

Standardized karyotype and idiogram of Thai's native cattle, *Bos indicus* (Artiodactyla, Bovidae) by convention staining, G-banding, C-banding and NOR-banding techniques

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ABSTRACT

Cytogenetics of Thai's native cattle (*Bos indicus*) at Rajamangala University of Technology Isan, Surin Campus, was studied. Blood samples were taken from two males and two females. After standard whole blood lymphocyte culture at 37 °C for 72 hours in presence of colchicine, the metaphase spreads were performed on microscopic slide and air-dried. Cytogenetic techniques including conventional staining, G-, C- and NOR-bandings were applied to the chromosomes. The diploid chromosomes number was $2n = 60$, the fundamental numbers (NF) were 61 in male and 62 in female. All autosomes were acrocentric with 18 large, 20 medium and 20 small acrocentric chromosomes. The X chromosome was a large submetacentric and the Y was the smallest acrocentric. From G-banding, each chromosome pair appeared with clearly differentiated. C-banding shown C-positive at centromeric region of all chromosomes. NOR-banding staining for nuclear organizer regions

(NORs) showed 6 NORs represented at telomeric end of chromosomes. The karyotype formula of Thai's native cattle was as follows:

$2n$ (diploid) $60 = L^a_{18} + M^a_{20} + S^a_{20} + \text{sex chromosomes}$

Keywords: Cytogenetics, Thai's native cattle (*Bos indicus*), karyotype

INTRODUCTION

Thai's native cattle or Zebu (*Bos indicus*) is member of order Artiodactyla (even-toed ungulates), family Bovidae. The family Bovidae has only 8 species, 4 genera in Thailand, namely gaur (*Bos gaurus* Smith, 1827), banteng (*Bos javanicus* D'Alton, 1823), cattle (*Bos taurus* Linnaeus, 1758), cattle (*Bos indicus* Linnaeus, 1758), swamp buffalo (*Bubalus bubalis* Linnaeus, 1758), river buffalo (*Bubalus bubalis* Linnaeus, 1758), goat (*Capra hircus* Linnaeus, 1758) and sheep (*Ovis aries* Linnaeus, 1758) (Lekagul and McNeely, 1977, 1988; Par *et al.*, 2003).



Figure 1 The female Thai's native cattle, *Bos indicus* (Artiodactyla, Bovidae).

Miniature Zebu cattle (Fig. 1) are one of the oldest known as cattle breeds dating back to 3,000 BC. Because of that little is known about their early history. But these tiny cows were believed to have come from Southern India and Sri Lanka in Asia. This is a very tough-hardy breed of cattle whose become a wonderful small family pet. At the withers, behind the hump, the height cannot exceed 100 centimeters at 3 years of age. Many are far smaller. Mature cows should weigh 300 to 500 kilograms. Bull from 500 to 600 kilograms. The colors that they come in are: steel gray to nearly white, cream, red, black or spotted and also available in paint colors and brindles. One advantage of the miniature Zebu is that they have better adaptation on heat than most European breeds. They require less space and care as they are extremely hardy and disease resistant. (<http://www.drzoolittle.net/zebuhistory.html>). (Fig. 1)

There are several reports on cytogenetic studies of *Bos indicus* including Wurster and Benirschke (1968); Evan *et al.* (1973); Potter and Upton (1979); James (1986); Mayr and Gruber (1986); Hayes *et al.* (1991) and Di Meo *et al.* (2005). In this work we studied on cytogenetics of Thai's native cattle and compared with previous

reports. The knowledge and information of this study will be useful for conservation of family Bovidae in Thailand.

MATERIALS AND METHODS

Blood samples of Thai's native cattles

The blood samples were collected from two males and two females the Thai's native cattle, kept in Rajamangala University of Technology Isan, Surin Campus, by aseptic technique. The samples were kept in 10 ml vacuum tubes containing heparin to prevent blood clotting and cooled on ice until arriving at the laboratory.

