IDENTIFICATION OF OVINE AND BOVINE MICROSATELLITES IN THE JAVANESE SHEEP GENOME

Muladno[1], B. Tiesnamurti and I. Inounu[2]

ABSTRACT

Five pairs of primers flanking 2 microsatellites in the ovine genome and 3 microsatellites in the bovine genome were used to identify the corresponding microsatellites in the Javanese sheep genome as an initial step to study genetic variability within highly- and lowly-prolific strains of the Javanese sheep. Each microsatellite was identified using Polymerase Chain Reaction (PCR) amplification technique with the following reagents: 50ng genomic DNA, 400 ng of each primer, 200 M dNTP's, 1.5-3.0 mM $MgCl_2$, 2.5 Unit *Taq* Polymerase and distilled water up to 25 l. A PCR thermocycler apparatus was programmed as follows: initial denaturation at 95 °C for 3 minutes; followed by 34 cycles, each of which comprised denaturation at 95 °C, annealing at various temperatures, DNA extension at 72 °C for 15 seconds respectively; then finalized by another extension step at 72 °C for 5 minutes. PCR products were electrophoretically separated in 1% normal agarose gel and visualized under Ultra Violet (UV) transillumination. Primer OarCP79 derived from the ovine microsatellite flanking regions and primers BL25 and BM2320 derived from the bovine microsatellite flanking regions successfully amplified single DNA segments in the Javanese sheep genome; the other ovine-derived primer OarFCB20 as well as bovine-derived primer BL37 are still being examined to amplify any DNA segment from the Javanese genome. This preliminary result indicated that primers flanking microsatellites isolated from both species and probably from other species are potential to generate a number of DNA markers for genetic studies in the Javanese sheep.

Key words: Identification, Microsatellites, Ovine, Bovine, Javanese sheep genome

INTRODUCTION

Microsatellite is an ideal DNA marker of choice for genetic studies in almost all species since it is highly polymorphic and abundantly scattered across genome. Because it is a simple DNA sequence with the total length of usually less than 100 basepairs and flanked by specific sequence, amplification of the microsatellite is efficiently carried out using a Polymerase Chain Reaction (PCR) technique. Since the discovery of microsatellites at the beginning of 1990s, a lot of genetic studies including construction of gene maps and genetic diversity are increasingly accumulated, for example: in cattle gene mapping (Bishop *et al.*, 1994); in sheep gene mapping (Crawford *et al.*, 1995); and in other species. Contribution of microsatellite as DNA marker for the construction of a gene map is substantial. In the case of pig gene mapping, for an example, number of marker and loci mapped is only less than two per year before 1992, totaling less than 60 markers mapped (Muladno, 1994). Using microsatellite in a gene-mapping project, a comprehensive pig gene map now consists of more than 2500 markers localized in individual chromosomes. Similar phenomena occur in other species of animals. It is clear that microsatellite is a powerful DNA marker for genetic studies, supported by advanced molecular technologies. Javanese sheep in Indonesia is one of the most prolific breed of sheep and it is due to a genetic factor (Bradford *et al*, 1990). Results from intensive research on the Javanese sheep have provided two extremely different

[1] Jurusan Ilmu Produksi Ternak, Fapet IPB dan PAU Bioteknologi IPB, Indonesia
[2] Balai Penelitian Ternak, Puslitbangnak, Bogor, Indonesia

populations being lowly- and highly prolific; and up to now they are maintained in the Animal Research Institute of Bogor. Given that thousands of microsatellites from different domestic animals such as cattle, sheep, and pig have been isolated and well documented through international research collaborations on the construction of linkage genetic maps, in this study we are trying to exploit such microsatellites for genetic studies of the two population of the Javanese sheep. For the initial step, objective of this study is to identify microsatellites in the Javanese sheep genome using primers flanking microsatellites isolated from sheep and cattle genomes.

MATERIALS AND METHODS

DNA samples extracted from highly and lowly prolific strains of Javanese sheep were used. DNAs were extracted using a standard procedure described by Sambrook *et al.* (1989) and stored in 4 °C, and concentration of the DNA was measured by a spectophotometer.

Five pairs of primer consisting of two pairs which flank ovine microsatellites (OarCP79 and OarFCB20F) and three pairs which flank bovine microsatellites (BL25; BL37 and BM2320) were used to identify corresponding microsatellites in the Javanese sheep genomes. DNA sequence and other information on those primers are available and extracted from internationally published journals (Crawford *et al.*, 1995 for ovine-derived primers and Bishop *et al.*, 1994 for bovine-derived primers). The primers were chosen based on three criteria, namely: a) it amplified microsatellites which have high degree of polymorphism; b). Sizes of PCR product were not less than 100 base pairs; c) Sizes of each primer ranged from 18 to 27 nucleotides.

