RESTRICTION DIGEST AND ELECTROPHORETIC ANALYSIS
OF MITOCHONDRIAL GENOME FROM JAMNAPARI GOATS

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Restriction Digest and Electrophoretic Analysis of Mitochondrial Genome from Jamnapari Goats

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This project is submitted in partial fulfillment of the requirements for the degree
Of Bachelor of Science with Honors
( Resource Biotechnology)

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LIST OF ABBREVIATION

% - Percentage
°C - Degree Celsius
A<sub>260</sub> - Absorbance at 260 wavelength
AGE - Agarose Gel Electrophoresis
bp - base pair
BSA - Bovine Serum Albumin
Cyt b - Cytochrome b
ddH<sub>2</sub>O - Deionized distilled water
dH<sub>2</sub>O - Distilled water
DNA - Deoxyribonucleic Acid
dNTPs - Deoxynucleoside triphosphate
EDTA - Ethylenediaminetetraacetic acid
EtBr - Ethidium Bromide
F-primer - Forward primer
g - gram
h - Hour (s)
HCl - Hydrochloric Acid
kb - Kilo base
kbp - Kilo base pair
KHCO<sub>3</sub> - Potassium Bicarbonate
KOAc - Potassium acetate
M - Molar
MgCl<sub>2</sub> - Magnesium Chloride
min - Minute (s)
mL - Milliliter
mM - Milimolar
mtDNA - mitochondrial Deoxyribonucleic Acid
Na<sub>2</sub>EDTA - Disodium ethylenediamine tetraacetate
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Meaning</th>
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<tr>
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<td>Sodium Chloride</td>
</tr>
<tr>
<td>NaOH</td>
<td>Sodium Hydroxide</td>
</tr>
<tr>
<td>NH$_4$Cl</td>
<td>Ammonium chloride</td>
</tr>
<tr>
<td>nm</td>
<td>nanometer</td>
</tr>
<tr>
<td>OD</td>
<td>Optical density</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
</tr>
<tr>
<td>PD</td>
<td>Primer dimer</td>
</tr>
<tr>
<td>pmole</td>
<td>Picomole(s)</td>
</tr>
<tr>
<td>R-primer</td>
<td>Reverse Primer</td>
</tr>
<tr>
<td>RBC</td>
<td>Red Blood Cell</td>
</tr>
<tr>
<td>RE</td>
<td>Restriction Endonuclease</td>
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<tr>
<td>rpm</td>
<td>Rotation per minute</td>
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<tr>
<td>SDS</td>
<td>Sodium Dodecyl Sulfate</td>
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<tr>
<td>$T_a$</td>
<td>Annealing temperature</td>
</tr>
<tr>
<td>TAE</td>
<td>Tris Acetate EDTA</td>
</tr>
<tr>
<td>$T_m$</td>
<td>Melting temperature</td>
</tr>
<tr>
<td>$\mu$g</td>
<td>Microgram</td>
</tr>
<tr>
<td>$\mu$l</td>
<td>Microliter</td>
</tr>
<tr>
<td>UV</td>
<td>Ultra violet</td>
</tr>
<tr>
<td>V</td>
<td>Volts</td>
</tr>
<tr>
<td>w/v</td>
<td>Weight per volume</td>
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Restriction Digest and Electrophoretic Analysis of Mitochondrial Genome from Jamnapari Goats

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ABSTRACT

Jamnapari is one of the domestic goats with a dual purpose whereby it is well known for high milk and meat production. As a preliminary step in establishing commercialization protocols for genotyping of the Jamnapari goats, DNA samples from these sources were tested for their feasibility in producing quality basic data in the lab and to obtain a gross profile of mitochondrial DNA (mtDNA) of Jamnapari goats. Hence, this study used simple strategies such as Restriction Endonucleases (RE) digestion and Agarose Gel Electrophoresis (AGE) to obtain the gross profile of cytochrome b (cyt b) of mtDNA from Jamnapari goat. The mitochondrial cyt b sequences of the goat samples were amplified by PCR using Universal Primer CYT b1 and CYT b2. PCR products of 359-bp were successfully obtained from the cyt b gene of these investigated samples. The differences observed between the investigated samples was detected by restricting the amplified product with RE. Hinf I RE were identified as potential RE to differentiate the investigated goat samples.

