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SEX-LINKED AMELOGENIN AND Y-LINKED DBY GENE SEQUENCE VARIATIONS IN *BOS GAURUS*

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SEX-LINKED AMELOGENIN AND Y-LINKED DBY GENE SEQUENCE VARIATIONS IN *BOS GAURUS*

BY

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A project report submitted to Faculty of Agriculture, Universiti Putra Malaysia, in fulfillment of the requirement of SHW 4999 (Final Year Project) for the award of degree of Bachelor of Agriculture (Animal Science)

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CERTIFICATION

This project entitled “Sex-linked Amelogenin and Y-linked DBY gene Sequence Variations in *Bos gaurus*” is prepared by Mok Pei Chyi and submitted to the Faculty of Agriculture in fulfillment of the requirements of the course SHW 4999 (Final Year Project) for the award of the degree of Bachelor of Agriculture (Animal Science).

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LIST OF ABBREVIATIONS

AMEL	amelogenin gene
bp	base pair
DBY	Y-linked DEAD Box Helicase 3
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleotide triphosphate
EtBr	ethidium bromide
g	gram
IUCN	International Union for Conservation of Nature and Natural Resources
KK	Kedah Kelantan cattle
$MgCl_2$	magnesium chloride
mL	milliliter
mM	millimole
NCBI	National Center for Biotechnology Information
ng	nanogram
PBS	phosphate buffer saline
PCR	polymerase chain reaction
rpm	revolutions per minute
SRY	sex determining region Y
TBE	Tris borate EDTA
UV	ultraviolet

V	volt
°C	degree Celcius
µL	microliter
µM	micromole

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ABSTRACT

The gaur, *Bos gaurus,* is classified as a vulnerable species as the population size is declining globally due to habitat loss, poaching and diseases from domestic cattle. Genetic studies on gaurs in Malaysia are limited; there is none on differentiating the two sexes using molecular markers. Molecular sexing is useful in identifying the gender of an animal, especially from the carcass parts, the meat, tissue and body fluid. This study was conducted to differentiate the male and female *B. gaurus* by the sequence variations of the sex-linked amelogenin gene and the Y-chromosome DEAD Box RNA Helicase 3 (*DBY*) gene. DNA from fecal samples of three male and three female gaurs were amplified using polymerase chain reaction (PCR) for these genes. A region of the amelogenin and *DBY* genes and amplicon sizes were estimated using electrophoresis as well as sequenced. Sequences were compared among themselves and with those of the *B. indicus* Kedah Kelantan (KK) which was used as a reference species. The fragment sizes and sequences for *DBY* did not show any variation between the males and females of both gaur and Kedah Kelantan; a sample DNA fragment of 180 bp was exhibited. *DBY* cannot be used to differentiate the two sexes for both gaur and *B. indicus* cattle. The amelogenin gene showed a single band pattern for the female KK (280 bp) and three bands (217, 280, and 340 bp) for the male KK but did give consistent results for the gaur samples.

VARIASI SEKUENS AMELOGENIN TERANGKAI-JANTINA DAN GEN DBY TERANGKAI-Y IN *BOS GAURUS*

OLEH

MOK PEI CHYI

ABSTRAK

Seladang, *Bos gaurus* diklasifikasikan sebagai spesies terancam disebabkan populasi seladang semakin berkurang pada tahap antarabangsa disebabkan oleh kehilangan tempat habitat, pemburuan haram dan penyakit daripada lembu tempatan. Kajian genetik terhadap seladang di Malaysia adalah terhad; masih tiada kerja yang dijalankan untuk membezakan jantina seladang dengan menggunakan penanda molekul. Penanda molekul berguna dalam mengenalpastikan jantina haiwan terutamanya daripada bahagian bangkai, daging, tisu dan fluid badan mereka. Kajian ini dijalankan untuk membezakan jantan dan betina *Bos gaurus* dengan variasi jujukan DNA dalam gen amelogenin dan gen DEAD Box RNA Helicase 3 (*DBY*). DNA daripada sampel najis tiga ekor seladang jantan dan tiga ekor seladang betina diamplifikasi dengan menggunakan Polymerase Chain Reaction (PCR). Sebahagian gen amelogenin dan *DBY* dan saiz amplikon dianggar dengan menggunakan elektroforesis serta disusun. Sekuens tersebut dibandingkan antara mereka dengan lembu Kedah Kelantan (KK) sebagai spesis rujukan. Analisis sekuens bagi *DBY* tidak mempamerkan variasi bagi kedua-dua jantina seladang ataupun lembu Kedah Kelantan; satu jalur DNA dengan 180 bp dipamerkan. *DBY* tidak boleh digunakan untuk membezakan jantina bagi seladang ataupun lembu spesis *B. indicus*. Gen amelogenin mempamerkan satu jalur bagi betina KK (280bp) dan tiga jalur (217, 280, 340 bp) bagi jantan KK tetapi tidak memberi keputusan yang konsisten pada sampel seladang.

