

Identification of a RAPD marker associated with weaning weight in Brakmas cattle

(Pengecaman penanda RAPD yang berkaitan dengan berat sapih lembu Brakmas)

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Key words: RAPD marker, weaning weight, cattle

Abstrak

Kaedah analisis segregan pukal dan teknik RAPD telah digunakan untuk menghasilkan penanda polimorf yang berkaitan dengan berat sapih anak lembu Brakmas. DNA genom daripada 20 anak lembu jantan Brakmas dibahagikan kepada dua kumpulan iaitu kumpulan (n = 10) yang mempunyai berat sapih yang tertinggi (>135 kg) dan kumpulan (n = 10) yang mempunyai berat sapih yang terendah (<75 kg). Sebanyak 100 primer rawak telah diuji dalam tindak balas PCR. Primer Gen 2-50-22 telah menghasilkan penanda polimorf antara dua sampel DNA terkumpul yang bersaiz 1.2 kb. Penanda RAPD ini telah dihasilkan daripada semua sampel DNA anak lembu yang mempunyai berat sapih yang tertinggi dan tidak dihasilkan daripada DNA anak lembu yang mempunyai berat sapih yang terendah. Ini menunjukkan penanda ini berkaitan dengan berat sapih lembu Brakmas.

Jujukan jalur RAPD ini telah dibandingkan dengan jujukan DNA yang berada di pangkalan data di GenBank. Homologi yang signifikan (99%) dengan beberapa jujukan gen hormon pertumbuhan yang ada di GenBank telah diteliti. Jalur RAPD ini berpotensi digunakan untuk mengenal pasti anak lembu Brakmas yang mempunyai berat sapih yang tinggi.

Abstract

Bulk segregant analysis and RAPD technique were used to generate a polymorphic marker that was associated with weaning weight in Brakmas cattle. Genomic DNA from 20 male Brakmas calves were pooled into two groups i.e. group (n = 10) with very high (>135 kg) and group (n = 10) with very low weaning weight (<75 kg). A total of 100 random primers were screened for polymorphisms between the two DNA pooled. Primer Gen 2-50-22 generated polymorphic markers of 1.2 kb size between the two pooled DNA samples. The marker was also generated from all individual DNA samples of Brakmas calves with very high weaning weight but was not generated from DNA samples of calves with very low weaning weight. This indicated that the RAPD marker was associated with weaning weight in Brakmas calves.

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The RAPD band sequence data was compared with the DNA sequences in the GenBank database. A significant homology (99%) was observed with some of the growth hormone gene sequences in the GenBank. The RAPD band generated has the potential to be used for identifying calves with high weaning weight.

Introduction

Permanent genetic improvement of livestock takes place through breeding and selection programme. An important component of any breeding programme is the identification of animals with the highest genetic merit that can be used as parents for the next generation. Currently most breeding programme utilized sophisticated selection procedures that estimate an animal's genetic potential through evaluation of its phenotypic performance as well as performance of its relatives (Mrode 1996). The accuracy of selection of superior individuals, however, is strongly dependent on the degree of data recording and environmental factors which influenced the phenotypic expression.

Advances in molecular biology in the 1980s had resulted in the development of molecular genetic technology which generated genetic markers in plant and animal genome. Among them are Restriction Fragment Length Polymorphism (RFLP, Botstein et al. 1980), Variable Number of Tandem Repeats (VNTR, Jeffrey et al. 1985), Random Amplified Polymorphic DNA (RAPD, Welsh and McClelland 1990; Williams et al. 1990) and microsatellite markers (Litt and Luty 1989; Weber and May 1989).

These genetic markers have wide potential application in animal and plant breeding. Markers have been used in animal breeding for the identification of breeds (Blott et al. 1999), parentage determination (Glowatzki-Mullis 1995) and the identification of loci linked to quantitative economic traits (Georges 1998). The genetic markers can also be included in the selection process to improve estimation of an animal's genetic potential and this is often referred to as marker assisted selection (MAS) (Kashi

et al. 1990; Bishop et al. 1995). MAS can increase genetic response by affecting accuracy and time of selection (Smith and Simpson 1986). The detection of genetic markers associated with quantitative traits loci (QTL) is the first step in MAS.

