Amplification and Sequencing Growth Hormone Genes in the Nurseri Center for PO Cattle on Balai Besar Inseminasi Buatan (BBIB) Singosari and Unit Perusahaan Aliansi (UPA) Pasuruan

Mariana Rengkuan[A], Aloysius Duran Corebima[B], Sutiman Bambang Sumitro[C], Mohamad Amin[B]
[A]Department of Biology, Manado State University, North Celebes, Indonesia ine_rengkuan@yahoo.com
[B]Department of Biology, Malang State University, East Java, Indonesia
durancorebima@yahoo.com, rizalamin@yahoo.com
[C]Department of Biology, Brawijaya University, East Java, Indonesia
sutiman@ub.ac.id

Abstract

This study aims to amplify the bovine growth hormone gene and learn the sequence of bases as well as the homology of growth hormone gene from the gene bank. This is important because as a Indonesia local cattle in this study will be a sperm-producing parental stock which will then be used by the farmers through insemination techniques. Related to our research goal, DNA sampels of 3 superior bull PO at BBIB Singosari, East Java and 10 PO cow of the parental stock at UPA Pasuruan, East Java had been analyzed. DNA isolation had been conducted by salting out technique on the total samples, followed by PCR-sequecing procedure. The process of amplification of bovine growth hormone gene carried by one pair of primer. The process of DNA amplification by PCR generating 329 bp long. Results showed that correct amplicon sequencing is the bovine growth hormone gene showed 99% homology with the GH gene Bos indicus Breed Butane that has been published in GenBank.
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Keywords: PO cattle, growth hormone gene, PCR, sequencing

INTRODUCTION

In recent times, it was developed the use of molecular technology in various field of science, for example field of animal husbandry in case of increasing of the productivity of PO Cattle. Related to the increasing of production of the meat, it is needed a gen-level study which control the growth characteristic (meat production). One of genes, that is indicated, influenced the meat production (growth) is Bovine Growth Hormone.

Bovine Growth Hormone is a single peptide with 22 KDa molecul-weight and codified by 191 amino acid (Wallis, 1973) with sequence length of nucleotide 2856 pb (Gordon et. al., 1983). Bovine growth homone gene consist of 5 exon dan seperated by 4 intron (Gordon et. al., 1983) and located at chromosome 19 (Hediger et. al., 1990). Growth hormone gene is growth hormone-marker which is produced by somatotropes, located in fore pituitary gland dan have some physiology activities. GH Gen has important role in case of regulate growth characteristics, reproduction, metabolism, lactation, and mamary glands growth (Cunningham, 1994; Hoj et. al., 1993).

Various research reported the relevance between GH gen with the milk production and the meat of foreigh cow (Lucy et. al., 1991, Hoj et. al., 1993, Schlee et. al., 1994a, Lagziel et. al., 1996, Sutarno et. al., 2000). In Indonesia, as reported Sutarno (2000) that GH gene can be a marker in process of selection of local cattle, including PO cattle by various research of GH gene.

Based on the analyze above, the analyze at the level of gene in this sense growth hormone gene need to be done by the offices which have duties as seed cattle supplier in

Indonesia in order to increase the quality and quantity of Indonesian cattle. One of the offices that has duty as supplier of seed cattle, including PO cattle is BBIB Singosari. BBIB Singosari is a technical implementer unit which carry out male replacement and production of superior seed sustainability by supplying frozen semen. In doing so, BBIB Singosari works with local state firm to supply the female cattle for the people. One of firms which collaborate with BBIB Singosari is UPA Pasuruan. UPA Pasuruan is a state firm which supply the female PO Cattle that will give descent and then will be used by the people. Until now, the effort of this two units in order to supply superior stock parental is selection based on morphology-marker. This marker still has many restrictiveness for the characteristic is more influenced by the surroundings and it is not generated to its descents (Sutarno et. al., 2005). Then, it is needed a study at the level of gene which control the growth characteristic (meat production) completing the morphology-marker.

The study at level of gene that control the growth characteristic (meat production) can be done by PCR-sequencing technique. Recently, PCR technique is one of methods which is easy relatively besides sequencing is a more powerful technique of measuring genetic diversity that has been innovated an automated recently, has been widely used in genetics to forensic. This research aims to detect the existence of growth hormone gene of PO cattle of all research samples by using PCR technique and sequencing.

MATERIALS AND METHODS

Sample

The sample used in this research is all bull PO (superior catlle) in BBIB Singosari. Those are amount 3 bull PO Cattle and 10 cow PO. The latest are used as cow-mother in the process of reproduction of PO Cattle in UPA Pasuruan. The blood samples were taken 3-5 ml through jugular vain by means vacutainer 9 ml contained EDTA (anti-coagulant) and shaken until homogeneous. The blood samples are kept in cold temperature.

