ORIGINAL ARTICLE

Comparative chromosome G-banding analysis of long-tailed macaque (*Macaca fascicularis*) and relationship to human (*Homo sapiens*)

Alongkoad Tanomtong[1], Sumpars Khunsook[2] and Wiwat Kaensa[3]

Abstract

Tanomtong, A., Khunsook, S. and Kaensa, W.

Comparative chromosome G-banding analysis of long-tailed macaque (*Macaca fascicularis*) and relationship to human (*Homo sapiens*)

Songklanakarin J. Sci. Technol., 2007, 29(3) : 583-589

This research is the first report of the comparative chromosome between long-tailed macaque (*Macaca fascicularis*) and human (*Homo sapiens*) using G-banding. Blood samples from four male and three female macaques were used. Their chromosomes were prepared using lymphocyte cultures at 37°C, 72 hours and detected using G-banding. The results showed the diploid chromosome number of 42, 18 metacentric and 22 submetacentric chromosomes. The satellite chromosome was on the short arm of chromosome 13. The X chromosome was a medium submetacentric and the Y was the smallest telocentric chromosome. By using G-banding, the macaque chromosome 5, 12, 13, 19 and X are identical to those of humans. The short arm and long arm of chromosome 13 of macaque were similar to chromosome 22 and 15 of human respectively. We indicate that macaque chromosome was split to two human chromosomes. The macaque and human chromosomes 1, 3, 6-11, 14, 17 and 20 were relatively similar. The macaque and human chromosome 1 is a pericentric inversion chromosome, indicating that the alternative construction of the chromosome cooperates with the centomere. There were 6 macaque chromosomes which were from different human, 2, 4, 15, 16, 18 and Y. All results demonstrate that the long-tailed macaque and human have are evolutionary relationship.

Key words : G-banding, long-tailed macaque (*Macaca fascicularis*), human (*Homo sapiens*)

---

[1]M.Sc. (Genetics), Asst. Prof., [2]Ph.D. (Molecular Genetics), [3]M.Sc. Student in Biology, Department of Biology, Faculty of Science, Khon Kaen University, Muang, Khon Kaen, 40002 Thailand.

Corresponding e-mail: tanomtong@hotmail.com

Received, 1 August 2006 Accepted, 3 December 2006

บทคัดย่อ

อลงกลด แทนออมทอง สัมภาษณ์ คุณสุข และ วิวรรธน์ แก่นสา

การเปรียบเทียบความสัมพันธ์ของโครโมโซมของลิงแสมและมนุษย์ ด้วยวิธีการย้อมแถบสีแบบจี

