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133 new gene localizations on the rabbit cytogenetic map

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Abstract. Rabbit *(Oryctolagus cuniculus)*, besides its interest for medical research and biotechnological applications, has a small agronomic production in southern European countries. However, it is still a "map-poor" species with about 80 genes mapped. Recently, useful tools for research on this species have been developed, such as heterologous human-rabbit chromosome painting data and a rabbit BAC library. In this study, our aim is to enrich the rabbit cytogenetic map using the FISH technique. Towards this, we have used cDNAs (rabbit and non rabbit) present in the public databases to determine intra-exon primers used to screen our three-genome equivalent BAC library, by standard PCR directly on DNA pools, and by hybridization of high-density filters. 133 BAC clones containing the genes of interest were isolated and FISH-mapped to the rabbit chromosomes. We present the localization of new genes on all rabbit chromosomes except OCU20 and OCUY and some preliminary data on the rabbit/human comparative map. In addition, this set of BAC clones quite regularly distributed on the rabbit genome will be useful to isolate microsatellites, in order to construct a first generation genetic map.

In contrast to other domestic mammalian species, rabbit *(Oryctolagus cuniculus)* is an important species in a variety of fields such as medical research, biotechnology, and animal production. Rabbit is an excellent model for the study of human diseases such as atherosclerosis (Mortensen et al., 1994) or carcinogenesis associated with papillomavirus infection (Breitburd et al., 1997). Since the 1990s, the development of transgenic rabbits has permitted the study of the physiological function of genes and their role in the mechanism of diseases and has also permitted large-scale production of foreign proteins for therapeutic purposes (Fan et al., 1999). Recently, Chesné and coworkers (Chesné et al., 2002) have succeeded for the first time in producing rabbit live somatic clones. Finally, in France and other southern European countries, rabbit breeding is a small but active animal sector for the production of meat, fur and wool.

Although rabbit has been widely used in research, it is still a "map-poor" species. In 1993, Fox published a rabbit genetic map with 39 loci. More recently, van Haeringen and coworkers (2001) have mapped a QTL for serum HDL cholesterol using the AFLP technology and Korstanje and coworkers (2001, 2003) have isolated a limited number of microsatellites from chromosome-specific libraries. Concerning the cytogenetic map, 55 genes have been precisely localized and 25 have been simply assigned to a specific chromosome (Zijlstra et al., 2002; Hayes et al., 2002; RABBITMAP, http://locus.jouy.inra.fr/cgi-bin/lgbc/mapping/common/intro2.pl? BASE=rabbit). Given the interest for this species, there is now a crucial need to devel-

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op high-densitytype I and type II marker maps, which will contribute to the identification of genes of interest and to the design of marker assisted selection procedures. Recently, tools which constitute valuable resources for the construction of high-density rabbit maps have been produced such as a bi-directional human–rabbit chromosome painting analysis (Korstanje et al., 1999) and a three genome-equivalent rabbit bacterial artificial chromosome (BAC) library (Rogel-Gaillard et al., 2001).

In 2002, Hayes and coworkers published an R-banded rabbit karyotype nomenclature in agreement with the 1981 G-banded standard nomenclature (Committee for Standardized Karyotype of *Oryctolagus cuniculus*, 1981), which will serve as reference for large-scale localization of type I and type II markers by fluorescent in situ hybridization (FISH) on R-banded chromosomes. In this work, our aim was to enrich the cytogenetic rabbit map and as a first contribution, we report the localization of 133 new genes.

Materials and methods

Choice of the genes and primer design

Genes distributed on all the human chromosomes were chosen from information in public databases (http://www3.ncbi.nlm.nih.gov/Entrez/index.html) (see Tables 1 and 2). The corresponding rabbit and bovine cDNAs and ESTs were identified using the Iccare program (code available at http://genopole.toulouse.inra.fr/Iccare/Inra/index_english.html), which provides similarities between the cDNAs and corresponding human genomic contigs in order to delineate the exons. For human cDNAs, the exon-intron organization was determined manually. PCR primers were further designed for each gene in exon segments with the Primer 3 program (Rozen and Skatlesky, 1998) (Code available at http://www-genome.wi.mit.edu/genome_software/other/primer3.html.). Tables 1 and 2 lists these primer pairs, the expected size of the PCR products and the cDNAs from which the primers were designed.

