



UNIVERSITI PUTRA MALAYSIA

**INVESTIGATION OF BOVINE GROWTH HORMONE (BGH) GENE
IN BUFALLO BULLS (*BUBALUS BUBALIS*) USING PCR METHOD**

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**INVESTIGATION OF BOVINE GROWTH HORMONE (BGH) GENE
IN BUFALLO BULLS (*BUBALUS BUBALIS*) USING PCR METHOD**

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CERTIFICATION

It is hereby certified that we have read this project paper entitled “Investigation Of Bovine Growth Hormone (bGH) Gene In Bufallo Bulls (Bubalus Bubalis) Using PCR Method” by Harizwan Bin Thomeeran and in our opinion it is satisfactory in terms of scope, quality and presentation as partial fulfillment of the requirement for the course VPD 4999 – Project.

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LIST OF ABBREVIATIONS

bGH	Bovine Growth Hormone
PCR	Polymerase Chain Reaction
DNA	Deoxyribonucleic Acid
bp	Base Pair
FAO	Food and Agriculture Organization
GH	Growth Hormone
EDTA	Ethylenediaminetetraacetic Acid
U.V	Ultraviolet
NCBI	National Center for Biotechnology Information
kDa	Kilodalton
rpm	Revolution per Minute
ml	Milliliter
μ l	Microliter
V	Volt
$^{\circ}$ C	Celsius
%	Percentage

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ABSTRAK

Abstrak kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 – Projek Tahun Akhir.

**SIASATAN TENTANG GENE HORMON PERTUMBUHAN BOVIN
PADA KERBAU JANTAN MENGGUNAKAN KAEDAH PCR**

Oleh

HARIZWAN BIN THOMEERAN

2017

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Kajian ini bertujuan untuk mengkaji keupayaan primers tertentu dalam pengesanan gen hormon pertumbuhan bovin (bGH) dalam kerbau jantan (*Bubalus bubalis*) menggunakan teknik PCR. Kajian ini telah dijalankan pada kerbau kacukan Murrah dan kerbau Sawah jantan yang ditempatkan di Pusat

Pembiakan dan Penyelidikan Kerbau, Jabatan Perkhidmatan Haiwan dan Perusahaan Ternak, Telupid, Sabah. Genomik DNA dari 12 ekor kerbau jantan yang sihat telah diekstrak dan ditingkatkan menggunakan primers yang direka daripada urutan gen hormon pertumbuhan lembu (GH). Kualiti DNA telah diperiksa dengan menjalankan sampel ke dalam 2% agarose gel elektroforesis. Kaedah PCR telah digunakan untuk meningkatkan bahagian hormon pertumbuhan. *GH1*, bahagian 428 bp gen hormon pertumbuhan telah ditingkatkan dengan urutan primer ke hadapan (5'-CGG ACC GTG TCT ATG AGA AGC TGA AG-3 ') dan belakang (5'-GTT CTT GAG CAG CGC GTC GTC A-3'). *GH2*, bahagian 211 bp gen hormon pertumbuhan telah ditingkatkan dengan urutan primer ke hadapan (5'-GCT GCT CCT GAG GGC CCT TC-3 ') dan belakang (5'-CAT GAC CCT CAG GTA CGT CTC G-3'). Keputusan PCR menunjukkan hasil negatif untuk kedua-dua bahagian *GH1* dan *GH2* dan primers yang tidak sepadan boleh menjadi salah satu faktor yang menyumbang. Kajian ini untuk mengenal pasti gen hormon pertumbuhan bovin (bGH) adalah tidak berjaya.

Kata kunci: Kerbau jantan, Hormon pertumbuhan bovin (bGH), PCR

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD 4999 – Final Year Project.