Leucocyte Isolation of Blood Sample

Isolation is made by saltingout technique. The blood sample from vacutainer are moved as much as 3 ml of each and put into polypropylene tube 15 ml sized, added 9 ml solution lysis RBC as much as one time and the tube then is inverted 2-3 times and incubated in room temperature during 10 minutes. Then, it is centrifuged with a speed of 1500 rpm during 10 minutes until the pellets and supernatant formed. The formed supernatant then disposed of then added 9 ml of RBC as much as one time and incubated in room temperature during 10 minutes then it is centrifuged with a speed of 1500 rpm during 10 minutes and supernatant is disposed of.

DNA Isolation from Leucocyte

The pellet that form leucocyte is added 750 µl cell lysis solution and then homogenized by the way of pipeting. It is incubted at level of 37^oC temperature during 15 minutes then it is added precipitation protein as much as 500 µl then the vortex process is made, centrifuged with a speed of 7000 rpm, temperature level 40^oC, during 15 minutes. The formed supernatant is moved to the new tube, added 2250 µl cool ethanol then the tube is inverted 25 to 30 times until the white DNA band showed. After that, it is centrifuged with a speed of 10.000 rpm, 4^0C temperature during 15 minutes and then supernatant disposed of. Continued by adding 3 ml of ethanol with 70% cold temperature, inverted for a few times, centrifuged with a speed of 10.000 rpm, 4^0C temperature during 15 minutes and then ethanol disposed of. Next treatment is the remain pellet then wind-dried at level of room

temperature then added 100 µl TE buffer, placed at the level of oven temperature 37^0C, during 10 minutes and kept at the level of -20^0C temperature.

Quantitative Measurement of DNA using Spectrofotometer

Purity measuring and DNA Concentration is isolated using spectrofotometer (Genesys 10). DNA Concentration is known by the way of measuring the absorbance value at length of wave 260 nm and 280 nm. The formula, that is used to measure the DNA consentration using spektrofotometer, is (Fatchiyah et., al., 2007):

Consentration (µg/ml) = A260 . fp . 50 µg/ml
A260 = OD 260 value in the measured DNA solution
fp = dilution factor
50 = OD 260 same with 1 mol then equal with 50 µg/ml

Whereas, the purity of DNA is measured by the way of comparing the absorbance at length of wave 260 nm and 280 nm.

Qualitative Measurement of DNA using Agarose Electrophoresis 2%

Agarose is weighed as much 0,3 g and fused into 15 ml of TBE and then heated until all fused. The solution gel is refregerated until warm (+45^{o}C) then added EtBr as much as 0,8 µL and moulded using gel mould which is set tool comb-like. Then let the gel condensed dan the tool like a comb is dropped, after that the gel is moved to electrophoresis chamber then it is filled with TBE until the gel damped. As much as 4 µL of DNA sample, the result of isolation, is added 3 µl loading dye, put into gel well. The electrophoresis is carried out using voltage 100 volt more or less 1 hour. The result of running then outstretched upon UV transluminator.

DNA Amplification using PCR

Amplification of hormon gene of PO Cattle growth is made by Polymerase Chain Reaction (PCR) method. PCR is made by mixing aquadest steril 4 µl and PCR mix 10 µl. The reaction is started by adding 2 µl DNA sample as template and 2 µl for each primer. Amplification is executed to PCR tool (gene cycler), programmed in accordance with used paired primer. The used primer is 5'-CCCACGGGCAAGAATGAGGC-3' as forward and 5'-TGAGGAACTGCAGGGGCCCA-3' as reverse, in which each primer can recognize gene sequence located in 2054-2074 bp for primer and gene sequence located in 2457-2337 bp (Sutarno, 2010). Gene cycler is programmed in accordance with used primer, that is hot start at 94^{o}C temperature during 1 minute, denaturation at 94^{o}C temperature during 1 minute, annealing at 60^{o}C temperature during 1 minute, extension at 72^{o}C temperature during 1 minute and ended with post extention at 72^{o}C temperature during 5 minutes. The amount of amplification are 30 cycles.

Sequencing PCR Result

The sample of the result of amplification is continued to the stage of sequencing, beforehand purified using ethanol method/EDTA precipitation. Then it is continued to the stage of sequencing with reagent ABI PRISM BigDye Terminator v3.1 cycle sequencing kit. This sequencing process is made by the analysis merit of the laboran of State Islamic University, Malang.