Key words: Jamnapari Goat, Mitochondrial DNA, PCR, Cytochrome b, RE, AGE

ABSTRAK

Jamnapari merupakan salah satu kambing ternakan yang terkenal dengan penghasilan kedua-dua susu dan daging yang tinggi. Sebagai langkah awal dalam mewujudkan protocol komersial bagi genotyping Jamnapari, sampel DNA daripada sumber tersebut dikaji untuk menghasilkan data yang berkualiti dan seterusnya menghasilkan profil mitokondria DNA Jamnapari. Jututu dalam kajian ini, strategi asas seperti Restriction Endonukleus (RE) dan Agarose Gel Electrophoresis (AGE) diaplikasikan untuk memperolehi profil cytochrome b (cyt b) mitokondria DNA daripada Jamnapari. Rangkaian nukleotida cyt b mitokondria diampifikasi dengan menggunakan Primer Universal CYT b1 dan CYT b2 melalui PCR yang memberikan produk PCR yang bersaiz 359-bp. Perbezaan antara sampel-sampel yang dikaji dapat dikesan menggunakan RE; Hinf I.

Kata Kunci: Kambing Jamnapari, Mitokondria DNA, PCR, Cytochrome b, RE, AGE
The *Capra Hircus* or domestic goat is known as “poor man’s cow” (MacHugh & Bradley, 2001) as it provides a good source of meat, milk, fiber, and skin. Jamnapari or Jamunapari is one of the domestic goats with a dual purpose whereby it produce both milk and meat. It is a breed of goat originating from India and Pakistan. According to research by Singh (1966), this breed is well known for milk production and probably the highest amongst the Indian goat breeds producing 201 kg/lactation and growth (reviewed in Digpal et al., 2006). However, the breed is showing endangerment trend in its native breeding region, presumably due to degradation of ecosystems and market forces (Gupta, 2000). Indiscriminate crossbreeding, uncontrolled intermixing and geographical overlap lead to the endangerment of breed purity, and potentially important caprine genetic material is being put to risk (Sivaselvam et al., 2009). Therefore, there is always a need for the conservation of livestock diversity and for the characterization of breeds and populations including their genetic differentiation and relationships (Henson, 1995) in order to development an accurate verification system for true breed line. Genetic profiling of these Jamnapari goats carries the important implication in the development of an accurate verification system for true breed line.

As a preliminary step in establishing commercialization protocols for genotyping of the Jamnapari goats, DNA samples from these sources must be tested for their feasibility in producing quality basic data in the lab and to obtain a gross profile of mitochondrial genomic DNA of Jamnapari goats. The general aim of this
study is to obtain a gross profile of mitochondrial genomic *cytochrome b* (*cyt b*) region from Jamnapari goat. Besides that, this study is also aimed to use simple strategies such as Restriction Endonucleases (RE) digestion and Agarose Gel Electrophoresis (AGE) to obtain the gross profile of *cyt b* of mitochondrial DNA (mtDNA) from Jamnapari goat.

MtDNA are widely used in phylogenetic studies as the fragment pattern analyses of mtDNA from several mammalian species have shown that individuals from the same population typically possess very similar mtDNA sequences, while individuals from different populations exhibit some mtDNA sequence divergence (Avise *et al.*, 1983). Animal mtDNA is a small (15-20 kb) circular molecule, which is composed of about 37 genes coding for 22 tRNAs, two rRNAs and 13 mRNAs and it is also consists of coding and non coding region (Desjardins & Morais, 1990). *Cyt b* is one of the 13 protein genes encoded by mtDNA and it is one of the best nine protein that make up Complex III of the mitochondrial oxidative phosphorylation system (Hatefi, 1985).

