# CLONING AND CHARACTERIZATION OF BOVINE GROWTH HORMONE (bGH) GENE FROM MALAYSIAN GAUR (*Bos gaurus hubbacki*)

by

MUHAMMAD HAFIZNUR BIN YUNUS

Dissertation submitted in partial fulfillment of the requirement for the degree of Bachelor of Health Sciences (Biomedicine)

October 2008

# CERTIFICATE

This is to certify that the dissertation entitled "CLONING AND CHARACTERIZATION OF BOVINE GROWTH HORMONE (bGH) GENE FROM MALAYSIAN GAUR (*Bos gaurus hubbacki*)" is the bonafide record of research work done by MR. MUHAMMAD HAFIZNUR BIN YUNUS during the period from July 2008 to October 2008 under my supervision.

Supervisor,

.....

Dr. Shaharum Shamsuddin
Lecturer
School of Health Sciences
Universiti Sains Malaysia
Health Campus
16150 Kubang Kerian
Kelantan
Malaysia

Date: 23/12/08

# ACKNOWLEDGMENTS

Alhamdulillah, all praise is to Allah, who has showered His greatest blessings and with that only I manage to complete this study.

First and foremost I wish to express my deepest gratitude to my main supervisor, Dr. Shaharum Shamsuddin who has contributed fruitful ideas, comments, continuous motivation and excellent technical assistance towards the completion of this thesis. I would also like to thank my co-supervisor, Dr. Zafarina Zainuddin for her assistance, guidance and supervision throughout the course of this undergraduate study, especially on the analysing the sequences and bio-informatics part of this study.

I also wish to express my sincere appreciation to the Director General of the Agro-Biotechnology Institute, Malaysia (ABI) for allowing me to use facilities at ABI during parts of the study. Special heartiest appreciation is also extended to all the staffs of ABI and Malaysian Agricultural of Research and Development Institute (MARDI) especially to the Head of Animal Biotechnology Division, ABI: Dr. Abas Mazni Othman. Special thanks for all the research officers involved in the field: Dr. Fazly Ann Zainalabidin, Mr. Asraf Noor Jamaluddin, Hj. Mohd Padzil A. Rahman, Mr. Fahrul Firdaus Jaffar, Ms. Noor Haiza Abdul Majid and Ms. Maslinda Mansor for their assistances and constant encouragements.

Appreciation is extended to Dr. Venugopal Balakrishnan for serving as mentors and helping me during this process of study. Thanks also go to my lab mates and collegues; Mr.

Amiruddin, Ms. Zawani, Hafiz, Hidayu, Wahida, Hidayah, Shobana,Wong and all who gave their support and helping hands that made this study successful.

Finally, thanks to my beloved father, Yunus Same'an, my mother, Rusidah Abdullah and all my families for love, encouragement and support in completing this study.

# TABLE OF CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

# LIST OF ABBREVIATIONS

The following abbreviations were used in the text:

| | |
|---|---|
| bGH | bovine growth hormone |
| GH | growth hormone |
| bST | bovine somatotropin |
| IUCN | International Union for Conservation of Nature |
| PCR | polymerase chain reaction |
| bp | base pair |
| °C | degree Celsius |
| µL | microlitre |
| mL | millilitre |
| DNA | deoxyribonucleic acid |
| nm | nanometer |
| OD | optical density |
| NCBI | National Centre for Biotechnology Information |
| URL | Uniform Resource Locator |
| mM | millimolar |
| (w/v) | weight per volume |
| V | Volts |
| LB | Luria-Bertani |
| g | gram |

| | |
|---|---|
| M | Molar |
| *E. coli* | *Escherichia coli* |
| BLAST | Basic Local Alignment Search Tool |
| E-value | Expected value |
| mRNA | messenger ribonucleic acid |
| cds | complete coding sequence |
| α | alpha |
| ω | omega |