ว. สงขลานครินทร์ วทท. 2550 29(3) : 583-589

เป็นรายงานครั้งแรกของการเปรียบเทียบความสัมพันธ์ของโครโมโซมของลิงแสมและมนุษย์ ด้วยเทคนิคการย้อมแถบสีแบบจี ใช้ตัวอย่างเลือดสัตว์เพศผู้ 4 ตัว และเพศเมีย 3 ตัว เตรียมโครโมโซมด้วยการเพาะเลี้ยงเซลล์เม็ดเลือดขาวที่อุณหภูมิ 37°C เป็นระยะเวลา 72 ชั่วโมง ทำการย้อมแถบสีโครโมโซมแบบจี ผลการศึกษาพบว่าลิงแสมมีจำนวนโครโมโซม 2n (diploid) เท่ากับ 42 แท่ง โครโมโซมร่างกายประกอบด้วยโครโมโซมชนิดเมทาเซนทริก 18 แท่ง และชนิดซับเมทาเซนทริก 22 แท่ง บนแขนข้างสั้นของโครโมโซมคู่ที่ 13 จัดเป็น satellite chromosome โครโมโซมเอ็กซ์เป็นชนิดเมทาเซนทริกขนาดกลาง และโครโมโซมวายเป็นชนิดเทโลเซนทริกขนาดเล็กมากที่สุด โครโมโซมของลิงแสมติดแถบสีจีเหมือนกับโครโมโซมของมนุษย์ 4 คู่ ได้แก่ คู่ที่ 5, 12, 13, 19 และโครโมโซมเอ็กซ์ พบว่าแขนข้างสั้นโครโมโซมคู่ที่ 13 ของลิงแสม จะมีความคล้ายกับโครโมโซมคู่ที่ 22 ของมนุษย์ และแขนข้างยาวจะเหมือนกับโครโมโซมคู่ที่ 15 ของมนุษย์ สันนิษฐานว่าโครโมโซมคู่ที่ 15 และ 22 ของมนุษย์เกิดจากการหักของโครโมโซมคู่ที่ 13 ของลิงแสม มีโครโมโซมที่มีการติดแถบสีจีคล้ายคลึงกับของมนุษย์ 11 คู่ ได้แก่ โครโมโซมคู่ที่ 1, 3, 6, 7, 8, 9, 10, 11, 14, 17 และ 20 พบว่าโครโมโซมคู่ที่ 1 ของลิงแสมจะมีส่วนที่สลับหัวท้ายกับโครโมโซมมนุษย์ สันนิษฐานว่าเกิดจากการต่อสลับกันของโครโมโซมที่มีส่วนของเซนโทรเมียร์ร่วมด้วย สำหรับโครโมโซมที่มีการติดแถบสีจีไม่เหมือนกันกับของมนุษย์มี 6 คู่ ได้แก่ โครโมโซมคู่ที่ 2, 4, 15, 16, 18 และโครโมโซมวาย ผลการศึกษาแสดงให้เห็นว่า ลิงแสมมีสายวิวัฒนาการร่วมกันกับมนุษย์

ภาควิชาชีววิทยา คณะวิทยาศาสตร์ มหาวิทยาลัยขอนแก่น อำเภอเมือง จังหวัดขอนแก่น 40002

The animals of order primate are separated into 13 families, 60 genera and 232 species (Wilson and Cole, 2000). There are 3 families, 5 genera and 13 species in Thailand. Five species are the macaques in genus *Macaca*, stump-tailed macaque (*Macaca arctoides*; Geoffroy, 1831), assam macaque (*M. assamensis*; McClleland, 1839), long-tailed macaque (*M. fascicularis*; Raffles, 1821), rhesus monkey (*M. mulatta*; Zimmermann, 1780) and pig-tailed macaque (*M. nemestrina*; Linnaeus, 1766) (Brockelman, 1981; Lekagul and McNeely, 1977, 1988). Monkey, macaque, gibbon and ape have the common ancestor with human.

Long-tailed macaque is classified as to kingdom Animalia, phylum Chrodata, class Mammalia, family Cercopithecidae, subfamily Cercopithecinae, genus *Macaca* and species *Macaca fascicularis*. There coat-color seems to be grayish-brown and reddish. The tail is usually longer than the length of head and body. The hairs on the crown sweep backward from the brow and often have a high point crest. They live near water along the coastline of South and East of Thailand especially in mangrove swamp. They swim expertly and feed in large groups, 10-45 individuals, often over 80 individuals (Brockelman, 1981; Lekagul and McNeely, 1977, 1988).

From previous study, the cytogenetics of the macaques in genus *Macaca* have been reported (Chiarelli,1962; Hsu and Benirschke,1967; Napier and Napier, 1976; Caballin *et al*., 1980; Small and Stanyon, 1985; Brown *et al*., 1986 and Hirai *et al*., 1991). In this study, we report the relationship between long-tailed macaque and human using G-banding.

Material and Methods

Blood samples from the jugular vein were collected from four male and three female macaques, which were kept in Nakhon Ratchasima Zoo, using aseptic technique. The samples were kept in 10 ml vacuum tubes containing heparin to prevent blood clotting and they were cooled on ice until arriving at the laboratory.