RE and AGE have been used to demonstrate extensive nucleotide sequence diversity in mtDNA within and between conspecific populations of rodents and other mammals and cleavage of mtDNA samples with a relatively small number of endonucleases provides information concerning the phylogenetic relatedness of individual organisms which cannot be readily obtained by any other type of molecular analysis (Lansman *et al.*, 1981).
1.1 **Objective and Rationale**

The general aim of this study is to obtain a gross profile of mitochondrial genomic DNA from Jamnapari goat by using simple strategies such as RE digestion and AGE on mtDNA from Jamnapari goat.

The specific objectives are:

i. To collect and prepare the hair and blood samples from Jamnapari goat before the isolation of total genomic DNA take place.

ii. To isolate total genomic DNA of Jamnapari goat by using phenol-chloroform method and salting out method

iii. To amplify *cytochrome b* gene from the total genomic DNA by using PCR

iv. To digest *cytochrome b* amplicons of Jamnapari goat by using several RE.

v. To detect the restriction digestion patterns of *cytochrome b* of mtDNA of Jamnapari goat by using AGE
CHAPTER 2

LITERATURE REVIEW

2.1 Jamnapari goat

India has 20 well-defined breeds of domestic goats (Capra hircus), representing a broad spectrum of genetic variability. It is known that within each Indian goat breed, the variation in phenotypes and production levels is mainly because of extensive management under low input, with little selection being practiced by the farmers (Ahlawat & Gupta, 2004). The genetic variability of native breeds is important for conserving valuable and unique genetic resources which may be applied to new productive demands.

The Jamunapari or Jamnapari goat is named after an area beyond the river Jamuna (Yamuna) where the breed is mainly found in the undulated land of Chakarnagar between the ravines of the Jamuna and Chambal rivers in the Etawah district (26.8° north latitude and 79.3° east longitude) of the Indian state of Uttar Pradesh (Acharya, 1982). It is a dual purpose milk and meat goat breed. The Indian Jamunapari goat is one of the ancestors of the American Nubian (Digpal et al., 2006). They were derived from crossing Jamunapari from India and Egyptian Zaraibi with native English goats, when they arrived in England on merchant boats and hence, this produced the Anglo-Nubian breed.

There are many features and characteristics that can be distinguished Jamnapari with other goats. Singh (1966), stated that the predominantly white-haired Jamunapari goat is probably the tallest and most handsome among the Indian goat
breeds and the other distinguishing feature of the breed is the presence of a thick
growth of long hair on the hindquarters (reviewed in Digpal et al., 2006). They also
have a large body, long and wide pendulous ears, a pronounced Roman nose, parrot
mouth, short and flat horns and the breed is known as prolific and non-seasonal
breeder (Devendra, 1985). The females possess large udders with big teats and are
well known for milk production because they produce the highest amounts of milk of
all Indian goat breeds, milk yield ranging from 1.13 to 3.63 kg d-1 or about 200 kg
per lactation (Acharya, 1982, Rout et al., 2004) and growth (Devendra, 1985).
Variation in milk yield has been reported to be associated with management
differences as well as variability in genetic makeup of the Jamnapari goats (Azevedo
et al., 1994). According to Singh (1966), Jamnapari are said to be hardy, active and
thrive well under rough village condition (reviewed in Digpal et al., 2006).

Therefore, it will be a great loss if this unique breed of goats is lost since the
concern for the preservation and improvement of this breed under field conditions is
very few. However, there is a worldwide recognition of the need for the conservation
of livestock diversity and for characterization of breeds and populations including
their genetic differentiation and relationships (Henson, 1995). These unique
characteristics are the result of evolutionary forces and their interactions over longer
periods of time. However, its small population of approximately less than 5000
(Acharya, 1982; Nivsarkar et al., 1996) makes it further vulnerable to the various
forces of genetic change and thus, modifying the basic genetic structure of the breed.

Hence, an investigation of genetic variation within the breed, and its structure
may help to assess these factors, and provide information to be used for conservation
and improvement of this goat. Therefore, genetic profiling of this commercial goat
breed carries the important implication in the development of an accurate verification
system for true breed line and thus, the maintenance of traits.