**INVESTIGATION OF BOVINE GROWTH HORMONE GENE IN
BUFALLO BULLS USING PCR METHOD**

By

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2017

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The aim of this study was to investigate the ability of specific primers in detection of the bovine growth hormone (bGH) gene in buffalo bulls (*Bubalus bubalis*) using PCR. The study was carried out in Crossbred Murrah buffalo and Swamp buffalo bulls maintained at Buffalo Breeding and Research Centre, Department of Veterinary Services and Animal Industry, Telupid, Sabah. Genomic DNA from 12 healthy buffalo bulls was extracted and

amplified using primers that were designed from the cattle growth hormone (GH) gene sequences. The DNA quality was checked by running the samples in 2% agarose gel electrophoresis. PCR method was used to amplify the growth hormone fragments. *GH1*, 428 bp fragments of the growth hormone gene was amplified with forward (5'-CGG ACC GTG TCT ATG AGA AGC TGA AG-3') and reverse (5'-GTT CTT GAG CAG CGC GTC GTC A-3') primer sequences. *GH2*, 211 bp fragments of the growth hormone gene was amplified with forward (5'-GCT GCT CCT GAG GGC CCT TC-3') and reverse (5'-CAT GAC CCT CAG GTA CGT CTC G-3') primer sequences. The amplification success for PCR showed negative results for both *GH1* and *GH2* fragments and the mismatch of the primers could be one of the contributing factors. This study to identify the bovine growth hormone (bGH) gene was unsuccessful.

Keywords: *Buffalo bulls, Bovine growth hormone (bGH), PCR*

1.0 INTRODUCTION

Buffalo provides mechanical power, meat and milk for human. The ability as draught power in wet condition can be utilized especially in agriculture. In Peninsular Malaysia, there was effort to use the buffalo as draught to carry the harvested oil palm fruit bunches in the early 80's. However, the interest in sustaining buffaloes in these oil palm plantations died out due to change in company policy and the work was taken over by small tractors (Liang *et al.*, 1985). Buffaloes are known to be better at converting poor-quality roughage into milk and meat. They are reported to have a 5% higher digestibility of crude fibre than high-yielding cows; and a 4-5% higher efficiency of utilization of metabolic energy for milk production (Mudgal, 1988).

Buffalo population has increased and is estimated to be approximately 177.247 million, spread through some 42 countries of which 171 million (97%) of them are found in Asia, while approximately 5.38 million (3%) are found in rest of the world (FAO, 2008). The buffalo population is distributed in major buffalo producing countries in Asia; therefore, Asian buffaloes dominate the world population, representing 96.4% of the worldwide population of 180.70 million as of 2008 (FAO, 2010).

The growth hormone genes are some of the genetic markers that are usually selected because of their biological significance on the quantitative traits of interest. Information of diversity using molecular approaches at the Malaysian buffalo population is still very rare. Potentially, the genetic marker-assisted selection can enhance progress in economic traits. Hence, the objective of this study was to identify

the bovine growth hormone (bGH) gene in buffalo bulls (*Bubalus bubalis*) using PCR technique.

1.1 HYPOTHESIS

The primers used are able to identify the bovine growth hormone (bGH) which can act as potential markers for selection of buffalo bulls in breeding programs.

1.2 OBJECTIVE

To investigate the ability of specific primers in detection of the bovine growth hormone (bGH) gene in buffalo bulls (*Bubalus bubalis*) using PCR technique.

2.0 LITERATURE REVIEW

2.1 Water Buffaloes (*Bubalus bubalis*)

Domestic water buffalo (*Bubalus bubalis*) is classified into swamp and river or dairy type (Murrah, Nilli Ravi breeds). The total population of buffaloes in Malaysia in 2004 is 138,098 heads and 95% being of swamp type. Over the past two decades, there has been an alarming decline in the buffalo population in Malaysia with an average rate of population decline of 1.2 percent per year (Abas Mazni *et al.*, 2006). This decline has been attributed to the displacement of buffalo by machinery for draught power in the rice fields, a low reproduction rate and a high extraction rate (FAO, 2005).

2.2 Growth hormone (GH)

Growth hormone (GH) is an anabolic hormone synthesized and secreted by cells of lobe somatotroph in the anterior pituitary (Ayuk and Sheppard, 2006). Growth hormone (GH) is necessary for tissue growth and fat metabolism, thus, it has an important role in reproduction, lactation and normal body growth (Beauchemin *et al.*, 2006, Curi *et al.*, 2006 and Yardibi *et al.*, 2009). Because of these important biological roles, GH is considered a promising candidate gene for improving milk and meat production in cattle and buffalo through marker-assisted selection programs (Othman *et al.*, 2012).