Cell culture and cytological preparation

The lymphocytes were cultured using the whole blood microculture technique from Rooney (2001) while the G-, Q-, and C-staining procedure used were adapted from Campiranon (2003).

Cell culture

The 5 ml of RPMI 1640 medium was prepared with 2% PHA (phytohemagglutinin) as a mitogen and kept in blood culture flasks. A blood sample of 0.5 ml was dropped into a medium bottle,

incubated at 37 °C under 5% of carbon dioxide environment and regularly shaken in the morning and evening. When reaching harvest time at the 72 hr of incubation, colchicine was added, followed by further incubation for 30 minutes.

Cell harvest

The blood sample mixture was centrifuged and the supernatant was discarded. Hypotonic solution (0.075 M KCl) was applied to the pellet for 30 minutes. KCl was discarded, cells were fixed in fresh cool fixative (3 methanol: 1 glacial acetic acid). Air-dried preparation was then made in the conventional manner.

G-banding method

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 sorenson buffer. The slide was stained with 20% Giemsa's solution for 30 min.

C-banding method

Slides were heated at 60 °C for 2-3 days, soaked in 0.2 N HCl for 10-15 min, rinsed with distilled water then soaked in 0.05 N Ba(OH)₂ for 15 min at 37 °C, rinsed with distilled water at 60 °C. After that soaked in 2X SSC at 60 °C for 1-2 hr. The slide was stained with 20% Giemsa's solution for 30 min.

NORs-banding method

Add 2 drops of 50% silver nitrate and 50% gelatin on slides, respectively. Then sealed with cover glasses and incubated at 60 °C for 3 hr. After that soaked in distilled water until cover glasses were separated. The slide was stained with 20% Giemsa's solution for 1 min.

Chromosomal counting, karyotyping and idiogramming

Chromosome counting was performed on mitotic metaphase cells under light microscope. Twenty clearly observable and well-cells spread chromosomes of each male and female were selected and photographed. The length of short arm chromosome (Ls) and the length of long arm chromosome (Ll) were measured and calculated to the length of total arm chromosome (LT, $LT = Ls + Ll$). The relative length (RL), the centromeric index (CI) and standard deviation (SD) of RL and CI were estimated. CI was also computed to classify the types of chromosomes according to Chaiyasut (1989). All parameters were used in karyotyping and idiogramming.

RESULTS AND DISCUSSION

The chromosome number of Thai's native cattle is $2n$ (diploid) = 60 (Fig 2, 3). This is the same chromosome number for the cattle (*Bos indicus*) as reported in previous studies (Wurster and Benirschke, 1968; Evan *et al.*, 1973; Potter and Upton, 1979; James, 1986; Mayr and Gruber, 1986; Hayes *et al.*, 1991; Di Meo *et al.*, 2005). The fundamental number (NF, number of chromosome arms) was 61 in male and 62 in female, which is same as the report of Wurster and Benirschke (1968); Mayr and Gruber (1986) and Popescu *et al.* (1996) that demonstrated the telocentric chromosome Y (has only one leg arm; account for 1) of male, while those of female shown the submetacentric chromosome X (has both of long and short arms; account for 2). According to the chromosome characteristics of other in family Bovidae, gaur ($2n = 58$, NF = 58), banteng ($2n = 60$, NF = 58), cattle, *B. taurus* ($2n = 60$, NF = 58), swamp buffalo ($2n = 48$, NF = 56), river buffalo ($2n = 50$, NF = 58), goat ($2n = 60$, NF = 58) and sheep ($2n = 54$, NF = 58), all

of it has the range of $2n = 48-60$ and most have $NF = 58$ except in swamp buffalo that has $NF = 56$ which its chromosome pair 1 derived from tandem fusion of chromosome pair 4 and 9 of river buffalo (Di Berardino and Iannuzzi, 1981).