Several methods are available for screening population for association between genetic markers and QTL (Beever et al. 1990; Darvasi and Soller 1993; Alfonso and Haley 1998). A rapid method to detect markers in the specific genome region is by bulk segregant analysis (BSA), also known as selective pooling or tail analysis (Michelmore et al. 1991). The objective of this study was to identify RAPD markers associated with weaning weight in Brakmas cattle using bulk segregant analysis.

Materials and methods

Sample collection and extraction of genomic DNA

A total of 85 male Brakmas calves were used in this study. The calves were selected from the Brakmas nucleus herd kept at Bukit Ridan MARDI Station, Pahang. Blood samples were collected from the calves in heparinized tubes and frozen at -20°C until analysed. Genomic DNA was extracted from the blood samples using Genomic DNA Purification Kit (Promega, CAT #A1120). The DNA concentration was measured with DyNA Quant 200 (Hoefer, Pharmacia Biotech., USA) and diluted with distilled water to 25 ng/ μL working solution for PCR amplification.

Two pooled DNA samples were made by mixing equal volume of DNA from 10 samples each from calves with high weaning weight and calves with low weaning weight. The calves weaning weight was adjusted to 205 days and calves with more than 135 kg weight were grouped into very high weaning

weight group while calves with less than 75 kg weight were grouped into very low weaning weight group. A total of 65 male Brakmas calves were selected at random from the nucleus herd.

Random amplification of genomic DNA

A total of 100 arbitrary primers were screened for polymorphisms between both DNA pools. Primers that generated polymorphism between pooled DNA samples were used to amplify individual DNA samples. Amplification was performed according to the method optimized for vertebrate DNA (Bielawski et al. 1995). The PCR reaction mixture consisted of the followings: 2.5 µL of PCR buffer 10X (10 mM Tris-HCL, 1.0% gelatine, 50 mM KCL), 2.0 µL of 2.0 mM MgCl₂, 0.5 µL of 0.2 mM dNTP mixed, 0.2 µL of *Taq* DNA polymerase (Promega), 2.0 µL of 25 ng of genomic DNA, 2.5 µL of 0.5 µM primers, and finally brought to a total volume of 25.0 µL with distilled water. A negative control containing all the components mentioned above except DNA was included in all amplification series to monitor contamination of reagents with DNA.

All amplifications were carried out in a Programmable Thermal Cycler (Biometra) at 94 °C for 30 sec, 36 °C for 30 sec and 72 °C for 2 min for 45 cycles. The amplification products were electrophoresed at 70 V in 1.5% agarose gel and stained in ethidium bromide. The RAPD fingerprinting was visualized and documented using Image Analyzer (Pharmacia Biotech. USA). Primer Gen 2-50-22 generated DNA fragment that was apparently polymorphic between the two pooled samples. The specific RAPD fragments from three individuals were cloned in plasmid vector pCR2.1 (Original TA cloning Kit, Invitrogen, USA) and the plasmid recombinants were subsequently sequenced automatically using PRISM Ready Reaction Dye Terminator Cycle Sequencing Kit in an ABI 377 DNA Sequencer (Perkin-Emer, USA).

Data analysis

Individual DNA band was scored as present (1) or absent (0) in each amplification profile. The frequency of RAPD bands scored was expressed as percentage of animals in each group showing visible bands. The specific RAPD sequences were compared for homology with the DNA sequences or protein sequence available in the GenBank database using BLAST software (Basic Local Alignment Search Tool) and significant homology is indicated by the high E value.