The bovine growth hormone is a 22 kDa single-chain polypeptide hormone produced in the anterior pituitary gland. The encoding gene is approximately 1800 bp and consists of five exons and four introns (Zhou *et al.*, 2005 and Sadeghi *et al.*, 2008). Growth hormone gene has been assigned to chromosome region 19q26 in bovine genome (Hediger *et al.*, 1990).

3.0 MATERIALS AND METHOD

3.1 Genomic DNA extraction

The total numbers of blood samples were taken from 12 buffalo bulls that were maintained at the Buffalo Breeding and Research centre, Department of Veterinary Services and Animal Industry, Telupid, Sabah. The blood samples were collected from the coccygeal vein of each animal in a sterile 10ml polypropylene vial containing 0.5 M EDTA as anticoagulant. The EDTA tubes were centrifuged at 3,000 rpm for 5 minutes and the buffy coats were obtained from the blood. The buffy coats then transferred to 1.5 ml microcentrifuge tube and were accomplished by reserving them at -20°C. Genomic DNA was extracted from the samples with GeneJET™ Genomic DNA purification Kit (Thermo Scientific, USA).

3.2 Polymerase chain reaction (PCR)

Amplification of bovine growth hormone (bGH) gene fragments was done by using polymerase chain reaction (PCR) method. *GH1*, 428 bp fragments of the growth hormone gene was amplified with forward (5'-CGG ACC GTG TCT ATG AGA AGC TGA AG-3') and reverse (5'-GTT CTT GAG CAG CGC GTC GTC A-3') primer sequences. *GH2*, 211 bp fragments of the growth hormone gene was amplified with forward (5'-GCT GCT CCT GAG GGC CCT TC-3') and reverse (5'-CAT GAC CCT CAG GTA CGT CTC G-3') primer sequences according to Reis *et al.* (2001) and Balogh *et al.* (2009) (**Table 1**). The PCR was performed in a 25 µl reaction mixture

containing 6 μ l PCR master mix, 5 μ l template genomic DNA, 2 μ l *GH1* and *GH2* primers, and 10 μ l nucleus free water.

3.3 Gel electrophoresis and documentation

The PCR products were electrophoresed on 2% agarose gel, stained with RedSafe™ at constant voltage (80V) for 50 minutes. The DNA quality check also was done with the same method as the PCR products (**Figure 1**). The result was visualized under UV light using Gel Doc™ system.

Table 1 The sequences and information of primers used in this study

Primers	Sequences 5'-----3'	PCR conditions (35 cycles)	PCR product size
<i>GH1</i>	CGGACCGTGTCTATGAGAAGCTGAAG GTTCTTGAGCAGCGCGTCGTCA	94°C 1 min 53°C 2 min 72°C 2 min	428 bp
<i>GH2</i>	GCTGCTCCTGAGGGCCCTTC CATGACCCTCAGGTACGTCTCG	94°C 1 min 62°C 2 min 72°C 2 min	211 bp

References: Reis et al. (2001) and Balogh et al. (2009)

