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Development and assignment of bovine-specific PCR systems for the Texas nomenclature marker genes and isolation of homologous BAC probes

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Abstract – In 1996, Popescu *et al.* published the Texas standard nomenclature of the bovine karyotype in which 31 marker genes, already mapped in man, were chosen to permit unambiguous identification and numbering of each bovine chromosome. However, specific PCR systems were not available for each marker gene thus preventing the assignment of part of these markers by somatic cell hybrid analysis. In addition, some difficulties remained with the nomenclature of BTA25, BTA27 and BTA29. In this work, specific PCR systems were developed for each of the marker genes except VIL1 (see results), from either existing bovine or human sequences, and a bovine BAC library was screened to obtain the corresponding BAC clones. These PCR systems were used successfully to confirm the assignment of each marker gene (except for LDHA, see results) by analysis on the INRA hamster-bovine somatic cell hybrid panel. The difficulties observed for LDHA and VIL1 are probably due to the fact that these genes belong to large gene families and therefore suggest that they may not be the most appropriate markers for a standardisation effort. This panel of BACs is available to the scientific community and has served as a basis for the establishment of a revised standard nomenclature of bovine chromosomes.

bovine / BAC library / cytogenetics / mapping / Texas standard

1. INTRODUCTION

The cattle genome is composed of 29 autosome pairs and two sex chromosomes. While X and Y chromosomes are submetacentric, all autosomes are acrocentric and with small size differences, therefore difficult to differentiate and impossible to identify without a banding method. Since the early 70s,

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several banding techniques have been applied to cattle chromosomes, resulting in different systems of cytogenetic nomenclature.

The first international nomenclature of the bovine karyotypes was established in 1976 during the Reading conference using G-banded metaphase chromosomes: this GTG standard karyotype created the basis for all subsequent nomenclature efforts [5]. With the development of prometaphase chromosome preparations and R-banding techniques, a second nomenclature was published in 1990 [4] in which correlations between G/Q- and R-banded chromosomes were proposed together with their diagrammatic representations. In the following years, some confusion in the bovine nomenclature led Popescu *et al.* [14] to define the Texas standard nomenclature during the third international meeting for the standardisation of cattle karyotype held in College Station (Texas). It resulted in the choice of 31 marker genes already mapped in man to permit unambiguous identification and numbering of each bovine chromosome.

However, part of these genes were cytogenetically mapped with heterologous probes and specific PCR systems were not available for each marker gene thus preventing PCR-based assignment using a somatic cell hybrid panel and the isolation of homologous probes from large insert genomic DNA libraries. In addition, some difficulties remained with the nomenclature of BTA25, BTA27 and BTA29.

In this work, PCR systems were developed from already published homologous or heterologous sequences for each of the marker genes and were used to assign the corresponding genes by analysis on the INRA hamster-bovine somatic cell hybrid panel [11] and to screen a bovine BAC library to obtain corresponding BAC clones.

2. MATERIALS AND METHODS

2.1. Primer design

When available, primers were designed from the bovine sequences stored in GenBank. When only the bovine mRNA sequences were reported, primers were designed either in the 3′ untranslated region because of its lower intron frequency [16] and lower similarity degree, or after comparison with the corresponding genes in human and mice, to infer gene structure. For CSN10 and LGB we used previously described primer pairs (see reference or Accession number in Tab. I).

2.2. PCR conditions

PCR reactions were performed on an MJ Research PTC-100 thermocycler in 15 μL reaction volumes with $1\times$ Mg^{2+} free buffer, 0.125 mM dNTP, 1.5 mM $MgCl_2$, 0.5 μM of each primer and 0.035 $U \cdot \mu L^{-1}$ *Taq* polymerase (Promega).

Samples were preheated for 5 min at 94 °C, subjected to 35 cycles of 94 °C for 20 s, optimal annealing temperatures ranging from 50 °C to 60 °C (see Tab. I) for 30 s and 72 °C for 30 s, and to a final extension step of 5 min.