All autosomes of Thai's native cattle are acrocentric, classified by size into 18 large, 20 medium and 20 small chromosomes. These features are similar to the report of Wurster and Benirschke (1968); Mayr and Gruber (1986) and Popescu *et al.* (1996). Animals in family Bovidae have many acrocentric chromosome lead to easily chromosome fusion at centromere (Robertsonian translocations). Robertsonian translocations have been well

documented in domestic cattle, with cases reported in numerous breeds (Popescu, 1984). Although various chromosomes have been shown to be involved in translocations (14/20, Logue and Harvey, 1978; 14/24, Di Berardino *et al.*, 1979; 16/20, Rubes *et al.*, 1996; 16/18, Iannuzzi *et al.*, 1993; 13/19, Molteni *et al.*, 1998; 1/21, Tateno *et al.*, 1994; 15/25, Iannuzzi *et al.*, 1992; 21/27, Berland *et al.*, 1988), the most commonly occurring fusion involves chromosomes 1 and 29, [rob(1;29)] has been found in more than 60 different cattle breeds (Popescu and Pech, 1991).

The sex chromosome of this study, the X was large submetacentric and the Y was the smallest

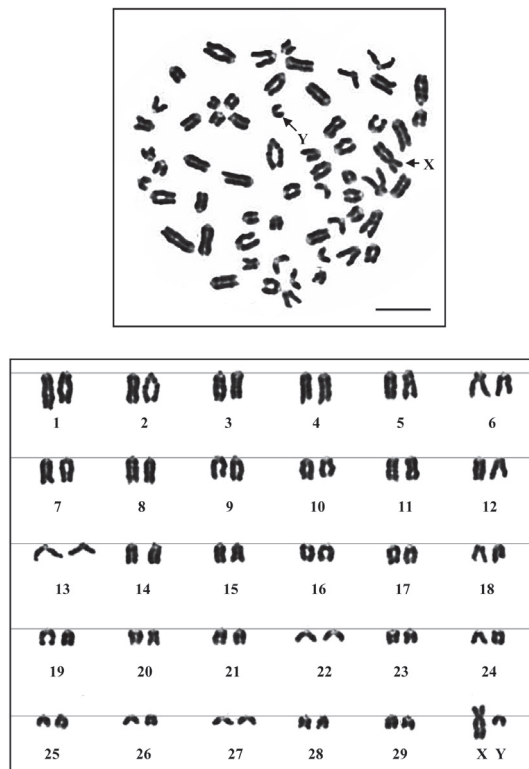


Figure 2 Metaphase chromosome plate (top) and karyotype (bottom) of male Thai's native cattle (*Bos indicus*) $2n$ (diploid) = 60 by conventional staining technique, showing sex chromosomes (arrows), scale bars = 10 μ m.

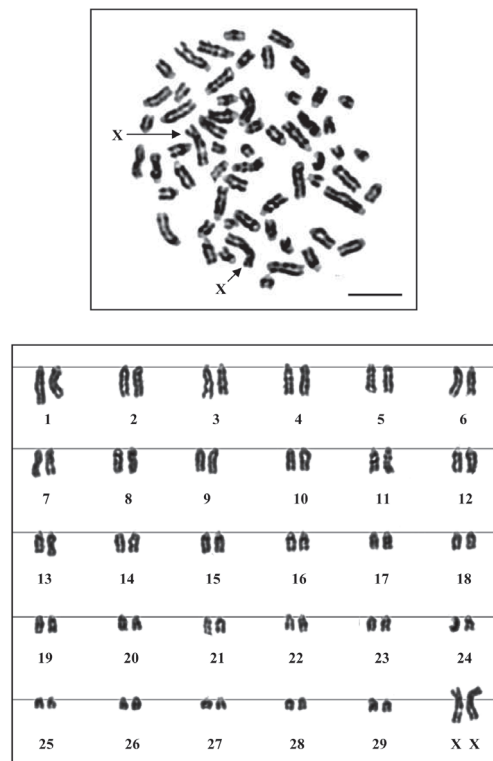


Figure 3 Metaphase chromosome plate (top) and karyotype (bottom) of female Thai's native cattle (*Bos indicus*) $2n$ (diploid) = 60 by conventional staining technique, showing sex chromosomes (arrows), scale bars=10 μ m.