Rabbit BAC library screening

The rabbit BAC library was screened by two methods i.e. by a PCR-based screening method and by hybridization.

For the PCR-based screening, PCRs were performed on DNA pools as described (Rogel-Gaillard et al., 2001). All reactions were performed with 1.5 mM $MgCl_2$ in a total volume of 15 µl with the program (94 °C 30 s; 55 °C 30 s; 72 °C 30 s). PCR with 10 ng of rabbit genomic DNA provided the positive controls.

For the screening by hybridization, gene specific probes were produced by PCR on genomic rabbit or bovine DNA. The PCR products were purified with ready-to-use columns (JETquick PCR purification spin kit Genomed). For each hybridization, 10 to 20 probes (50 ng of each probe) were mixed together in a maximum volume of 30.5 µl and labeled by terminal labeling with 125 µCi of [γ^{32}P]-ATP, 20 units of T4 polynucleotide kinase (New England Biolabs) for 30 min at 37 °C. The reaction was stopped by adding 2 µl of EDTA 0.5 M pH 8 and the labeled products were purified with commercial columns (Nick™ column, Pharmacia Biotech), in conditions recommended by the manufacturer.

The whole BAC collection was spotted on four high-density filters by the INRA Resource Center (http://www.jouy.inra.fr/unites/lreg/site_francais/CRB/index.html), which were hybridized in homologous or heterologous conditions with mixtures of rabbit or bovine probes, respectively. Hybridization procedures corresponded to standard protocols (Sambrook et al., 1989). Each pool of BAC clones identified by hybridization was then screened by PCR with the adequate primers. Although more than one BAC clone was recovered in most cases, only one clone per gene was used in this study.

Sequencing of the PCR products

DNA from each selected BAC clone was amplified by PCR with the corresponding primer pair. The PCR products were either purified with ready-to-use columns (JETquick PCR purification spin kit Genomed) or isopropanol-precipitated. 5–10 ng of purified PCR product was directly sequenced on one strand with the forward primer using the automatic sequencers ABI 377A or Megabace. The sequences were checked using the standard nucleotide-nucleotide BLAST (BLASTN) program (Altschul et al., 1990) and the nucleotide query-protein database (BLASTX) program (Altschul et al., 1997).

R-banded chromosome preparations

Chromosome spreads were prepared from rabbit fibroblast cell cultures from normal New Zealand female embryos as described (Hayes et al., 1991). To obtain R-banded chromosome spreads, cell cultures were synchronized with an excess of thymidine and treated with 5-bromodeoxyuridine (BrdU) during the second half of S phase (Hayes et al., 1991).

Probe preparation for fluorescence in situ hybridization (FISH)

BAC DNA extracts were prepared according to standard protocols and purified with the S.N.A.P.™ K1900-01 Miniprep kit (Invitrogen). DNA was then labeled by nick-translation with biotin-14-dATP (BioNick™ 18247-015 labeling system, Invitrogen), mixed with 100× total sonicated herring sperm DNA and 100× total sonicated rabbit DNA, ethanol precipitated, slightly dried and resuspended in hybridization buffer.

FISH on R-banded chromosomes

Fluorescent in situ hybridization, signal detection and R-banding were performed as previously described (Hayes et al., 1992) with 50–100 ng biotin-14-dATP labeled probe per slide. Before hybridization to the chromosomes, probes were denatured at 100 °C for 10 min and pre-hybridized at 37 °C for 30–60 min. Slides were examined under a Zeiss Axioplan 2 epifluorescence microscope and the Applied Imaging Cytovision (version 2.7) software was used for image capturing and analysis. Chromosome and band numbering were as described (Hayes et al., 2002).