2.3. Sequencing

The sequencing reactions were performed on the PCR products directly using a Dye Terminator kit (Perkin Elmer). For IGH@, the PCR product was cloned in the vector PGEMT (Promega) and sequenced with a universal sequencing kit (Perkin Elmer).

The sequencing products were run on an ABI377 sequencer. The resulting sequences were compared to existing sequences using the BLAST program and submitted to GenBank.

2.4. Chromosomal assignment using the INRA hamster-bovine somatic cell hybrid panel

The panel was constructed by Heuertz and Hors-Cayla [9] and is composed of a total of 38 hamster-bovine cell lines. A more complete description of the panel is given in Laurent *et al.* [11]. A correlation coefficient of 0.69 was used as the threshold for confident assignment of a marker to a chromosome [3]. PCR-based assignments were performed according to Laurent *et al.* [11].

2.5. Bovine BAC identification and preparation

A 4-fold genome equivalent bovine BAC library containing 105 984 clones was constructed in pBeloBAC11 (Eggen *et al.*, submitted). Clones were pooled in 46 primary superpools of 2 304 clones each and in secondary pools consisting of pools of plates, columns and rows using a 3D strategy. PCR-based screening was performed as described in Eggen *et al.* (submitted).

The BAC clone DNA mini-preparations were performed according to Birnboim and Doly [2].

2.6. Fluorescent In Situ Hybridisation (FISH) experiments

The BAC containing LDHA was hybridised on R-banded bovine chromosomes (according to ISCNDA 1989, [4]) using the same protocol as described in Hayes *et al.* [7].

3. RESULTS

3.1. Primer design

Homologous primers were designed from existing bovine sequences for every gene of our study except IGH@, PGK1, VIL1 and ZFY. Description of

Table I. Loci list and description of PCR systems. Primer pairs in bold characters are heterologous and Accession number in bold characters corresponds to fragments sequenced during this study. All chromosome localizations are those given in the Bovmap database and gene names are according to the HUGO Nomenclature except BOLA-DYA and LGB for which no human equivalent gene is known.
BOVMAP database: http://locus.jouy.inra.fr/
HUGO Gene Nomenclature: www.gene.ucl.ac.uk/nomenclature/. *(continued on the next page)*

Gene Symbol	Gene Name	Localization	Forward primer	Reverse primer	Accession number	Annealing temperature (°C)	Product size (bp)	Published Marker Highly Correlated (Correlation coefficient)	Bovine BAC Adress
SOD1	superoxide dismutase 1, soluble	1q12-q14	GTTTGGCCTGTGGTGTAATTGGAA	GGCCAAAATACAGAGAGATGAATGAA	M81129	58	273	DIK70 (0,89)	426H12
VIL1MS	villin 1 microsatellite (see text)	2q43	GAGTTGGGGAGAAAATCAAGGTTG	ACACTCACACACACACAAAGCCTC	[12]	55	~300	INRA232 (0,93)	406B1
HSD3B1	hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 1	3q12	AAGACGGCCGTGAAGAAGAGC	TGGATGTTTGGGACCTTTTTGG	X17614	55	334	RM065 (1,00)	219F9
INHBA	inhibin, beta A	4q26	AGTCACCATCCGTCTCTTTCAACAG	CCCTTCCCCCTCCTCTTCTTTCT	U16238	55	289	CSSM014 (0,94)	455E12
IFNG	interferon, gamma	5q22-q24	AGTAACTAGGCAAGTCTATGGGATT	GAGATGCTATGTTTTTGTCAGG	Z54144	55	278	INRA240 (0,73)	946H5
CSN10	casein, kappa	6q31	ATCATTTATGGCCATTCCACCAAAG	AGACAATGTCTCTTCCGCTTTACCCG	[13]	55	350	INRAK (0,94)	505F4
RASA1	RAS p21 protein activator	7q24-qter	GGGCCACAGCCCAGGATCGGGAG C	CCCTTCCGCTTTAGTGCAGCCAG	X12602	55	187	ILSTS006(1,00)	982C12
IFN1@	interferon, type 1, cluster	8q15	CATCTCTGTGCTCCACGAGGTGA	GGCTCTCATGACTTCTGCTCTGACA	M10952	55	294	INRA129 (0,85)	70B4
IGF2R	insulin-like growth factor 2 receptor	9q27-q28	CTCCGACCAAATCCGATGGGA	CACGCTCAAGGTGAGGGCA	J03527	58	191	UWCA09 (1,00)	293G9
CYP19	cytochrome P450, subfamily XIX	10q26	GCCATGGTGATGATGAAGGTCGTC	TAGCGCTCGAGGCACTTGTCTGAAT	Z69250	55	183	INRA071 (0,86)	963H2
LGB	lactoglobulin, beta	11q28	TGTGCTGGACACCGACTACAAAAA G	GCTCCCGGTATATGACCACCCTCT	X14710	55	300	ILSTS049 (0,83)	286F8
RB1	retinoblastoma 1	12q13	GAATGAGGAAGTGAGAT	CTTTGTTTAAACTGAGAGT	**AF304439**	50	137	INRA209 (0,83)	783G1
IL2RA	interleukin 2 receptor, alpha	13q13-q14	GGATTGCTATAAATGATGCTCCACA	ACATTAGATGTACTGCTCCCTTATC	M20818	55	133	TGLA006 (0,80)	283G8
TG	thyroglobulin	14q12-q15	GGCCCTGGCCCTATGGGTC	AAAGATGTTGGCGGATGCC	X05380	55	63	RM11 (0,94)	1004F9