1. Cell preparation

The lymphocytes were cultured using the whole blood microculture technique adapted from Kampiranont (2003).

Cell culture

The RPMI 1640 medium was prepared with 2% PHA (phytohemagglutinin) as a mitogen and kept in blood culture bottles of 5 ml each. A blood sample of 0.5 ml was dropped into a medium bottle and well mixed. The culture bottle was loosely capped, incubated at 37°C under 5% of carbon-dioxide environment and regularly shaken in the morning and evening. When reaching harvest time at the 72nd hour of incubation, colchicine was introduced and well mixed, followed by further incubation for 30 minutes.

Cell harvest

The blood sample mixture was centrifuged at 1,200 rpm (92 g) for 10 minutes and the supernatant was discarded. Ten ml of hypotonic solution (0.075 M KCl) was applied to the pellet and the mixture was incubated for 30 minutes. KCl was discarded with the supernatant after centrifugation again at 1,200 rpm (92 g) for 10 minutes. Cells were fixed by fresh cold fixative (methanol : glacial acetic acid = 3 : 1) gradually added up to 8 ml before centrifuging again at 1,200 rpm (92 g) for 10 minutes, and the supernatant was discarded. The fixation was repeated until the supernatant was clear and the pellet was mixed with 1 ml fixative. The mixture was dropped onto a clean and cold slide using a micropipette followed by the air-dry technique. The slide was conventionally stained with 20% stock Giemsa's solution for 30 minutes.

2. G-banding method

G-banding technique was adapted from Kampiranont (2003). The slide was well dried and then soaked in working trypsin (0.025% trypsin EDTA) at 37°C before the termination of trypsin activity by washing the slide with 10% fetal calf serum (FCS) or phosphate buffer. FCS was washed out by 50% methanol and the slide was stained with 10% Giemsa's solution for 30 minutes and compared with human chromosome obtained from G-banding according to Rooney (2001)

Results

By using T-lymphocytes culture and G-banding, the results showed that long-tailed macaques have 2n (diploid) = 42. Their chromosomes included 40 autosomes and the X and Y chromosome (Figure 1 and 2). The fundamental number (NF) was 83 in male and 84 in female. There were 2 types of autosome, metacentric and submetacentric. The large, medium and small metacentrics were chromosomes 6, 4 and 8 and the large, medium and small submetacentrics were chromosomes 8, 12 and 2 respectively (Tanomtong *et al.*, 2006). The X was a medium metacentric chromosome and the Y was the smallest telocentric chromosome. Chromosome 13 possesses a satellite in which the nucleolar organizer region (NOR) was on the short arm. The largest chromosome was the metacentric autosome 1 and the Y smallest was the telocentric chromosome.

Bands of chromosome 1 haploid set consisted of autosomes and sex chromosomes. G-banding was used in the metaphase chromosome and the result showed 269 bands (Figure 3). The macaque chromosomes 5, 12, 13, 19 and X were identical to human chromosome in terms of G-banding patterns (Rooney, 2001). While the macaque chromosomes 1, 3, 6-11, 14, 17 and 20 were relatively similar to human chromosome, their chromosomes 2, 4, 15, 16, 18 and Y were different from those of humans (Rooney, 2001) (Figure 4). The results showed that the macaque chromosome 1 was a pericentric inversion to human chromosome and the short arm and long arm of macaque chromosome 13 was similar to human chromosome 22 and 15 respectively. We hypothesize the

