行政院國家科學委員會專題研究計畫 成果報告

衛星 DNA 之著絲點功能及核型演化的研究
研究成果報告(精簡版)

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報 告 附 件 ：出席國際會議研究心得報告及發表論文

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Introduction:

The human genome project is moving toward to new a phase focusing on comparative and functional genomics. Thus attempts and efforts will be made to understand the function of our genes, their regulation and expression and their complex interactions, primarily as they pertain to evolutional biology, human health and medicine. It is important to resolve the evolutionary aspect of the human genome, as this has affected both genome organization and chromosomal architecture. Comparative G-banding studies (Shi et al. 1980; Yunis et al. 1980), somatic cell hybrid mapping (Lalley et al. 1978; O'Brien et al. 1997), and Zoo-FISH mapping (Wienberg and Stanyon 1995; Yang et al. 1995; Fronicke and Scherthan 1997; Muller et al. 2000) had been used to infer homologies of whole chromosomes or subregions between species and even across mammalian orders. The genomic organizations of eight phylogenetically distinct species from five mammalian orders had been compared. These comparative gene-mapping data collected to date indicated that most of mammalian chromosomes presented as a highly conserved synteny, whereas some other chromosomes appeared to have more extensive rearrangement in each mammalian lineage. In multispecies studies, the sequence alignment analysis of an evolutionary breakpoint (an interval region between two homologous synteny blocks) showed that nearly 20% chromosome breakpoint regions were reused during karyotypic evolution; these reuse sites were also enriched for centromeric sequences that were associated with the formation of acrocentric centromeres in other species (Murphy et al. 2005). Furthermore, sequence analyses at the junctions of human syntenic segments exhibited a preponderance of highly repetitive elements the junctions (Puttagunta et al. 2000). The highly repetitive elements at the evolutionary breakpoints might be the remnant of ancient acrocentric centromeres, by product of tandem fusion in the karyotypic evolution process. Our previous FISH studies using the telomeric and centromeric satellite DNA as probes showed the interstitial telomere and centromeric satellite DNA signals on the Indian muntjac chromosomes. These studies suggested that the chromosome fusion events occurred in the karyotypic evolution of Indian muntjac (Lin et al. 1991; Lee at al. 1993; Scherthan 1995; Li et al. 2000). In human, several *de novo* "neocentromeres" found in abnormal chromosomes (Amor and Choo 2002). At one point it was thought that the neocentromeres might have derived from a cryptic interstitial centromere at the evolutionary breakpoint/fusion site. This observation addressed a possible mechanism for chromosome evolution and the demonstration of reuse breakpoints, whereby these evolutionary breakages preferentially occur at sites of ancestral centromeres or cryptic interstitial centromeric stes in independent lineages. Alternatively, reuse breakpoints may represent unstable chromosomal sites that, after breakage, will tend to form a new centromere or telomere. Taken all data together, it affirms that the centromeres are dynamic, rapidly evolving structures that can be repositioned themselves among closely related species and have evolutionary importance in karyotype evolution and speciation.

Almost as a rule, the repetitive satellite DNAs is the main composition of centromere and centromeric DNA sequences diverse among species. Generally, most similar satellite DNAs are restricted to closely related species. In other words, the related (or congeneric) species share a "library" of similar related satellite sequences, some of which could be amplified onto a major satellite DNA in some particular species (Salser et al. 1976; Meštrović et al. 1998). The evolutionary dynamics of satellite DNAs could be a driving force for the speciation process, thus forming a species-specific profile of satellite DNAs (Ugarković and Plohl 2002). The species-specific profile of centromeric satellite DNA is possible to be demonstrated by means of comparative genomic studies that showed a large fraction of centromeric sequences were repositioned either by independent chromosomal rearrangements or by *de novo* centromere emergence in the independent lineage to become unique to a species (Murphy et al. 2005). After a sufficient evolutionary time, the DNA composition of the repositioned centromere might be continuously originated and amplified to form a new highly repetitive DNA. Nijman and Lenstra (2001) also proposed that in the course of satellite DNA evolution, newer or younger satellite

sequences could have derived from pre-existing DNA sequences to replace or coexist with the old satellites via the three-phases of evolution processes.   In summary, satellite DNA has a specific mode of evolution, species profile, and distribution in the genome; therefore, by itself it was recognized as a valuable marker for phylogenetic relationship and karyotypic evolution studies (Laursen et al 1992; Garrido-Ramos et al. 1999, Li et al. 2000 and 2005).   In order to further appreciate the "library" hypothesis, it will be essential to collect and to identify more novel satellite DNAs in the related species.   The mapping and sequencing of satellite DNAs from phylogenetically closely or divergent species will provide a better understanding on the relationship of extant species, and thus frame evolutionary hypotheses.   Additionally, it can elucidate an important structural and functional role of breakpoint regions in a broader range of mammalian taxa. With the use of comparing the sequence among breakpoint regions and centromeric satellite DNAs, it will shed more light on the mechanism for centromeric satellite DNA evolution.   Furthermore, it could also unravel the puzzle of a functional conserved centromere in the face of rapidly evolving centromeric DNA sequence.

Comparative studies of chromosome content and organization are providing insight into fundamental questions of genome evolution and speciation.   BAC library has tremendous potential to facilitate such studies to shed light on the temporal relationships of extant species by comparative sequence analysis and cytogenetic mapping of genome rearrangement, expansion, and loss (Gomyo et al. 1999; Srinivasan et al. 2002).   In addition to decipher genome evolution, BAC resources could be used to address specific questions pertaining to centromeric and pericentromeric evolution, and the mechanisms of karyotypic change between closely related species.   The Indian muntjac (*Muntiacus muntjac vaginalis*) has the lowest chromosome number in mammalian species with 6 chromosomes in female and 7 in male.   Recent molecular cytogenetic studies landed support to the hypothesis that the karyotype of the Indian muntjac could evolve from an ancient deer species with a karyotype 2n=70 through extensive tandem fusions and several centric fusions (Li et al. 2000). Moreover, the centromere of X+3 chromosome of the species is compound and unique.   Therefore, it is an ideal species for studying chromosome rearrangement, comparative genomic and karyotype evolution.   Thanks to the constructed BAC library of Indian muntjac in our lab; therefore, it is availability to explore the following studies: the centromeric evolution and function, the underlying mechanism of tandem fusion of Indian muntjac chromosomes, the chromosomal rearrangement, the karyotype evolution.