Many studies regarding Jamnapari goat have been carried out and presented. These include the genotyping studies of the goat. Attempts have been made in microsatellite (Ganai & Yadav, 2001; Behl et al., 2003; Kumar et al., 2005) and mitochondrial markers (Joshi et al., 2004) to detect genetic variation within and among three Indian breeds of goat as part of their conservation.

According to Ganai and Yadav (2001), the mean number of alleles observed in three Indian goat breeds using heterologous 16 cattle microsatellite markers are 4.12 (Sirohi), 4.00 (Jamnapari) and 3.37 (Barbari). From the study, a phylogenetic tree constructed from inter-individual distances revealed that the individuals clustered according to the breed to which they belonged, and the Jamnapari and Barbari goats formed a cluster. Other than that, it has been revealed that the divergence times between Sirohi and Jamnapari, and Sirohi and Barbari were approximately 2000 years, while its value between Barbari and Jamnapari goats was approximately 1370 years.

2.2 Mitochondrial DNA and Cytochrome b Gene

Animal mtDNA is a small, approximately 15kb to 20 kb circular molecule, composed of about 37 genes coding for 22 tRNAs, two rRNAs and 13 mRNAs, and the latter codes for proteins mainly involved in the electron transport and oxidative phosphorylation of the mitochondrial (Desjardins & Morais, 1990). Mitochondrial DNA contains highly informative polymorphic sites and its simple maternal inheritance without recombination makes it useful for population studies in many organisms (Manceau et al., 1999; Luikart et al., 2001). Apart from that, mtDNA does
not recombine, although some evidence of recombination events has recently been reported (Hatefi, 1985). A worldwide survey of domestic goat mtDNA diversity have been carried out by Luikart et al., (2001) and have identified three major mtDNA lineages. Loci in the mitochondrial genome among mammals are more strictly conserved.

**Figure 1: Diagram of Typical Mitochondrial DNA and location of Cytochrome b gene**

Source: http://www.clinsci.org/cs/107/0355/cs1070355f01.gif

Cytochrome b is localized in mitochondrial genome of mammals and it is the only cytochrome coded by mtDNA. Other than that, According to Esposti et al., (1993), cyt b is one of the cytochromes involved in the electron transport in the respiratory chain of mitochondria and it contains eight transmembrane helices connected by intramembrane or extramembrane domains (reviewed in Meyer, 1994).
Besides that, cyt b is present in much high copy number compared to nuclear DNA. This makes it a very useful tool for phylogenetic analysis (Meyer, 1994). The detection of cyt b gene is applied as an identifying marker. Although it evolves slowly in terms of non-synonymous substitutions, the rate of evolution in silent positions is relatively fast (Irwin et al., 1991). The wide use of cyt b has created a status as a universal metric, in the sense that studies can be easily compared (Edward & Arctande, 1997). Other than that, cyt b is thought to be variable enough for population level questions, and conserved enough for clarifying deeper phylogenetic relationships and it is a highly applicable DNA marker for molecular identification of species (Esposti et al., 1993). The locus of cyt b has been well characterized among different vertebrate groups (Irwin et al., 1991). The cyt b gene has been used in numerous studies of phylogenetic relationships within mammals, and it is the gene for which the most sequence information from different mammalian species is available (Irwin et al., 1991). Hence, the sequence variability of cyt b makes it most useful for the comparison of species in the same genus or the same family and for addressing general questions on intra-specific diversity (Meyer, 1994). Although the use of cyt b has some consequences such as cyt b gene is under strong evolutionary constraints as some parts of the gene are more conserved than others due to functional restrictions, but it is still could somehow be the best choice for resolving relatively recent evolutionary history (Castresana, 2001)

So far, cyt b has been the most prevalent source of sequence data in avian studies and it have been successfully used to identify taxonomic groups even at subspecies level (reviewed in Castresana, 2001)
2.3 **Universal Primer CYT b1 and CYT b2**

Primers of wide range of reactivity are very useful to amplify DNA from an unknown origin. Korcher *et al.*, (1989) identified universal primers CYT b1 and CYT b2 which consistently amplified a fragment of 359 bp of the *cyt b* gene. Hence, a number of studies have adopted the universal primers introduced by Kocher *et al.*, (1989). According to Kocher *et al.*, (1989), by employing universal primers, it eliminates the requirement for an internal control, which is otherwise use to monitor the success of DNA amplification.