**CLONING AND CHARACTERIZATION OF BOVINE GROWTH HORMONE (bGH) GENE FROM MALAYSIAN GAUR (*Bos gaurus hubbacki*)**

# ABSTRACT

Total genomic DNA from Malaysian gaur (*Bos gaurus hubbacki*) was isolated from whole blood. The genomic DNA was then subjected to PCR amplification using specific primers for bovine growth hormone gene (bGH). This PCR product which represents part of the bGH gene was cloned into pCR® 2.1 TOPO® cloning vector before sent for sequencing analysis. The nucleotide sequence of the gene coding regions was found to be identical with that of GenBank database sequence of *Bos gaurus* growth hormone mRNA sequence except for two nucleotides position (position 101 and 128). The current database of *Bos gaurus* growth hormone mRNA sequence at GenBank showed one unknown nucleotide at position 128 which denoted as N in the sequence. The finding of this study determined that the nucleotide base at position 128 is cytosine (C). The entire cloned gene sequence is 665 bp in length and contains 333 bp coding regions that code for 110 amino acid residue. The *in-silico* restriction mapping site analysis carried out in this study revealed that 70 enzymes produce unique site when they cut the sequence. A comparison of the cloned Malaysian gaur bGH gene with *Bos taurus* (local cattle) growth hormone mRNA sequence also showed a high homology between both sequences.

**PENGKLONAN DAN PERINCIAN PERWATAKAN GEN HORMON PERTUMBUHAN BOVIN DARIPADA GAUR MALAYSIA (*Bos gaurus hubbacki*)**

# ABSTRAK

Keseluruhan DNA genomik daripada gaur Malaysia (*Bos gaurus hubbacki*) telah dipencilkan daripada darah utuh. DNA genomik kemudian diamplikasi melalui PCR dengan menggunakan primer spesifik terhadap gen hormon pertumbuhan bovin (bGH). Produk PCR ini yang mewakili sebahagian gen bGH kemudiannya telah diklon ke dalam vektor pengklonan pCR® 2.1 TOPO® sebelum dihantar untuk analisis penjujukan. Jujukan nukleotida yang mengekodkan kawasan gen tersebut didapati menyerupai dengan jujukan mRNA hormon pertumbuhan *Bos gaurus* daripada pangkalan data GenBank kecuali pada 2 tapak bes iaitu tapak 101 dan tapak 128. Pengkalan data mRNA hormon pertumbuhan *Bos gaurus* terkini daripada GenBank, menunjukkan bahawa nukleotida pada tapak 128 tidak diketahui dan dilabel sebagai N dalam jujukan tersebut. Hasil daripada kajian ini menunjukkan bahawa bes nukleotida pada tapak 128 adalah sitosin (C). Keseluruhan jujukan gen yang diklonkan bersaiz 665 bp dan mengandungi 333 bp bahagian pengekodan yang mengkodkan untuk 110 amino asid. Analisis penyekatan pemetaan tapak secara *in-silico* yang dijalankan menunjukkan 70 enzim yang terhasil menghasilkan tapak yang unik apabila jujukan tersebut dipotong. Perbandingan antara gen bGH gaur Malaysia dengan mRNA hormon pertumbuhan *Bos taurus* (lembu tempatan) juga menunjukkan homologi yang tinggi antara kedua-dua jujukan.

# CHAPTER 1

# INTRODUCTION

## 1.1 Bovine Growth Hormone (bGH)

Growth hormone is a protein of 22000 Dalton single chain polypeptide that synthesized in anterior pituitaries. Its production is tissue specific and occurs predominantly within a specialized subset of cells in the anterior pituitary gland (Woychik *et al.*, 1982). Bovine growth hormone (bGH) which is a growth hormone that produce from anterior pituitary of cattle composed of a single polypeptide of 191 amino acids (Etherton and Bauman, 1998, Keshet *et al.*, 1981). bGH also well-known as bovine somatotropin (bST) and those terms (bGH and bST) are used interchangeably.

bGH is among the first biotechnology products for animal production. bGH is administrated exogenously as to increase the food output (meat or milk) per unit of feed resource input in animal agricultural industry (Etherton and Bauman, 1998). With the advances of technology, the production of large quantities of recombinant bGH for commercially used are now become possible.