Data Analysis

The data of PCR result is used to know the existence of growth hormone gene, by observation of the photo of agarose electrophoresis result, whereas the sequencing data is used to confirm the sequence of PCR result and each sample is traced and confirmed

between the result of forward primer sequence and reverse primer sequence to each gene by using software sequence scanner v.10 and software Bioedit. Then, the result of sequencing is confirmed by using BLAST (Basic Local Aligment Search Tool) programme which can be found in NCBI website. It is used to know whether growth hormone gene in GenBank has similarity with gene sequence of growth hormone of cattle which is the sample of this research.

RESULT AND DISCUSSION

The result of amplification of GH Gen fragment is showed figure 1, whereas the result of sequencing is showed figure 2. Growth Hormone Gene is a single peptide with nucleotide sequence length 2856 pb, consist of 5 exon and seperated by 4 intron (Gordon et al., 1983). Based on the used primer pair, the length of the product of the result of gen GH fragment amplification is 329 pb, located at exon 3 and 4. The length of this fragmen, that has similarity with the result of amplification (Zhou et al., 2005 and Sutarno et al., 2010), is 329 pb with formation of a single DNA band. The success of GH gene amplification is determined by the annealing conditions on the target gene and condition of thermocycler (denaturation temperature, annealing, and extention). Beside, it is determined by the interaction of the component of PCR reagent in appropriate concentration (Viljoen et al., 2005). Annealing temperature that is used in this research is 60^0C during 1 minute. It is different as suggested by Mitra et al. (1995) and Sutarno (2005) that the primer annealing occurs at 60^oC temperature during 40 and 45 seconds. That annealing time can not be used in this research for if it is used the level of success of amplification of growth hormone gene to this cattle show less optimum results because of there are some DNA bands.

Then the result of PCR is continued by sequence process using reagent ABI PRISM BigDye Terminator v3.1 Cycle Sequencing Kit. The data of sequence result of each sample is traced and confirmed between the result of forward primer sequence with reverse primer to each gen by using software sequence scanner v.10 and Bioedit. Then, based on that data, the homology is confirmed with the growth hormone gene of cattle by using BLAST (Basic Local Aligment Search Tool) programme which can be found in NCBI website. It is used to know whether growth hormone gene in GenBank has similarity with gene sequence of growth hormone of cattle.

The result of alignment (table 1) shows that gene sequence of growth hormone of PO cattle in BBIB Singosari and UPA Pasuruan have homology value that reach 99% with 5 samples of growth hormone gene in GenBank. Based on this homology value, it is concluded that the gen of amplification result is a correct growth hormone gene of cattle and can be used for further research.

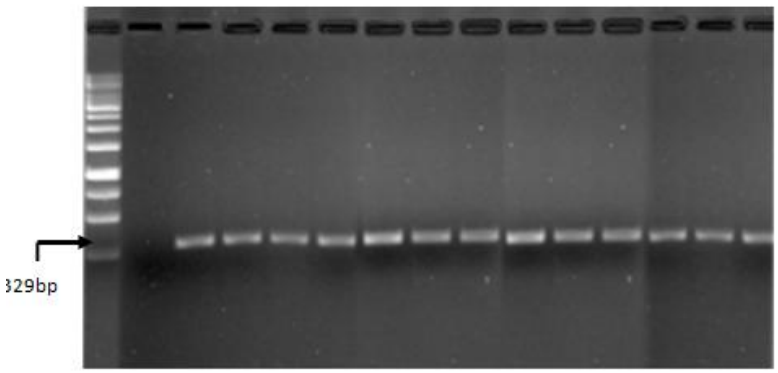

Fig. 1. Result of amplification of growth hormone gene of PO cattle using Gel Agarose 1,5%
Explanation: M: Marker; K: Kontrol (Control); 1-13: Samples