Results and discussion

Choice of genes and definition of primer pairs

Two criteria were used to choose the genes localized in this work (see Tables 1 and 2) i.e. (1) their localization on human chromosomes to have a distribution as regular as possible on rabbit chromosomes and (2) the possibility of isolating a corresponding rabbit BAC clone as probe for FISH. To meet with the first criterion, we based our search for genes on the human/rabbit bi-directional chromosome painting data already published (Korstanje et al., 1999). It allowed us to select genes relatively to their localization in man and with hypotheses on their putative localization in rabbit. To screen the rabbit BAC library, three types of primer pairs were used: rabbit, human and bovine primers (see Table 1). First, we benefited from the fact that many rabbit ESTs and cDNAs are stored in GenBank because physiological functions have been much studied in this species. Thus, homologous primer pairs could be designed for 120 genes and BAC clones were recovered for 81 genes. Secondly, for the PHKA2 and SMCX genes on chromosome X, since no rabbit sequence was found in GenBank, we designed primer pairs from the corresponding human cDNAs. Finally, we had the opportunity to collaborate with another project in our laboratory aiming at exploiting a source of 473 bovine probes for genes well distributed over the human genome. Among the bovine primer pairs, 148 gave a PCR product and were also used in the work presented here. Only the primer sets which permitted identification of BAC clones are listed in Table 1.

Table 1. Primers used, species and GenBank accession number of the sequences from which primers were developed and size PCR product