Specific Aim:
To study the karyotypic evolution among muntjac species and Human
Recently, comparative genome analyses among over 40 diverse mammalian species had been done; and showed that during karyotypic evolution, a highly degree of the chromosomal synteny conservation occurred and the reused chromosome breakpoint regions were enriched for centromeric sequences (Murphy et al. 2001 and 2005).   A preponderance (60%-70%) of highly repetitive elements at the evolutionary breakpoints were observed in human and mouse genome (Puttagunta et al. 2000).   Moreover, our previous studies in localization of telomeric and centromeric sequences at the fusion points of Indian muntjac's chromosomes by molecular cytogenetic mapping (Lee et al. 1993; Li et al. 2000 and 2005).   Most likely, these fusion sites are evolutionary breakpoints as defined by Murphy et al. (2005).   These highly repetitive elements at breakpoints/ fusion sites might drive chromosomal rearrangements.   However, it is hard to discriminate more recent rearrangements from older events based on the breakpoints/ fusion sites. Therefore, an evolutionary age of breakpoints/fusions should be taken into considerations by more precise alignment of ordered gene maps from multiple mammalian genomes and by interpretation of these maps with regard to phylogeny (Murphy et al. 2001).   Comparative molecular analyses are also helpful to determine the mechanisms involved in the formation of these fusions.   On the other hand, multispecies sequences alignments had revealed that the more frequent

cancer-associated chromosome aberration sites fell within or near evolutionary breakpoint regions three times as often as did the less frequent cancer-associated aberration sites (Murphy et al. 2005). Chromosomal rearrangements in evolution are probable correlated with common chromosomal translocations that cause neoplastic transformation. Karyotypic differences between distantly related species also indicate the dynamic nature of the genome, evolving through rearrangement, invasion, duplication, and loss (Wienberg and Stanyon 1997; Koonin 2000; Eichler 2001; Murphy 2001; Ostertag and Kazazian 2001; Pevzner and Tesler 2003). Therefore, study of karyotype evolution is good approach not only to shed light on the mechanism of chromosome rearrangements but also to aid understanding of the phylogenetic relationship within a given taxonomic group. Additionally, precisely defining the conservation of chromosomal segments among many diverse species might make up the karyotype of the ancestral mammalian karyotype. As a result, the evolutionary age of breakpoints/fusion sites would be unraveled and further shed light on the mechanisms and consequences of karyotypic change between closely related species. Moreover, it is possible to find the breakpoints/fusion site-related cancer-associated aberration sites for understanding the mechanism of chromosomal rearrangements in cancer cells. The first step is to define the conservation of chromosomal segments using Zoo-FISH, also known as cross-species FISH (Chowdhary et al. 1998).

Method:
Mapping of BAC clones by FISH:
The BAC clones will be labeled with either biotin or DIG. The labeled BAC DNA will be hybridized to metaphase chromosomes of Indian muntjac cells, Formosan sambar deer, and human. The biotin signals are observed by means of incubating with Cy3-conjugated avidin, biotinylated anti-avidin and Cy3-conjugated avidin, sequentially. The digoxigenin signals are detected using mouse anti-DIG antibody, cy5-conjugated rabbit anti-mouse antibody and cy5-conjugated goat anti-rabbit antibody, sequentially. Subsequently, samples are mounted in antifade mounting medium with DAPI (vector). The FISH images are captured by a CCD camera (Photometrics, Sensys) equipped on a fluorescence microscope (Olympus, BX51).

Results and discussions:
I. Construction of an Indian muntjac BAC library and production of the most highly density FISH map of the species
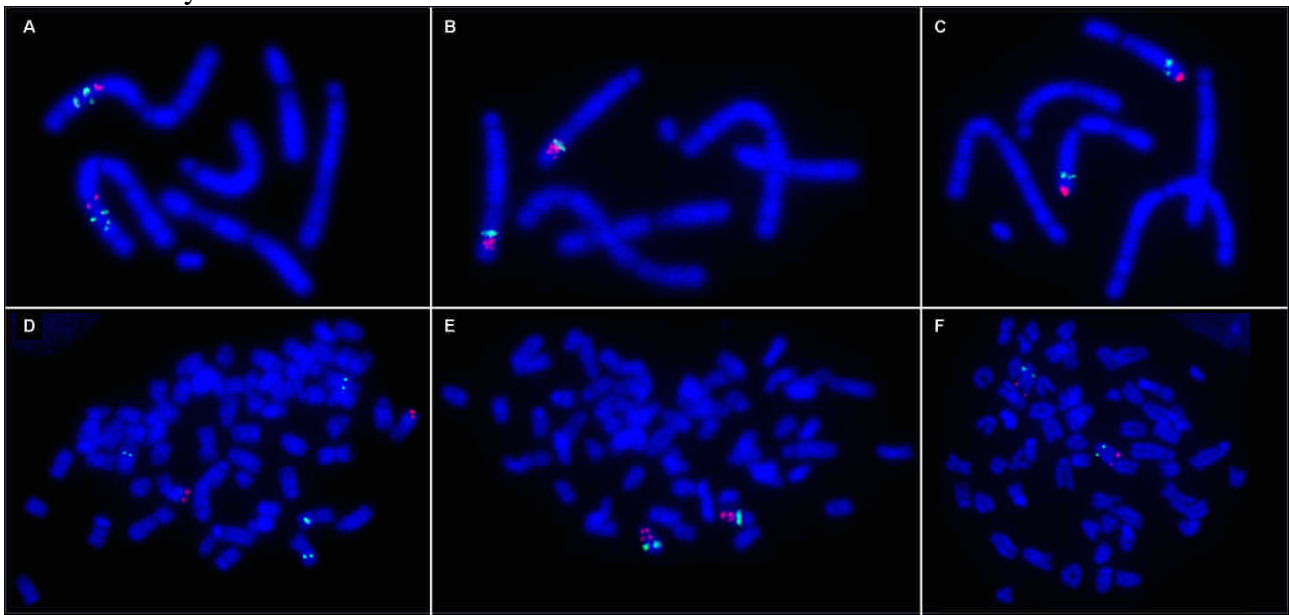   Following the completion of genome sequences of some mammalian species, comparative genome studies in mammals have been actively conducted for assessing gene change or for identifying the synteny conservation during evolution. The Indian muntjac (*Muntiacus muntjac vaginalis*) (2n=6 in female / 7 in male) could have evolved from an ancient deer species with a karyotype 2n=70 through extensive chromosome rearrangements creating the lowest chromosome number of a mammalian species. Therefore, the species has become a good resource for studying the synteny conservation among deer species. An Indian muntjac BAC library that contains a total of 126,336 individual BAC clones with an average insert size of 80 kilobases was obtained in this study. The frequency of clones with inserts was 88% and thus this library corresponds to approximately 4X coverage of Indian muntjac genome. The individual chromosomal location of 1619 BAC clones on the Indian muntjac metaphase chromosomes were identified by fluorescence in situ hybridization (FISH). Among these clones, 1517 BAC clones were mapped onto specific loci and 102 BAC clones were mapped onto the centromeric region. This provided the most high density FISH BAC clone map for the species. This dense ordered map could be used as a blueprint for comparative FISH mapping studies of other deer species in order to understand the mechanism of genomic rearrangement and karyotypic evolution. Moreover, the centromeric BAC clones will provide an excellent resource for studying the structure and function of mammalian centromeres.
The result has been accepted by Zoological Studies

II. Defining the karyotype evolution of Formosan sambar deer by comparative FISH mapping

About 100 clones were selected and mapped onto the Formosan Sambar deer by comparative FISH mapping, the results showed that large synteny segments were conserved between Indian muntjac and Formosan sambar deer (figure 2-1).   Comparison of the orders of mapped BAC clones on Formosan Sambar deer and Indian muntjac enabled us to determine the orientation of the more ancestral chromosomal segments on the Indian muntjac chromosomes that could subsequently define the mode of tandem fusions in the events of karyotypic evolution of the Indian muntjac. The partial results had been presented in the 56th annual meeting of American Society of Human Genetics, New Orleans, Oct. 2006, abstract program no. A840.   A manuscript about this study is in preparation for submitting to *Animal Genetics*.