acrocentric. These features are similar to the report of Wurster and Benirschke (1968); Mayr and Gruber (1986) and Popescu *et al.* (1996) that revealed Thai's native cattles have submetacentric X chromosome and acrocentric Y chromosome. In comparison with the other ruminant species in the genus *Bos* in Thailand, the X chromosomes of gaur (*B. gaurus*), banteng (*B. javanicus*) and cattle (*Bos taurus*) are submetacentric chromosome and the Y chromosome of all those species are metacentric, submetacentric and submetacentric chromosome, respectively (Wurster and Benirschke, 1968).

G-banding provide a clearly chromosome band which represent in dark and light regions on chromosome. The level of G-banding technique (band numbers) is defined as its visible and in haploid set which compose of autosomes, X and Y chromosome (Yunis, 1976). Thus, the haploid set of Thai's native cattle is consist of 29 autosomes include X and Y chromosome. However, some chromosome can not clearly identify because of the variable of some bands according to Figure 4 and 5. The chromosome band scoring is representing by approximate band that can observe. This result demonstrated that the chromosome band number of Thai's native cattle from G-banding technique of metaphase chromosome is 435 bands (Fig. 4, 5, 10). Moreover, Yunis and Prakash (1982) reported that chromosome band number from high-resolution technique of prometaphase chromosome of human and ape are over 1,000 bands per haploid set.

C-banding demonstrated dark bands (C-positive) on all centromeric region of autosomes, the representative of constitutive heterochromatin. However, there is no dark band (light or C-negative) on the X and the Y chromosome (Figs 6, 7). According to the report of Stranzinger *et al.* (2007)

which study on the polymorphism of chromosome Y in various breeds of cattle (*Bos taurus*) in Switzerland that shown the negative X chromosome from C-banding and the positive short arm of Y chromosome. For the present study, there is no heterochromatin on chromosome Y of Thai's *Bos indicus* that may influence from the variation of chromosome Y heterochromatin or chromosome Y heterochromatin polymorphism which exist in human that reported by Rooney (2001). The dark bands those appear by C-banding technique are obviously arises on centromeres, telomeres and some parts of its regions (Campiranon, 2003).

The six nucleolar organizer regions (NORs) were located on the long arm near telomere of the pair autosomes. In contrast, Mayr and Gruber (1986) indicated that NORs of the cattle (*B. indicus*) appear on eight positions of the long arm of the pair autosomes 2, 3, 4 and 28. Di Berardino and Iannuzzi (1981) reported that NORs of genus *Bubalus* in Thailand such as swamp buffalo (*B. bubalis*) and river buffalo (*B. bubalis*), were located on long arms of the pair autosomes 4, 8, 20, 22, 23 and 3, 4, 8, 21, 23, 24 respectively.

Metaphase cells and karyotypes of Thai's native cattle in male and female by conventional staining, G-, C- and NORs-bandings are shown in Figure 2, 3, 4, 5, 6, 7 and 8. The chromosomes length in centimeters of twenty cells (males and females) in mitotic metaphase was measured. The mean length of short arm chromosome (Ls), length of long arm chromosome (Ll), total length of arm chromosome (LT), relative length (RL), centromeric index (CI), standard deviation (SD) of RL, CI, size and type of presented in Table 1. The idiogram of Thai's native cattle shows gradually decreasing length of the autosomes and sex chromosomes (Fig. 9, 10, 11).

