PHYSICAL MAPPING OF THE BOVINE 18q24-q26 CHROMOSOMAL REGION


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INTRODUCTION
Synteny conservation of homologous genes in species from diverse mammalian orders has provided the basis for comparative genome mapping (Womack, 1996; Lyons et al., 1997). However, it is clear that synteny maps will not be sufficient for genomic comparisons among species because of locus order rearrangement within conserved segments (Georges and Anderson, 1996). RH mapping and contig construction are powerful tools to determine gene order and to facilitate comparative studies between human genome resources and those of other species such as cattle (Yang et al., 1998). We have constructed a high definition comparative map of the region surrounding the bovine $\alpha_2$-fucosyltransferase gene cluster located on BTA18q24distal. Over a 10 Mb segment, in addition to rearrangements previously described in the $\alpha_2$-fucosyltransferase gene cluster (Saunier et al., 2001), we have found a discrepancy concerning another gene cluster. The gene order of 25 other genes located in the BTA18q24-q26 region appears conserved in comparison with the human counterpart HSA19q13.2-q13.4.

MATERIAL AND METHODS
Choice of loci. Genes were selected from the HSA19q13.2-q13.4 region and microsatellites were chosen according to previously published BTA18 genetic maps (BOVMAP database at http://locus.jouy.inra.fr). For each locus, a PCR system was determined, and the sequence of the amplification product was controlled by Blast analysis on the NCBI server.

BAC library. A bovine BAC library (Eggen et al., 2001) of 105984 clones representing a 4 genome-equivalent coverage and organized in a 3 dimension format was screened by PCR.

Radiation hybrid panel. A 12000 rad panel of 180 bovine-rodent radiation hybrids (Rexroad et al., 2000) was analyzed using the same PCR conditions as for BAC library screening.

Mapping and size estimation of BACs. FISH was performed as previously described (Hayes, 1995). BAC size was estimated by FIGE (Schibler et al., 1998).

Contig construction and ordering. Contigs were constructed and ordered taking into account all the information obtained by FISH mapping, BAC size determination and individual content analyses (genes, microsatellites, and STS), and data from the RH12000 map that was established.
RESULTS AND DISCUSSION
The PCR screening of the BAC library and characterization of BAC contents permitted the construction of 8 contigs (table 1). Altogether, they span 2.66 Mb of the q24-q26 region of BTA18 (figure 1). Among the 27 genes mapped by FISH, 22 are present in contigs containing 4 to 15 BACs, whereas for \textit{TGFB1}, \textit{LU}, \textit{MIA}, \textit{PTGIR} and \textit{TNNI3} only singleton BACs have been identified.

Table 1. Characteristics and content of BAC contigs

<table>
<thead>
<tr>
<th>Contig</th>
<th>Number of BACs</th>
<th>Estimated size (^a) (kb)</th>
<th>Genes</th>
<th>Microsatellites (^b)</th>
<th>STS (^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>7</td>
<td>260</td>
<td>\textit{APOE}, \textit{APOC2}, \textit{SWAP2}</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td>4</td>
<td>180</td>
<td>\textit{ERCC2}, \textit{ERCC1}, \textit{FOSB}</td>
<td>-</td>
<td>\textit{510C8.1}</td>
</tr>
<tr>
<td>C</td>
<td>6</td>
<td>200</td>
<td>-</td>
<td>\textit{RME01}</td>
<td>-</td>
</tr>
<tr>
<td>D</td>
<td>15</td>
<td>500</td>
<td>\textit{PSCD2}, \textit{DBP}, \textit{CA11}, \textit{sec1}, \textit{butf2}, \textit{butf1}, \textit{BAX}, \textit{NUCB1}, \textit{GYS1}, \textit{LHB}, \textit{SNRP70}</td>
<td>\textit{KS13}, \textit{KS5}</td>
<td>-</td>
</tr>
<tr>
<td>E</td>
<td>13</td>
<td>630</td>
<td>\textit{KCNC3}, \textit{NR1H2}</td>
<td>\textit{UWCA5}, \textit{RM128}</td>
<td>\textit{549B9.1}, \textit{609H11.1}</td>
</tr>
<tr>
<td>F</td>
<td>8</td>
<td>270</td>
<td>\textit{ETFB}, \textit{LIM2}</td>
<td>-</td>
<td>\textit{99H1.2}</td>
</tr>
<tr>
<td>G</td>
<td>15</td>
<td>400</td>
<td>\textit{PRKCG}</td>
<td>\textit{BM2078}</td>
<td>-</td>
</tr>
<tr>
<td>H</td>
<td>6</td>
<td>220</td>
<td>-</td>
<td>\textit{BM6507}</td>
<td>-</td>
</tr>
</tbody>
</table>