Table I. Continued.

Gene Symbol	Gene Name	Localization	Forward primer	Reverse primer	Accession number	Annealing temperature (°C)	Product size (bp)	Published Marker Highly Correlated (Correlation coefficient)	Bovine BAC Adress
FSHB	follicle stimulating hormone, beta polypeptide	15q25-qter	TCAAGGAGCTGGTCTACGAGA	CCGCTGCTCTTTATTCTTTGAT	M13383	55	188	INRA224 (0,71)	462D8
PIGR	polymeric immunoglobulin receptor	16q13	GACCGGATTTCAATCAGGAGCTAC C	TCTGGAGAGGCTCCCATGTTGTCAC	L04797	55	100	INRA013 (0,71)	349E11
FGG	fibrinogen, gamma polypeptide	17q12-q13	GGTCGGAGTAGAACATCACGTT	GGGAATACAGTCCAAAGTGAGT	X15556	55	192	INRA193 (0,93)	111B7
GPI	glucose phosphate isomerase	18q22-q24	GGCCGCTACTCGCTGTGG	CCAGTGAGCTCCTGAGAGCAGC	AF043228	58	92	BM2078 (0,79)	651C8
GH1	growth hormone 1	19q22	TATGAGAAGCTGAAGGACCTG	GTGCCATCTTCCAGCTCCTG	M57764	55	337	CSSM065 (1,00)	993G12
MAP1B	microtubule-associated protein 1B	20q14-q15	TTTCTCCACCAGACTTCTCCCCTAA	AGTGGGCCCGTTTTCAGTGATA	Z29520	60	178	INRA36 (0,94)	713A1
IGH@	immunoglbulin heavy polypeptide gene cluster (V,D,J,C)	21q23-q24	**GGACTGGTGAAGGCTTCGGAGAC**	**ACAGAGCTCAGCCTCAGGGAGAACT**	**AF304438**	50	227	TGLA122 (0,86)	355H4
LTF	lactotransferrin	22q24	GATTCAAGCTGAGGCATTCC	CCAAAGTGGCCAATTTGAC	L19986	55	164	BM4102 (1,00)	336B8
BOLA-DYA	major histocompatibility complex, class II,DY alpha	23q12-q13	TCCCTGAAGTGGCTGTGTTTCC	ACTCTTGGGGTAGAAGGTGGTCTCA	M30118	55	167	BM47 (0,89)	712H8
DSC@	desmocollin genes cluster	24q21-q22	CAGTGACTGGCACAACTT	AGTGTGTCCTCTAATGGATT	X56968	58	811	TGLA351 (0,83)	810H10
ELN	elastin	25	CGGGAACAGGGCCAGCAGC	CTGGGTCTGACTGGACTGGAC	M58652	55	161	ILSTS063 (0,83)	513H8
TNFRSF6	tumor necrosis factor receptor superfamily, member 6	26q13	GGGCTAAAATGGCAAATATTAGGTA AG	TATCTTTGCAACTTTTCTGTAAGTCGA A	U24240	55	200	INRA081 (0,93)	839E10
DEFB1	defensin, beta 1	27q13-q14	CATTTCCTTCTGCATAGAAAA	AAGCCCATGCTTGGCCTG	AF016539	56	912	INRA016 (0,75)	312A6
CGN1	conglutinin 1	28q18	CTGCTCCTGCTCACACAG	TCTCCCATCTTGTCCATCAT	U06854	55	143	BM2515 (1,00)	196G4
LDHA	Lactate dehydrogenase A	29qter	ATGAGGTGATCAAACTGAAA	CCTTAATCATGGTGGAAATC	D90143	55	119	Unassigned	39C7
#IGF2	insulin-like growth factor 2	29qter	GACCATCCAGCCGCATAAA	GGGGGTGGCACAGTAAGTCT	Z68151	55	100	ILSTS081 (1,00)	953A11
PGK1	phosphoglycerate kinase 1	Xq21-q22	**GGATGTTCTGTTCTTGAAGG**	**CTTGTTCCCAGAAGCATCTT**	**AF304436**	55	177	INRA242 (0,74)	327D2
ZFY	zinc finger protein, Y-linked	Yp13	**GGTTATTAAATCGCCACCTT**	**GTTTTCACATGCGTCTTCA**	**AF304437**	55	198	see text	852D12