Each pair of primers were used to amplify corresponding microsatellites (if any) in the genomic DNAs of Javanese sheep, using Polymerase Chain Reaction (PCR) technique. The reaction was performed in a 0.2 ml-tube containing 50 ng genomic DNA, appropriate amount of each primer, 200 M dNTP's, 1.5-3.0 mM $MgCl_2$, 2.5 Unit *Taq* DNA Polymerase, and distilled water up to 25 l. PCR was programmed as follows: initial denaturation at 95 °C for 3 minutes followed by 34 cycles each of which consist of denaturation at 95 °C for 15 seconds, annealing at various temperatures for 15 seconds, extension at 72°C for 15 seconds, then finalized by a further extension at 72 °C for another 5 minutes. PCR products were electropheretically separated in 1% normal agarose gel using an electrophoresis apparatus (MUPID, made in Japan), visualized under Ultra-Violet (UV) transillumination, and photographed with a Polaroid camera.

RESULT AND DISCUSSION

Determination of optimal condition in PCR technique for DNA amplification was observed by justifying $MgCl_2$ concentration ranging from 1.5 to 3.0 mM and annealing temperatures ranging from 55 to 69 °C with interval of 1 °C. Concentration of primers applied is either 200 ng/l or 400 ng/l. In this PCR analysis, genomic DNAs from three individuals were used. If PCR product was not generated after all possible justification, it indicated that microsatellites flanked by primers are not present in the genome of Javanese sheep.

Careful examination on each pair of primer showed that primers OarCP79 and BL25 successfully amplified single bands in the genomic DNA of Javanese sheep. As expected, size of PCR products amplified by the primers is within range of those reported by Crawford *et al.* (1995) and Bishop *et al.* (1994). All PCR condition applied for the two primers are the same, but annealing temperatures are 68 °C and 58 °C for primer OarCP79 and BL25 respectively. With low intensity of single band generated, primer BM2320 also successfully amplified a region in the genome of Javanese sheep (data not shown). Two other primers including OarFCB20 and BL37 are still being examined in the same genome. Table 1 presents detail information on analysis of PCR amplification using five pairs of primers as well as PCR condition applied for each pair of the primer.

Table 1. DNA sequence of individual primers used for PCR analysis on genomic DNA extracted from Javanese sheep, PCR condition and size of the PCR products amplified.

Primers	DNA sequence (5'-3') (1st line = Forward; 2nd line = Reverse)	AT (°C) reported *)	AT (°C) used in this study	Size of PCR products (bp)
BL25	AACAgTggCAATggAAgTgg AgTCAggATCTAgTggggTgAgTg	58	58	approx. 170
BL37	gCAATCCCACTCTCCAggTg CATTCATgTTgCTgTAAATggC	58	??	??
BM2320	ggTTCCCAgCAgCAgCAgTAgAg ggCCAgCACTACTCTTATAgCCAg	58	58	approx. 140
OarCP79	AAAACCATCTCAAAAgATCACCgTCCC ggCCAgCACTACTCTTATAgCCAg	65	68	approx.160
OarFCB20	AAATgTgTTTAAgATTCCATACAgTg ggAAAACCCCCATATATACCTATAC	63	??	??

* AT (= Annealing Temperature) reported in Crawford *et al* (1995) and Bishop *et al* (1994).

Further PCR analysis on populations of lowly (FF) and highly-prolific Javanese sheep (++) confirmed that each of the three primers consistently amplified single bands in genomic DNA of almost all individuals tested. As an illustration, Figure 1 depicts PCR products amplified by a bovine-derived primer BL25 in genomic DNAs extracted from eight individuals of each of the two populations. For unknown reason yet, a single band was not generated by one of the eight individuals used in this analysis. In this particular primer, number of alleles in microsatelite flanked by the primer BL25 in a cattle population is six with the degree of heterozygosity of 93% (Bishop *et al.*, 1994). Using 1% normal agarose gel as shown in the Figure 1, allelic differences of microsatellite amplified in this population cannot be detected due to low resolution of the gel.

Figure 1. PCR products amplified using bovine-derived primer BL25 in genomic DNAs extracted from Javanese sheep. The PCR products were electrophoretically separated in 1% normal agarose gel. Lanes 1-8: genomic DNAs from lowly prolific population of Javanese sheep; lane 9: molecular weight marker; and lanes 10-17: genomic DNAs from highly prolific population of Javanese sheep.

