

Assignment of PCR markers to river buffalo chromosomes

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Abstract – In the process of developing a buffalo physical map using somatic cell hybrids and the cattle gene map as a template, ten PCR primers designed for four coding genes: F10, FSHB, HBB, and CYM and six DNA segments: TGLA9, TGLA227, UWCA5, CSSM6, CSSM47 and RF131 were tested on a panel of 47 buffalo–hamster somatic cell hybrids. F10-TGLA9, FSHB-HBB and UWCA5-TGLA227, respectively, were found to segregate together forming three syntenic groups. These three syntenic groups have also been reported in cattle, where they have been assigned to chromosomes BTA 12, BTA 15 and BTA 18, respectively. Comparative mapping predicts the assignment of these syntenic groups to river buffalo chromosomes BBU 13, BBU 16 and BBU 18, respectively.
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buffalo / chromosome / synteny / PCR markers / gene mapping

Résumé – **Assigination de marqueurs PCR aux chromosomes du buffle de rivière.** Dans le but de développer la carte génétique physique du buffle en utilisant l'hybridation cellulaire somatique et la carte génétique bovine comme référence, dix amorces PCR correspondant à quatre gènes codants : F10, FSHB, HBB et CYM et six segments d'ADN : TGLA9, TGLA227, UWCA5, CSSM6, CSSM47 et RF131 ont été testés sur une série de 47 hybrides cellulaires somatiques entre buffle et hamster. F10-TGLA9, FSHB-HBB et UWCA5-TGLA227, respectivement, ont été trouvés ségréger ensemble pour former trois groupes synténiques. Ceux-ci ont été aussi observés chez les bovins où ils ont été assignés aux chromosomes BTA 12, BTA 15 et BTA 18, respectivement. Les cartes comparées permettent de prédire une assignation respective des trois groupes synténiques aux chromosomes BBU 13, BBU 16 et BBU 18 du buffle de rivière. © Inra/Elsevier, Paris

buffle / chromosome / synténie / marqueurs PCR / carte génétique

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1. INTRODUCTION

The establishment of a physical gene map of the buffalo genome is an important step towards identifying and cloning loci controlling physiological and quantitative traits. There is a high level of syntenic and chromosomal conservation among members of the family Bovidae [38]. Therefore, inferences can be made concerning the expected location of markers in one species from information available in another species. Several genes previously assigned to cattle syntenic groups and/or chromosomes have been assigned to buffalo chromosomes [6, 7, 10, 11, 18, 19, 23, 24, 28].

The aim of the present work was to investigate the syntenic relationship of ten markers in buffalo, and to map them to buffalo chromosomes on the basis of the comparative banding homoeology between buffalo and cattle.

2. MATERIALS AND METHODS

Ten PCR primers designed to amplify bovine specific sequences were used. The PCR primers represented four coding genes: coagulation factor X (F10), follicle stimulating hormone (FSHB), beta hemoglobin (HBB), and prochymosin pseudogene (CYM), in addition to six DNA markers, namely: TGLA9, TGLA227, UWCA5, CSSM6, CSSM47 and RF131.

Forty-seven somatic hybrid cell lines were developed as described previously [6] from fusion between buffalo lymphocytes and the Chinese hamster cell line wg3h [9]. Genomic DNA was extracted from buffalo lymphocytes, the parental hamster cell line and the 47 hybrid somatic cell clones, according to established protocols [4].

The PCR was performed using Taq polymerase obtained from Promega and the buffer recommended by the manufacturer. For each locus the PCR was carried out in a 25 μ L reaction mixture consisting of 0.2 mM dNTPs, 10 mM Tris, 50 mM KCl, 1.5 mM MgCl₂, 0.01 % gelatin (w/v), 0.125 % units Taq polymerase and 1 μ M upper and lower primers. It was distributed into PCR tubes with 100 ng DNA of buffalo, hamster or hybrid cells. The reaction mixture was overlaid with sterile mineral oil and was run in a Coy Temp Cycler II under the optimum conditions for each primer (*table I*). PCR reaction products were subjected to 3 % agarose gel electrophoresis, stained with ethidium bromide, and scored for the presence or absence of river buffalo-specific PCR products.