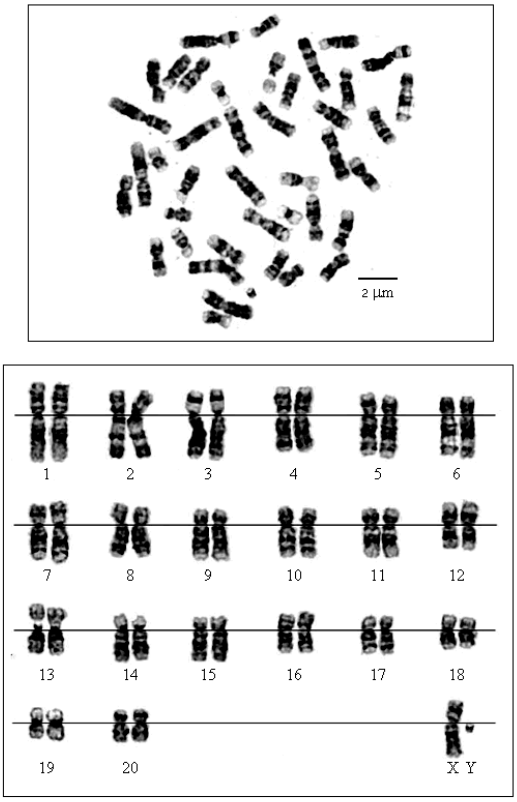

Figure 1. Metaphase chromosomes and karyotype of male long-tailed macaque (*Macaca fascicularis*) 2n (diploid) = 42 using G-banding technique.

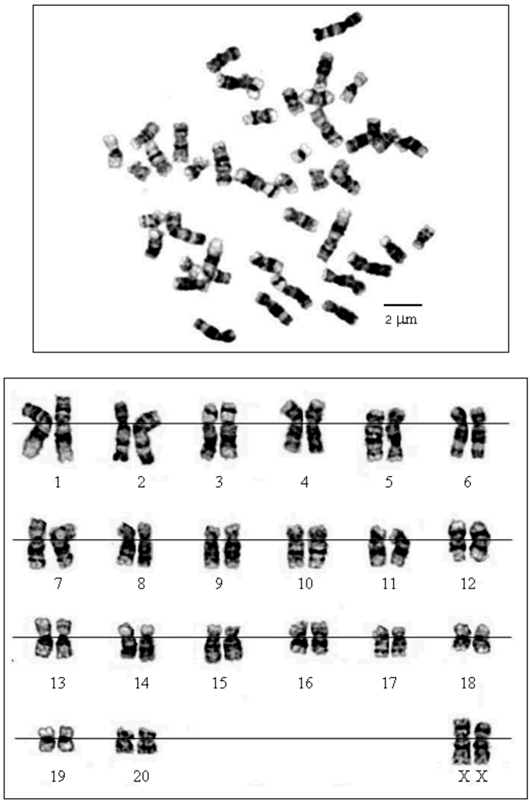

Figure 2. Metaphase chromosomes and karyotype of female long-tailed macaque (*Macaca fascicularis*) 2n (diploid) = 42 using G-banding technique.

pericentric inversion resulted from the alternative construction of the chromosome cooperating with the centomere (Figure 5).

Discussion

Long-tailed macaques have 2n = 42, 40 autosomes and 2 sex chromosomes, X and Y. The fundamental number is 83 in male and 84 in female. Human has 2n = 46, 44 autosomes and 2 sex chromosomes, X and Y. The fundamental number is 92 (Rooney and Czepulkowski, 1986, 2001; Miller, 1977; Mitelman, 1995). Human has chromosome number and fundamental number more than macaque.

Long-tailed macaques have only two types of autosome. They have large, medium, small metacentric and submetacentric, 6-4-8 and 8-12-2 respectively (Tanomtong *et al.*, 2006). Human has 6 large, 6 medium and 4 small metacentric, 4 large, 8 medium and 6 small submetacentric. Moreover there are 6 medium and 4 small acrocentric chromosomes in human (Rooney and Czepulkowski, 1986, 2001). The results show that long-tailed macaque has more conservative chromosome than those of human because there is no acrocentric in macaque. This result agrees with Jones *et al.* (1994) who report the chromosome numbers of ape and the other macaque in the same genus are similar to the ancestor, 2n = 42.

