

## PCR-RFLP Analysis Of Insulin-Like Growth Factor-I Gene In Bali Cattle

(Analisis PCR-RFLP dari Gen Insulin-Like Growth Factor-I pada Sapi Bali )

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### ABSTRAK

Penelitian ini bertujuan mengungkap polimorfisme gen *insulin-like growth factor-I* (IGF-I) pada sapi Bali menggunakan teknik PCR-RFLP. Apabila sifat polimorfik dari gen-gen ini dapat dideteksi maka efeknya terhadap pertumbuhan dapat dipelajari dan kemungkinan dapat dipergunakan sebagai *marker* genetik untuk seleksi pertumbuhan sapi Bali. Sampel darah diambil dari tiga populasi sapi Bali: sapi Bali di Pusat Perbibitan Sapi Bali (P<sub>3</sub>Bali) dan sapi Bali di luar wilayah P<sub>3</sub>Bali di pulau Bali maupun di luar pulau Bali (Kalimantan Selatan), berturut-turut sebanyak 111, 85 dan 46 sampel. Identifikasi polimorfisme gen IGF-I dilakukan dengan mendigesti fragmen DNA berukuran 249 bp yang berlokasi pada 5'-*flanking region* dari gen IGF-I menggunakan enzim *Sna*BI. Hasil penelitian menunjukkan bahwa pada ketiga populasi sapi Bali tersebut hanya ditemukan alel B, dan tidak ditemukan alel A atau alel lain. Disimpulkan bahwa gen IGF-I tidak bersifat polimorfik pada sapi Bali dan genotipe IGF-I adalah homosisgot BB.

**Kata kunci :** *polimorfisme, gen, insulin-like growth factor-i, sapi bali*

### INTRODUCTION

Insulin-like Growth Factor-I (IGF-I) or somatomedin C is a protein and affected by Growth Hormone (GH). Its structure and function similar to insulin, but they have greater stimulant effect (Hadley, 1992). IGF-I of cattle consist of 70 amino acids (Judith *et al.*, 1990 in Anonymous), synthesized at the liver tissue and the transcription of IGF-I gene is controlled by GH (Lamberson *et al.*, 1995). The effect of IGF-I is skeletal muscle cell (Florini, 1987 in Hong and Forsberg, 1994), therefore IGF-I is essential in controlling the metabolism of mammal growth (Werner *et al.*, 1994). IGF-I of cattle is coded by gene which content of five exons separated by introns and located at chromosome 5 (NCBI, 2005).

Polymorphism of bovine IGF-I gene occur in 5'-*flanking region*. The sequence of DNA fragment (GenBank accession number AF10383) showed the transition of one nucleotide T (A allele) to C (B allele) (Ge *et al.*, 1997; 2001). Ge

*et al.* (2001) reported that there was correlations between IGF-I gene polymorphism and growth performance in Angus cattle, where BB genotype showed a faster growth at the first twenty days of post weaning. Polymorphism of IGF-I gene also correlated to birth weight (Moody *et al.*, 1996). Those explanation indicated that IGF-I gene polymorphism and its effect on growth aspect was not similar among cattle breeds. The study was conducted to evaluate the polymorphism of IGF-I gene in Bali cattle.

### MATERIALS AND METHODS

#### Animals and Blood Sample

The number of animals were 242 Bali cattle, consisted of 111 Bali cattle from Bali Cattle Breeding Centre at Bali Cattle Breeding and Improvement Project or also called is P<sub>3</sub>Bali (Proyek Pembibitan dan Pengembangan Sapi Bali), 85 Bali cattle from Bali island, and 46 Bali cattle from South Kalimantan. About 3 ml venous blood was collected under sterile conditions from

the jugularis vein of the animals into a sterile tube containing K<sub>3</sub>EDTA as anticoagulant.

### DNA Preparations

The DNA genome was isolated from the blood samples following phenol-chloroform extraction method described by Sambrook *et al.* (1989). The quality of DNA was tested by taking ratio of O.D. at 260 and 280 nm in the spectrophotometer. The samples having O.D. ratio between 1,5 to 2,3 were selected and used for PCR study. The quantity of DNA was estimated by spectrophotometry taking O.D. 260 nm.

### PCR-RFLP *Sna*BI

A 249 bp fragment of IGF-I gene is located in 5'-flanking region was amplified by IGF-forward: 5'-ATTACAAAGCTGCCTGCCCC-3', IGF-reverse: 5'-ACCTTACCCGTATGAAAGGAATATACGT-3' (Ge *et al.*, 2001). Both primer were ordered from Cyber Gene AB. The DNA samples for amplification were prepared by adding 19 µl dH<sub>2</sub>O, 2 µl DNA solution (50 – 100 ng) and 2 µl (16 pmol) primer into tube of 0,2 ml Ready-To-Go PCR Bead (Amersham Biosciences). The amplification of DNA fragments were done by using Thermal Cycler machine. The PCR machine was programmed for initial denaturation at 95°C for 5 minutes, and followed by 35 cycles of denaturation at 95 °C for 30 seconds, primer annealing at 65 °C for 30 seconds, primer extension at 72 °C for 30 seconds, then followed by final extension at 72 °C for 5 minutes.