## 1.2 Malaysian gaur (*Bos gaurus hubbacki*)

Gaur cattle which is including Malaysian gaur is considered one of the highly endangered wild cattle species (Bongso *et al.*, 1984). The worldwide population of gaur are in decline as there are only a few thousand head still survive in tropical forests and woodlands in India, Indochina and the Peninsular Malaysia which corresponds to the remaining large forested areas (Vadhanakul *et al.*, 2003). In Malaysia, as an effort to protect Malaysian gaur, the wildlife department of Malaysia has started seladang management units at the National Park (Taman Negara) and the Krau game reserve (Bongso *et al.*, 1984).

### 1.2.1 Taxonomy of Malaysian gaur

Gaur cattle belong to the genus *Bos* (subgenus, *Bibovinae*) of the Bovidae family. There marked anatomical, physiological and behavioral differences amongst the members of genus *Bos* (Bongso *et al.*, 1984). Under the genus *Bos*, there has several subgenera, such as the *Bisontinae* (Bison, wisent, yak), *Bubalinae* (Asian and European water buffaloes), *Syncerinae* (African buffalo), *Taurinae* (European and zebu cattle) and the *Bibovinae* (Banteng, gaur, gayal and kouprey) (Bongso *et al.*, 1984). There are three recognized wild subspecies of gaur as reported by Vadhanakul *et al.* (2003) which are the Indian gaur (*Bos gaurus gaurus*), the Indochinese gaur (*Bos gaurus readei* or *Bos gaurus laotiensis*) and the

Malaysian gaur (*Bos gaurus hubbacki*). The abbreviated lineage of Malaysian gaur is shown in Figure 1.1.

| | | |
|---|---|---|
| Superkingdom | : | Eukaryota |
| Kingdom | : | Metazoa (Animalia) |
| Phylum | : | Chordata |
| Subphylum | : | Craniata |
| Class | : | Mammalia |
| Order | : | Cetartiodactyla |
| Suborder | : | Ruminantia |
| Family | : | Bovidae |
| Subfamily | : | Bovinae |
| Genus | : | *Bos* |
| Species | : | *Bos gaurus* |
| Subspecies | : | *Bos gaurus hubbacki* |

Figure 1.1 Taxonomy of Malaysian gaur

### 1.2.2 Characteristic of Malaysian gaur

Malaysian gaur is among the biggest of the bovines. It has muscular body with huge head, and sturdy limbs. There is a shoulder hump which is especially pronounced in adult males. Malaysian gaur also has a narrow dewlap under the chin and between the front legs. Besides, it also has light colored forehead and yellowish or white stockings. It has horns which are crescent shaped, creamy yellow, and taper to a sharp point, which is usually

tipped in black. Malaysian gaur also excretes an oily, aromatic sweat which is unique characteristic to this species. It gives the animals a strong bovine smell and may be an adaptation for keeping away insects. The phenotypic characteristic of gaur is shown as in Figure 1.2.

At the habitat where gaur has not been disturbed, they are basically diurnal, being most active in the morning and late afternoon and resting during the hottest time of the day. However, where population has been interfered by human populations; the gaur has become largely nocturnal, rarely seen in daytime. While gaur is dependent on water for drinking, it does not seem to bathe or wallow.

Naturally, gaur feed on grass and herbs or shrubs such as *Hibiscus lampas*, *Grewia aspera*, *Grewia hirsute*, *Helicteres isora*, *Butea parvifloara* etc. Gaur also browses on leaves, soft twigs of tall shrubs and trees, young bamboo shoots and some fruits. However, at Seladang Conservation Centre, South Jenderak, Pahang (placed where sample was obtained), the seladang mainly feeds on *Erythrina* sp. (pokok dedap), *Trema tomentosa* (daun mengkirai), *Artocarpus heterophyllus* (daun nangka), *Pennisetum purpureum* (daun napier), besides pellet (dedak). Gaur generally drinks at least once a day.