The X chromosome of long-tailed macaque and human is medium metacentric. The Y chromosome of long-tailed macaque is the smallest telocentric but, in human, it is the smallest acro-

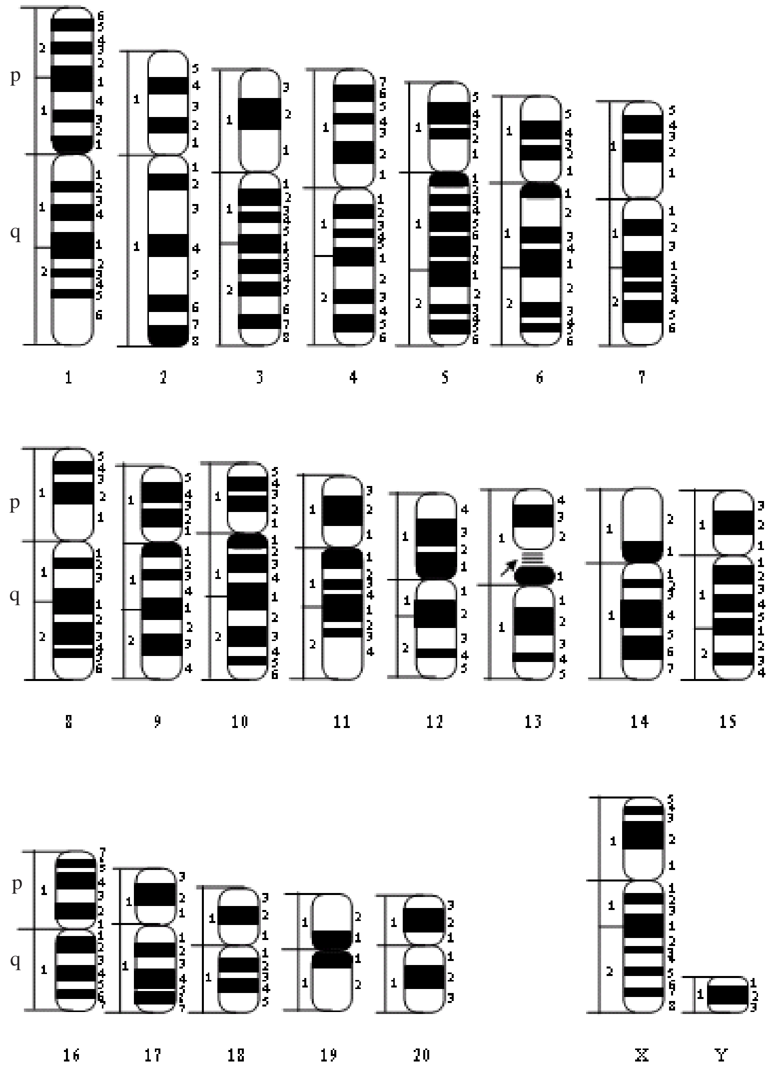

Figure 3. Idiogram of long-tailed macaque (*Macaca fascicularis*) metaphase chromosomes 2n (diploid) = 42 using G-banding technique. The satellite chromosome was on the short arm of chromosome 13 (arrow).

centric (Rooney and Czepulkowski, 1986, 2001; Miller, 1977; Mitelman, 1995). Rooney (2001) reported the shift of long arm of human Y chromosome. By using G-banding, there is the different band size. Thus there is a different size of heterochromatin.

The satellite chromosome of long-tailed macaque is similar to that of human. However, long-tailed macaque chromosome marker is chromosome 13 and human chromosome markers are chromosomes 13, 14, 15, 21 and 22. The largest long-tailed macaque and human chromosome is metacentric chromosome 1. The smallest long-tailed macaque chromosome is telocentric chromosome. The smallest in human is acrocentric.

