APPLICATION OF MICROSATELLITE MARKERS IN IDENTIFICATION AND PARENTAGE ASSIGNMENT OF SHEEP

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ABSTRACT

The accuracy of the parentage reference is the early step of information needed for genotyping study. Inaccurate data of recording sheep numbers is often occurred. Sometimes, we found sheep or lambs with uncertain original parents. This study was designed to identify the parentages of sheep and to find the putative parents of lambs. Using an advance DNA technology in molecular genetics particularly in genotyping study, those purposes can be clarified. DNA of sheep were isolated from fresh blood samples in EDTA using a high salt method. A concentration of 50ng/(il DNA was used for amplification. Several microsatellite markers were applied in the amplification. Using a DNA analyser and sequencer, PCR products were analysed to look at the figures of alleles (bands). Results showed that using microsatellite markers could inform that parents of several experimental lambs were incorrect. It was found that putative parents of some lambs were generated from incorrect parents. This study suggests that microsatellite marker can be used to clarify unknown original parents.

Key words: Microsatellite marker, DNA sheep, identification, parentage check.

INTRODUCTION

In breeding program, it always involves a high population, accuracy recording therefore is often to be the main obstacle in the field. It is due to there are many animals should be recorded with correct identity (ID). In sheep herd, a number of animals is often reared at the same flock. During lambing season, farmers often find difficulty to distinguish pedigree of their progeny. In flocks, small lambs often move to another group while recording for pedigree morphology is routinely performed. Therefore, it can not be avoided from incorrect data.

Before techniques in biology molecular were discovered, breeder only depends on morphological measurements or physically observations. At present, techniques in molecular biology are available and develop from time to time. These techniques provide plenty of ways to solve doubts in recording. Moreover in molecular genetic research, the correct pedigree of high population is a prominent step to be performed. As reported by Li (1997), there are at least three more advantages of using molecular techniques compared to those using morphological data or physiological data. First, DNA and protein sequences generally develop to many ways than morphological characters. Second, molecular data is more amenable to quantitative treatments than morphological data. Third, molecular data provides abundant information.

More specific in molecular genetics, genetic marker are now more familiars and very useful to facilitate in selection of breeding program. Microsatellite marker is one of genetic markers found in DNA genome. As stated by LI (1997).this marker is one of five classes of Tandem Repetitive Sequence (TRS). The other four classes are satellites, minisatellites, short tandem repeats (STR), and Alu Tail Arrays. The term microsatellite refers to the smallest repeat units around five bases or less, *e.g.,* ACCGG, ATTT, GGC, AC or T (Nicholas, 1996). The microsatellites can be found throughout the genome. Van der Werf (2000) mentioned that microsatellite is a DNA region with a short number of repetitive region and flanked by a unique sequence. Microsatellite marker is extremely polymorphic (Nicholas, 1996) for individual animal. Microsatellites are the markers chice since they can be visualized using PCR and sequencing gels (Fries, 1993). When PCR product amplified from unique-sequence primers only one or two bands are produced from animal (Nicholas, 1996). In addition, each pair of primers corresponds to a specific site on a chromosome, and there are many different alleles at each site. As a family consists of ram and ewe, an offspring will content of contribution alleles of its father and mother.

Recently, there are many studies in molecular genetics using microsatellite markers to identify

genetic distance, relationship or genetic variation of Europe cattle breed (MacHugh *et al.,* 1994; MacHugh *et al.,* 1998) also in evolution, domestication or phylogenetic cattle breed (MacHugh *et al.,* 1997).

In the field, we often fail to find the correct data of morphological performance or quantitative measurements. A correct pedigree is very important and necessary for further molecular genetics research. Thus, the correct pedigree of population should be concerned at the very beginning. At present, molecular techniques provide 'tool' that can provide data more accurate. Therefore, this study was designed to clarify incorrect pedigree of lambs using macrosatellite genetic markers.