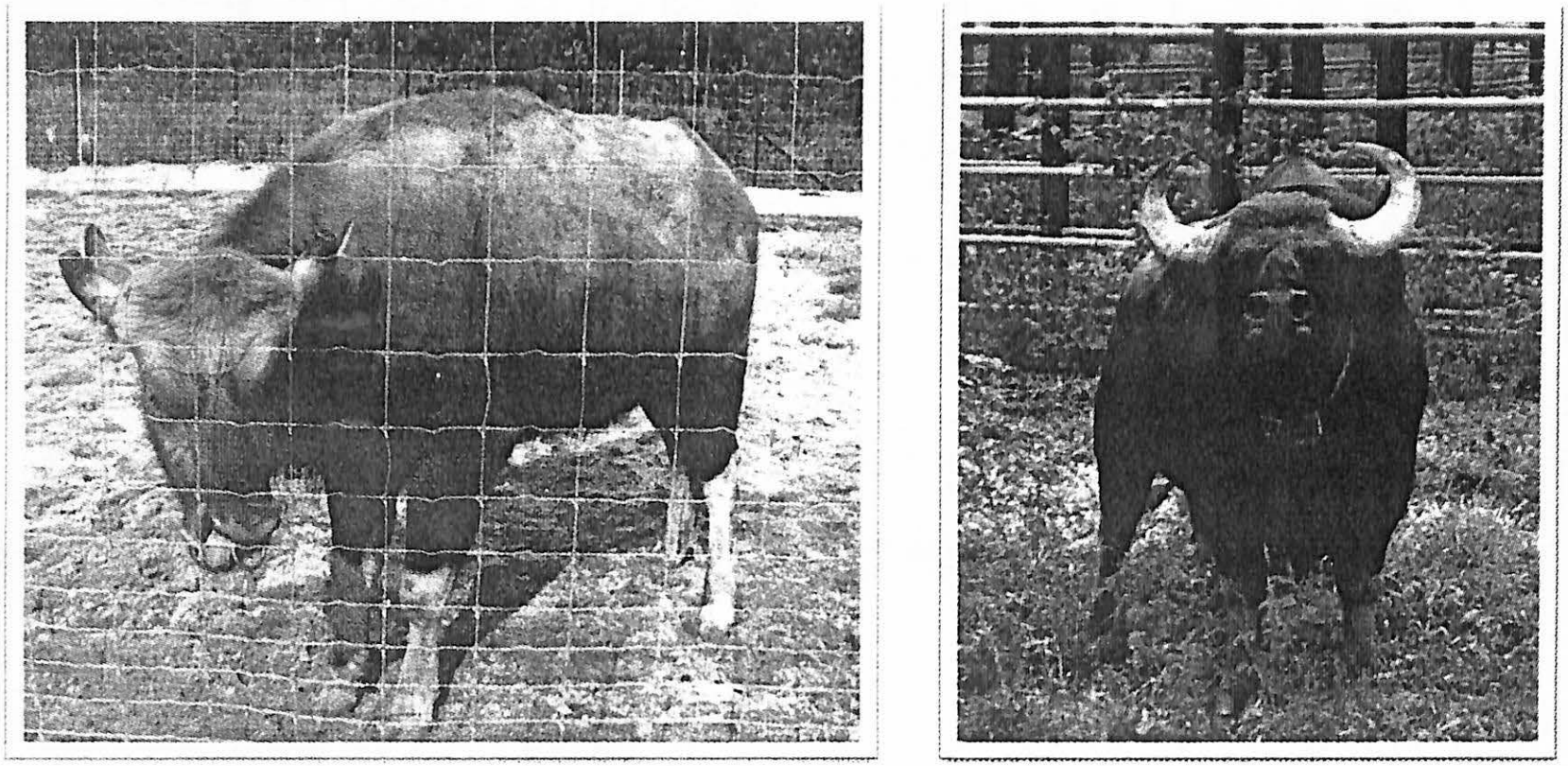

Figure 1.2 Phenotypic of Malaysian gaur at Seladang Conservation Centre, South Jenderak, Pahang.