Using G-banding, the metaphase chromosomes, show 269 bands. When compared with Rooney (2001), the identical long-tailed macaque and human chromosomes are chromosomes 5, 12, 13, 19 and X. The results agree with Miller (1977) who reported that 8 ape chromosomes are similar to human.

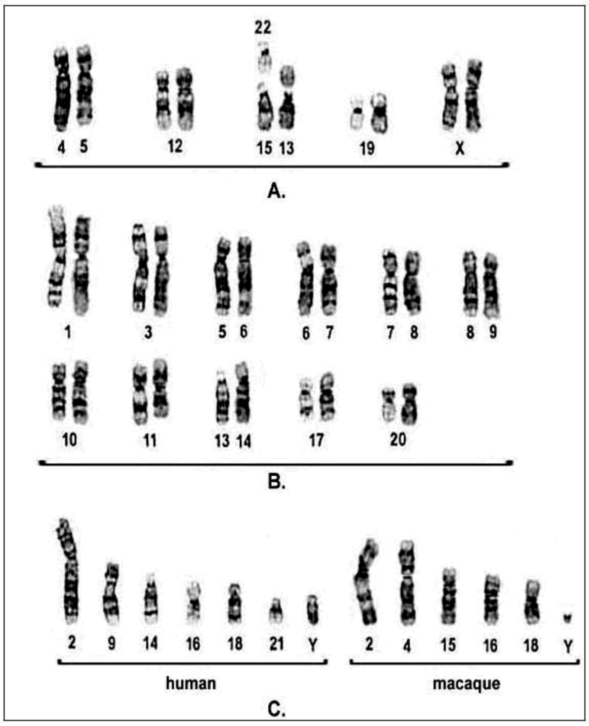

Figure 4. The comparison of the chromosome pair between human (left) and long-tailed macaque (right), 5 long-tailed macaque chromosomes are identical to human (A), 11 long-tailed macaque chromosomes are relatively similar to human (B) and 6 long-tailed macaque chromosomes are different from human (C).

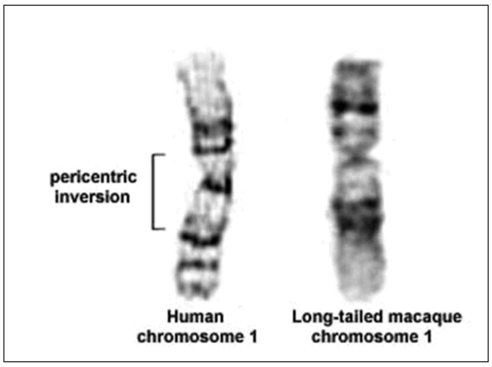

Figure 5. The chromosome 1 of long-tailed macaque is a pericentric inversion of human chromosome.

Short arm and long arm of long-tailed macaque chromosome 13 are similar to human chromosome 15 and 22 respectively. We suggest that the evolution of human and long-tailed macaque involved the fission or fusion of the chromosome. Most of the long-tailed macaque chromosome bands are similar to human those of but some bands are different. Then long-tailed macaque chromosomes 1, 3, 6-11, 14, 17 and 20 are similar to those of human.

The comparison of long-tailed macaque and human chromosomes was done using G-banding patterns. The results show that chromosome 1 of long-tailed macaque is a pericentric inversion to human chromosome. This result agrees with Brown *et al*. (1986) who reported the pericentric inversion of chromosome 5 of rhesus macaque and long-tailed macaque using R-banding.

We found the alternation of chromosome i.e. human chromosome 4, 5, 6, 7, 8 and 13 are similar to long-tailed macaque chromosome 5, 6, 7, 8, 9 and 14 respectively. Long-tailed macaque chromosomes 2, 4, 15, 16, 18 and the Y are different from those of human. Jones *et al*. (1994) found that the G-banding pattern of human chromosome is similar to that of monkey in genus *Macaca* and baboon, 2n = 42 more than gibbon, 2n = 44. This result contradicts the morphology and molecular biology. Human morphology is more similar to gibbon than is macaque morphology. Moreover, the social behavior and ecology are closely related to the evolution rate of monkey chromosome. Using only chromosome evolution data it is difficult to arbitrate the similarity or difference in monkey. Thus other data are needed for the decision. In this research, we hypothesize that long-tailed macaque has a close evolutionary relationship with human.