CHAPTER 1

INTRODUCTION

The Gaur, *Bos gaurus*, is a wild member of Bovini tribe. The IUCN Red List of Threatened Species has classified the gaur as a vulnerable species. Traditionally, three subspecies of Gaur were generally recognized, namely, the *B. gaurus gaurus* (the Indian Gaur), *B. gaurus laosiensis* (found in the Indochinese region) and *B. gaurus hubbacki* (Malayan Gaur, also known as Seladang) (Duckworth *et al*., 2008), but currently only two subspecies, the Indian Gaur and Malayan Gaur, are recognized. The Gaur which is considered as the wild cattle differs from the domestic cattle both morphologically and genetically. The gaur and cattle have chromosome complements of 2n = 56 (Maslinda *et al*., 2010) and 2n = 60, respectively. The Malayan gaur is mainly distributed in the tropical woodlands of Peninsular Malaysia and Southern Thailand (Lydekker, 1907). Malayan gaur can be identified by their dark brown coat, lower legs being white to tan in color and weights over 1000 kg in males and 700–800 kg in females.

In Malaysia, the population of the gaur was stated to be less than 500 individuals and it is thought to be declining (Read *et al*., 1994). Several studies indicate that the population size of gaur is declining globally, due to habitat loss, poaching and diseases from domestic cattle (Conry, 1981, 1989; DWNP, 2005; IUCN, 2008; Nguyen *et al*., 2007). An improvement in the management system for genetic conservation via semen collection, and cryopreservation and in situ breeding would help increase the number of gaurs in the wild (Hafiz *et al.*, 2010; Iswadi *et al*., 2010, 2012). In addition, increased research activities on breeding management programs, such as synchronization of estrus, improvement of semen collection, and cryopreservation,

genetic studies and use of assisted reproductive biotechnologies, could be useful in increasing gaur production (Fazly Ann *et al*., 2010, 2011; Hafiz *et al*., 2011, Iswadi *et al*., 2011, 2012; Rosli *et al*., 2011). As with other bovidae, chromosome studies indicate that the gaur evolved from a wild ancestor, differentiating the domestic taurine and zebu cattle (Buckland and Evans, 1978; Da Paixao Scavone *et al*., 2000; Gallagher and Womack, 1992; Iannuzzi *et al*., 2009; Wurster and Benirschke, 1968).

Molecular sexing is one of the methods to identify the gender of an animal, especially from the carcass with anatomical structure not intact, the meat, tissue and body fluid (Khaledi, 2008). In mammals, the amelogenin gene is present on both the X and Y chromosomes. They occur as two single copy genes located on the short arms of the two chromosomes (Nakahori *et al*., 1991). The DEAD Box RNA Helicase 3 gene (DBY or DDX3Y), located in the non-homologous region of the Y chromosome, may serve as a mammalian male sex marker while females are identified by absence of amplification (Lahn and Page, 1997).

Some methods of sex determination for animals rely on the detection of Y-chromosome specific DNA sequences, such as sex determining region Y (SRY) (Mara *et al*., 2004; Yamauchi *et al*., 2000) and testis-specific protein Y-encoded (TSPY) (Lemos *et al*., 2005) genes. However, the failure of detection does not necessarily mean that the sample is of female origin (Pajares *et al*., 2007). Sometimes, experimental errors can also lead to negative results. Thus, the detection of X- and Y-chromosome specific sequences is very important to confirm the gender of the animal and, therefore, advantageous.

1.1 Research Problem

The studies on Gaur only focus on habitat, ecology, threats and conservation strategies. The scientific works in Malaysia are limited. The works reported include semen collection, analysis and cryopreservation (Iswadi *et al*., 2012) and mitochondrial gene analysis (Rosli *et al*., 2011). There is no working done on the differentiating the two sexes using molecular markers. Sex identification by using genomic DNA extracted from fluid sample or tissue sample is an important analytical tool in forensic science and in sexing of embryos.

1.2 Research Hypothesis

The genetic sequences on the males and females of *B. indicus* and *B. gaurus* can be differentiate by the sequence variations of amelogenin gene and the Y-chromosome DBY gene. These sequences will be different from those of the males and females of *B. indicus.*

1.3 Objectives

The general objective of the study was to determine and compare the amelogenin gene and the Y chromosomal DBY gene sequence variations of males and females of *B. gaurus* and *B. indicus* using polymerase chain reaction (PCR) and electrophoresis techniques.