Results and discussion

The random amplified polymorphic DNA (RAPD) technique and pooled samples procedure provide an alternative technique for identification of genetic markers associated with quantitative trait in cattle. Out of the 100 primers used in the PCR amplification, two primers designated as Gen 2-50-22 and Gen 1-50-09 amplified polymorphic bands between the two pool DNA samples (*Plate 1*). The sequence of the two primers were: Primer Gen 1-50-09

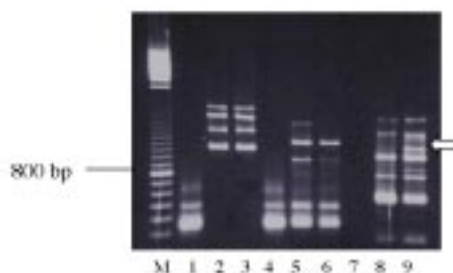


Plate 1. RAPD fingerprinting of pooled samples

- M = 100 bp marker
- 1– 3 = Primer Gen 1-50-20 fingerprinting
- 1 = Control
- 2 = Low weaning weight pooled sample
- 3 = High weaning weight pooled sample
- 4– 6 = Primer Gen 1-50-09 fingerprinting
- 4 = Control,
- 5 = Low weaning weight pooled sample
- 6 = High weaning weight pooled sample
- 7– 9 = Primer Gen 2-50-22 fingerprinting
- 7 = Control
- 8 = Low weaning weight pooled sample,
- 9 = High weaning weight pooled samples

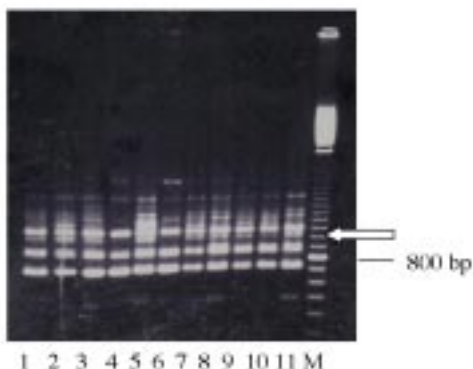


Plate 2. RAPD fingerprinting of individual samples generated by Primer Gen 2-50-22

1 = Low weaning weight individual
2–11 = High weaning weight individuals
M = 100 bp marker

5'AGAAGCGATG and Primer Gen 2-50-22
5'CGAAACAGTC.

Primer Gen 1-05-09, however, generated the specific fragment (about 1.5 kb) in 2 DNA samples in low weaning weight pool and one DNA sample in the high weaning weight pool. Primer 2-50-22 generated specific DNA fragment of about 1.2 kb in 8 individual DNA samples in high weaning weight pooled and in 2 individual DNA samples in the low weaning pooled (Plate 2). To confirm the finding, RAPD reactions using primer Gen 2-50-22 were performed on 65 individual DNA samples of Brakmas male calves selected at random from the nucleus herd. The primer (Gen 2-50-22) generated the same specific band in 39 out of 65 DNA samples.

Grouping these Brakmas calves into three groups of high (>135 kg), medium (90–130 kg) and low weaning weight (<70 kg), the specific fragment was present in all calves grouped in the high weaning group and was not observed among the calves in the low weaning weight group. The fragment was present in 71.2% of the calves in the middle weight group (Table 1). This result thus showed that the specific RAPD marker was associated with weaning weight of Brakmas cattle.

Table 1. Frequency of specific RAPD markers in Brakmas male calves

Groups	N	Frequency of RAPD marker (1.2 kb)
High weaning weight (>135 kg)	10	1.0
Medium weaning weight (90–130 kg)	45	0.71
Low weaning weight (<70 kg)	10	0

The specific RAPD fragment was cloned and was subsequently sequenced. The resulted DNA sequence data confirmed that the size of the DNA fragment was 1 200 bp (Figure 1). The DNA sequence of the RAPD fragment was compared for homology with DNA sequences in the GenBank database. BLAST search indicated significant homology with a number of growth hormone gene sequences available in the Genbank with very high E value (Figure 2).