Figure 2-1. BAC clones mapped onto the metaphase chromosome of Indian muntjac and Formosan sambar deer by FISH

Clone 0127E6 (red), 0130E6 (green), and 0072H12 (green) hybridizing onto the chromosome 1p of Indian muntjac (A) and its homologous segment in three different pair chromosomes of the Formosan sambar deer (D). Clone 0016A1 (green on the 2q35 of IM), 0744H12 (red on the 2q37 of IM) and 0015A1 (red on the 2q38 of IM) (B) were presented at the single homologous chromosome of Sambar deer (E). Clone 0124A1 (green) and 0105H12 (red) both located to the single homologous chromosome of Sambar deer (F) that mapped onto the 3q41 and 3q44 of Indian muntjac, respectively (C).

III. Defining synteny conservation studies between Human and Indian muntjac by Zoo-FISH

      Thanks to our constructed Indian muntjac's BAC library and near 2000 BAC clones mapped onto Indian muntjac, we could perform the Zoo-FISH experiment of BAC clones to several mammalian species for defining the synteny conservation during karyotype evolution.   First, we selected 3 to 5 BAC clones on each G-banded of Indian muntjac and mapped onto human metaphase chromosomes by Zoo-FISH mapping.   More 30 mixes of BAC clones, which locus located on Indian muntjac chromosome 1, had been mapped onto human metaphase chromosome (Figure 3-1).   The FISH signals of two mixes, located on the muntjac's 1p17 and 1q24, were observed on the telomere/centromere of human chromosomes.   The muntjac's 1p17 is also one of the C5 satellite interstitial sites.   This result suggested that a putative satellite DNA sequence could be conserved during karyotype evolution.   Furthermore, single BAC FISH was performed to confirm the result of Zoo-FISH mapping using the BAC clone mixes (Figure 3-2).   The FISH mapping of more BAC clone mixes will be performed on human chromosomes.   The result of this study will shed more light on the gene ordered in the conserved synteny between human and Indian muntjac.

Figure 3-1: BAC clone mixes mapped onto the human metaphase chromosomes by Zoo-FISH mapping.   The chromosome ideogram in black and white displayed the banding patterns of Indian muntjac chromosome 1 generated by DAPI staining.   The black circles along the left side of the black-white ideogram represented the relative position of interstitial C5 satellite signals.   The human locus corresponding to the muntjac locus shown between two chromosomal ideograms were identified by Zoo-FISH using muntjac's BAC clone mixes.   The coloured ideogram indicated the synteny conservation of human and Indian muntjac.
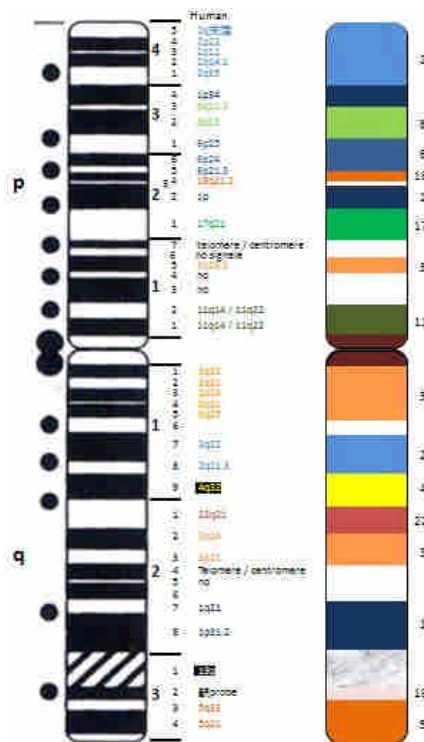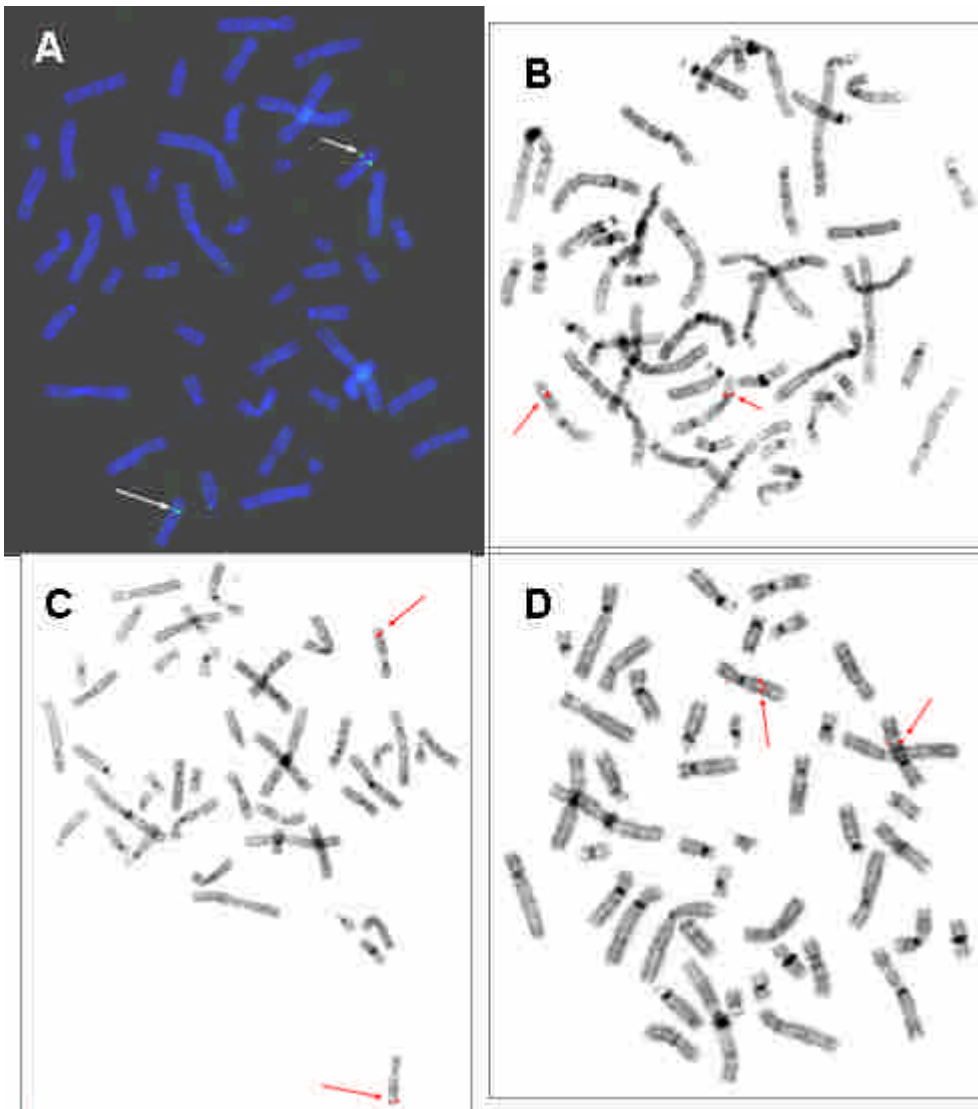

Figure 3-2.   BAC clones mapped onto the metaphase chromosome of human metaphase chromosomes by FISH.   A. Clone 0512E6 on (green on the 10q24 of human)   B. Clone 127A1 (red on the 11q22.3 of human)   C. Clone 443E6 (red on the 12q24.2 of human)   D. Clone 123E6 (red on the 7q22 of human)

Self-evaluation:

In this year's project, our aim is to studying the evolution of karyotype using BAC-FISH mapping. We had constructed 4X coverage of Indian muntjac BAC library at last project. In this study, we have mapped 1619 BAC clones onto the Indian muntjac metaphase chromosomes by fluorescence in situ hybridization (FISH). This part of result has been accepted by Zoological studies for publication. 100 of 1619 BAC clones have been mapped onto the Formosan sambar deer metaphase. This result would enable us to determine the orientation of the more ancestral chromosomal segments on the Indian muntjac chromosomes that could subsequently define the mode of tandem fusions in the events of karyotypic evolution of the Indian muntjac. More 50 of 1619 BAC clones have been mapped onto human metaphase chromosomes by Zoo-FISH mapping. This result would shed light on the synteny conservation during karyotype evolution. Therefore, our studies meet the aim of the original project and achieve the progress of the project. Totally, we have one accepted paper for publication and one preparing paper during carrying out this project.