MATERIALS AND METHODS

Materials:

- A number of uncertain sheep were used in this identification of pedigree.A number of 15 merino sheep was used for identification of rams, it involved Lamb or called Bart, 1 Dam and 13 suspected Rams. For clarification of pedigree, *i.e.* Fl (ID 1262 and ID 1265) was used 6 sheep. Both experiments involved Merino, Garut, Sumatera, Crossbreed sheep: Local sheep (either male Garut or male Sumatera sheep) crossed with female Merino.
- Blood was collected in vacutainer tubes containing 0.117ml of 15% (Ka) EDTA solution. Blood sheep was collected at a volume of 10ml per head
- Microsatellite markers were BM81124; BMS772 and CSM4 for identification of Bart lamb and OARFCB128; OARHH47 andBM17132 were for parentage checking of both lambs Fl 1262 and lambFl 1265.
- PCR primers, tandem repeat units of microsatellites can be detected using PCR with primer corresponding to the unique-sequence DNA that flanks the tandem repeats. Using electrophoresis, it allows band to be distinguished which differs in its size.

Methods: n

- DNA Extraction, a method of high salt concentrate was used to collect white blood cells. This method was a slightly modified method of Montgomery and Sise (1990). DNA was collected in TE solution. Quantification of DNA was performed by using spectropho-tometer based on reading wave length of DNA at A260 and A 280.
- Amplification of DNA. Using a technique of Polymerase Chain Reaction (PCR), a single molecule DNA was amplified to produce million copies of fragment DNA or called PCR products. The PCR was set up for 35 cycles, with DNA genome concentration of 50ng/ul was used as DNA template. A PCR machine used was a programmable thermal controller MJ-50 LICOR
- Gel acrylamide analysis, PCR products were analyzed in 6% acrylamide, to determine specific allele. This step is called genotyping. By evaluation of specific alleles of parentage which contribute to the lamb, clarification of incorrect parents either father or mother can be performed. As suggested by Crawford *et al.,* 1995, evaluation of specific allele should be done at least by 2 researchers. It can be also evaluated by using a specific software for genotyping analysis.
- In this study, evaluation of specific alleles by two researchers was used. A machine of LI-COR IR^2 Sequencer is a semi-automated DNA sequencer, was used to analyze the PCR products. Fluorescent labeled DNA molecules are detected during electrophoresis as they pass through a fixed point on an acrylamide gel.

RESULT

Identification of a correct Ram

Three markers of BM81124, BMs772 and CSSM4 were used in PCR to identify of the correct ram. The PCR products were analyzed to genotype of candidate rams. Alleles of each genotype of candidate rams were arranged in order as follow: Lamb (Bart), Dam, Rams (from ID 11 up to ID 24), see Figure 1, 2, and 3 for markers BM81124, BMS772 and CSSM4, respectively.

