Characterization Of Novel Microsatellite Markers In *Bos Taurus* Autosome

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Introduction

In the last decade, extensively quantitative trait locus (QTL) mapping have been implemented to detect QTLs with large effect on the milk production traits of dairy cows. Several independent experiments have reported the presence of QTLs affecting milk yield and composition at 10-44 cM on BTA3 (Ashwell, M.S., Heyen, D.W., Sonstegard, T.S. *et al*. (2004), Heyen, D.W., Weller, J.I., Ron M., *et al*. (1999), Khatkar, M.S., Thomson, P.C., Tammen, I. *et al*. (2004), Olsen, H.G., Gomez-Raya, L., Våge, D.I. *et al*. (2002), Plante, Y., Gibson, J.P., Rodriguez-Zas, S.L., Southey,B.R. Heyen D.W. *et al*. (2002), Viitala, S.M., Schulman, N.F., de Koning, D.J. *et al*. (2003)). The availability of the draft genome sequence of bovine (Btau_4.0, 2007) has boosted the researches on positional cloning of QTLs that may underlie economically important traits. To improve the marker density and lay foundation for fine mapping of these QTLs, additional markers in this region are needed.

Materials and methods

Microsatellite isolation. The sequence between *URB006* and *DIK5036* was obtained from the bovine genome sequence assembly Btau_4.0 (http://www.ensembl.org). With SSRHunter1.3 (Li, Q. and Wan, J.M. (2005).), eighteen dubious microsatellites were found, of which the repeat numbers were higher than 9. Primers were designed in the flanking regions using Oligo6.0, and all the 18 microsatellites were detected. PCR was performed in a final volume of 20 μl containing 100 ng genomic DNA, 0.5 μM of each primer, 2.5 mM $MgCl_2$, 200 μM dNTPs, and 1U Taq polymerase (TaKaRa). Annealing temperature ranged from 55°C to 62°C for 35 cycles. PCR products for homozygous genotypes at each locus were excised from 1.0% agarose gel and sequenced on an ABI 3730XL DNA sequencer.

Microsatellite amplication and genotyping. To examine the polymorphism of each locus, a total of 1,276 Chinese Holstein cows, from 21 herds in Beijing Dairy Cattle Center in Beijing, Dairy Association of China, were genotyped. Forward primers were end-labelled with dyes (6-FAM or HEX). The PCR condition was the same as described above. Fluorescent dye-labelled PCR products were separated using an ABI 377 DNA sequencer, and allele sizes were determined against the internal size standard 400HD (Applied Biosystems) with GeneMapper3.0.

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Linkage analysis. CRI-MAP2.4 (http://linkage.rockefeller.edu/software/crimap/) was carried out to estimate the recombination distances between the microsatellite markers within 10~44 cM on BTA3 under a daughter design. The corresponding reference population was constructed with 11 bull families each having the averaged daughters of 35-145.

Results and discussion

With SSRHunter1.3, eighteen dubious microsatellites were found, of which the repeat numbers were higher than 9. All the 18 microsatellites were detected by PCR amplification, 7 out of which were picked out for further investigation based on amplification efficiency, named *CCM2*, *CCM4*, *CCM5*, *CCM6*, *CCM7*, *CCM17*, and *CCM18* (GenBank accession numbers: EU807941-EU807947). Their primer sequences and other characteristics are given in Table 1. Sequencing results showed that the repeat unit of *CCM2*, *CCM4*, *CCM5*, *CCM6*, *CCM7*, *CCM17* and *CCM18* was $(TG)_{21}$, $(CA)_{20}$, $(CA)_{23}$, $(TG)_{17}$, $(TG)_{23}$, $(TA)_{20}$, and $(CA)_{19}$, respectively.

The polymorphism of each novel microsatellite locus was detected in a population of 1276 Chinese Holstein cows. The seven microsatellites were revealed to by polymorphic. The observed (N_O) and effective (N_E) number of alleles, observed (H_O) and expected (H_E) heterozygosities , polymorphic information content (PIC), and Shannon information index (I) were calculated using PopGene3.2. The results showed that the observed allele number per locus ranged from seven (*CCM18*) to 18 (*CCM7*, *CCM17*) and H_E from 0.32 to 0.86 and PIC from 0.30 to 0.85, while Shannon information index ranged from 0.63 to 2.13.