References:

Amor DJ, Choo KH (2002) Am J Hum Genet 71:695-
Chowdhary BP, et al. (1998) Genome Res. 8:577-589.
Fronicke L, Scherthan H (1997) Chromosome Res 5:254-261.
Garrido-Ramos MA, et al. (1999)Mol Phylogenet Evol 12:200-204.

Koonin EV, Aravind L (2000) Cell 101:573-576.

Lalley PA, et al. (1978) Nature 274:160-163.

Laursen HB, et al. (1992) EMBO J 11: 2367–2372.

Lee C, et al. (1993) Cytogenet. Cell Genet. **63:** 156-159.

Li YC, et al. (2005) Chromosoma 114:28-38.

Li, Y.C., et al. (2000) *Chromosome Res* **8**:363-373.

Lin CC, et al. (1991)Chromosoma 101:19-24.

Meštrović N, et al. (1998) Mol Biol Evol 15:1062-1068.

Muller S, et al. (2000) Proc. Natl. Acad. Sci. USA 97:206-211.

Murphy WJ, et al. (2005) Science 309:613-617

Murphy WJ, Stanyon R (2001) Genome Biol 2:review S0005.

Nijman IJ, Lenstra JA. (2001) J. Mol. Evol. **52:** 361-371.

O'Brien SJ, et al. (1997) J Hered. 88:408-414.

Ostertag EM, Kazazian HH (2001) Annu Rev Genet 35:501-538.

Pevzner P, Tesler G (2003) Genome Res 13:37-45.

Puttagunta R, et al. (2000) 10:1369-1380.

Salser W, et al. (1976) Fed Proc Fed Am Soc Exp Biol 35:23-35.

Scherthan H: Kew chromosome Conf IV:267-280 (1995).

Shi LM, et al. (1980) Cytogenetic Cell Genet 26:22-27.

Ugarković D, Plohl M. (2002)EMBO J 21:5955-5959.

Wienberg J, Stanyon R. (1995) Curr. Opin. Genet. Dev. 5: 792-797.

Wienberg J, Stanyon R. (1997) Curr Opin Genet Dev 7:784-791.

Yang F, et al. (1995) Chromosoma 103:642-652.

Yunis JJ, et al. (1980) Science 208:1145-1148.

行政院國家科學委員會補助國內專家學者出席國際學術會議報告

<div style="text-align: right">附件三</div>

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會議 時間 地點	95 年 10 月 9 日至 13 日 美國紐奧良	本會核定 補助文號	NSC-95-2311-B-040-001
會議 名稱	（中文）2006 第五十六屆美國人類遺傳學會年會 （英文）56th Annual American Society of Human Genetics Meeting		
發表 論文 題目	（中文）高密度印度山羌 BAC DNA 的定位及確立台灣水鹿的核型演化 （英文）High density BAC clone mapping and defining the karyotype evolution of Formosan sambar deer		

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報告內容應包括下列各項：

一、參加會議經過

第一天早上辦理報到、海報的張貼。

第二天早上聽了一系列有關目前 array CGH 在細胞遺傳學上的臨床應用報告；下午則聽了一場有關 epigenetics 和複雜的性狀表徵的演講。

第三天上午觀賞海報內容；下午展示海報及聽了一場有關在早期哺乳動物發育過程中 epigenetic reprogramming 的演講。

第四天聽了一系列有關目前 non-coding RNA 在不同遺傳疾病中的致病機制的研究報告。

二、與會心得

The American Society of Human Genetics (ASHG), founded in 1948, is the primary professional membership organization for human geneticists in the Americas. The annual meeting mainly provides venues to bring investigators opportunities to share their research findings in the many areas of endeavors in human genetics; facilitating interactions between geneticists and other communities including policy makers, industry, educators, and patient and public advocacy groups.

I listens three topics of speech: 1. array-CGH application in cytogenetics; 2. regarding epigenetics; 3. effects of non-coding RNAs. In the session of array-CGH application in cytogenetics, I realized the good or bad of this array CGH developed from different company and how efficiency in clinical cytogenetics.   This information could be a base to establish this kind of technique future in my lab.   Lately, I was interested in the epigenetics issue.   Therefore, I listens two speeches regarding the epigenetics.   I truly gained a lot of knowledge in this issue.   It is helpful for my research and lecture present in future.   Additionally, I learned more pathogenesis of genetic disorders caused from non-coding RNA that I never learned before.   It is truly a great annual meeting.

I also met and discussed with several directors of cytogenetics in US.   This discussion also let me realize that array CGH technique had been applied to diagnose the genetic disorders in the some cytogenetic labs in state.   I think that our government should support and fund the cytogenetic lab in Taiwan to set up this array CGH technique to survey the whole genome of individuals with unknown and rare genetics disorders. This will speed up understanding the key genes that associated with the rare genetics disorders in Taiwan.

Appendix： 壁報論文

High density BAC clone mapping and defining the karyotype evolution of Formosan sambar deer

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Abstract

Comparative studies of chromosome content and organization are providing insight into fundamental questions of genome evolution and speciation. BAC library has tremendous potential to facilitate such studies to shed light on the temporal relationships of extant species by comparative sequence analysis and cytogenetic mapping of genome rearrangement, expansion, and loss (Gomyo et al. 1999; Srinivasan et al. 2002). In addition to decipher genome evolution, BAC resources could be used to address specific questions pertaining to centromeric and pericentromeric evolution, and the mechanisms of karyotypic change between closely related species. The Indian muntjac (*Muntiacus muntjac vaginalis*) has the lowest chromosome number in mammalian species with 6 chromosomes in female and 7 in male. Recent molecular cytogenetic studies landed support to the hypothesis that the karyotype of the Indian muntjac could evolve from an ancient deer species with a karyotype 2n=70 through extensive tandem fusions and several centric fusions (Li et al. 2000b). Moreover, the centromere of X+3 chromosome of the species is compound and unique. Therefore, it is an ideal species for studying chromosome rearrangement, comparative genomic and karyotype evolution. In an attempt to explore the centromeric evolution and function, and to elucidate the fusion orientations of conserved chromosome segments and the underlying mechanism of tandem fusion of Indian muntjac chromosomes, we therefore constructed a BAC library of Indian muntjac.