Gene symbol	Forward primer	Reverse primer	Species	GenBank accession no.	Size (bp)
ENO1	ACCCCTTTAACCAGGGTGAC	CCGATCTGGTTGACCTTCAG	rabbit	AF260259	163
FLJ11838	CATCAAGTGGTGGTGGCTAAT	AAAGGCTTTTTACCTCAGTTTTTG	bovine	BE476488	197
PGM1	GATCCTCTGCGAAGAACTCG	GAAGTCATGCTCTCCGGACT	rabbit	M97663	145
JAK1	CCATGGCTTTCTGTGCTAAAA	CTCCTCCGCCGTGTACTCT	bovine	AV606622	143
FUBP1	TGTGTAGAAATGAAAATTGGTTTG	TGTAAAGCACAAAACAGGCATT	bovine	BE236721	159
EDG7	TCTTTGCTGGAATCGCCTAC	CTCCACAGCAATAACCAGCA	rabbit	AF404276	149
ADORA3	GTCATCAGCCTGGGAATCAC	ATCTGACCGTGAGCTTGACC	rabbit	AF145438	142
BCAS2	CTGGGTATCCCTGGTCAGTAA	TCTTTGTTTGCTTCTCCGTGT	bovine	BF606731	105
S100A4	CCTCCTGATCGGCATCTTC	GATGGTGAGCTCCTTTTGGA	rabbit	D10885	103
CD1B	AGCAGGAGAAGTTGGCTCAG	CCTAGACTGCTGTGCTTCACC	rabbit	AF276979	133
CRP	TCAAAGCCTTCACTGTGTGC	AGGTGAGTTGGATCCACAGG	rabbit	L47237	199
MGST3	GGAATACGGTTTCGTGCTTC	TGTACTTCTTGCGAGCCTTG	rabbit	AY050567	92
SIP	GGGTGGAATCAAGAGAGAAGC	CTCCAGCAGGAAATGGAGAAT	bovine	BE664145	196
GNPAT	AAAGACCGGGTGATTCTGAAA	TCCTGAACCCAGGTGTCATC	bovine	BG358927	172
HNRPU	GTCAGAAGCCATTGAGTCAGC	TCAACCCACAAAGCTTCTAAAAA	bovine	AW461707	185
FSHR	GTGACACCAAGATAGCCAAGC	TTGGTGAAGATGGCATAGAGG	bovine	BF075894	196
PELI1	GATCATTTGGGTTCACTCAGG	CACAGGACTTGATTTCCTTGC	bovine	BE666542	172
IL1B	TGTAGACCCCAACCGTTACC	CAGGAAGACGGGCATGTACT	rabbit	D21835	151
ACTR3	GCTGGTTTTGAACCTGACTTG	CAAAATACCAGGGCACAGAAA	bovine	BF042466	190
TTN	GAGACAAGGAAGCCAAGTGC	ACGCTTCCGCAATCAGTAAG	rabbit	Y18102	155
ATIC	GAGCAGGACAGCAGTCTCGTA	TCGCCGATAGTTCCAGTAACA	bovine	AW463391	169
UGT1A@	ACATGAGCTTCCTGCAAAGG	ATCCACCAACGACACCTCTC	rabbit	U09101	131
GPX1	CTCTTCCAGAAGTGCGAGGT	TTCTCGAAGCTCCAGGAAAC	rabbit	X13837	167
ITIH3	GATGGAGTACCCCAAGAACG	TCCGCCTTAAAGCTGTTCAT	rabbit	AB050593	126
ITIH4	ACGGAGATCCAGAGGAACG	AGCTTCTCCAGGAAGGGGTA	rabbit	AB050594	101
GBE1	GGTAATGAATTTGGGCATCCT	TGAAAGCCAACCACATCTTTC	bovine	AV667636	183
RAB7	GGCCTTCTACAGAGGTGCAG	CCAACACAACAAAGGGGAAG	rabbit	AF050174	146
STAG1	CAGTGGTGATTATCCCCTTACC	AAAAGCTCTGACCTGGGAGTC	bovine	AW653226	181
MYNN	AGGAAGCATAGTGGAGAGAAGC	TAGAGACAGCAAATGCCTTCC	bovine	AW481645	148
FXR1	CAGCGTACTCCAGGAGAAGAA	ACTGAAAGTGCGATGGAAAGA	bovine	BF774832	196
HES1	AGCGTGCTGGGGAAGTACC	TGGATAGGTCATGGCGTTGAT	bovine	AW465398	156
APOD	TCCCTCCTGAAACAGTGACC	AGCGAAGCAGGAGAAGTGAC	rabbit	L42979	160
CSN3	CCATTATGAGCTAAATTTCT	TTTGTTGAACTGAAACTCCCA	rabbit	U44058	300
ALB	CTGAACAGGTTGTGCGTGTT	GGAAGGTGAATGTTTCAGCA	rabbit	U18344	160
ART3	GCAAAAGTCAAGTGGGAAGC	GCCATATCCACAGCTTTGCT	rabbit	AJ291432	149