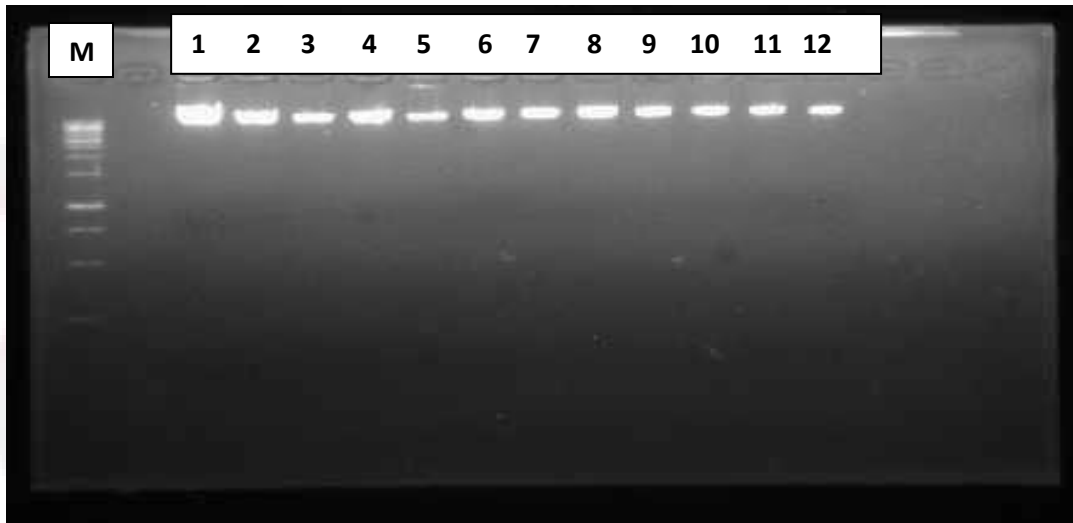


Figure 1: DNA quality check using 2% agarose gel electrophoresis. Lane M DNA ladder (1kb). Lane 1-12 DNA samples.

4.0 RESULTS

4.1 PCR product of bGH amplified using *GHI* primers.

The PCR products of bGH gene with size of 428 bp, amplified with primer *GHI*. The product was electrophoresed on 2% agarose gel stained with RedSafe™ at constant 80V for 50 minutes. Lane M shows DNA ladder (1kb). PCR was visualized under UV light using Gel Doc™ system (Figure 2).

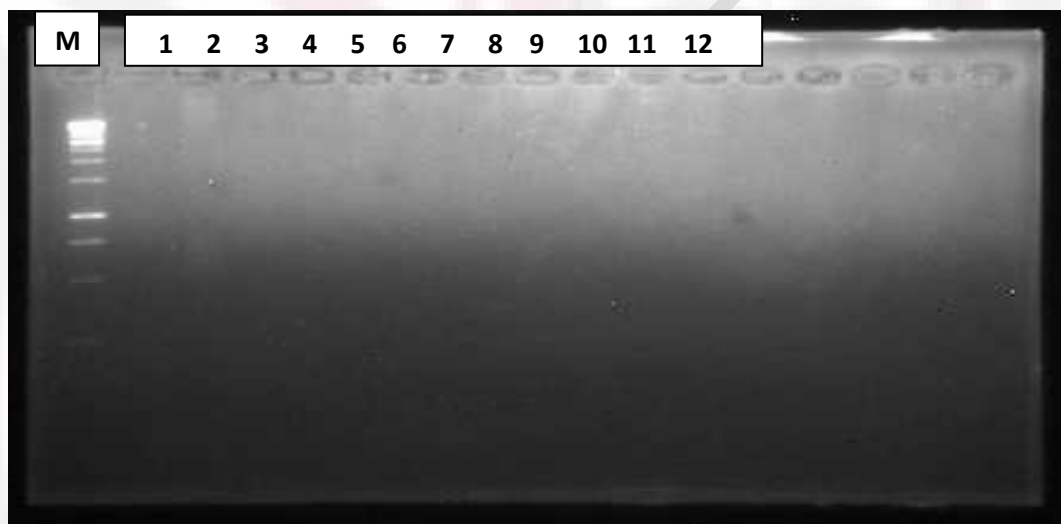


Figure 2: The PCR products of bGH with size of 428 bp, amplified using *GHI* primer. Lane M DNA ladder (1kb). Lane 1-12 PCR product for each DNA samples.

4.2 PCR product of bGH amplified using *GH2* primers.

The PCR products of bGH gene with size of 211 bp, amplified with primer *GH2*. The product was electrophoresed on 2% agarose gel stained with RedSafe™ at constant 80V for 50 minutes. Lane M shows DNA ladder (1kb). PCR was visualized under U.V light using Gel Doc™ system (**Figure 3**).