In this study, the insert DNA fragments of BAC were generated from a male Indian muntjac fibroblast cell line (ATCC) and were treated with *Eco*RI/*Eco*RI methylase. The high-molecular-weight DNA fragments were cloned into the *Eco*RI site of pCC1BAC (Copy ControlTM cloning system). In total 126,336 individual BAC clones have been obtained. The frequency of clones with inserts is 88% and the average size of the inserts was estimated at 80 kilobases by analyzing ~545 randomly chosen clones using *Not*I digestion followed by Pulsed Field Gel Electrophoresis (PFGE). Assuming that the Indian muntjac genome contains 2.2X109bp, the total library constructed corresponds to 4X genome coverage. The chromosomal location of 591 BAC clones was each mapped to the Indian muntjac metaphase chromosomes by fluorescence in situ hybridization (FISH), thus providing a high density FISH BAC clone map never been achieved of the species. Among these clones, 31 BAC clones were mapped onto the centromeric region. The genomic organization of satellite DNA in these clones should shed light on the structural aspect of centromeric satellite DNA. Furthermore, the centromeric BAC clones will also provide an excellent resource for the isolation of new centromeric satellite DNA sequences and their role in centromere function that can be evaluated by an artificial-chromosome assay. This could lead to the construction of function artificial mammalian chromosomes with gene therapy potential. 21 clones were selected and mapped onto the Formosan Sambar deer by comparative FISH mapping, the results showed that large synteny segments were conserved between Indian muntjac and Formosan sambar deer. Comparison of the orders of mapped BAC clones on Formosan Sambar deer and Indian muntjac enabled us to determine the orientation of the more ancestral chromosomal segments on the Indian muntjac chromosomes that could subsequently define the mode of tandem fusions in the events of karyotypic evolution of the Indian muntjac. This study was supported by grants from the National Health Research Institute (NHRI-EX94-9207SI) and from the National

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Material and methods

    Construction of a Indian muntjac genomic BAC Library.   Male Indian muntjac fibroblast cells (CCL-157, American Type Culture Collection) were used to prepare the high molecular weight (HMW) genomic DNA. Briefly, 8X106 cells (corresponding 40ug of DNA) were embedded in low-melting agarose (0.5%) in PBS for each plug.   The cells in the plug would be digested by proteinase K to extract the genomic DNA. After extraction, the genomic DNA in the plug would be run a 1% agarose gel using a PFGE apparatus in 0.4X TBE buffer at 12°C and 120V/cm for 10hrs with a 5-sec pulse time to remove the mitochondria DNA. Subsequently, the genomic DNAs in plugs were partial digested with *Eco*RI/*Eco*RI methylase (New England Biolabs) at 37℃ water bath for 16hrs. The partially digested DNAs were double-size fractionized by pulsed-field gel electrophoresis (PFGE) in a low-melting agarose to obtain HMW genomic DNA. Gel slices containing the DNA fragments in the length range of about 60-100kb and 100kb-150kb were excised.   The HMW genomic DNAs were eluted and were ligated with   pCC1BACTM *Eco*RI cloning-ready vector (Epicentre, U.S.A.) by   Fast-LinkTM DNA Ligase (Epicentre, U.S.A.). Ligation were drop dialyzed against 5% PEG or TE buffer with Millipore VS 0.025 uM membranes for 1hr. The dialyzed ligation DNAs were electroporated into 33ul of *E*. coli DH10B competent cells (Epicentre, U.S.A.). After electroporation, cells were incubated in 600ul 2XLB medium containing 1mM MgCl2 and 20mM glucose at 37℃ with gentle shaking for 1hr and spread on 2XLB plates containing chloramphenicol (12.5ug/ml), X-gal (40ug/ml) and IPTG (100ug/ml). The plates were incubated at 37℃ overnight. Blue and white color selection was used to identify the recombinants. Approximately 20 transformations were carried out to obtain >6,000 BAC clones. White positive BAC clones were picked manually to 96-well microtiter plates containing 100ul freezing media (0.5% w/v NaCl, 1% w/v Bacto-Tryptone, 0.5% w/v Bacto-extract, 13mM KH2PO4, 36mM K2HPO4, 1.7mM sodium citrate, 6.8mM (NH4) SO4, 4.4% v/v glycerol, 0.4mM MgSO4 · 7H2O and 12.5ug/ml chloramphenicol). The microtiter plates were incubated at 37℃ with 350rpm shaking for 18-20hrs, two copies of each 96-well microtitre plate were prepared and stored at -80℃ at different locations. The detailed protocol was described in Peterson et al. (2000).

Characterization of the BAC Library.   **Insert size estimation.**   Randomly picked recombinant clones were pre-grown in 2ml 2XLB medium containing 12.5ug/ml chloramphenicol for 5hrs and then 2ul of 1000X CopyControl Induction Solution (Epicentre, U.S.A.) was added.   Inoculation continued at 37℃ with 250 rpm shaking for another 18-20hrs. The bacteria broth was collected for isolating the BAC DNA.   The BAC DNA was isolated by the standard alkaline lysis protocol. The purified BAC DNA was digested by *Not*I to excise the insert.   The insert was fractionated by running pulsed-field gel electrophoresis in 0.4X TBE buffer at 12℃ and 6.0V/cm for 15.5hrs with 1- to 15-sec pulse time using a PFGE apparatus.   **Fluorescence in situ hybridization (FISH)** was used to evaluate the degree of chimerism in this library and physical mapping of BAC clones. Metaphase chromosomes were prepared from the male Indian muntjac cell line and the BAC DNA probes were labeled with either digoxigenin-11-dUTP or biotin-16-dUTP (Roche, Basel, Switzerland) by nick-translation. The procedures for denaturation, hybridization, post hybridization washing and signals were described in detail elsewhere (Lee et al. 1999). Fluorescent signals were

captured on a Leica ALM fluorescence microscope equipped with appropriate filter sets and a cooled charge-coupled device (CCD) camera. The images were normalized and enhanced using the FISH software (Applied Spectral Image, Isrel).

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Results:

Characterization of the BAC Library.

We have constructed a bacterial artificial chromosome (BAC) library of Indian muntjac with pCC1BAC vector (8.1kb). The complete library consists of 126,336 clones arranged in 1316 of the 96-well microtiter plates. A total of 545 randomly chosen BAC clones were sized using NotI digestion followed by Pulsed Field Gel Electrophoresis (PFGE). There are 480 BAC clones with insert and 65 BAC clones without insert. The frequency of clones with inserts is 88%. The PFGE result showed that an average size of BAC DNA with insert is 80 kb (Figure 1). Assuming that the Indian muntjac genome contains 2.2×10^9 bp, the total library constructed corresponds to 4x genome coverage.

Chromosomal mapping of BAC clones by FISH.