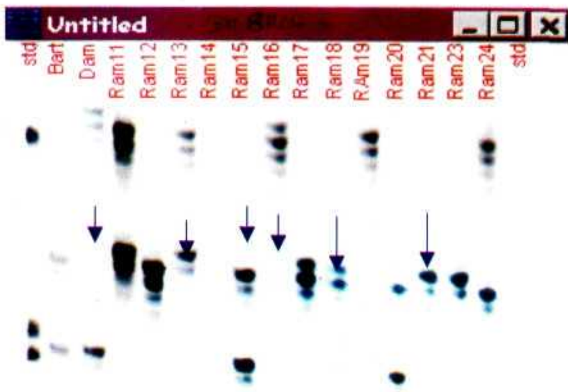

Figure 1. Genotyping result by using microsatellite marker of BM81124

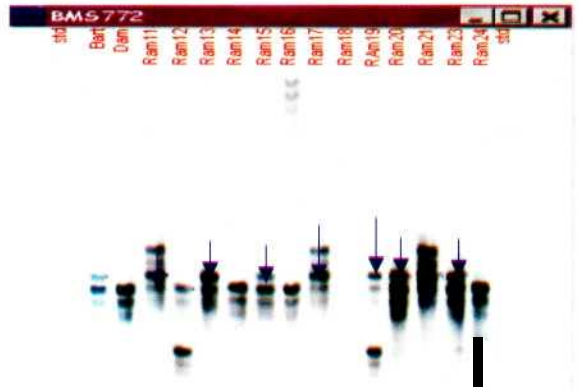

Figure 2. Genotyping result by using microsatellite marker of BMS772

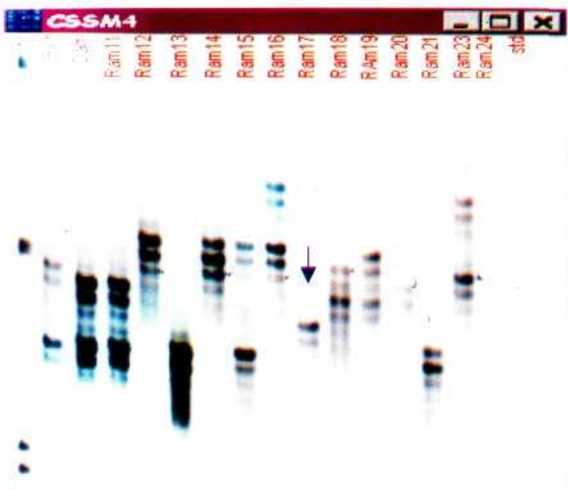

Figure 3. Genotyping result by using microsatellite marker of CSSM4

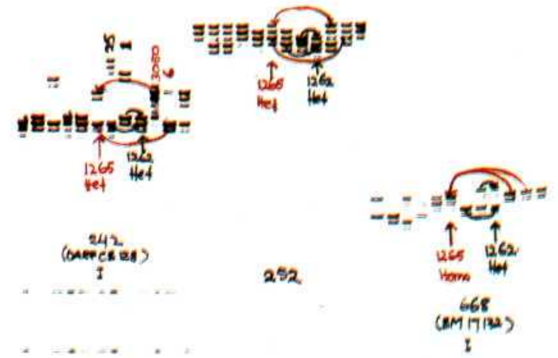

Figure 4. Genotyping results of micosatellite markers OARFCB128, OARHH47 and BM17132 to clarify the correct pedigree

Clarification of Pedigree

Three markers of OARFCB128, OARHH47 and BM17132 were used in PCR to check the correct pedigree. The result of analyzing of the PCR products was presented in Figure 4.

DISCUSSION

Identification of a correct Ram

Using a microsatellite marker of BM 81124 and evaluation of contribution specific alleles (bands), suspected rams as father were rams with ID 12, 15,

17,18,20 and 24 (Figure 1). While using microsatellite marker of BMS772, specific alleles were found at suspected rams with ID 11, 13, 15, 17, 19, 20, 21, 23 (Figure 2). And by using microsatellite marker of CSSM4, specific allele which contributed to the Bart alleles was only ram with ID 20 (Figure 3).

Clarification of Pedigree

By using gel analysis, 3 microsatellite markers (OARFCB128; OARHH47 and BM17132) showed the same of allelic contribution (Figure 4). The figure 4 showed that there was swapping of father (sire) and dam (mother) positions to the position of lamb 1262 and 1265. It can be seen that contribution alleles from Sire and Dam did not match to the allele distribution of both lambs (1265 and 1262. It can be clarified that Sire 25 and Dam 1 were not parents of lamb 1265. The correct parents of lamb 1265 were Sire 3050 and Dam 6 (see the arrows). The same confirmation was also occurred in lamb 1262. The correct parents of lamb 1262 were Sire 25 and Dam 1 and not Sire 3050 and Dam 6 (see arrows).

Allele is an alternative form of gene (Hartl, 1988). In addition the term 'gene' is a general term usually used in the sense of 'locus'. If the two alleles at a locus are chemically identical, the individual is said to be homozygous. In contrary, if two alleles at a locus are chemically different, the individual is said to be heterozygous.

At figure 4, most microsatellite markers showed heterozygous individual of Fl (1262 and 1265), except for marker BM17132 resulted homozygous in individual F1 1265. It is an approval that microsatellite marker is high polymorphic or hyperpolymorphic marker and it is very informative at family level and animal crosses (Nicholas, 1996). Therefore, microsatellite markers are often used in linkage map of different organisms. In the following research of looking for inheritance traits or interest gene, the creation of heterozygous individuals are an important step in establishment of reference family. It is therefore, genotyping should be extensively performed using many microsatellite markers to find heterozygous individuals (ACIAR report, 2001). Genotyping is aimed to see a consistency of allelic availability from ancestor.

By using molecular analysis, it can be identified the correct father (Ram) and be clarified the correct pedigree. Molecular data are often much more amenable to quantitative treatments than those morphological data (Li, 1997). In the field, sometime phenotypic data confuse in recording. Therefore, molecular analysis should be performed to clarify unsure recording.

CONCLUSION

1. Microsatellite can be used to clarify incorrect pedigree
2. The correct sire (father) of Bart was ram with ID 20
3. The correct parents of Fl 1262 was Sire with ID 25 and Dam with ID 1 and the parents of Fl 1265 was Sire with ED 3050 and Dam with ID 6
4. Correct pedigree is important to be identified at the very beginning before starting to genotype progeny.

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