The PCR product with specific fragment (249 bp) of IGF-I gene was digested with *Sna*BI (*Sphaerotilus natans*) enzyme. The 10 µl of PCR product was put into 1,5 tube then added 2 µl of 10X *Sna*BI Bufer, 2 µl of 0,1% BSA and 0,5 µl *Sna*BI enzyme (10 unit/ µl), and H<sub>2</sub>O until the volume reached 20 µl, and the tube was incubated at 37<sup>0</sup>C for 2 hours for digestion.

The digested product was electrophorized in 2% w/v agarose gel in TBE bufer at 100 V for 30 minutes. A 100 bp DNA ladder was used as DNA marker. The result of electrophoresis was

visualized under the ultraviolet light, then photographed using Polaroid film. The AA genotype was identified by two bands of 233 bp and 26 bp; the AB genotype by three bands of 249 bp, 223 bp and 26 bp; and the BB genotype by one band of 249 bp (Ge *et al.*, 2001).

## RESULTS AND DISCUSSION

### Identification of IGF-I Genotype

Specific DNA fragment of size 249 bp in the first exon of IGF-I gene was amplified by using a pair of primer: IGF-forward and IGF-reverse. The result of the digestion of PCR product by *Sna*BI enzyme showed that only one allele, namely B allele was found this indicated that the *Sna*BI failed to recognize the restriction site, because the transition of nucleotide C to be nucleotide T (yACGTA, to CACGTA) was not found (Ge *et al.*, 1997; 2001). Consequently, the size of PCR product before and after digested by *Sna*BI enzyme remain constant of 249 bp.

Genome DNA was double helix (diploid: 2n). If *Sna*BI enzyme recognized sequence in both helix of DNA in PCR product, it mean that two A alleles obtained and the animal was AA genotype. If *Sna*BI enzyme only recognized one sequence in one of two helix of DNA in PCR product, then the animal was AB genotype. If *Sna*BI enzyme did not recognized sequence in both helix of DNA in PCR product, then the animal was BB genotype. In this study, both genotypes: AA and AB, were not found in Bali cattle. Figure 1 showed the BB genotype of IGF-I in Bali cattle.

### Allele and Genotype Frequency

The allele and genotype frequencies of IGF-I of Bali cattle population at P<sub>3</sub> Bali, from Bali island, and from South Kalimantan were similar. The frequency of IGF-I allele was 100% B, so the frequency of BB genotype was 100 %.

The results indicated that IGF-I gene of Bali cattle (*Bos sondaicus*) was non polymorphic, and all Bali cattle were BB genotypes. Non-polymor-

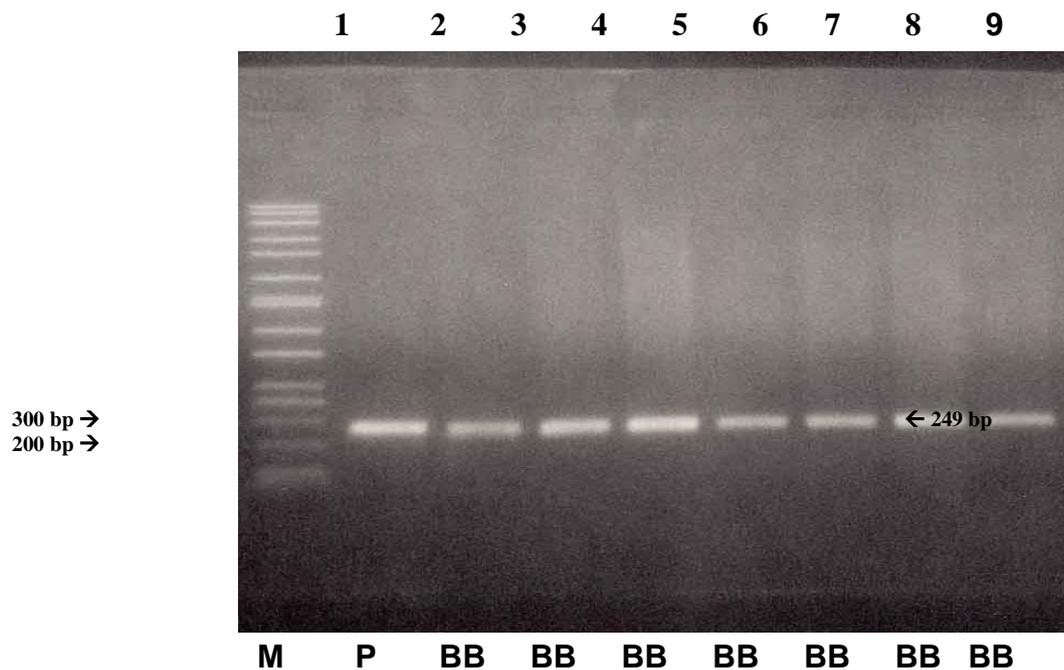


Figure 1. *Sna*BI RFLPs of IGF-I Gene in Bali Cattle  
 Lane 1 is 100 bp DNA ladder (M); lane 2 is PCR product (P=249 bp);  
 Lane 3 until 9 are BB genotype (249 bp).

phic IGF-I genes of Bali cattle might indicate that there was no mutation of IGF-I gene or no migration from other breeds. By neglected the possibility of gene mutation in future, the non polymorphic of IGF-I gene in Bali cattle could be used as an indicator that the population was never subjected to crossing, especially with *Bos taurus*.

### CONCLUSION

The IGF-I gene of Bali cattle was non polymorphic, only B allele for the IGF-I was found. The non polymorphic of this gene could be used as one of indicators that crossing has never been practiced in the population studied.

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