A total of 591 randomly picked BACs were mapped onto the metaphase chromosome of Indian muntjac by fluorescence in situ hybridization FISH. None of the BAC clones were observed with FISH signal at more than one site indicating that the chimeric frequency was roughly 0%. The detailed chromosomal assignments of BAC clones mapped on the chromosomal bands of the Indian muntjac constructed based on a published high-resolution G-banded karyotype of Indian muntjac (Li et al. 2000) were summerized in Table 1. Among these clones, there are 260 clones mapped to chromosome 1 of Indian muntjac, 148 clones mapped to chromosome 2 of Indian muntjac, 134 clones mapped to chromosome 3 and 18 clones mapped to chromosome X. Totally, 560 clones mapped onto specific chromosomal position (Figure 2) almost covered all bands of ideogram except for band 2q22. The remaining 31 clones mapped to the centromere regions of Indian muntjac. Eight types of centromeric distribution were observed (Figure 3). One of the distribution patterns of centromeric signals was presented similar to that of the C5 probe (cervid satellite I) which located on the centromeric and interstitial regions of Indian muntjac chromosomes(Figure 3A). Another type of patterns was observed to have similar patterns like the signals of Mmv-0.7 probe (cervid satellite II) also with centromeric and interstitial signals (Figure 3B). 5 clones were only mapped onto the two sites of compound centromere of X+3 (Figure 3C and D). 4 clones located to the Yq and centromeric region of chromosome 3 in Indian muntjac (Figure 3E). A number of BAC clones mapped onto all centromere regions, some of which appeared as kinetochore signals which parallel to two sides of centromere (Figure 3F), some BAC clones occupied all centromeric heterochromatin region (Figure 3G), and some of which located in the middle region of centromere especially X+3 (Figure 3H). 21 clones were selected and mapped onto the Formosan Sambar deer chromosomes by comparative FISH mapping. For example, clones 0124A1 and 0105H12 both located to the same chromosome of Sambar deer that mapped onto the 3q41 and 3q44 of Indian muntjac, respectively (Figure 4C and F). 0016A1 (on the 2q35 of IM), 0744H12 (on the 2q37 of IM) and 0015A1 (on the 2q38 of IM) were presented at the same chromosome of Sambar deer (Figure 4B and E). The results showed that large synteny segments were conserved between Indian muntjac and Formosan sambar deer. Comparison of the orders of mapped BAC clones on Formosan Sambar deer and Indian muntjac enabled us to determine the orientation of the more ancestral chromosomal segments on the Indian muntjac chromosomes. This could lead to the further

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definition of the mode of tandem fusions that occurred during the karyotypic evolution of the Indian muntjac.

Figure 1: A) The insert size distribution of the Indian muntjac BAC library.   Insert sizes were determined for 545 BAC clones by performing PFGE after *Not* I digestion.   The horizontal axis refers to the size ranges in kb, while the vertical axis indicates the number of clones. (B) Typical examples of *Not* I restricted BAC clones after PFGE analysis (Lane 3 to 17). Lane 1 and 19 denoted the PFGE standard DNA marker; Lane 2 and 18 were λ -*Hin*dIII marker; The row of vector bands was at 7.5 kb (denoted by arrow).
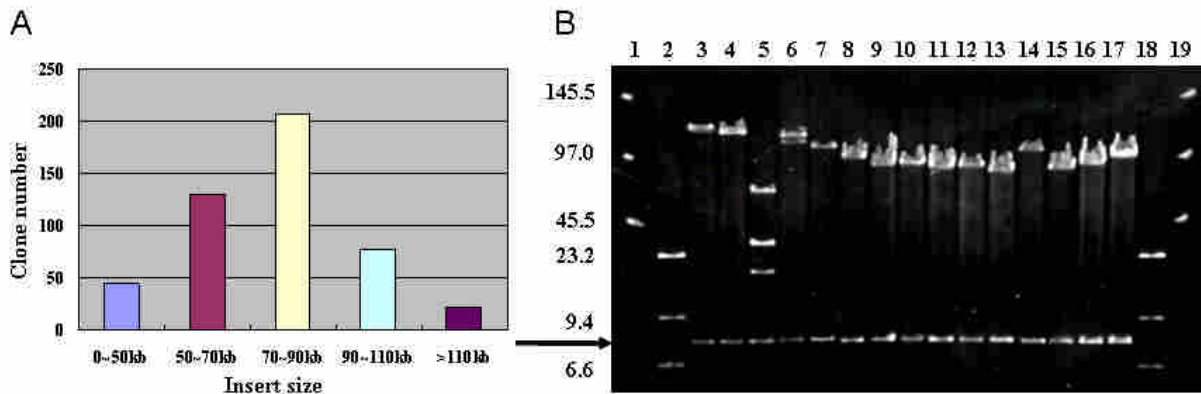
Figure 2: BAC clones mapped on the specific chromosomal sites of Indian muntjac by FISH.
(A) Clone 119A1 mapped onto the 1q23 (green) and clone 122A1 mapped onto the 1p14 (red).   (B)
Clone 121A1 mapped onto the Xp15 (red) and 124A1 mapped onto the 3q41(green). (C) Clone
41E6 mapped onto the 1p12 (red) and 50A1 mapped onto the Xp15(green). (D) Clone 3E6 mapped
onto the 2p11 (red) and 4E6 mapped onto the 3q31(green). (E) Clone 127E61 mapped onto the
1q21 (red) and 130E6 mapped onto the 1q25(green). (F) Clone 15A1 mapped onto the 2q37(red)
and 16A1 mapped onto the 2q35(green). (G) Clone 1121H12 mapped onto the pseudo-autosomal
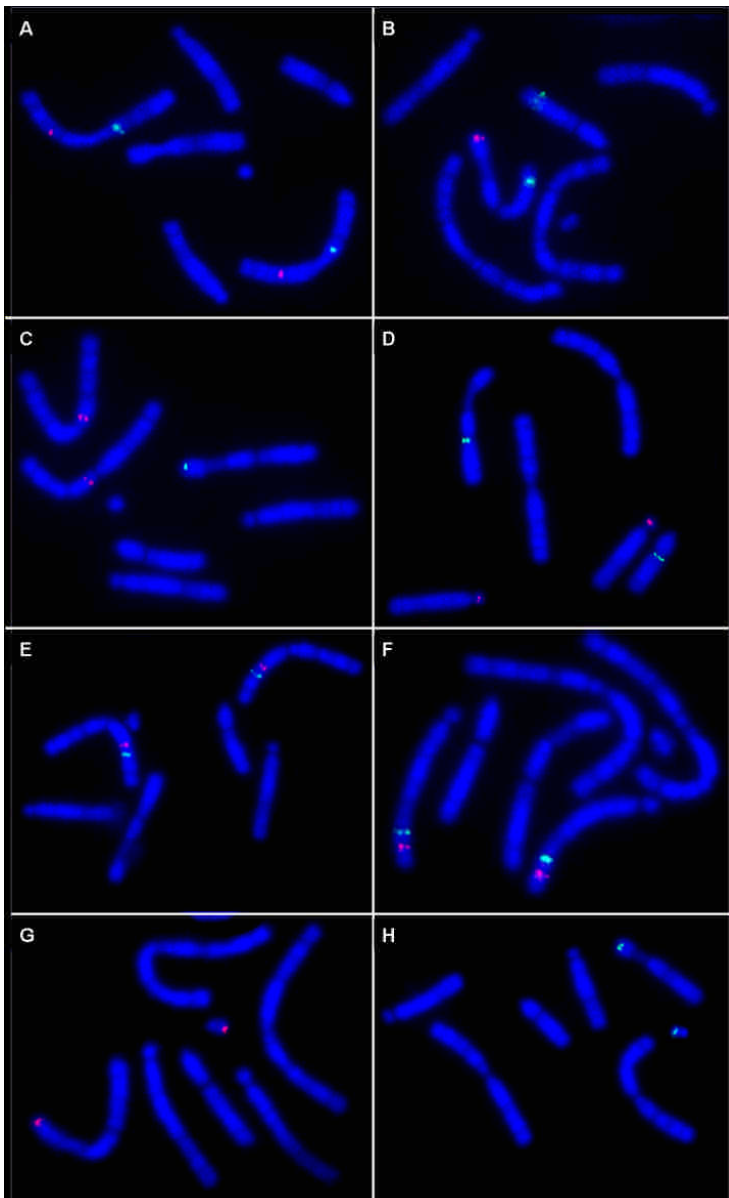region of X and Y (red). (H) Clone 1154E6 mapped onto the pseudo-autosomal region of X and Y
(green).