TACR3	AAGCGTATGAGGACCGTCAC	AAGTTCTGGAAGCGGCAGTA	rabbit	AF133908	149
EDNRA	CAACTACTGCCCACAGCAGA	TCCTCATGCACTTGTTCTGG	rabbit	AF311974	140
F11	TTGTTGGAGGATCTGCCTCT	CCCATAGAAGCAATGAGCAG	rabbit	AF395821	143
CDH10	TGACATTCATGCCACAAGAAG	ACAACCGACATTTCAGGAACA	bovine	BF655207	198
NNT	CCAGATTTCGGATTTACCTCA	ATAGGCAACCAGAGACCCACT	bovine	AV608263	199
PPAP2A	CTTCAAGGCATACCCCCTTC	CCACCTAATAACGCATAAGATATGG	rabbit	AF404277	100
RASA1	TGCTGGAACTGTTGAAAGAAAA	ATGGTTGAATGGGTAACATGC	bovine	BE668821	210
CD14	TACTGAACATTGCCCAAGCA	GGAACTTGTGGGGACAGAGA	rabbit	D16545	144
HARS	TTGCTCGAGGACTGGACTACT	AGATCCGCTCTACTCCAATGC	bovine	AV618341	198
PRO1331	AGAGTTGTCGGGGATTTTTGT	TTTGGAAGCAGTTTTAATGGAGA	bovine	AW356076	195
STK10	AGATGCAGCGCTACAACCAG	CTTGATCTTCTCACGCTGCTC	bovine	BE589473	176
SLC34A1	AGGTGGCTGGTGACATCTTC	ATGCTGACGATGATGGAGGT	rabbit	U20793	127
DSP	GTTCCAAGCCATGAACAAAGA	GGGTCAAAGAAGCCTTTGGTA	bovine	BE721681	204
LOC285849	GGAAGGCCCTCACCTACTTC	ATGAACTCGGGTCTCTCGTG	rabbit	AF198089	103
RNGTT	CCGGTTGGAGATTGTGATTTT	TGGCTTATGTCTGACACTGAATG	bovine	BF231301	132
ARG1	AACCCATCTCTGGGGAAAAC	GTCAATTGGCTTGTGATTGC	rabbit	AF365403	114
FLJ20323	TGTCTGCATGCACGTGTAAAT	TTTTCCCATTACAGGAAAGTCA	bovine	BE665716	191
AQP1	CCATGACCCTCTTCGTCTTC	TGGGCGATGATGTACAGGAC	rabbit	AF000311	244
STK17A	TTTGCAGTGGTGAGGAAATG	CAATTCAAGGACGGCAATTT	rabbit	AB042195	129
PPIA	GTGACTTCACACGCCACAAT	TGGCAGTGCAGATGAAGAAC	rabbit	AF139893	159
PON1	GAATGACATTGTCGCTGTGG	TCTGCCACTACTCGAACATCA	rabbit	AF220943	153
DLX5	CAGGTGAAAATCTGGTTTCAG	GGGACTGCGGTGAGTTACAC	rabbit	AB073104	124
CFTR	GCTAGCGTCTCCTGGAATGA	GGCCAGTGTTGATCATGTTG	rabbit	U40227	253
GPR37	CCTCCTCTTCTGTCTCTGCAA	GCAATGAGTTCCAACAGAAGC	bovine	AW656929	199
PDGFRL	AAAGGAGCCAGGAGAGAACAG	TTCCTTTACATCGAAGCTCCA	bovine	BE590257	206
SNAI2	ACAAGCAGCTGCACTGTGAC	TGAGTTCTGATGTGTCCTTGC	bovine	BE476033	184
ASPH	GTGCCAAGACCAAGGAAAAC	GCTGGTTAGAACTTCGGCTTT	rabbit	AF198966	111
BIG1	TCGTAGGGCTTTGAAAGATGA	AAATCTAACCTCCCCAAACCA	bovine	BG224162	209
JPH1	GAATGCGGTCAGAGGATTGT	ACGAAACGTTCTCTCCCAGA	rabbit	AB023447	153
HAS2	AGGGCCTGCCAGTCTTATTT	GGTTGCCCATAAATTCCTGA	rabbit	AB055978	121
MYC	AGAGAAGCTGGCCTCCTACC	AGTGGGTAGGGGAAGACCAC	rabbit	AB019241	158
IFN1@	CATCTCTGTGCTCCACGAGGTGA	GGCTCTCATGACTTCTGCTCTGACA	rabbit	NM_174085	295
TEK	ATCACCATCGGAAGGGACTT	TGTTGACGCATCTTCATGGT	bovine	AW354935	200
ACO1	CAGGAATCATCCACCAAGTG	AAACCGTCAATCATGGTCGT	rabbit	M95815	121
AQP7	AGCTGTGTCTCTTAGCCATCG	CAGCGAGGAAGGTGAAGAAG	bovine	BF073478	174
TPM2	AGTCCGTCCGGTCCTCAC	GGTCCTCAGCTTGCTTCTTG	rabbit	AF399638	154