The specific objectives of the study were:

1. To estimate and differentiate the size of the PCR amplified amelogenin gene in the male and female *B. gaurus*.

2. To compare the nucleotide sequences of the amelogenin gene on the X and Y chromosomes.

3. To determine the nucleotide sequences of the Y chromosome DBY gene of the *B. gaurus*.

4. To compare and differentiate the nucleotide sequences variations of the X and Y chromosome amelogenin genes and the Y chromosome DBY gene of *B. gaurus* and *B. indicus.*

REFERENCES

Ahrestani, F.S. (2011). Life-history traits of gaur *Bos gaurus*: a first analysis. *Mammal Review, 41:* 75-84.

Barbosa, A.M. & J. Carranza (2010). Lack of geographic variation in Y-chromosomal introns of red deer (*Cervus elaphus*). *Journal of Negative Results - Ecology & Evolutionary Biology*, 7: 1–4.

Barbosa, A.M., J.L. Fernandez-Garcia & J. Carranza (2009). A new marker for rapid sex identification of red deer (*Cervus Elaphus*). *Hystrix Italian Journal of Mammalogy,* 20(2): 169-172.

Bryja, J. & A. Konecny (2003). Fast sex identification in wild mammals using PCR amplification of the Sry gene. *Folia Zoological*, 52(3): 269–274.

Carlos, F., III. Barbas, D.R. Burton, J.K. Scott & G.J. Silverman (2007). *Quantitation of DNA and RNA. Cold Spring Harbor Protocol,* 2007: doi:10.1101/pdb.ip47.

Chen, C.M., C.L. Hu, C.H. Wang, C.M. Hung, H.K. Wu, K.B. Choo & W.T.K. Cheng (1999). Gender determination in single bovine blastomeres by polymerase chain reaction amplification of sex-specific polymorphic fragments in the amelogenin gene. *Molecular Reproduction and Development,* 54: 209-214.

Deuter, R., S. Pietsch, S. Hertel & O. Muller (1995). A method for preparation of fecal DNA suitable for PCR. *Nucleic Acids Research*, 23.18: 3800-3801.

Dewey, F.E., S. Pan, M.T. Wheeler, S.R. Quake & E.A. Ashley (2012). DNA sequencing: Clinical applications of new DNA sequencing technologies. *Circulation*, 125: 931-944.

Fernando, P. & D.J. Melnick (2001). Molecular sexing eutherian mammals. *Molecular Ecology Notes*, 1: 350-353.

Garibyan, L. & N. Avashia (2013). Polymerase chain reaction. *Journal of Investigative Dermatology,* 133*,* e6. doi:10.1038/jid.2013.1.

Gokulakrishnan, P., R.R. Kumar, B.D. Sharma, S.K. Mendiratta, D. Sharma & O.P. Malav (2012). Sex determination of cattle meat by polymerase chain reaction amplification of the amelogenin (*AMELX*/ *AMELY*) gene. *Veterinary World,* 5.9: 526-529.

Gurgul, A., A. Radko & E. Slota (2009). Characteristics of X- and Y-chromosome specific regions of the amelogenin gene and a PCR-based method for sex identification in red deer (*Cervus Elaphus*). *Molecular Biology Report,* 37: 2915-2918.

Hamilton, C.K., A. Combe, J. Caudle, F.A. Ashkar, A.D. Maaulay, P. Blondin & W.A. King (2011). A novel approach to sexing bovine blastocysts using male-specific gene expression. *Theriogenology,* 77: 1587-1596.

Hellborg, L. (2004). Evolutionary studies of the mammalian Y chromosome. *Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology* 951: 1-55.

Hellborg, L. & H. Ellegren (2002). Y chromosome conserved anchored tagges sequences (YCATS) for the analysis of mammalian male-specific DNA. *Molecular Ecology,* 12: 283-291.

Hellborg, L. & H. Ellegren (2004). Low Levels of Nucleotide Diversity in Mammalian Y Chromosomes. *Molecular Biology and Evolution*, 21(1): 158–163.

Iswadi, M.I., Z.F. Ann, M.M. Hafiz, M.D. Hafiz, F.J. Fahrul, H. Hajarian, H. Wahid, I. Zawawi, M.S. Khairiah & O.A. Mazni (2012). Collection, analysis and cryopreservation of semen from Malayan gaur (*Bos gaurus hubbacki*): A preliminary study. *Open Veterinary Journal, 2*: 109-114.

Iswadi, M.I., Z.F. Ann, M.M. Hafiz, M.D. Hafiz, A.A. Samad, F.J. Fahrul, I. Zawawi, M.S. Khairiah, H. Wahid & O.A. Mazni (2013). Seme Collection: A Malayan gaur bull experience. *Malaysian Journal of Veterinary Research,* 2: 55-59.

Khaledi, K.J., J.M. Panandam, A.A. Maheran & S.S Siraj (2009). Reliable and fast molecular sexing in *Bos indicus* cattle using amelogenin gene primers. *Journal of Applied Animal Research,* 35: 83-85.