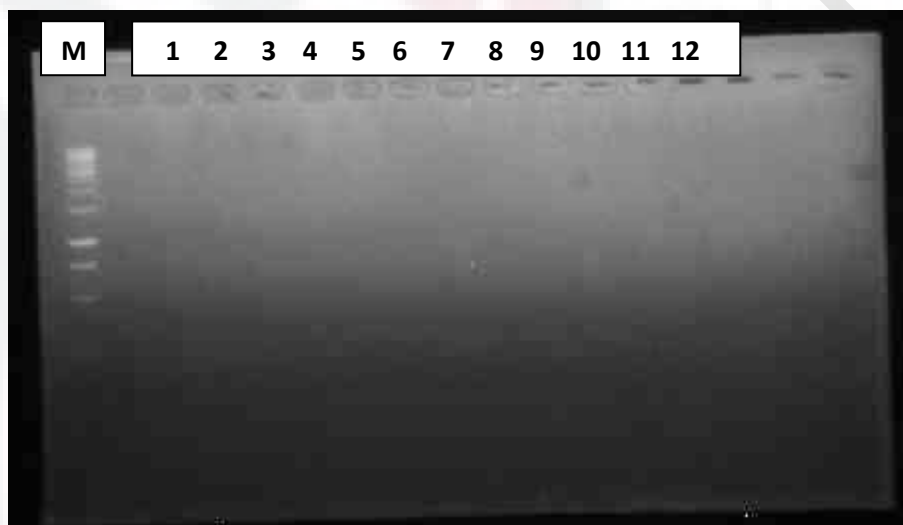


Figure 3: The PCR products of bGH with size of 211 bp, amplified using *GH2* primer. Lane M DNA ladder (1kb). Lane 1-12 PCR product for each DNA samples.

5.0 DISCUSSION

In this study, we failed to amplify the bovine growth hormone (bGH) gene using both *GH1* and *GH2* primers. This might be due to several factors including poor DNA extraction and purification, PCR protocol optimization, and ability of the primers to amplify the growth hormone fragments (Gonçalves-de-Albuquerque *et al.*, 2014).

DNA quality check can be done by using ultraviolet spectrophotometry and also using standard gel electrophoresis. DNA concentration and purity can be checked using ultraviolet spectrophotometry. However, in this study the DNA quality only be checked by using gel electrophoresis. The gel electrophoresis was done using 2% agarose gel stained with RedSafe™ and 1kb ladder. The results showed that the DNA length were more than 1kb for all extracted DNA samples (**Figure 1**).

The cycle conditions were changed to optimize the annealing temperature for both *GH1* and *GH2* primers. In theory, the difference in the guanine and cytosine (GC) contents of primers used could interfere in the annealing temperatures for each primer pair and eventually affect the PCR performance (Gonçalves-de-Albuquerque *et al.*, 2014). In this study, different annealing temperatures were performed in the Eppendorf Mastercycler gradient (Eppendorf AG, Germany) as in Table 2. However, the optimum annealing temperature were failed to be detected (Figure 4 and Figure 5). The denaturation and extension temperature were maintained at 94°C and 72°C respectively and the cycles were maintained at 35 cycles.

Table 2: Different annealing temperature gradient (Eppendorf Mastercycler gradient (Eppendorf AG, Germany) for *GHI* primers.

Temperature range: 52°C - 57°C	
Column	Temperature
1	51.1°C
4	52.2°C
5	53.0°C
7	54.6°C
9	56.1°C
11	57.0°C



Figure 4: Gradient PCR with range of temperature from 52°C to 57°C. Lane M DNA ladder (1kb). Lane 1, 4, 5, 7, 9, 11 columns with different temperature.

Table 3: Different annealing temperature gradient (Eppendorf Mastercycler gradient (Eppendorf AG, Germany) for *GH2* primers.

Temperature range: 59°C - 64°C	
Column	Temperature
1	59.1°C
4	60.0°C
6	61.3°C
7	62.0°C
9	63.3°C
11	64.0°C



Figure 5: Gradient PCR with range of temperature from 59°C to 64°C. Lane M DNA ladder (1kb). Lane 1, 4, 6, 7, 9, 11 columns with different temperature.