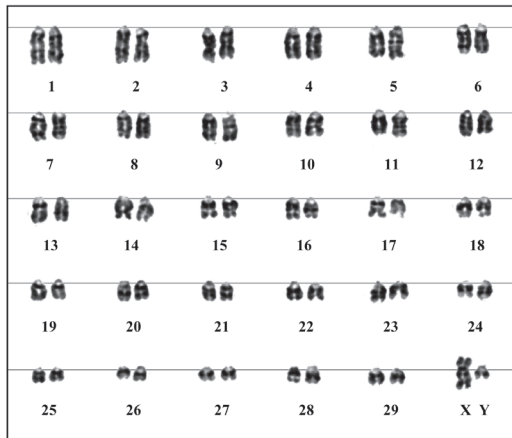
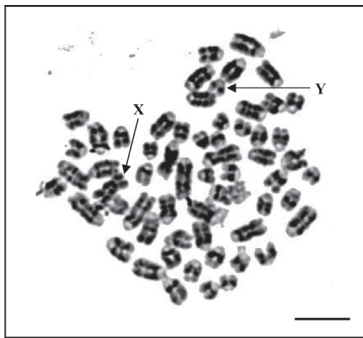


Figure 4 Metaphase chromosome plate (top) and karyotype (bottom) of male Thai's native cattle (*Bos indicus*) $2n$ (diploid) = 60 by G-banding technique, showing sex chromosomes (arrows), scale bars = 10 μm .

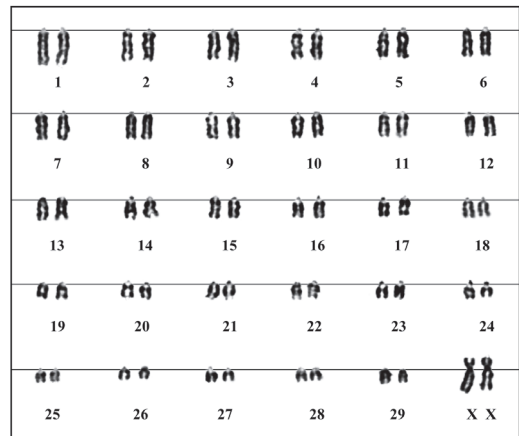
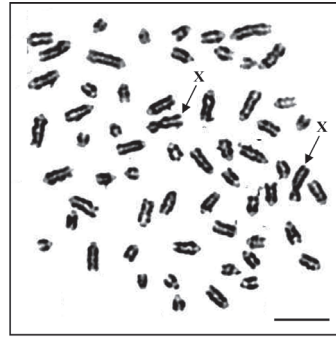


Figure 5 Metaphase chromosome plate (top) and karyotype (bottom) of female Thai's native cattle (*Bos indicus*) $2n$ (diploid) = 60 by G-banding technique, showing sex chromosomes (arrows), scale bars = 10 μm .

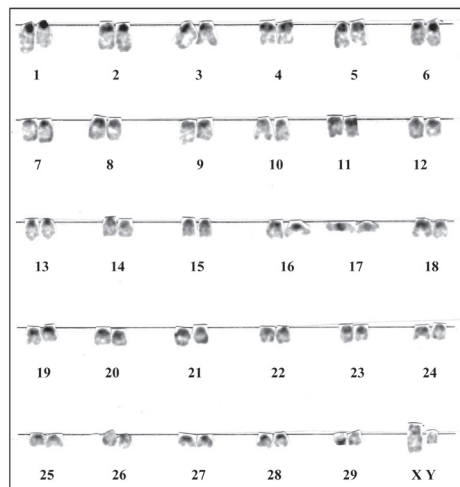
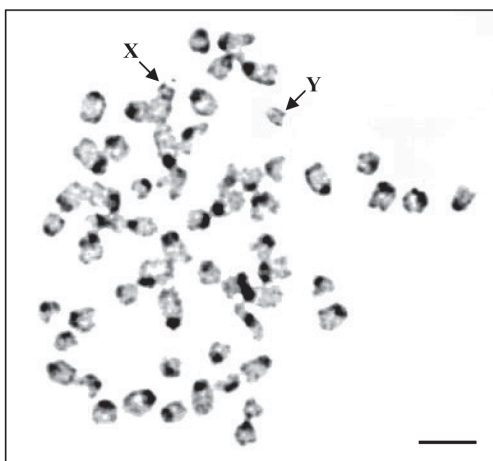