Acknowledgements

We appreciate to the Zoological Park Organization under the Royal Patronage. We would like to thank Mr. Sophon Dumnui (the director of the Zoological Park Organization), WG.CDR.Kravee Kreethapon (the director of Nakhon Ratchasima Zoo) and Dr. Wachirawit Somsa (the veterinarian of Nakhon Ratchasima Zoo) for the blood samples. Thanks to the authorities and officers of these zoos for good cooperation.

References

Brockelman, W. 1981. The primates in Thailand. Office of the welfare promotion commission for teachers and educational personnel (Kurusapa Business Organization), Bangkok.

Brown, C.J., Dunbar, V.G. and Shafer, D.A. 1986. A comparison of the karyotypes of six species of the genus *Macaca* and a species of the genus *Cercocebus*. Folia. Primatol. 46(3): 164-172.

Caballin, M.R., Miro, R., Ponsa, M., Florit, F., Massa, C. and Egozcue, J. 1980. Banding patterns of the chromosomes of *Cercophithecus petaurista* (Schreber, 1775): comparison with other primate species. Folia. Primatol. 34: 278-285.

Chiarelli, B. 1962. Comparative and morphometric analysis of primate chromosomes: The chromosomes of genera *Macaca, Papio, Theropithecus* and *Cercocebus*. Caryolo. 15: 401-420.

Hirai, S., Terao, K., Cho, F. and Honjo, S. 1991. Chromosome studies on cynomolgus monkeys (*Macaca fascicularis*). Primatol. Tod. 24: 619-622.

Hsu, T.C. and Benirschke, K. 1967. An Atlas of Mammalian Chromosomes. Springer Verlag Press, New York.

Jones, S., Martin, R. and Pilbeam, D. 1994. The Cambridge Encyclopedia of Human Evolution. Cambridge University Press, Cambridge.

Kampiranont, A. 2003. Cytogenetics. 2nd ed. Department of Genetics, Faculty of Science, Kasetsart University, Bangkok.

Lekagul, B. and McNeely, J.A. 1977. Mammals of Thailand. 1nd ed. Kurusapha Ladprao Press, Bangkok.

Lekagul, B. and McNeely, J.A. 1988. Mammals of Thailand. 2nd ed. Sahakarn Bhaet, Bangkok.

Miller, D.A. 1977. Evolution of primate chromosomes. Sci. 198: 1116-1124.

Mitelman, F. 1995. An international system for human cytogenetic nomenclature. Karger, Basel.

Napier, J.R. and Napier, P.H. 1976. A Handbook of Living Primates. John Wiley and Sons, New York.

Rooney, D.E. 2001. Human cytogenetics constitution analysis. Oxford University Press, Oxford.

Rooney, D.E. and Czepulkowski, B.H. 1986. Human cytogenetics. IRL Press, Oxford.

Roos, C. and Geismann, T. 2001. Molecular phylogeny of the major hylobatid divisions. Mol. Phylogenet. and Evol. 19: 486-494.

Small, M.F. and Stanyon, R. 1985. High-resolution chromosome of rhesus macaque (*Macaca mulatta*) Am. J. of Primatol. 9: 63-67.

Tanomtong, A., Khunsook, S., Kaensa, W. and Bunjonrat, R. 2006. Cytogenetics of long-tailed macaque (*Macaca fascicularis*) in Thailand by conventional staining. KMUTT Resea. and Develop. J. 29(1): 3-15.

Wilson, D.E. and Cole, F.R. 2000. Common Names of Mammals of the World. Smithsonian Institution Press, Washington.