Since the difference size between one allele of microsatellite to another is only few nucleotides (1-4, depending on type of microsatellite), DNA sequencer is the only means to detect such differences. Unfortunately, this facility is not yet available in our laboratory at the moment.

Possibility of using primer-flanking microsatellite in one species to amplify corresponding microsatellite in another species is well known. Sequence conservation of microsatellites and their flanking regions between cattle, goat, and deer has been reported and 40 per cent of microsatellites isolated from cattle have been very useful to characterize caprine genome (Pepin *et al.*, 1995). Thus, the same type of microsatellite as well as its flanking regions is present in more than one species.

Identification of microsatellite as DNA markers is prerequisite to study genetic variation within population in order to construct a gene map or a phylogenic tree. In the case of constructing a gene map, identification of microsatellites in a animal's genome helps to localize gene(s) responsible for quantitative traits or singly qualitative traits such as the one responsible for genetic disease. Several genes and QTLs in cattle have been localized using microsatellite as DNA markers (see for example Andersson *et al.*, 1994).

Number of microsatellites in the genome is abundant and thousands of microsatellite have been mapped in individual chromosomes and publically accessible for scientific purpose. Eventhough new microsatellites may be isolated from the genome of Javanese sheep using available molecular techniques already developed, exploitation of microsatellites characterized and mapped by international research collaboration efforts either in sheep genome or others is more efficient for genetic studies in the population. This approach is suitable for Indonesia since we have a diversity of animals which need to be evaluated and at the same time international efforts have provided DNA markers isolated and characterized from a number of species.

Toward the construction of a sheep gene map in Indonesia in the near future, more microsatellite and possibly other DNA markers need to be identified and examined. In addition, degree of heterozygosity for each microsatellite that has been identified need to be estimated so that decision to whether or not identified microsatellites are potential for gene mapping studies can be made.

CONCLUSION

By exploitation of primers flanking microsatellites isolated from sheep and cattle genomes which are publically accessible, at least three microsatellites have been isolated from the Javanese sheep genome and will be expectedly useful for genetic studies in the population. It is worth trying to utilize primers flanking microsatellites isolated from species other than sheep and cattle genomes for identification of more microsatellites in the Javanese sheep genome.

ACKNOWLEGDMENTS

Thanks to ARMPII (Agricultural Research Management Project) Department of Agriculture, Republic of Indonesia for financial support. To Dwi Astuti (Puslitbang Biologi LIPI and Abdilla (Puslitbang Bioteknologi LIPI) who assisted for laboratory work, authors greatly appreciated.

REFERENCES

Andersson L., Haley C.S., Ellegren H., Knott S.A., Johansson M., Andersson K., Andersson-Eklund L., Edfors-Lilja I., Fredholm M., Hansson I., Hakansson J. and Lundstrom K. 1994. Genetic mapping of Quantitative Trait Loci for growth and fatness in pigs. *Science*, 263:1771-1774.

Bishop M.D., Kapes S.M., Keele J.W., Stone R.T., Sunden S.L.F., Hawkins G.A., Toldo S.S., Fries R., Grosz M.D., Yoo J. and Beattie C.W. 1994. *A Genetic Linkage Map for Cattle. Genetics*, 136:619-639.

ISSN 0126-4400

Bradford G.E., Inounu I., Iniguez L.C., Tiesnamurti B. and Thomas D.L. 1990. The Prolificacy gene of Javanese Sheep. In: *Major Genes for Reproduction in Sheep.* Eds: J.M.Elsen, L.Bodin and J.Thimonier. Toulouse, July 16-18, 1990.pp:67-73.

Crawford A.M., Dodds K.G., Ede A.J., Pierson C.A., Montgomery G.W., Garmonsway H.G., Beattie A.E., Davies K., Maddox J.F., Kappes S.W., Stone R.T., Nguyen T.C., Penty J.M., Lord E.A., Broom J.E., Buitkamp J., Schwaigner W., Epplen J.T., Matthew P., Matthews M.E., Hulme D.J., Beh K.J., McGraw R.A. and Beattie C.W. 1995. An Autosomal genetic linkage map of the sheep genome. *Genetics,* 140:703-724.

Muladno. 1994. DNA markers for pig gene mapping. PhD Thesis. University of Sydney.

Pepin L., Amigues Y., Lepingle A., Berthier J-L., Bensaid A. and Vaiman D. 1995. Sequence conservation of microsatellites between Bos taurus (cattle), *Capra hircus* (goat) and related species. Examples of use in parentage testing and phylogeny analysis. *Heredity,* 74: 53-61.

Sambrook J., Fritsch, E.F. and Maniatis T. 1989. *Molecular Cloning: a Laboartory Manual.* Cold Spring Harbour Laboratory Press.