Figure 6 Metaphase chromosome plate (top) and karyotype (bottom) of male Thai's native cattle (*Bos indicus*) $2n$ (diploid) = 60 by C-banding technique, showing sex chromosomes (arrows), scale bars = 10 μm .

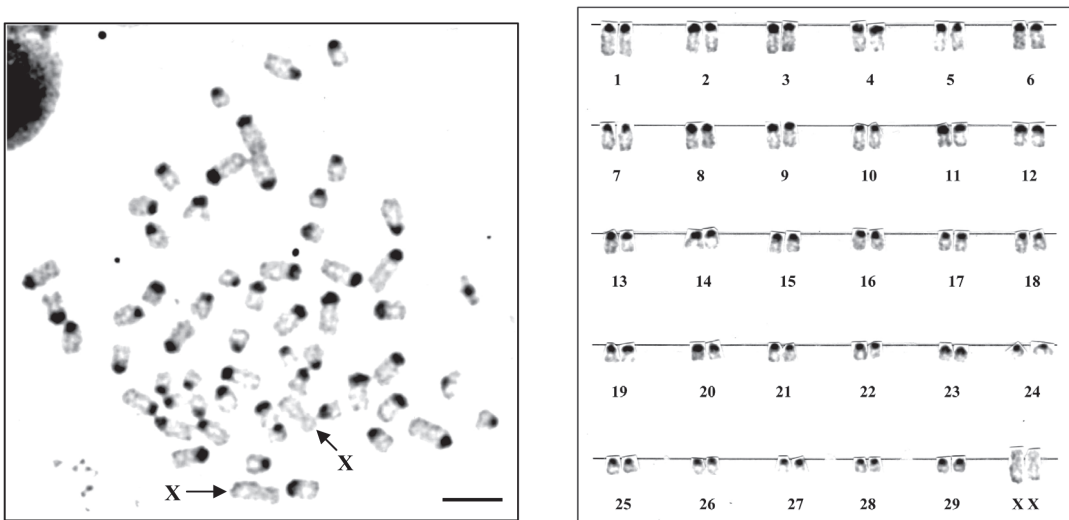


Figure 7 Metaphase chromosome plate (top) and karyotype (bottom) of female Thai's native cattle (*Bos indicus*) $2n$ (diploid) = 60 by C-banding technique, showing sex chromosomes (arrows), scale bars = 10 μ m.

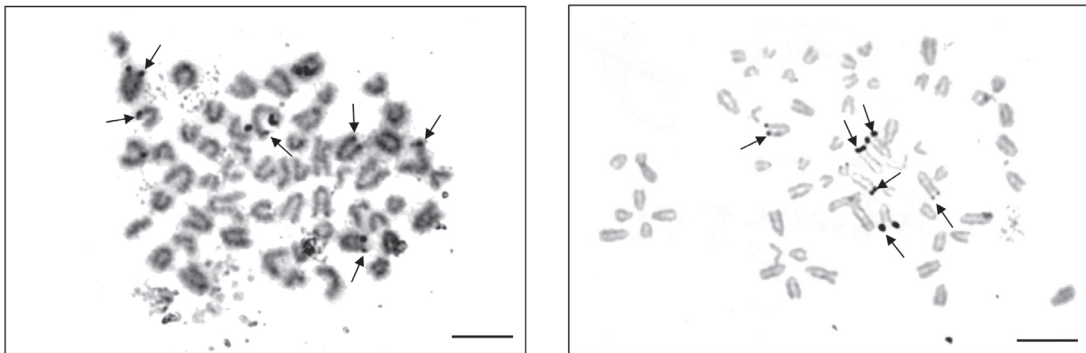


Figure 8 Metaphase chromosome plate of male (top) and female (bottom) Thai's native cattle (*Bos indicus*) $2n$ (diploid) = 60 by NOR-banding technique, showing satellite chromosomes (arrows), scale bars = 10 μ m.