Kohn, M.H. & R.K. Wayne (1997). Facts from feces revisited. *Trends in Ecology & Evolution*, 12.6: 223-227.

Malik, H.N., D.K. Singhal, A. Mukherjee, N. Bara, S. Kumar, S. Saugandhika, A.K. Mohanty, J.K. Kaushik, S. Bag, B.C. Das, S.K. Bhanja & D. Malakar (2013). A single blastomere sexing of caprine embryos by simultaneous amplification of sex chromosome-specific sequence of *SRY* and amelogenin genes. *Livestock Science,* 157: 351-357.

Mamat-Hamidi, K., M. Halmi, I. Idris, D. Di Berardino & L. Lannuzzi (2012). Chromosome evolution of the Malayan gaur (*Bos gaurus Hubbacki*). *Caryologia: International Journal of Cytology, Cytosystematics and Cytogenetics*, 65: 34-39.

Nowacka, J., M. Switonski, M. Mackowski, E. Slota, A. Radko, T. Zabek & K. Urbaniak (2004). The ambiguity of freemartinism diagnosis in cattle revealed by cytogenetic and molecular techniques. *Czech Journal of Animal Science,* 49: 239-243.

Parati, K., G. Bongioni, R. Aleandri & A. Galli (2006). Sex ratio determination in bovine semen: A new approach by quantitative real time PCR. *Theriogenology,* 66: 2202-2209.Xhang, B.W., M. Li, L.C. Ma & F.W. Wei (2006). A widely applicable protocol for DNA isolation from fecal samples. *Biochemical Genetics,* 44 (11-12): 503-512.

Patelia, E.M., R. Thakur & J. Patel (2013). Sex Determination using Polymerase Chain Reaction. *Open Access Scientific Reports*, 2: 717 doi: 10.4172/scientificreports.717.

Pfeiffer, I. & B. Brenig (2005). X- and Y-chromosome specific variants of the amelogenin gene allow sex determination in sheep (*Ovies aries*) and European red deer (*Cervus elaphus*). *BioMed Central Genetics*, 6: 16.

Romaino, S.M., Z.A. Fazly-Ann, S.S. Loo, M.M. Hafiz, M.D. Hafiz, M.I. Iswadi, P. Kashiani, M.K.A. Rosli, S.M.F. Syed-Shabthar, B.M. Md-Zain & O. Abas-Mazni (2014). Species identification of Malayan Gaur, Kedah-Kelantan and Bali cattle using polymerase chain reaction-restricted fragment length polymorphism. *Genetic and Molecular Research,* 13.1: 406-414.

Rosli, M.K.A., S.M.F. Syed-Shabthar, P. Abdul-Patah, Z. Abdul-Samad, S.N. Abdul, M.N. Burhanuddin, N.A. Zulkifli, M.N. Shukor, K. Budsabong, S. Changtragoon, T. Sekiguchi, H. Sasaki & B.M. Md-Zain (2014). A new subspecies identification and population study of the Asian small-clawed otter (*Aonyx cinereus*) in Malay Peninsula and Southern Thailand based on fecal DNA method. *The Scientific World Journal,* 14: 11 pages.

Rosli, M.K.A., S.S. Zakaria, S.M.F. Syed-Shabthar, Z.Z. Zainal, M.N. Shukor, M.C. Mahani, O. Abas-Mazni & B.M. Md-Zain (2011). Phylogenetic relationships of Malayan gaur with other species of the genus *Bos* based on cytochrome *b* gene DNA sequences. *Genetics and Molecular Research,* 10(1): 482-493.

Sasaki, S. & H. Shimokawa (1995). The amelogenin gene. *International Journal of Development Biology*, 39: 127-133.

Sekiguchi, T., H. Iida, J. Fukuruma & T. Nishimoto (2004). Human DDX3Y, the Y-encoded isoform of RNA helicase DDX3, rescues a hamster temperature-sensitive ET24 mutant cell line with a DDX3X mutation. *Experimental Cell Research,* 300: 213– 222.

Sembon, S., S. Suzuki, D. Fuchimoto, M. Iwamoto, T. Kawarasaki & A. Onishi (2008). Sex identification of pigs using polymerase chain reaction amplification of the amelogenin gene. *Zygote,* 327-332.

Taberlet, P., S. Griffin, B. Goossens, S. Questiau, V. Manceau, N. Escaravage, L.P. Waits & J. Bouvet (1996). Reliable genotyping of samples with very low DNA quantities using PCR. *Nucleic Acids Research*, 16: 3189–3194.

Yamauchi, K., S. Hamasaki, K. Miyazaki, T. Kikusui, Y. Takeuchi & Y. Mori (2000). Sex Determination Based on Fecal DNA Analysis of the Amelogenin Gene in Sika Deer (*Cervus nippon*). *Journal of Veterinary Medical Science,* 62(6): 669–671.