GENETIC AND PHYSICAL MAPPING OF FOUR COSMID-DERIVED MICROSATELLITES IN CATTLE

MAPEO FÍSICO Y GENÉTICO DE CUATRO MICROSATÉLITES DERIVADOS CÓSMIDOS EN BOVINO

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Additional keywords

Genetic markers. Linkage groups.

Palabras clave adicionales

Marcadores genéticos. Grupos de ligamiento.

SUMMARY

The isolation of microsatellites from cosmids permits the integration of the meiotic and cytogenetic maps. The precise localisation of genetic markers is a powerful tool to assign and orientate linkage groups.

In the present work, we report the isolation of four polymorphic microsatellites (INRAZARA 232, INRAZARA 233, INRA 238 and INRA 241) from four cosmids (cos AE21, cosAE33, cosAE44 and cosPL139), screened for TG/TC motifs and located by FISH on cattle chromosomes 2q45, 2q45, l9ql5 and 1lq28, respectively. Sequences flanking microsatellites were characterised by subcloning into pGEM4Z vector. Primers were designed and specific amplification products were achieved.

These markers were analysed in the animals belonging to the International Bovine Reference Panel (IBRP) and analysis for linkage was performed using the CRI-MAP program in the Cattle Geneotypic Database. The results obtained are in correspondence to the physical location. Furthermore, the polymorphism of two of these microsatellites was evaluated on 40 unrelated animal belonging to different autochtonal Spanish breeds and showed the following number of alleles and PIC values: 9/0.796 for INRAZARA232 and 10/0.805 for INRAZARA233.

RESUMEN

El aislamiento de microsatélites a partir de cósmidos, permite la integración de los mapas meióticos y citogenéticos. La localización precisa de los marcadores genéticos es una potente herramienta para asignar y orientar los grupos de ligamiento.

En el presente artículo se presenta el aislamiento de cuatro microsatélites polimórficos (INRAZARA 232, INRAZARA 233, INRA 238 and INRA 241) procedentes de cuatro cósmidos (cos AE21, cosAE33, cosAE44 and cosPL139), estudiados por motivos TG/TC y localizados mediante FISH en los cromosomas bovinos 2q45, 2q45, l9ql5 y 1lq28 respectivamente. Las secuencias de flanqueo de los microsatélites fueron caracterizadas mediante subclonamiento en vector pGEM4Z. Se designaron los primeros y se consiguieron productos específicos de amplificación.

Esos marcadores fueron analizados en los animales pertenecientes al International Bovine Reference Panel (IBRP) y el análisis del ligamiento fue realizado empleando el programa CRI-MAP en la base de datos Cattle Geneotypic. Los resultados obtenidos estan de acuerdo con la localización física. Además el polimorfismo de dos de esos microsatélites fue evaluado sobre 40 animales no relacionados,

pertenecientes a diferentes razas autóctonas españolas mostrando el siguiente número de alelos y valores PIC 9/0.796 para INRAZARA232 y 10/0.805 para INRAZARA 233.

INTRODUCTION

Microsatellites have been identified in all eukaryotic species studied so far. On average, their number is estimated at 100,000 in mammals. They are distributed through the euchromatin displaying a high polymorphism, with a median Polymorphic Information Content (PIC) of 0.60 (Vaiman *et al.*, 1994). It is possible to follow the segregation of microsatellite alleles in pedigrees because of their relatively low mutation rate.

These simple tandem repetitive sequence motifs have become the most popular markers for gene mapping projects in human (Weissenbach *et al.*, 1992), mouse (Dietrich *et al.*, 1992) and also in several livestock species (Archibald *et al.*, 1995, Crawford *et al.*, 1995). In cattle more than six hundred of DNA microsatellites have been characterised and mapped by linkage analysis (Barendse *et al.*, 1994; Bishop *et al.*, 1994; Barendse *et al.*, in preparation).

Localization by FISH (Fluorescence *In Situ* Hybridization) of large-insert clones to chromosomes makes the cosmid-derived microsatellites to be a very efficient system for obtaining highly polymorphic markers to be placed on the genetic and physical maps (Solinas Toldo *et al.*, 1993; Ferreti *et al.*, 1994; Eggen and Fries, 1995). We report here the physical mapping by FISH of 4 cosmid-derived microsatellites. These markers have been also placed on the bovine linkage map. The correspondence between linkage groups and chromosomes was confirmed.

MATERIALS AND METHODS

ISOLATION OF COSMIDS CONTAINING MICROSATELLITES

A cosmid library was obtained from high-molecular-weight bovine DNA. Sau3A partially digested and dephosphorylated DNA was ligated into the BamHI site of the SuperCos 1 cloning vector (Stratagene Inc., La Jolla, CA, USA). Cosmids were plated and screened with $(TG)_{10}$ and $(TC)_{10}$ probes 5' end labeled with $\gamma[^{32}P]ATP$ and T4 polynucleotide kinase, following standard protocols. Positive cosmids were digested with Sau3AI, fragments were dephosphorylated and cloned into the BamHI site of pGEM4Z. After transformation, bacteria were plated and screened in the same way as described above. Positive subclones were sequenced with an ABI 373 sequencer.