In this study, we have checked the primers compatibility using analysis tool web services from the EMBL-EBI (Clustal Omega, UK). The primers used in this study (*GH1* and *GH2*) were compared with the DNA sequences of *Bubalus bubalis* gene encoding growth hormone available in GenBank (accession: AJ011533) retrieved from NCBI in FASTA format. Although the primers used were already published as reported in Reis *et al.* (2001) and Balogh *et al.* (2009), however the results showed that the primers were mismatched as in Figure 6 (*GH1*) and Figure 7 (*GH2*).

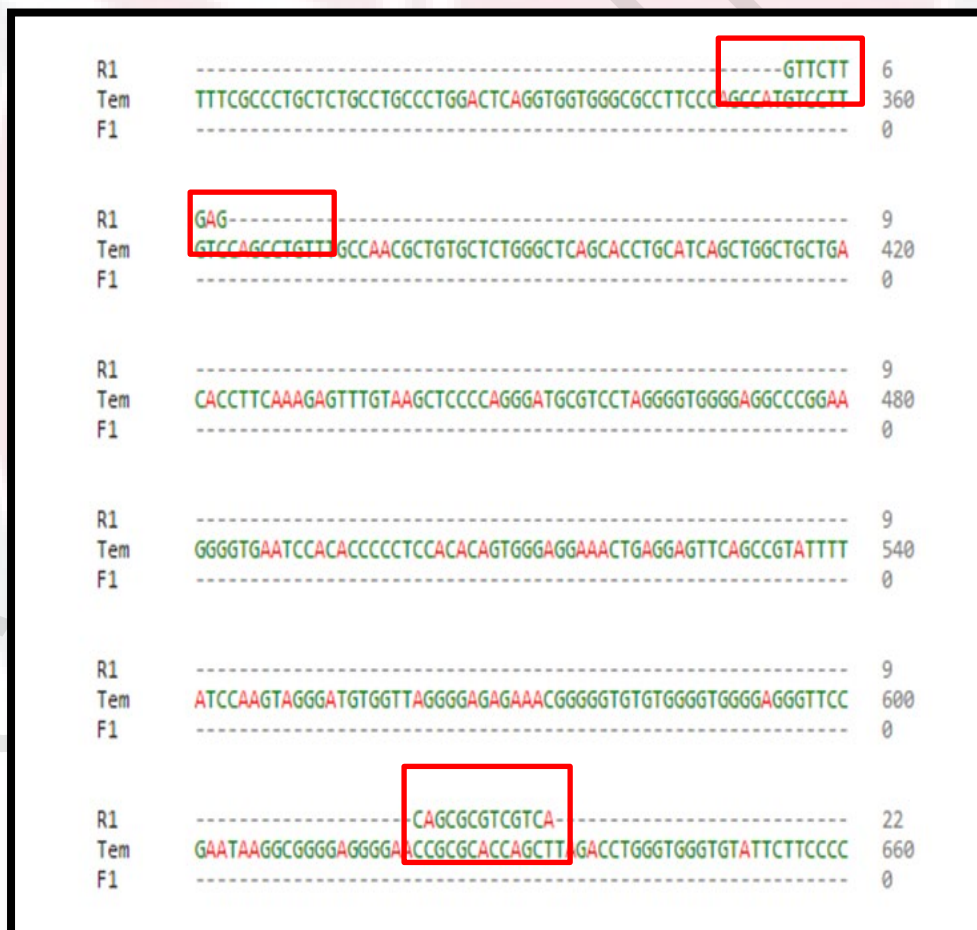


Figure 6: Compatibility check for GH1 primer with DNA sequences of bGH for buffalo from GenBank (accession: AJ011533). R1 for reverse primer. Temp for DNA template. F1 for forward primer. Region of mismatched.