the primer pairs is given in Table I as well as the GenBank Accession numbers of the sequences they were designed from.

For ZFY, IGH@ and PGK1, heterologous primers were designed respectively from the buffalo sequence (X99826), and the corresponding human genes (L03677 and M11961 respectively). PCR products were sequenced to confirm homology and sequences were submitted to GenBank (see accession number in Tab. I).

For VIL1, no specific primers could be obtained. As a result, we decided to use primers specific for a microsatellite derived from a phage vector containing the bovine gene [12] and which serves as a reference for the establishment of the Texas nomenclature [14].

For RB1, heterologous primers:

RB1F: CTTGTGTGATTAACTTATTTAGAG
and RB1R: AATGTGAACTTAGTAGCAAAAGAC

derived from the human sequence L11910 were used to amplify bovine genomic DNA. Unfortunately, as these PCR primers amplify a product of similar size in cattle and hamsters, the assignment on the hamster-bovine somatic cell hybrid panel of this gene was not possible with the heterologous primers. Therefore, the bovine-specific fragment obtained was sequenced and the resulting sequence (GenBank accession number AF 304439) was used to define specific homologous bovine primers (see Tab. I).

3.2. Chromosomal assignments

Clear chromosomal assignments were obtained for each marker gene except LDHA and ZFY. Correlation coefficients with the first published marker are given in Table I and vary from 0.71 to 1.00, always above the significant threshold (see Materials and Methods).

For LDHA, although a bovine sequence (D90142) was used to design several primer pairs, giving a product of the expected length and sequence, no clear assignment could be obtained.

No correlation coefficient could be obtained for ZFY because no other marker of the Y chromosomes was found in the non-pseudo autosomal region. As a result, ZFY itself will serve as a marker of the Y chromosome in our panel.

3.3. Isolation of bovine BAC clones

For each marker gene, at least one BAC clone was identified after screening the primary and the secondary pools. The presence of the gene of interest was confirmed by PCR on the BAC DNA. For the three BAC clones identified using