2.1. Statistical analysis

Pairwise analysis, based on percentage concordances and correlation coefficients [5] of the segregation profiles of buffalo-specific PCR products, was carried out on the 47 buffalo-hamster somatic cell hybrids.

Table I. DNA sequences of primers used.

Primer	Sequence 5' - - - - -3'	References	Annealing (°C)	PCR product size (bp)
F10	TGTCAGCAGCCTTACCATTACGC C GCCTTCATGATCTTGTCCGATCCAC T	Fung et al. [15] Dietz et al. [8]	70	229
TGLA9	GAACTTCATATGTTAAGAAAAGCC A ACCTTTAGTAACTAGGTGAGAAT A	Barendse et al. [2]	60	267
UWCA5	CTTCCCTGTGTTAGGCTATGT CAAGAGAAAGACAAGACTACAG A	Barendse et al. [2]	60	123
TGLA227	CGAATTCCAAATCTGTTAATTTGCT ACAGACAGAAACTCAATGAAAGCA	Fries et al. [14]	55	157
CYM	CCAGGGCTTCTGTACCAGTGGCTT C TGAGAAATCATCTGTCTGGAACCT C	Neibergs et al. [31]	67	253
FSHB	TGGATATAGACTTAGTGGC CAGTTTCTAAGGCTACATGTT	Heriz et al. [21]	65	195
HBB	ACAGCTCCTGGGCAACGTGCT A GAAAGGAGCTTAATGATATC AG	Schimenti and Duncan [32]	58	115
CSSM6	AGCTTCTGACCTTTAAAGAAAATG AGCTTATAGATTTGCACAAGTGCC	Moore et al. [30]	55	193
CSSM47	TCTCTGTCTCTATCACTATCTGGC CTGGGCACCTGAAACTATCATCAT	Moore et al. [30]	55-60	150
RF131	GTGGACTATAGATCATAAGGT C GCTGTGATGGTCTACGAAATGA	Steffen et al. [36]	50	153

3. RESULTS AND DISCUSSION

The present investigation deals with ten PCR primers designed for either coding genes or microsatellites. Microsatellites constitute a powerful tool for mapping the bovine genome [2, 3, 12], since these DNA markers of unknown function can be exploited as a basis to map genes of economic interest [37]. PCR gene mapping with primers designed from different types of bovine sequences (exons as well as non-coding regions) is very useful when amplifying relatively short fragments (up to 600 bp), with the annealing portion of the reaction run at the highest possible temperature [8].

The presence or absence of the PCR amplified fragment in each of the 47 hybrid clones determined the assignment of each marker. Results (*table II*) revealed syntenic relationships between F10–TGLA9, HBB–FSHB and TGLA227–UWCA5, for which the percentage agreement and correlation coefficient, φ , values exceeded 95.7 % and 0.81, respectively. The results presented in *table II* showed that some markers, despite the fact that they had a high percentage agreement (> 90 %) were not syntenic because their estimated φ values did not exceed 0.04. The correlation coefficient, φ , is considered to be the best discriminating factor for synteny when its value exceeds 0.69 (in 40 independent clones) and it recognizes about twice as many true syntenic pairs as a simple discordant ratio calculation [5].

The six loci investigated, representing three syntenic groups in buffalo, have also been found to be syntenic in cattle. F10 [8] and TGLA9 [2] have been assigned in cattle to syntenic group U27, which has been assigned to cattle chromosome BTA 12 [29]. FSHB and HBB have been mapped to cattle syntenic group U19 [1] and FSHB has been localized on chromosome BTA 15q24-qter [20]. Fries et al. [14] have assigned TGLA227 and UWCA5 to cattle syntenic group U9 and to cattle chromosome BTA 18. TGLA227 and UWCA5 have also been found to be genetically linked [2].