PCR AMPLIFICATION

Primers were designed from the sequences (see **table I**). Amplification was carried out in 10 µl containing 20-30 ng bovine DNA, 5 pmol of each primer, 2mM of each dNTP, lx PCR buffer with 1.5 mM $MgC1_2$ and 0.5 units Taq polymerase. Thermocycling was performed by initial denaturation at 94°C for 5 min, 30 cycles of 15 s at 94QC, 15 s at 58-64°C (see **table I**) and 20 s at 72°C and a final extension of 10 min at 72°C. Polymorphism was found by PCR performed in the presence of lµCi of $a[^{32}P]$-dCTPs. PCR products were analyzed by denaturing polyacrylamide

gel electrophoresis. Size of the alleles were determined by comparison to M13mpl8 DNA sequence ladders.

Polymorphism was evaluated in 40 unrelated animals belonging to several Spanish autochtonous breeds. Polymorphism Information Content (P.I.C., Botstein *et al.*, 1980) value was calculated.

MENDELIAN INHERITANCE AND LINKAGE

Codominant segregation was observed in the families of the International Bovine Reference Panel. Typing data were analyzed against the CGD (Cattle Genotypic Database) using the CRI-MAP program.

PHYSICAL LOCATION

Localization of cosmid clones containing microsatellites by fluorescence in situ hybridization (FISH) was made following the technique described by Bahri Darwich *et al.* (1994). R-banding was induced in the metaphase spreads. Biotinylation of the cosmid DNA was achieved by random-priming in the presence of biotin-11-dUTP (Sigma) and Klenow DNA polymerase I.

RESULTS AND DISCUSSION

ISOLATION OF MICROSATELLITES

Four positive clones containing cosmids (cosAE21, cosAE33, cosAE44 and cosPL139) were selected for further analysis. Sequence of microsatellite regions INRAZARA232, INRAZARA233, INRA238, INRA241 were obtained from cosAE21, cosAE33, cosAE44 and cosPL139 cosmids respectively. **Table I** shows primer sequences, optimal annealing temperature, product size and number of alleles obtained for each microsatellite.

The polymorphism found in these markers allowed us to analyze the IBRP families for linkage analisis. Because of the high number of alleles found at INRAZARA232 and 233, these microsatellites were tested on 40 animals belonging to three Spanish breeds (Pire-

Table I. *Characterization of four bovine microsatellites.* (Caracterización de cuatro microsatélites bovinos).

Microsatellite	PCR primers	Pr. size	Ann.T.	N. Alleles	PIC
INRAZARA232	GGTGCCTGCATGTGCATGCACAGC GATCACTGCCTAAGTTCAAGAGTG	97 bp	60°C	9	0.796
INRAZARA233	GACAGTCTAGAGGATCTAAAG CCTGTGATAGTGTGGGCAGAGC	309 bp	58°C*	10	0.805
INRA238	GCTTAAGGAGACAGGATGGAT GTACTCTGTATTGTGCACTGG	150 bp	62°C	3	-
INRA241	GCTTCAAACCCAGGCGTTTCG CTGGGTCCTAAAACTTGCTCAC	134 bp	61°C	3	-

* A Touch Down PCR was neccesary to amplify this microsatellite (Touch DownTemperature).

naica, Rubia Gallega and Asturiana de los Valles). **Table I** shows the obtained PIC values which are informative enough to consider these markers as good candidates for identification and paternity testing.

GENETIC MAPPING

Families from the IBRP were tested for these four new markers, linkage data maps INRAZARA232 and INRAZARA233 at BTA2, INRA241 at BTAll and INRA238 at BTAl9 (Barendse, pers. comm.). Recombination fractions will be published in somewhere else as a part of the Second Bovine Genetic Map (Barendse *et al.*, in preparation).

PHYSICAL MAPPING

Parallel at linkage studies, physical location by FISH was realised for the four cosmids. The R-banding allowed us to localize all the specific spots on chromosome 2 at q45 for INRAZARA 232 and 233, on chromosome 11 at q28 for INRA 241 and on chromosome 19 at qlS for INRA238.

Physical assignation by FISH of INRAZARA232 and INRAZARA233 confirms the linkage group orientation of BTA2, previously orientated by the location of IDVGA 2 marker. INRA241 also confirms the linkage group orientation of BTAll, previously done by the location of INRA177 at 1 lql6 and INRAl 15 at 1 lq25. The last new marker, INRA238, confirms the linkage group orientation of BTAl9, this chromosome was previously orientated with ETH3 marker, which maps in the telomeric region of this chromosome, ETH12 has been also located using FISH but this marker was not placed at genetic mapping. Using cosmid derived microsatellites, we have stablished four new anchor sites between physical and genetic maps in three bovine chromosomes in confirming the linkage groups orientations.

ACKNOWLEDGEMENTS

This work is part of the BovMap project financed by the EU (Biotechnology contrast N.BI02-CT92-0359)

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