### 1.2.3 Conservation status of Malaysian gaur

The world wide of gaur population is estimated to lie within 13000 to 30000 animals (IUCN, 2008). A camera-trapping (suitable in methodology and intensity to find gaur if any significant numbers were present) methodology used by Lynam *et al.* (2007) did not record gaur at a fourth sector of Taman Negara in 1999. There was no gaur photographed at all in eight other potential tiger survey areas during 1997–1999 (Lynam *et al.*, 2007). In their study, the areas that were selected are the areas most likely to support tigers but, as tiger-oriented camera-trapping elsewhere in Southeast Asia usually yields photographs of gaur, this study proves that only very low numbers of gaur remain in Malaysia.

Corlett (2007) reported that all the tropical Asian wild cattle are currently threatened by trade-induced hunting activities for meat and trophies. This activity is due to increase in market demand rapidly for trophies such as gaur heads and samba antlers, and meat of ungulates to supply emerging wildlife restaurants that encircle the sanctuary (Steinmetz *et al.*, 2006).

The population of Malaysian gaur has been declining drastically throughout its original habitat due to human disturbances. In Malaysia, the Malaysian gaur or seladang is a fully protected animal under the Wildlife Protection Act No. 76 of 1972.

## 1.3 Objectives of study

The objective of this study is to amplify the bovine growth hormone (bGH) gene from Malaysian gaur (*Bos gaurus hubbacki*) genomic DNA derived from leukocytes by PCR. The amplified bGH product will then be cloned into pCR® 2.1 TOPO® (Invitrogen Inc.) cloning vector. The recombinant plasmid produced will then sent for sequencing to obtain the sequence for the bGH gene. This bGH gene nucleotide sequence will further being analyzed and characterized.

The overview of the study is shown as in Figure 1.3.