With linkage analysis, the recombination distances between the seven new markers isolated here as well as 5 known markers (*DIK4842*, *NLBCMK38*, *DIK1057*, *DIK4103* and *BMS2522*) within 10~44 cM on BTA3 have been estimated. We found that the order of five known microsatellite markers achieved (Table 1) was consistent with those of MARC map (Ihara, N., Takasuga, A., Mizoshita, K., *et al*. (2004),), but the total coverage in the study was much longer. This is likely due to the limited recombination information found in our family structure and the number of daughters from each sire. Based on the estimated order of 12 markers, their physical positions obtained from Btau_4.0 as well as bovine linkage maps (Ihara, N., Takasuga, A., Mizoshita, K., *et al*. (2004), Arias, J.A., Keehan, M., Fisher P. *et al*. (2009)), we estimated the approximate positions of five new isolated microsatellite markers (Table 1), of which discrepancies were observed between the marker order in this study and their physical positions (Btau_4.0). Similar discrepancies were also found in other linkage maps, and could be attributed to spurious information in the bovine assembly Btau 4.0 (Arias, J.A., Keehan, M., Fisher P. *et al*. (2009)).

Table1: Information of seven microsatellite loci

Locus	Genbank accession no.	Repeat type	Position (cM)*	Physical position (Build 4.0)
CCM2	EU807941	$(TG)_{21}$	11	3892213
DIK4842	AB165967	$(TG)_{14}$	12.2	7401665
CCM4	EU807947	$(AC)_{20}$	14	4232566

NLBCMK38	AB166576 AB166577		15.7	
CCM5	EU807943	$(CA)_{23}$	16	10298343
DIK1057	AB164720	$(CA)_{18}$	17.4	10935417
DIK4103	AB165431	$(TG)_{11}$	17.4	11139637
CCM6	EU807942	$(GT)_{17}$	18	10935406
CCM7	EU807944	$(TG)_{23}$	19	9770243
BMS2522	G18949	$(CA)_{13}$	28.8	18429752
CCM17	EU807945	$(AT)_{20}$	40	29003780
CCM18	EU807946	$(CA)_{19}$	41	29615233

*The relative positions of the seven known microsatellite markers were obtained from the MARC map (Ihara, N., Takasuga, A., Mizoshita, K., *et al*. (2004),).

Conclusion

Although advances in genotyping technology of high-density SNPs have dramatically increased in cattle, microsatellite markers positioned in linkage maps play critical roles in QTLs mapping because of their high polymorphism and the feature of easily high-throughout detection, especially in the regions where QTLs have been reported. The developed microsatellites here improved the map in the region of 10~44 cM on BTA3.

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References

Arias, J.A., Keehan, M., Fisher P. *et al.* (2009). *BMC Genet.*, 10: 18.

Ashwell, M.S., Heyen, D.W., Sonstegard, T.S. *et al*. (2004). *J. Dairy Sci.,* 87: 468-475.

Heyen, D.W., Weller, J.I., Ron M., *et al*. (1999). *Physiol. Genomics*, 1: 165-175.

Ihara, N., Takasuga, A., Mizoshita, K., *et al*. (2004). *Genome Res,*. 14: 1987-1798.

Khatkar, M.S., Thomson, P.C., Tammen, I. *et al*. (2004). *Genet. Sel. Evol.*, 36:163-90.

Li, Q. and Wan, J.M. (2005). Yi Chuan, 27: 808-810.

Olsen, H.G., Gomez-Raya, L., Våge, D.I. *et al*. (2002). *J. Dairy Sci*., 85: 3124-3130.

Plante, Y., Gibson, J.P., Nadesalingam, J. *et al.* (2001). *J. Dairy Sci*., 84: 1516-1524.

Rodriguez-Zas, S.L., Southey,B.R. Heyen D.W. *et al*. (2002). *J. Dairy Sci.,* 85: 2681-2691.

Viitala, S.M., Schulman, N.F., de Koning, D.J. *et al*. (2003) *J. Dairy Sci.,* 86: 1828-1836.