So far, Universal Primer CYT b1 and CYT b2 have been successfully used in various studies, for an example in differentiating meat from animal origin (Ong *et al.*, 2007)
CHAPTER 3

MATERIALS & METHODS

3.1 Sample Collection

In this study, samples such as hair and blood from 10 Jamnapari goats were collected at random. All the samples were taken from Handalas Farm, Matang in Sarawak. The details of the sample are as stated in Table 2. Blood sample was collected using the vacutainer tube containing EDTA as an anticoagulant. These samples were stored at -20°C while the hair samples were stored in falcon tubes at room temperature till further processing.

Table 2: Details of the sample used

<table>
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<tr>
<td>Hair</td>
<td>L0XX, R411, C26, C25, T453, L387, L457</td>
</tr>
<tr>
<td>Blood</td>
<td>T454, T459, T470</td>
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3.2 Total genomic DNA isolation

DNA extraction was carried out according to the protocol from Ausubel et al., (2001). The materials needed for the genomic DNA extraction are Digestion buffer, Phenol:Chloroform 1:1, Proteinase K, Absolute Ethanol, 70% ethanol, Nuclease free water, 6M Sodium Chloride, 3M Sodium Acetate, Red Blood Cell Lysis (RBC) Buffer, Isopropanol, SE buffer and Sodium dodecyl sulfate (SDS) 10%. All the reagents were prepared prior to the isolation of total genomic DNA (Appendix A).
Hair samples: The collected and stored hair samples were treated identically. DNA was extracted from the samples using Phenol-Cloroform (Figure 2) and Salting Out method (Figure 3). Around 50 hairs from each individual goat were processed by careful excision of hair samples (approximately 1cm lengths) that contained the root end by using scissors which has been sterilized with 70% ethanol. The hair segments containing the root end were placed in a microcentrifuge tube and 500µl of digestion buffer with 25 µl of Proteinase K was added. The samples were incubated overnight at 55°C or 37°C. After the incubation, equal volume of Phenol-Chloroform 1:1 6M NaCl was added followed by centrifugation at 13000 rpm for 15 minutes. The supernatant were transferred into a fresh eppendorf tube and 1/10 volume of NaOAc with equal volume of absolute ethanol were added. The samples were then centrifuged at 13000 rpm for 15 minutes to pellet the DNA. The supernatant were discarded and the pellet was washed with 1ml of 70% ethanol. Lastly, the pellet were air dried, diluted in 40µl of nuclease free water and stored in -20°C until further processing.

Whole Blood: Whole goat blood was collected by venipuncture and stored in EDTA-coated Vacutainer® tubes. For processing, 300µl of blood was placed in a microcentrifuge tube, and 500µl of RBC Lysis buffer was added. The sample was mixed by inversion and incubated on ice for 30 minutes. The sample was centrifuged at 10000 rpm for 10 minutes at 4°C, and the supernatant was carefully removed and discarded. RBC Lysis Buffer (250µl) was added, and the tube was vortexed for 10 seconds to resuspend the cell pellet. The sample was centrifuged at 10000 rpm for 10 minutes at 4°C, and the supernatant was carefully removed and 200µl of SE-buffer was added. These steps were repeated. Twenty five µl of Proteinase K with 100µl of 10% SDS was then added and the mixture was incubated for 2 hours at 37°C. Then,
100μl of SE buffer with equal volume of phenol-chloroform 1:1 was added. The sample was mixed by inversion and incubated on ice for 5 minutes. The supernatant were transferred into a fresh eppendorf tube and 1/10 volume of 3M NaOAc with equal volume of isopropanol were added. The samples were then inverted gently until the DNA was precipitated. By using a bore tip, the precipitated DNA was carefully captured and transferred into a fresh eppendorf tube. The pellet was washed with 1ml of 70% ethanol. Lastly, the pellet were air dried, diluted in 40μl of nuclease free water and stored in -20°C until further processing.