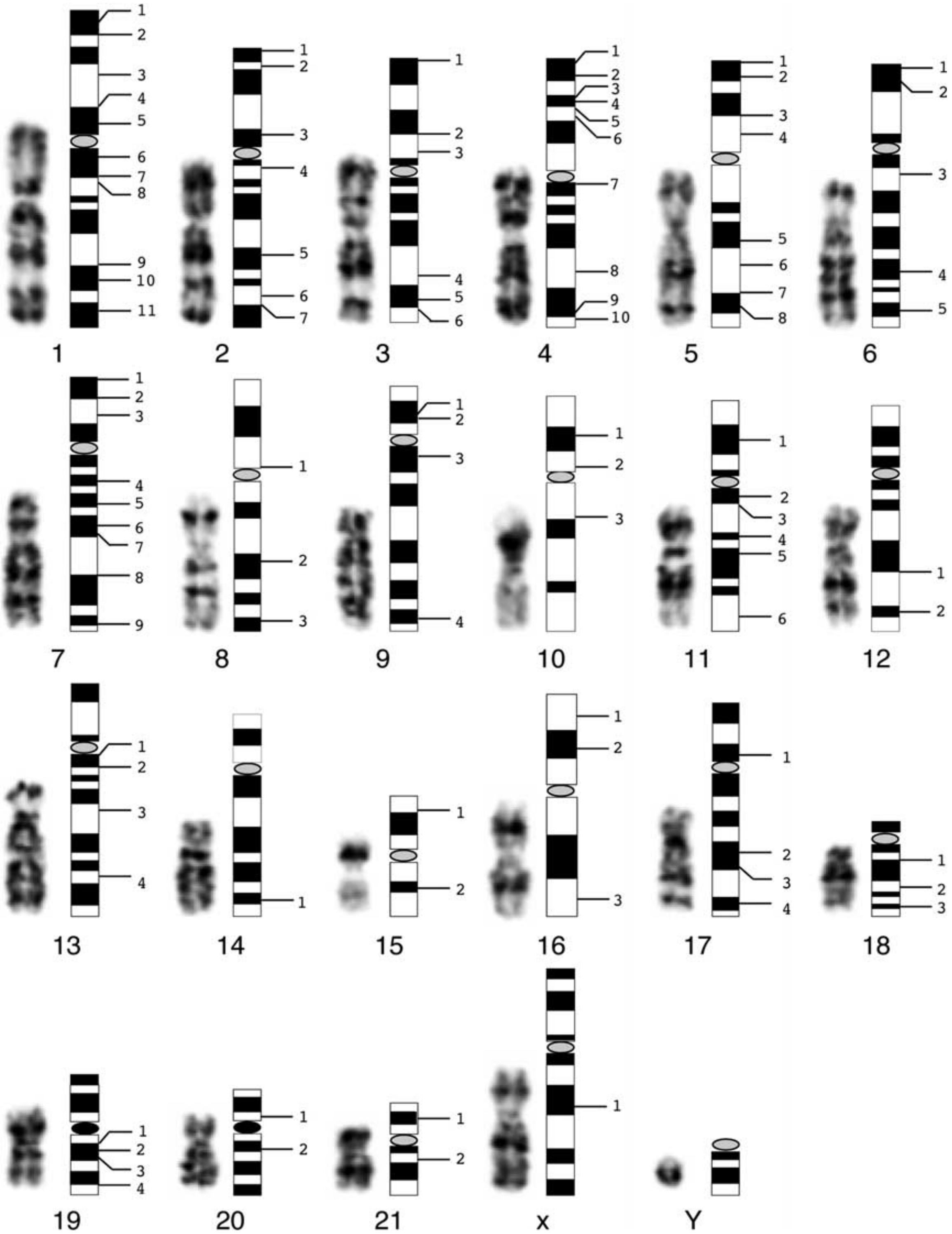

Figure 6. The G-banding karyotype of a male Yunnan snub-nosed monkey and the idiogram with the mapped BAC clones. The clone ID of each mapped BAC can be found in Table 2.

Table 2. The blast search and FISH mapping results of the 100 BAC clones.