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Figure 3: BAC clones mapped on the centromeric region of Indian muntjac by FISH.
(A) Clone 1128A1 (green) mapped onto the centromeric and interstitial regions of Indian muntjac chromosomes. (B) Clone 1158A1 (green) was observed to have similar patterns like the signals of Mmv-0.7 probe (cervid satellite II) also with centromeric and interstitial signals. (C and D) Clones 1189E6 and 1179H12 were only mapped onto the two sites of compound centromere of X+3.   (E) Clone 1189A1(red) located to the Yq and centromeric region of chromosome 3 in Indian muntjac. (F) Clone 1296A1 (green) mapped onto all centromere regions, some of which appeared as kinetochore signals which parallel to two sides of centromere. (G) Clone 1241H12 (red) occupied all centromeric heterochromatin region. (H) Clone 747A1 (red) located in the middle region of centromere especially X+3.
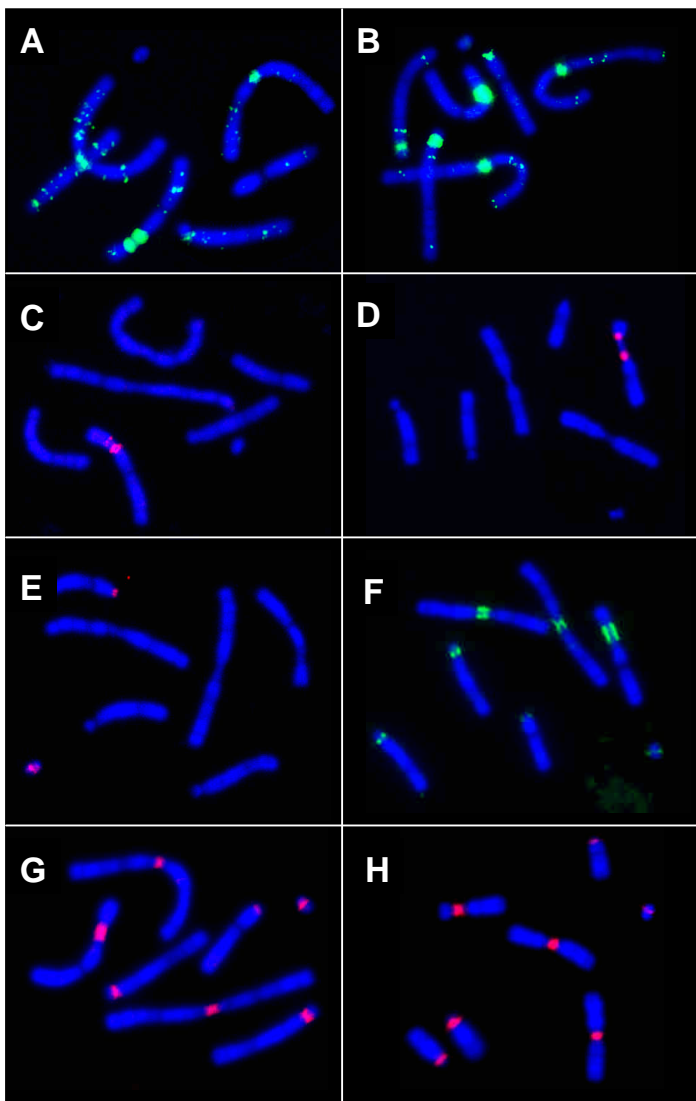
Figure 4: BAC clones mapped onto the metaphase chromosome of Indian muntjac and Formosan sambar deer by FISH.

Clone 0127E6 (red), 0130E6 (green), and 0072H12 (green) hybridizing onto the chromosome 1p of Indian muntjac (A) and its homologous segment in three different pair chromosomes of the Formosan sambar deer (D). Clone 0016A1 (green on the 2q35 of IM), 0744H12 (red on the 2q37 of IM) and 0015A1 (red on the 2q38 of IM) (B) were presented at the single homologous chromosome of Sambar deer (E). Clone 0124A1 (green) and 0105H12 (red) both located to the single homologous chromosome of Sambar deer (F) that mapped onto the 3q41 and 3q44 of Indian muntjac, respectively (C).
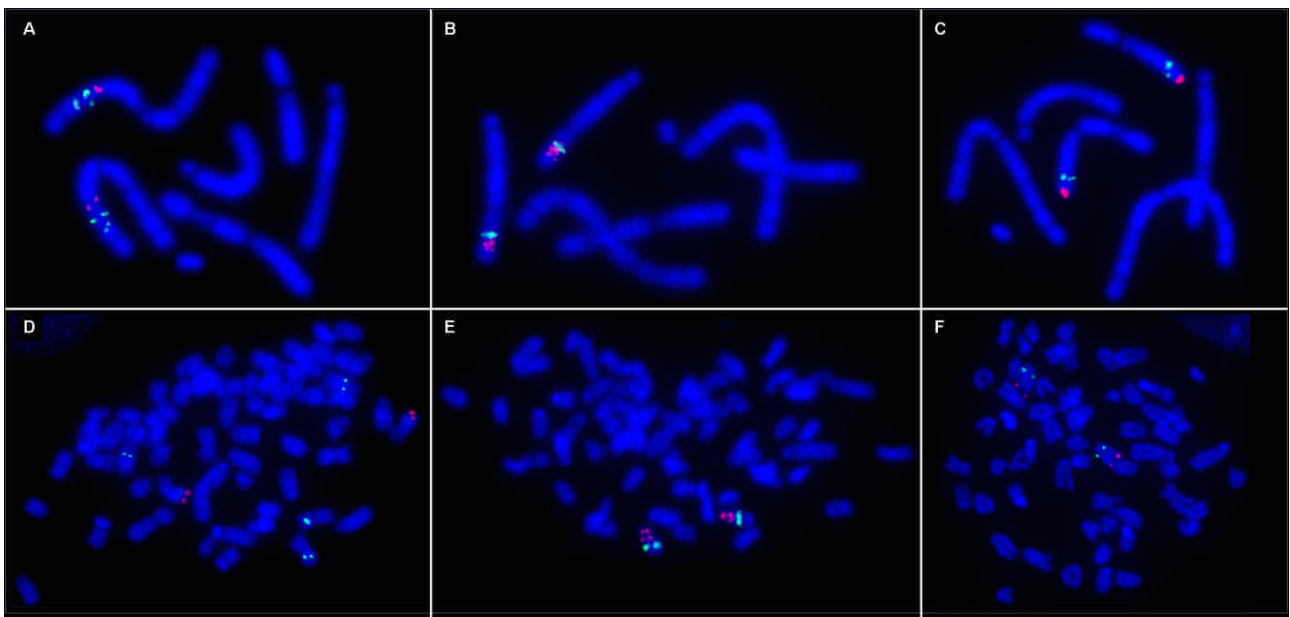
Table 1 Summary of 591 bacterial artificial chromosome (BAC) clones mapping to the Indian muntjac by FISH