The other markers investigated (CYM, CSSM6, CSSM47 and RF131) have been found to segregate independently from each other and from the three syntenic groups. In cattle, these markers are assigned to different syntenic groups and consequently to different chromosomes. CYM has been assigned to bovine syntenic group U6 and to chromosome BTA 3 [16]. CSSM6 and CSSM47 have been assigned to U12 and U18, respectively [30]. Syntenic group U12 has been mapped to BTA 22 [35] while U18 has been mapped to both BTA 8 [24, 33] and BBU3q [33]. RF131 has been mapped to cattle syntenic group U4 [36], which has been located on BTA21 [13, 17].

The present study confirms syntenic conservation between cattle and buffalo as previously demonstrated [6, 7, 11, 32]. Chromosomal conservation between cattle and buffalo has also been demonstrated, based on the banding homoeology between cattle and river buffalo chromosomes [22]. Genes assigned to specific cattle chromosomes have been assigned to homoeologous buffalo chromosomes [6, 7, 10, 11, 18, 19, 23–28]. Chromosomal conservation between cattle and buffalo suggests that the localization of the syntenic loci F10–TGLA9, FSHB–HBB and TGLA227–UWCA5 could be on buffalo chromosomes BBU 13, BBU 16 and BBU 18, respectively. Comparative mapping also predicts the assignment of CYM, CSSM6, CSSM47 and RF131 to river buffalo chromosomes BBU 6, BBU 21, BBU 3q and

Table II. Percent concordance and correlation coefficients (φ) of the segregation of ten markers tested on 47 buffalo-hamster somatic cell hybrids.

	CYM	F10	TGLA9	CSSM6	HBB	CSSM47	FSHB	RF131	UWCA5	TGLA227
CYM										
%	-	-	-	-	-	-	-	-	-	-
φ	-	-	-	-	-	-	-	-	-	-
F10										
%	80.8	-	-	-	-	-	-	-	-	-
φ	0.28	-	-	-	-	-	-	-	-	-
TGLA9										
%	80.8	95.7	-	-	-	-	-	-	-	-
φ	0.28	0.87	-	-	-	-	-	-	-	-
CSSM6										
%	82.2	88.8	88.8	-	-	-	-	-	-	-
φ	0.3	0.67	0.67	-	-	-	-	-	-	-
HBB										
%	91.4	76.6	76.6	77.7	-	-	-	-	-	-
φ	0.04	0.086	0.08	0.09	-	-	-	-	-	-
CSSM47										
%	91.5	73.9	76.0	80.0	89.1	-	-	-	-	-
φ	0.032	-0.11	-0.11	0.07	-0.06	-	-	-	-	-
FSHB										
%	93.6	78.7	78.7	80.0	97.9	89.4	-	-	-	-
φ	-0.03	0.15	0.15	0.16	0.80	-0.05	-	-	-	-
RF131										
%	70.0	72.3	72.3	73.3	70.2	71.7	72.3	-	-	-
φ	0.22	0.26	0.26	0.29	0.03	0.10	0.11	-	-	-
UWCA5										
%	80.8	82.9	82.9	86.6	80.1	78.7	82.9	76.6	-	-
φ	0.28	0.04	0.49	0.58	0.29	0.16	0.39	0.38	-	-
TGLA227										
%	80.8	78.7	78.7	86.6	85.1	80.9	87.2	72.3	95.7	-
φ	-0.07	0.32	0.21	0.55	0.34	0.18	0.47	0.23	0.87	-

Two markers are syntenic if $\varphi > 0.69$ with an error $Q = 0.025$ and a probability of correct decision $P = 0.96$.

BBU 20, respectively. The results presented here need to be confirmed by in-situ hybridization localizations on the river buffalo chromosomes.

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