Clone ID.	Human chr.	Monkey chr.
243L1	3q22–23	1p (3)
330E1	3q26–27	1p (4)
330P3	3p24.3	1p (1)
243B1	3q21–22.3	1p (2)
243D4	3q26.32	1p (5)
330N2	3p26.1–25	1q (8)
183B4	3p26.3–25	1q (7)
183B5	3p26–25	1q (6)
243A6	3q13.3–21.1	1q (9)
243O5	3q13.1–13.3	1q (10)
243P2	3q12–13.11	1q (11)
183A2	5p15.32	2p (1)
183C5	5q12–13	2p (3)
243N6	5p15.3–15.2	2p (2)
330A6	5q31.2–33	2q (7)
330B1	5q23.3	2q (6)
330G6	5q34	2q (5)
330J6	5p14–13	2q (4)
183K3	6q25.2	3p (1)
243B6	6q25–25.1	3p (3)
243F5	6q16.1–16.3	3p (2)
243I5	6p25.3–24.1	3p (6)
183D3	6p23–22.3	3q (5)
243H4	6p22–21.1	3q (4)
183E4	4p13–12	4p (5)
183J5	4p15.31–15.2	4p (2)
243A5[a]	11,15,1,13	4p (6)
243C5	4p14	4p (3)
243L4	4p16–15.33	4p (1)
243E3	4p14–13	4p (4)
183M1	4q12–13.1	4q (7)
243A4	4q28.3	4q (8)
330B2	4q32	4q (9)
243O4	4q13.3	4q (10)
183I5	15q22–22.32	5p (4)
243N2	15q11–11.2	5p (1)
330K1	15q11–12	5p (2)
243K3	15q26.3	5p (3)
183M4	14q23.3–24.1	5q (8)
243C1	14q22.1	5q (5)
243P6	14q31–31.1	5q (7)
330B5	14q24.3	5q (6)
183E2[b]	10,13,9,7	6p (2)
330A3	7p14.1	6p (1)
183P2	7p13–12.2	6q (3)
243D1	7q22.3–31.1	6q (4)
330H1	7q35-qter	6q (5)
183O1	8p23–22	7p (1)
243L3	8p 21.3–21	7p (2)
243M6	8p21.3–21	7p (3)
243J6	8q24.13–24.21	7q (9)
243M1	8q21.11	7q (6)
330A1	8q21.1–21.11	7q (4)
330G3	8q22.3	7q (8)
330J3	8q22.1–23	7q (7)
330P6	8q21.1–21.11	7q (5)
330E2	1q21.2–22	8p (1)
243H5	1q31–31.1	8q (2)
330L4	1q42.3	8q (3)
243A3[c]	3,2,7,9,17	9p (1)
243J4	2q21–21.2	9p (2)
243G2	2q36–37	9q (4)
330K3	2p12–11.2	9q (3)
330N3[d]	1,9,7,3,X	10p (1)
330D1[e]	19,12,1,10	10p (2)
243K1	1q41	10q (3)
243J5	12p 13.1	11p (1)
183B3	12q15–21	11q (4)
243B4	12q14–21.1	11q (5)
330J1	12q15	11q (3)
330K2	12q13–14	11q (2)
330P2	12q22–24.31	11q (6)
330E6	10q26–26.13	12q (2)
330I6	10q23.31	12q (1)
243C4	9q21.12–21.32	13q (4)
243D5	9q31.1–32	13q (2)
243M4	9q22.1–22.33	13q (1)
330O3	9p22.2–22.1	13q (3)
243E1	2q35	14q (1)
243K4	21q21.3–22.1	15p (1)
243J2	22q11.21–11.23	15q (2)
243E4	11p11.2	16p (1)
330M6	11p14.1	16p (2)
330M5	11q23.3	16q (3)
243K2	17p12–11.2	17p (1)
183F2	17q12–21.1	17q (2)
243L2	17q23–24.3	17q (4)
330D6	17q23.3–24.1	17q (3)
183N3	20q11.1–11.21	18q (2)
243J1	20ptel-c15bt7	18q (1)
330D3	20q13.13	18q (3)
243C2	13q22–22.2	19q (3)
243F1	13q32.3	19q (4)
243F2	13q21.1	19q (2)
243L6	13q21.31	19q (1)
243G3	16p12.1–11.2	20p (1)
243H2	16q23.1	20q (2)
243E5	18q22.3–23	21p (1)
243P1	18q12.1–12.2	21q (2)
183M3	Xq22–23	Xq (1)

Note: The numbers in parenthesis indicate the serial numbers of the approximate chromosomal locations in Figure 6. The chromosomal locations of the five BAC clones with multiple hits on human chromosomes are: [a]243A5: 11p13, 15q23–24, 1q41–42, 13q12; [b]183E2: 10p12, 13q12, 9q32–34.11, 7q11–22; [c]243A3: 3q21.1, 2q37, 7q36, 9p23–22.1, 17q25; [d]330N3: 1p31, 9q22, 7p13–12, 3p14, Xp22.2–22.11; [e]330D1: 19q12, 12q13, 1q23.1–24.1, 1q24.

The filters and BAC clones of the Yunnan snub-nosed monkey BAC library is available upon request. It is distributed by the Key Laboratory of Cellular and Molecular Evolution, Kunming Institute of Zoology, CAS.

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