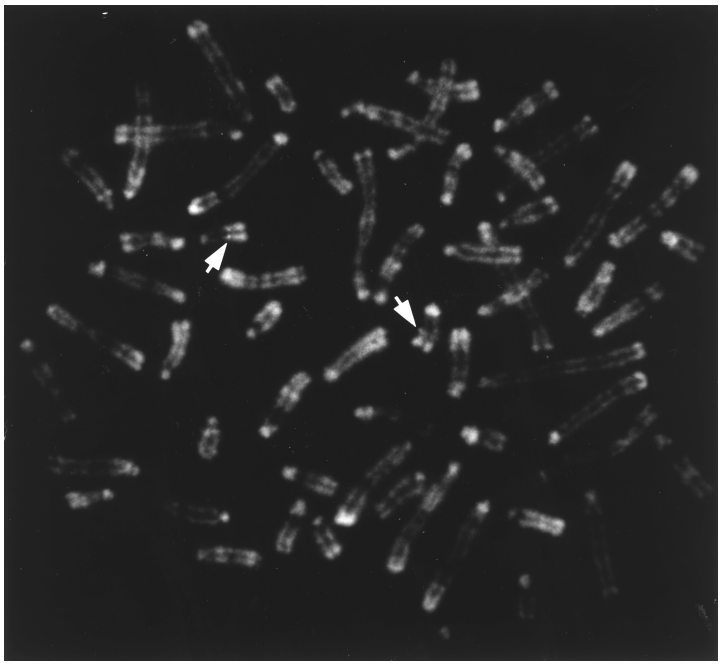

Figure 1. Metaphase spread of bovine R-banded chromosomes showing specific hybridization of LDHA BAC clone to bovine chromosome 29q22. Arrows indicate the specific hybridization signal.

heterologous primers (355H4, 327D2 and 852D12 containing respectively IGH@, PGK1 and ZFY) specific PCR-amplified fragments were sequenced to confirm the presence and the homology with the corresponding gene.

Bovine BAC addresses proposed as probes for further cytogenetic studies are given in Table I.

3.4. FISH localisation of LDHA

Because of difficulties with chromosomal assignment of LDHA on the INRA somatic cell hybrid panel, the BAC isolated with specific primer pairs was hybridised on R-banded bovine chromosomes. This revealed that LDHA is physically mapped to BTA29q22 (see Figs. 1 and 2).

4. DISCUSSION

Problems encountered for the assignment and the design of specific primer pairs for VIL1 and LDHA can be explained by the fact that these genes belong to large gene families with or without pseudogenes. Thus they may not be

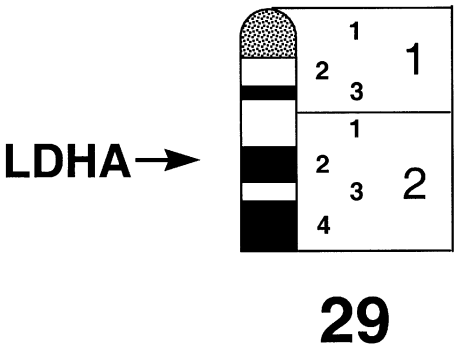

Figure 2. Ideogram of bovine R-banded chromosome 29 indicating the position of the LDHA gene.

the most appropriate marker genes for standardisation. The difficulties with VIL1 have been solved using the microsatellite isolated in the same phage as the gene [12]. The isolated BAC is currently being studied to confirm the presence of the VIL1 gene and to describe a specific coding sequence. For BTA29, as LDHA could not be assigned to the somatic cell hybrid panel despite the fact that homologous primers were chosen [11], we proposed to solve the difficulties in assignment by choosing another marker gene for BTA29, IGF2. Both LDHA and IGF2 have been localised by radioactive ISH at the same telomeric end of BTA29 [15] and IGF2 has been mapped to BTA29 using the INRA somatic hybrid cell panel [11].

The panel of BACs obtained in this study constitutes an essential tool to solve the remaining ambiguities of the bovine karyotype nomenclature, particularly concerning BTA25, BTA27 and BTA29, and could be used as a standard for cytogeneticists using different banding techniques (G, R and Q). Each BAC has just been recently localised by FISH on R-banded and G-banded bovine chromosomes [8].

These BAC clones could also serve as chromosome markers in other cytogenetic studies which require to trace a specific chromosome, for example X and Y [6], and the specific primers developed here could serve as an efficient tool to calibrate different existing hybrid somatic panels [1, 10, 11, 17].

The panel is available upon request to the entire scientific community and has served as a basis for the establishment of a revised standard nomenclature [8] based on homologous probes.

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