The DNA sequence of this RAPD band was 99% identical to the complete sequence of the *Bos primigenius indicus* growth hormone gene that was in the GenBank. This homology result indicated that the specific RAPD fragment could be the segment of one of the quantitative trait loci (QTL) for growth performance or the DNA sequence that flank the QTL for growth trait in cattle or be part of the sequence of the growth hormone gene in cattle which is composed of five exons, coding for a 786 long mRNA, spread over 4 kb in the genome (Woychik et al. 1982). These results thus indicated that the specific RAPD marker generated by primer Gen 2-50-22 is associated with the growth trait in beef cattle.

Marker assisted selection technology (MAS) has been successfully applied and used in important agriculture plant and animal breeding programme and is a potential technique for accelerating the genetic gain in plant and livestock species (Bishop et al. 1995; Spelman and Garrick 1997; Ribaut and Hoisington 1998). In beef

TTCCAGGATCCCAGGACCCAGTTCACCAGACGACTCAGGGTCTGTGGACAGCTCACCAGATGA
TGGCTGCAGGGCCCCCGGACCTCCCTGCTCCTGGCTTTCGCCCTGCTCTGCCTGCCCTGGACTCA
GTGGTGGGGCGCTTCCCAGCCATGTCTCTTGCTCCGGCGCTGTTTGCCAACGCTGTGCTCCGGGCTCA
GCACCTGCATCAGCTGGTGTCTGACACCTTCCAAAGATTTGACGCCCGACTCCTATAGGGCGAAT
TGGGCCCTCTAGATGCATGCTCGACGGCGCCGACGTGTGATGGATATCTGCAGAATTCGCCCTTG
TCCTACTCGCTGCGAGTAATTATTTAACTTTCTGATCACCTGCATTTTGGCAGGCAAGACGGACTG
CCACCTTTTACAATAATCAGCTATAAAATCCTCATGATTGTGTACACTTTTATGCAGCAGTAATCT
GAACAAATTTGGCTGCTGACAGCTGTGGTAAATTTGCTAAGAAATGATGCTGTGCAGAGACAAGT
TGTTTTTATGACAGTGCAGAATAAGATAAATCCCATGTCTAAGCAATTTCAATTCAGTCGGAGTTATTT
GCAATCCAATATAGCAAGATTTATAGCATTATTTATTAGTGTGCAAAATGCTTGTTTATCTTTAAAG
TGGCATTGATTTGGTGCCATATATTTTCGGTTGGTGGATTGCTCCTAACTTTATTTGCTGCGTTG
TTATCAGGGACAAGACATTCAACCTACATCCCCGAGGGACAGAGATACTCCATCCAGAACACCCA
GGTTGCCCTTCTGCTTCTGAAACCATCCCCGCCGCCACGGGCAAGAATGAGGCCAGCAGAAAT
CAGACTGGAGTGTCTTCGCATCTCACTGCTCTCATCCAGTCGTGGCTTGGGCCCTGCAGTTC
CTCAGCAGAGTCTTACCAACAGCTTGGTGTGTTGGCACCTCGGACCGTGTCTATGAGAAGCTG
AGGACCTGGAGGAAGGCATCCTGGCCCTGATGCGGGAGCTGGAAGATGGCACCCCCGGGCGT
ACCTGAGGGTCTATGAAGTGCCGCGCTTCGCGGGAGGCCAGCTGTGCCTTCTAGTTGCCAGCCAT
CTGTTGGTTTGCCTCTCCCCGTGCCTTCCTTGACCTGGAAGGTGCCACTCCCAGTGTCTTTCTT
AATAAAATGAGGAAATTCATCGC

Figure 1. DNA sequence of the RAPD bands associated with weaning weight in Brakmas cattle