1p45	96 E6,111 E6,122H12,124H12,137H12,139A1, 1161H12
1p44	735A1,118H12,839H12, 1260H12, 1218H12
1p43	97 E6,743A1, 1165H12, 1198E6
1p42	92A1,811A1,821 E6, , 1238A1, 1147H12, 1218A1, 1218H12
1p41	746 E6,110A1,135 E6,158 E6,822 E6, 1196E6
1p34	113H12,121H12,125 E6, 1196A1
1p33	734A1,38A1,31 E6,12H12,116A1,145 E6
1p32	750A1,89A1,101H12,845 E6, 1176H12, 1151A1, 1225H12, 1216E6
1p31	752H12,40H12,737H12,749 E6,752A1,152A1,822H12
1p26	730A1,84A1,118A1,160A1,834 E6, 1155A1
1p25	1141H12, 1184H12
1p24	726 E6,144 6,818 E6,827 E6,831A1, 1254E6, 1236H12
1p23	732 E6, 1174H12
1p22	5A1,115H12,816H12,837A1, 1170E6, 1139A1, 1226A1, 1212H12
1p21	744A1,747H12,16 E6,826 E6, 1169H12
1p17	825 E6, 1228H12, 1195A1, 1191A1
1p16	60H12, 1152E6
1p15	725H12,6H12,65 E6, 1195A1, 1165A1, 1143H12
1p14	739H12,51A1,95A1,122A1,129H12,157H12,824 E6
1p13	729H12,739A1,3H12,12A1,754H12, 1158A1, 1161E6, 1141E6
1p12	738A1,8H12,41 E6,93 E6,833 E6, 1227E6, 1190A1, 1184A1
1p11	740 E6,111A1,147 E6,820 E6,843 E6, 1254A1, 1145E6, 1234E6
1q11	3A1,41H12, 1171A1
1q12	12H12,33H12, 1183E6
1q13	124 E6, 1222E6, 1200H12
1q14	724H12,38A1
1q15	829A1
1q16	1219A1, 1156H12, 1256H12
1q17	734 E6,728H12,741A1,2 E6,4A1,51H12,151A1,837 E6,842H12, 1258E6
1q18	116 E6,118 E6
1q19	725 E6,47 E6,88H12,126A1,136 E6,144H12,159 E6,826H12,845A1, 1182A1, 1152A1, 1179E6
1q21	727A1,127 E6,829 E6, 1262A1, 1240E6, 1155H12, 1200A1
1q22	002H12, 005 E6, 7H12,101 E6,108 E6,140A1, 1134A1, 1217A1, 1194A1
1q23	29A1,87H12,119A1,827A1
1q24	39A1,154 E6,845H12, 1150E6
1q25	736 E6,130 E6
1q26	723 E6,36 E6,141H12, 1191E6, 1175H12
1q27	1 E6,13 E6,72H12,840H12, 1199H12, 1190E6
1q28	726H12,732A1,743 E6,744A1,751A1,38 E6,100H12,106H12,290H12,122 E6, 155H12,813E6,151H12,833H12, 838 E6, 1166H12, 1157A1, 1154H12, 1183A1
1q31	727H12,144A1,145H12,157A1,1300H12
1q32	1300E6, 1260A1
1q33	69A1,94H12,844 E6
1q34	737 E6,28H12,1H12,740A1,40 E6,828A1,828 E6,150 E6, 1195E6,1216A1

2p12	13H12, 1316A1, 1174E6
2p11	3 E6,155 E6,836 E6
2q11	64 E6,821H12, 1262H12, 1232A1
2q12	41A1,130A1,812H12, 1220H12
2q13	731 E6,16H12,58A1,84 E6,89 E6,109H12,154 E6, 1153E6
2q14	733H12,738H12,64H12
2q15	89H12,151 E6
2q16	1260E6
2q17	730H12,736H12,30A1,33A1,99 E6,136H12,842A1, 1198A1
2q18	736A1,744 E6,725A1,82A1,98H12,119H12,843A1
2q21	741H12,24A1,8 E6,94A1,121 E6,136H12,823A1, 1164E6
2q22	
2q23	102A1,841 E6, 1187H12, 1224H12, 1190H12,
2q24	841H12, 1256E6
2q25	815 E6, 1187A1, 1194E6

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2q26	726A1,91A1,104A1, 1227A1
2q27	15 E6,112A1,134H12, 1188E6, 1184E6
2q28	1193A1
2q29	735H12,104 E6,106A1,111H12,846H12
2q210	723H12,8A1,123 E6,104A1, 1225E6, 1220E6
2q31	735 E6,62H12,123H12,818A1, 1302H12
2q32	1149A1
2q33	745H12,86H12,110 E6, 1161A1, 1173A1, 1177H12
2q34	1254H12, 1167H12
2q35	16A1,48H12,65A1,34H12,84H12,119 E6,154H12,817 E6,821A1
2q36	7A1,147A1,163 E6, 1158H12, 1158H12, 1186E6
2q37	5H12,15A1,15H12,744H12,290A1,127A1,836A1,1242E6, 1143A1, 1197E6, 1188H12
2q38	742A1,113 E6,119A1, 1220A1
2q39	64A1,34 E6,157 E6,810A1,145A1,819A1,1164H12, 1230A1

Xp15	751 E6,50A1,121A1,160 E6, 1181A1
Xp14	86A1
Xp13	742H12,753 E6,750 E6,1156A1, 1224A1,
Xp12	130H12,67 E6
Xp11	746A1,739 E6,830 E6, 1219H12
3q11	732H12,17A1,127H12,815A1,835A1,841A1,1302E6,1141A1,1219E6, 1197H12, 1214 H12
3q12	35A1,810H12,843H12, 1183H12
3q13	6 E6,10A1,37A1,7 E6,93H12,104H12,115H12, 1192A1
3q14	740H12,810 E6
3q15	736A1,91H12,146A1,155A1,823H12, 1559H12, 1216H12
3q21	82H12,831 E6, 1163H12, 1181H12, 1173E6
3q31	733E6,749A1,749H12,4E6,57E6,63H12,92H12,723A1,21H12,146H12, 1156 E6
3q32	754A1,811 E6, 1159A1, 1222A1
3q33	738 E6,752 E6,6A1,67A1,90 E6,117A1,141 E6,148 E6,825A1,825 H12,844A1,1238E6, 1153H12, 1188A1
3q34	38A1,85H12,91 E6,96H12,141A1,844H12, 1162H12
3q35	741 E6,748H12,753H12,58H12,112 E6,140 E6,838A1,839A1,839 E6
3q36	730 E6,32H12,160H12,813A1, 1163E6, 1162A1
3q37	734H12,140H12, 1172H12
3q38	123H12,820H12
3q39	728A1,59H12,742E6,729A1,87E6,107H12,1164A1,1153A1,1154A1,1197A1, 1186H12,
3q41	55A1,124A1, 1225A1, 1169E6
3q42	753A1,751H12,99H12,848 E6
3q43	747 E6,84 E6, 1199A1
3q44	727E6,754E6,748E6,61A1,88A1,115E6,82A1,88 E6,105H12,137A1, 1145H12, 1152H12, 1143E6, 1169A1,1170A1

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