\(^a\)the average BAC size is 103 kb  
\(^b\)\textit{KS5} and \textit{KS13} are two newly isolated microsatellites  
\(^c\)BAC end sequences (STS) are named by the BAC number followed by .1 or .2

On the 618 cR bovine \textit{RH\textsubscript{12000}} map (figure 1), constructed using a lod7 threshold value, genes and markers are distributed in 5 independent groups (RH1 to RH5). Three groups contain more than one contig: RH1 includes contigs A and B, RH4 contigs D to F, and RH5 contigs G and H. \textit{APOC2} and \textit{PRKCG} are separated by 515.4 cR in cattle and by 10.4 Mb on the human physical map. The interval between \textit{RME01} and \textit{BM6507} spans 9 cM and 484.6 cR on the genetic (BOVMAP database) and \textit{RH\textsubscript{12000}} maps respectively. The ratio may be evaluated at 18.6 kb/cR assuming that 1 cM is equivalent to 1 Mb. Thus the calculated physical distance between \textit{APOC2} and \textit{PRKCG} is 9.69 Mb in bovine. The gene order is similar except for a possible minor discrepancy in the \textit{APOE-APOC2} order that is inverted between man and cattle.
Figure 1. Alignment of chromosomal, radiation and physical maps of the BTA18q24-q26 region and comparison with its human counterpart HSA19q13.2-q13.4.
- To date, SNRP70 (contig D) and MIA (BTA18q24median) are not precisely positioned on the human map.
- Two BACs (449H3 and 753G11) have been mapped by FISH and only one was positioned on the RH12000 map (RH3).
CONCLUSION

Forty loci (27 genes, 7 microsatellites and 6 STS) have been finely located on the BTA18q24-q26 region by FISH and RH12000 mapping. Comparison of the bovine map with its human counterpart (HSA19q13.2-q13.4 region) shows that the genes are distributed on a similar distance (about 10 Mb) with a conserved order except for APOC2 and APOE. This inversion could be due to a local chromosomal rearrangement. In this region, rearrangements such as retrotransposition have already been described (Saunier et al., 2001). They lead to the inactivation of one α2-fucosyltransferase gene (Sec1) which encodes a fully active enzyme in bovine (Barreaud et al., 2000) whereas in man it is a pseudogene.

The 18.6 kb/cR ratio illustrates the potential provided by the RH12000 panel to construct a bovine high resolution physical and comparative map which will permit efficient use of the rich human genome resources to search for genes of interest in cattle and for their positional cloning. For example, the EAC bovine blood group loci located within the region studied here constitute a highly polymorphic multigenic system. Interestingly, the corresponding human region contains several gene clusters which could constitute positional candidates but their precise organization needs to be established in cattle. For this purpose, panels generated after higher irradiation doses could be used but they present some difficulties recently evidenced by Robic et al. (2001). Therefore, the 12000 rad panel represents a good compromise to order markers in small subregions of the genome. Ultimately, sequencing of regions of interest spanning several kb remains the most appropriate approach to study gene organization and to understand gene regulation and function in different species.

REFERENCES