Sequences producing significant alignments:	Score (bits)	E Value
gi 6063097 gb AF177289.1 AF177289 Bos primigenius indicus g...	581	e-172
gi 2708610 gb AF034386.1 AF034386 Bos indicus growth hormon...	573	e-170
gi 163119 gb M23813.1 BOVGROWP Bos taurus adrenocorticotrop...	573	e-170
gi 163089 gb M27325.1 BOVGHC Bovine growth hormone mRNA, co..	573	e-170
gi 15451666 gb AC012312.9 Homo sapiens chromosome 5 clone ...	557	e-165
gi 9558573 gb AC008534.5 AC008534 Homo sapiens chromosome 5...	557	e-165
gi 399 emb V00111.1 BTGROW Bovine mRNA for growth hormone	553	e-164
gi 6063095 gb AF177288.1 AF177288 Bubalus arnee bubalis gro...	549	e-163
gi 975 emb Y00767.1 CHGHR Goat mRNA for growth hormone	549	e-163
gi 973 emb X07035.1 CHGH Goat mRNA for growth hormone	549	e-163
gi 6063093 gb AF177287.1 AF177287 Capra hircus growth hormo...	541	e-160
gi 1666693 gb U49063.1 OAU49063 Ovis aries placental growth...	541	e-160
gi 609665 emb X15976.1 OOGHM6 Ovine GH mRNA for growth h...	541	e-160
gi 261622 gb S50877.1 S50877 growth hormone [sheep, pituita...	533	e-158
gi 296142 emb X72947.1 BBGHMRNA B.bubalis mRNA for growth h..	525	e-156

Figure 2. BLAST search result showing significant homology of RAPD sequence with gene sequence in the GenBank as indicated by the high Score bits and E value

cattle however, the application of MAS technology is still in infancy. This is due to the lack of genetic markers associated with or linked to economic traits in beef cattle reported or mapped in the bovine genome map. The identification of genetic markers associated with or link to quantitative trait loci (QTL) is thus the first step in the application of marker assisted selection technology in beef cattle breeding.

There are a number of techniques used to identify genetic markers link to QTL in livestock (Michelmore et al. 1991; Plotsky et al. 1993; Lamont et al. 1996; Alfonso and Haley 1998). The most popular method is the reference family and linkage mapped analysis (Beever et al. 1990; Creighton et al. 1992; Alfonso and Haley 1998). This method however, is expensive and time consuming.

An alternative technique, bulk segregant analysis and RAPD technique for the identification of genetic markers associated with or linked to specific gene in the genome was described by Micheltore et al. (1991). Bulk segregant analysis and RAPD technique is as an efficient technique to screen markers linked to specific regions of the genome. Georges (1998) in a review on the use of molecular markers for genetic mapping indicated that the above approach also reduces the number of samples to be analysed and therefore has the potential to accelerate and reduce the cost of the whole genome scan and saturate the gene map. The technique was successfully used to identify genetic markers associated or linked to specific gene or quantitative trait loci in livestock (Schrum et al. 1995; Tarcic et al. 1998.) Tarcic et al. (1998) using the above technique were able to identify three RAPD markers associated with growth rate in chicken but BLAST search did not indicate significant homology of any of the markers with any DNA sequence published in the GenBank.

In the present study, the use of bulk segregant analysis and RAPD technique successfully identified RAPD marker associated with weaning weight in Brakmas cattle. BLAST search indicated that the DNA sequence of the specific RAPD fragment had high identity to the sequences of the *Bos primigenius indicus* growth hormone gene in the GenBank database. The RAPD marker could be used to differentiate between high and low weaning weight Brakmas calves. The marker has the potential to be used for MAS technology for breeding and selection of beef cattle population.

Conclusion

The present study used bulk segregant analysis and RAPD technique to successfully generate genetic marker associated with weaning weight in Brakmas cattle. This RAPD marker of 1.2 kb generated by primer Gen 2-50-22 could be

useful for identifying Brakmas calves with high and low weaning weight. This specific RAPD marker associated with weaning weight would apparently be useful for marker assisted selection of Brakmas cattle for growth performance. The use of this marker in conjunction with other markers available in the genome map would not only increase the efficiency of selection of Brakmas for growth rate but also reduce the cost of selection for breeding bull in central performance test station.

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