MICROSATELLITE ANALYSIS
IN BIOTECHNOLGY OF ANIMAL REPRODUCTION

Siti Darodjah Rasad

Animal Reproduction Laboratory, Animal Husbandry Faculty,
Padjadjaran University, Bandung-Indonesia
e-mail : sd_rasad@unpad.ac.id

Abstract

In year 1970 was found nucleotide sequenz which have repeated sequenz of nucleotide. with high polymorh and using PCR could be amplified. That sequenz of nucleotide called Microsatelite. Microsatelite consist of 1 – 6 repeated nucleotide, which is CA repeated as mostly a repeated DNA in the animal [39].

Based on difference of long and amount ofrepeated nucleotide, there are three kind of DNA satelite, midi-, mini- and microsatelite [28]. Microsatelite analysis was used to analyse of paternity and identity of animal, which was done as a conventional analysis with blood group analysis. The advantage of microsatelite analysis compare to blood group system are the exclution probability was high (EXP 99.9%), needs small sampel (tissues, sperm or follicel of hair), could be use for all animal without special age and possible for died animal.

Key words: microsatelite analysis, paternity, reproductive biotechnolgy

INTRODUCTION
Description of Microsatelite Marker

The character and function of microsatellite marker which was repeated could not clear to explain. Many researchers estimated that repeated was done along as replicated process which was deviation polymerase chain in default of nucleotide formation [26]; [37]. [14] was succesfull to look structure of hair with Okazaki method. There was unbalanced chromosome DNA-position through crossing over during replication process, as sama as extension of DNA-chain.

The purpose of the microsatellite was to arrangement of gene expression [30], and the function same as a recombinant of DNA [33]. [12], [23] and [40] indicated the relationships between microsatellite and health or disease.

Structure of Microsatellite

Microsatellite as a DNA sequence in genome which was repeated (*Short Tandem Repeat/STR*) from mono - hexanucleotide Motive, and mostly in non coding Genome. Microsatellite have polymorph character with average 11 – 12 alleles, and the single allele was variation depending on the different of amount of the repeated [39]; [27]; [43].

Every microsatellite could be formatted the repeat nucleotide sequence combination in motive, and mostly appeared was CA-CA-CA-GT-GT-GT sequences [16]; [39]; [45].

The inheritance of microsatellite character was known after "Mendelian Law" was settled and there was mutation index about 10^{-3} [22]. Thereby mutation index for DNA sequence of $(CA)_n(GT)_n$ dimer of human was 5×10^{-4} [10].

Base on repeated type, Microsatellite divided into three types of microsatellites, complete repeated, incomplete repeated, combination complete and incomplete repeated [44]. By Complete repeated, there was no ceasing of DNA sequence, and incomplete repeated was inverse of complete repeated, there was ceasing of DNA sequence in the middle of the sequencing and followed nucleotide repeated in other part. By third type of microsatellite was combination both of types.

Expression of Microsatellite

Technically, expression of microsatellite alleles sequence could be seen with PCR (*Polymerase Chain Reaction*). After allele replication with PCR, the separation of the allele could be seen with helping of

electrophoreses Gel. Result of that process, the specific of nucleotide sequencing was formed and could be seen and compared with primer starter oligonucleotide complement as a control in PCR reaction (Mullis and Faloona, 1987; Saiki *et al*, 1988). The fragment of PCR result could be displayed amount of the repeated [27]; [43].

To convinced the observation, fluorescent technique could be used and the PCR product of electrophoreses could be seen more clearly [38]; [8]; [32]. Followed by computer programs, coloration product like band could be converted in to value as a fragment value. Thereby, with this method, the genotype analysis of many sample could be counted [7]; [46].

Application of Microsatellite Analysis as Parentage Test by Pig

Microsatellite analysis could be used in order to look parentage test for livestock animals, because microsatellite have co-dominant character and will be inherited in the offspring. Thereby, amount of allels could be expressed and the microsatellite as a informative genetics marker for the parentage test [21]. [3] said, microsatellite as a standard for estimate of genetics diversity in livestock. This condition as same as researches of [9]; [2]; [35]; [1] which were analyzed by Goat. By Pigs, microsatellite was used in biodiversity commercial and wild animals [15].

Using microsatellite analysis as a parentage test by Horses, was done as a routine in many laboratory and research institutions. By Cattle and Dog, this analysis was done as a commercial analysis. Many research was done, especially about advantages and possibility microsatellite analysis as an tools for parentage test in livestock. For instant, by Horse [6] and [17], Cattles [13]; [41] and [18], Dogs [4]; [11] and [24] and Pigs [19] and [42].

Microsatellite analysis in three Pig breeds was done by [34] using 25 Microsatellites marker, and result of that research were three groups PCR-marker (Multiplex-PCR) consist of five microsatellite marker each group for parentage and identity test of three Pigs breeds (Deutsche Edelschwein/DE, Deutsche Landrace/DL dan Pitrain), with exclusion probability value (EXP) 99,9%. Microsatellite marker could be seen in Table 1.

Superiority of Microsatellite Analysis

The superiority of Microsatellite analysis compared with blood test analysis, level of accurasy higher with using small marker and exclusion probability value (EXP) 99,9% then blood test analysis. Threby, more economic and with simple method without blood sample as a sample test. This analysis was possible for animal product test like meat [19]; [25].

Table 1. Microsatellite Marker for Parentage and Identity Test by Pig

Multiplex-PCR 1	Multiplex-PCR 2	Multiplex-PCR 3
SW936	SW240	S0026
SW857	S0101	SW951
S0115	S0355	S0225
CGA	SW122	S0227
S0068	SW911	S0178

Another advantage, sample for microsatellite analysis method, could be used sperm cell, tissues or hair follicle. There was no limitation age of animals, on the contrary died animals could be used. Thereby, sample for analysis was easy to find, small amount of sample and using PCR, DNA-sequenzer and computer program, parentage and identity test using Microsatellite analysis will be done [19].

Calculation of Microsatellite Analysis Product

PCR product by microsatellite analysis was analysed using DNA-sequencer, and allel sequences as a nucleotide band. From that analysis, every specific allels could be expressed from sample, because co-dominant allels comes from 50% mother and 50% from father. Thereby, allel expressed of the offsprings comes from mother (50%) and father (50%).

Allel band of the offspring could be compared with DNA sample form its parents (mother and father), and the allels sequence from the offspring could de identified and analysed and how far level of parentage of the offsprings. Based on the allels, level of accuracy of the test could be convinced the result almost 100%

Furthermore, to look for the probability of microsatellite marker as a parentage and identity test, the EXP (Exclusion Probability) dan combination EXP (cEXP) was analysed [5]. The formula of EXP were;

$$EXP = \sum_{u=1}^{z} Pu(1 - Pu)^2 - 1/2 \sum_{u=1}^{z-1} \sum_{v=u+1}^{z} Pu^2 Pv^2 (4 - 3Pu - 3Pv)$$

Z : amount of Allele

P_u, P_v : Level of relative allele u and v in locus

$$H_e = \frac{2N\left(1 - \sum_{i=1}^{k} P_i^2\right)}{2N - 1}$$

This formula could be followed by heterozygosity of the population from frequent and amount of allele with [31], Note :

k : amount of allele

P_i : allel frequent from Alel$_i$

N : Amount of Sample animls

CONCLUSIONS
1. Microsatellite analysis could be used for pedigree and parentage test of livestock
2. Microsatellite analysis have a higher level of accuracy with simple analysis, and using small sample
3. Application of Microsatellite analysis could be used for livestock selection process, economic and needs short time

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