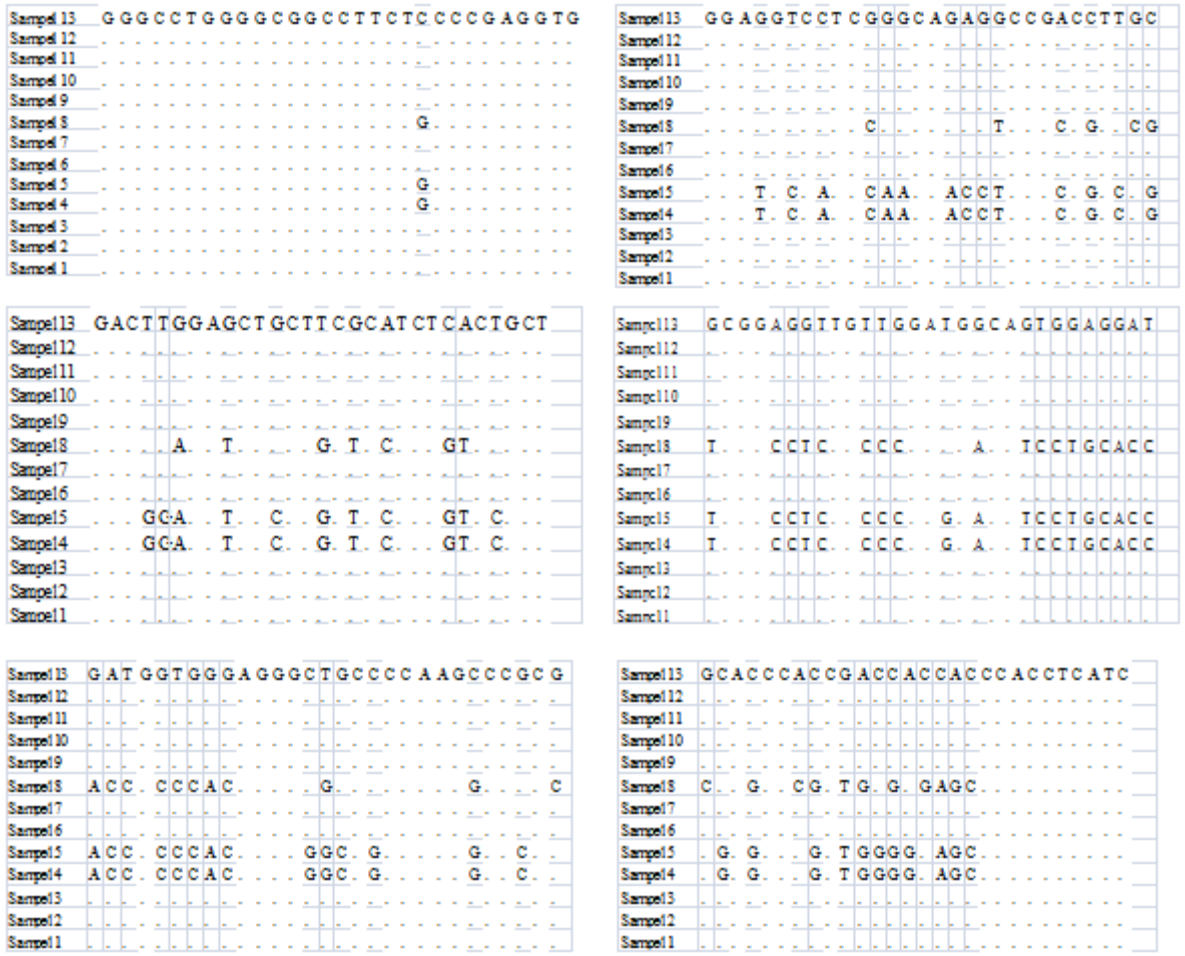

Fig. 2. The result of sequencing of growth hormone gene
Explanation: samples numb. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10: from UPA Pasuruan and numb.11, 12, 13: from BBIB Singosari

Table 1. Sequence similarity value of growth hormone gene of superior bull PO Cattle in BBIB Singosari and mother-cattle in UPA Pasuruan with growth hormone gene

Sample	Sequence of Growth Hormone Gene (Source: *NCBI*)				
	J00008.1	EF592534.1	EF592533.1	AF529184.1	AY271297.1
Sample A	99%	99%	99%	99%	99%
Sample B	99%	99%	99%	99%	99%
Sample C	99%	99%	99%	99%	99%
Sample D	99%	99%	99%	99%	99%
Sample E	99%	99%	99%	99%	99%
Sample F	99%	99%	99%	99%	99%
Sample G	99%	99%	99%	99%	99%
Sample H	99%	99%	99%	99%	99%
Sample I	99%	99%	99%	99%	99%
Sample J	99%	99%	99%	99%	99%
Sample K	99%	99%	99%	99%	99%
Sample L	99%	99%	99%	99%	99%
Sample M	99%	99%	99%	99%	99%

Note. Sample A-C: superior bull PO Cattle from BBIB, sample D-M: mother-PO cattle from UPA, J00008.1: bovine growth hormone (presomatotropin), EF592534.1: Bos indicus breed Kenana growth hormone gene, EF592533.1: Bos indicus breed Butana growth hormone gene, AF529184.1: Bos grunniens growth hormone precursor, AY271297.1: Bos grunniens growth hormone gene.

ACKNOWLEDGEMENT

Thanks to State Islamic University which allows the implementation process in their sequencing laboratory and to Higher Education Directorate, the National Education Department of Indonesia financing this research through a doctoral research grant program

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