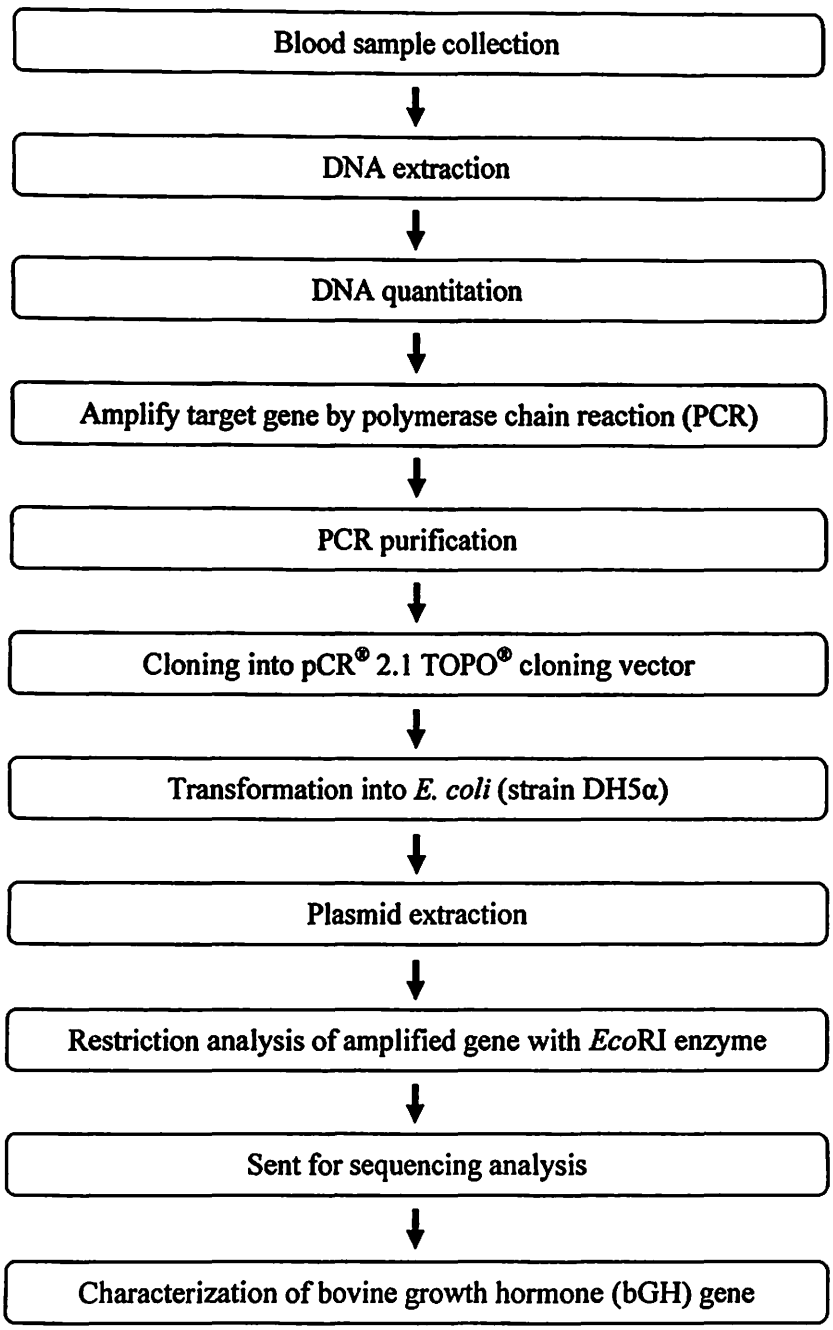


Figure 1.3 Flow chart of the study.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Background of growth hormone

Growth hormone or somatotropin is a protein hormone, that is, a chemical produced by one organ or cell and then transported to another target tissue or organ to cause a biological effect. Growth hormone is produced by the anterior pituitary, which is a small gland located at the base of the brain (Bauman, 1992). From there, it is transported to various body organs by blood where it has biological effects. The secretion of growth hormone is regulated by two well-characterized hypothalamic peptides that act either to stimulate or inhibit release of growth hormone from pituitary gland (Etherton and Bauman, 1998). The peptide that stimulates the secretion of growth hormone is known as growth hormone-releasing factor, while the one which inhibit the secretion is known as somatostatin (Etherton and Bauman, 1998).

Growth hormone has to be administrated by injection method to make it become biologically active as orally administration will not give any effects because it will be broken down to amino acids in the digestive process just like any other dietary protein (Bauman, 1992). This biologically effects of growth hormone can be broadly classified as either somatogenic or metabolic (Etherton and Bauman, 1998). The somatogenic effect of growth hormone is presented by its action of stimulating cell proliferation. For the metabolic effects, growth hormone produces direct action that involve a variety of tissues

and the metabolism of all nutrient classes (Etherton and Bauman, 1998). Growth hormone play a role in the regulation of carbohydrate, lipid (Paladini *et al.*, 1973), protein and minerals metabolism (Etherton and Bauman, 1998).

## 2.2 Characterization of bovine growth hormone (bGH)

A study by Gordon *et al.* (1983) revealed that the bGH gene contains approximately 1793 nucleotides and consists of five exons separated by four intervening sequences. This study has supported the previous study done by Woychik *et al.* (1982) which state that the entire bGH gene is approximately 1800 bp long and contains four intervening sequence. The length of the intervening sequence are 248 bp, 227 bp, 229 bp and 274 bp respectively (Woychik *et al.*, 1982).

A TATAAA sequence is found in the 5' flanking region and probably is involved in facilitating transcription initiation (Gordon *et al.*, 1983, Woychik *et al.*, 1982). This sequence which is similar to the TATA box found in the 5' flanking regions of a number of eukaryotic genes probably functions as part of promoter sequence (Woychik *et al.*, 1982).

The bGH produced by the pituitary gland can either be 190 or 191 amino acids long and there are four variants of bGH produced naturally (Bauman, 1992, Etherton and Bauman, 1998). bGH can have either a leucine or valine substitution at position 127 and an alanine or phenylalanine at the $NH_2$ terminus (Etherton and Bauman, 1998, Miller *et al.*, 1980).