The Thai's native cattle revealed that the chromosome marker is the chromosome pair 1, which is the largest acrocentric chromosome. According to the Table 1, the chromosome pair 1 is the longest chromosome. The important chromosome marker of Thai's native cattle is the asymmetrical karyotype, which is all two types of

chromosomes were found (submetacentric and acrocentric chromosome). The largest and smallest chromosomes show difference size (approximately 4 folds by calculation asymmetrical karyotype from Table 1). The karyotype formula of Thai's native cattle was as follows:

$$2n \text{ (diploid) } 60 = L_{18}^a + M_{20}^a + S_{20}^a + \text{sex chromosomes}$$

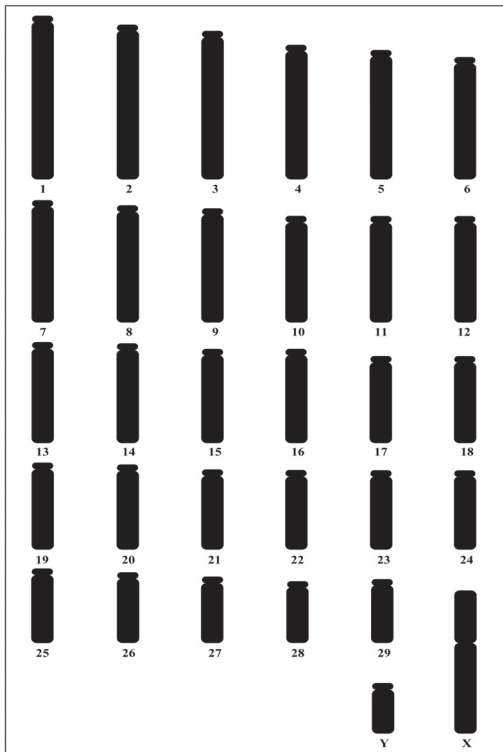


Figure 9 Idiogram of Thai's native cattle (*Bos indicus*) $2n = 60$ by conventional staining technique.

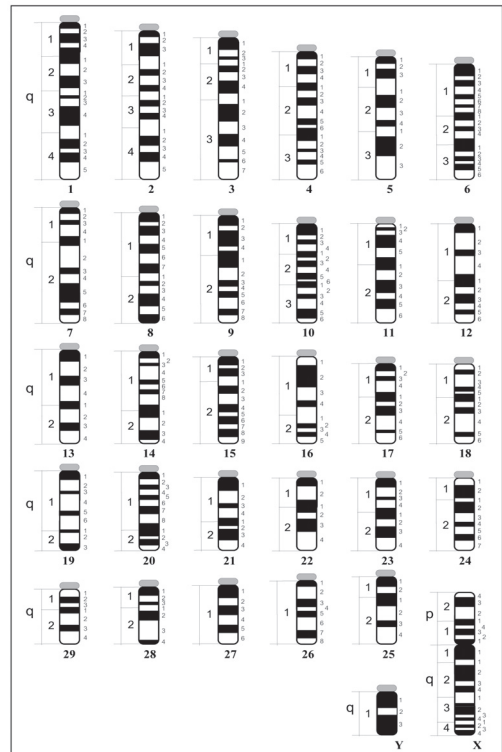


Figure 10 Idiogram of Thai's native cattle (*Bos indicus*) $2n = 60$ by G-banding technique.