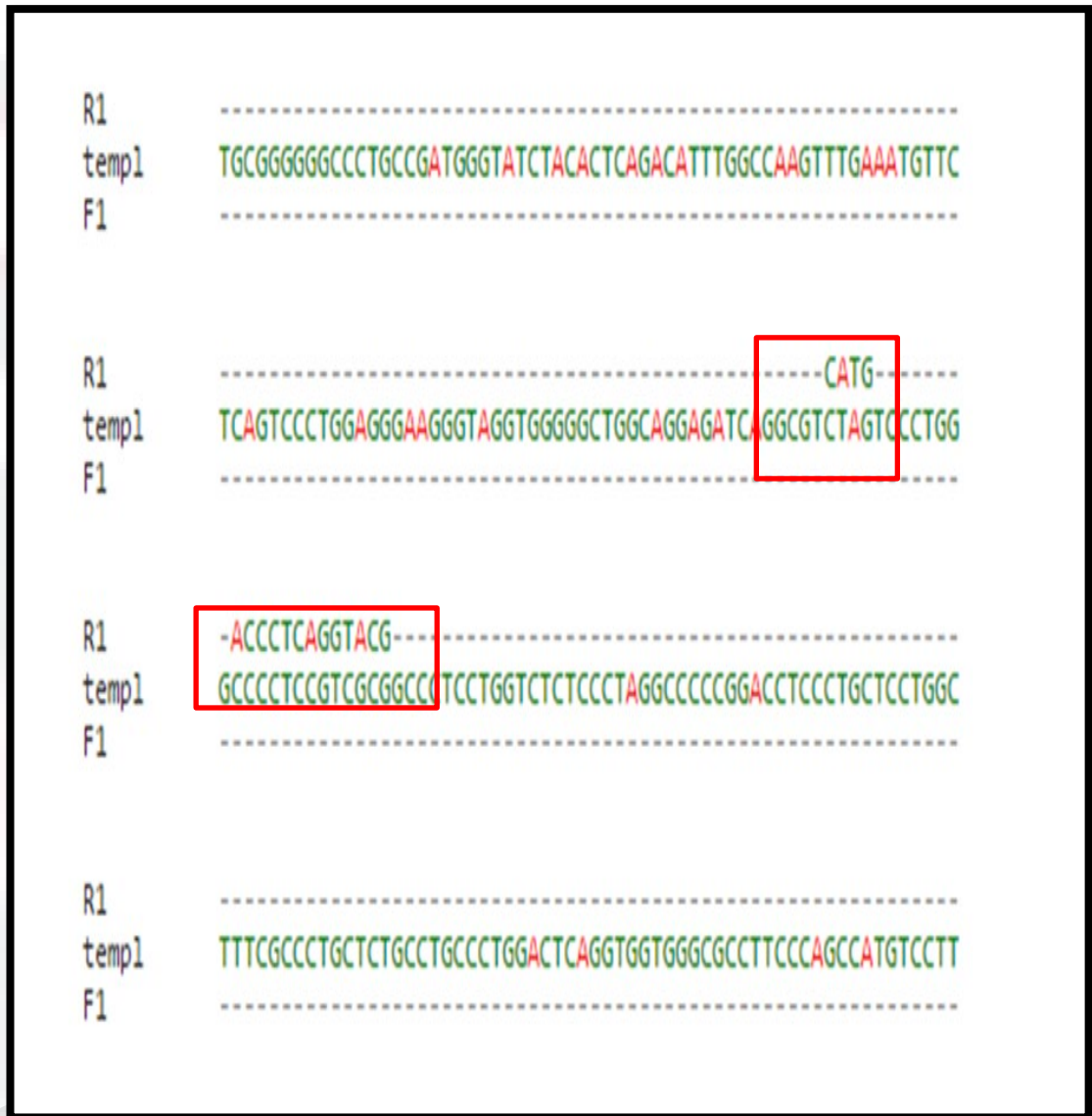


Figure 7: Compatibility check for *GH2* primer with DNA sequences of bGH for buffalo from GenBank (accession: AJ011533). R1 for reverse primer. Temp for DNA template. F1 for forward primer. Region of mismatched.

6.0 CONCLUSION

In conclusion, in this study, the bovine growth hormone (bGH) gene could not be identified. It is recommended to design new sets of primer using software primer designing tool from NCBI primer BLAST.

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