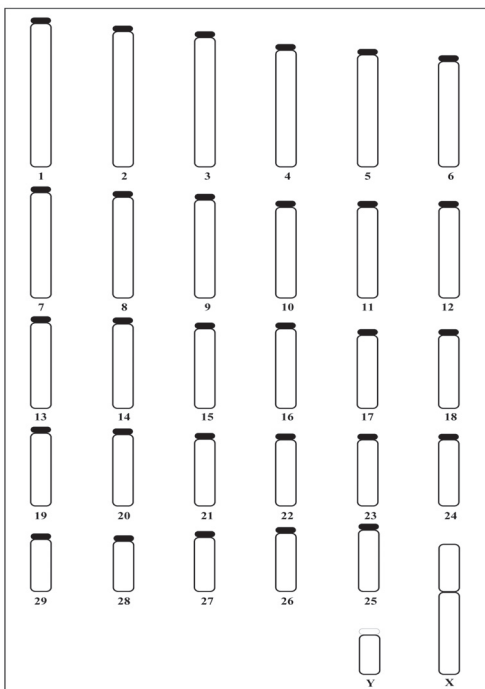


Figure 11 Idiogram of Thai's native cattle (*Bos indicus*) $2n = 60$ by C-banding technique.

Table 1 Mean of the short arm chromosome length (Ls), the long arm chromosome length (Ll), total arm chromosome length (LT), relative length (RL), centromeric index (CI), chromosome size and chromosome type from metaphase chromosomes of 20 cells in male and female the Thai's native cattle (*Bos indicus*), $2n$ (diploid) = 60.

Chromosome Pair	Ls (cm)	Ll (cm)	LT (cm)	RL	CI	Size	Type
1	0.00	1.62	1.62	0.052	1.000	L	a
2	0.00	1.50	1.50	0.048	1.000	L	a
3	0.00	1.42	1.42	0.045	1.000	L	a
4	0.00	1.36	1.36	0.043	1.000	L	a
5	0.00	1.31	1.31	0.042	1.000	L	a
6	0.00	1.25	1.25	0.040	1.000	L	a
7	0.00	1.21	1.21	0.039	1.000	L	a
8	0.00	1.17	1.17	0.038	1.000	L	a
9	0.00	1.13	1.13	0.036	1.000	L	a
10	0.00	1.09	1.09	0.035	1.000	M	a
11	0.00	1.06	1.06	0.034	1.000	M	a
12	0.00	1.02	1.02	0.033	1.000	M	a
13	0.00	0.98	0.98	0.031	1.000	M	a
14	0.00	0.94	0.94	0.030	1.000	M	a
15	0.00	0.90	0.90	0.029	1.000	M	a
16	0.00	0.87	0.87	0.028	1.000	M	a
17	0.00	0.83	0.83	0.026	1.000	M	a
18	0.00	0.80	0.80	0.026	1.000	M	a
19	0.00	0.78	0.78	0.025	1.000	M	a
20	0.00	0.75	0.75	0.024	1.000	S	a
21	0.00	0.75	0.75	0.024	1.000	S	a
22	0.00	0.69	0.69	0.022	1.000	S	a
23	0.00	0.66	0.66	0.021	1.000	S	a
24	0.00	0.62	0.62	0.020	1.000	S	a
25	0.00	0.49	0.49	0.016	1.000	S	a
26	0.00	0.53	0.53	0.017	1.000	S	a
27	0.00	0.57	0.57	0.019	1.000	S	a
28	0.00	0.60	0.60	0.019	1.000	S	a
29	0.00	0.63	0.63	0.020	1.000	S	a
X	0.57	1.04	1.61	0.051	0.643	L	sm
Y	0.00	0.41	0.41	0.013	1.000	S	a

Notes: L = large chromosome, M = medium chromosome, S = small chromosome, a = acrocentric chromosome, and sm = submetacentric chromosome.

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