Table 1 (continued)

Gene symbol	Forward primer	Reverse primer	Species	GenBank accession no.	Size (bp)
TJP2	ATAGCTGCCTTCCAGGACTGT	GGCTTTGCATAAAGCTTCAAA	bovine	BF652482	169
DAPK1	ATGGTGTTGGTCAGTGAGGTC	GACGGAGAAGATCAAGTGCTG	bovine	AW314328	210
FBP1	GGCCATTGGTGAGTTCATTT	GGGGGAACTTTTTCTTCTGG	rabbit	AJ300657	137
PTCH	TCCTTTGCAGTGGACAAACTT	CAGTCTGGATCAGCTGGATTG	bovine	AW314277	156
ITIH2	CTCCGCAATGTTCAGTTCAA	CTGCCACCACAATCTCTGAA	rabbit	AB050592	100
KCNMA1	TTCCCGTTCTCCCCTCTAGT	TATTTGCCATCGTGCTCAAG	rabbit	AF321818	121
MINPP1	GAAGAATTCCGAGTGCAGATG	TCACATTCTTCACTGGTATGACAA	bovine	BE487165	140
TNFRSF6	AAGTCCAGCTGCTGCGTAAT	TGAATTTTCTCCGCAAGAGC	rabbit	AB021299	112
CYP2C18	AGGAGATCGAGCGTGTGATT	TGTTGGGGACGAGGTTAATG	rabbit	D00190	120
GSTTLp28	GGCATGAAGTCATCAACATCAA	TTTGGCAAGCTTTCTCATAGG	bovine	BF075251	198
FGFR2	CAGGGGAGTCGCTAGAGTTG	CTCCCCAATAAGCACTGTCC	rabbit	AF184968	110
ART1	CAGATGCCGTCAGGTGTTC	ACTGCTGGGCTGCTACATTT	rabbit	AF291444	122
PARVA	GCATGTAATGGAAGTGCCCTA	CGCCAGAAATGGTCTAAATGA	bovine	BE477207	215
SLC29A2	CTCTTCATGCTGTGCCATGT	ATGGTGAGGGACACCAGGTA	rabbit	AF323951	125
RBM4	GGGTTATGGGGAATCCATGT	TGCGTAGTTGTATGCGGAAG	rabbit	AF233063	124
HTR3B	CCTTCCTGAGGAGCAGAGAA	CTCTGCAGGATGCCGTACAT	rabbit	AF305700	106
SLC6A12	CGAGAAACTGGAGCAGGAAG	AGGAAACCTCCAGACATTGC	rabbit	AF26341	124
SSPN	ACCGGGATGTGACTGACTGT	CCCCCACATAAAAGACCTGA	rabbit	AF120276	150
ERBB3	GGGACTCAGGTGTACGATGG	AGCTGAGTGAAGCGGAGCTG	rabbit	AF333179	98
TMEM4	CCTCACAGAGCTGCTAGAGGA	AACTTGAGGGTGCCACTGAT	bovine	BF043303	174
PMCH	GGATGGCAAAAATGAGTTTCTC	ATATTGCTCCAGGGAAGGAAC	bovine	BI681754	200
DAO	TGCATCCATGAGCTCTACCA	TTGCTGGGGTCAGAGAGGTA	rabbit	D12494	131
GCN1L1	TGGAGAAGTTGGAGAAGCTGA	TGGAGAAGTTGGAGAAGCTGA	bovine	BM087294	166
DACH	TGAATTCATGTCCCCATTTTC	GCTGCTACCAATGCAGCTATC	bovine	BE756347	126
C13ORF10	ACGAGGGATTCTTCATCTGGT	AAATGCAGAAATCTCCAATGC	bovine	BG358668	153
SLC15A1	AGCACTCCTGATTCTGTACTTCA	GATGAGAGCTCCGAGAATGG	rabbit	U13707	118
DHRS4	GGCCCACGTGGTCATCAG	ACGGTGCCCGTCACACTC	rabbit	AB045133	93
GMFB	TCAGGTTACTATGCTGAATTCCAA	CAGTCAAACAACTTTTTCCCTAA	bovine	AW346299	101
RAD51	ACCGCCCTCTACAGAACAGA	CTCATCAGCAAGTCGCAGAA	rabbit	AF017729	102
SLC28A2	CTCTTCATGCTGTGCCATGT	ATGGTGAGGGACACCAGGTA	rabbit	AF323951	125
CA12	GGAGAGGAGCTGGTTCAAGA	GTCGGCAGACACATTGTAGC	rabbit	AF263367	136
RHCG	TGATGGTCTTCTTGGGCTTC	TACTGGAACCAGCCTTGCAT	rabbit	AY013263	133
MYH11	TGTTAGCCGAGGAAAAGAACA	CTTGGAGCTGACGAGGTCTT	rabbit	J03614	194
PRKCB1	GACACCTCCAATTTCGACAAA	ACACTCCAGGCTTACAATGGA	bovine	BF600191	174
EIF3S8	TACGTGGAGCACCTGAAGGA	TGGGCCTTGTAATCGAACTT	rabbit	C82605	152
SULT1A1	ATGGAGCTCATCCAGGACAC	GACTTGGGGTAGGTGCTGAT	rabbit	AF360872	146
FTS	AAGCTGACCAATGACAAAGGA	AAGCTTCAAGGTCCCCAATAA	bovine	BF890067	198
LCAT	AGGAGATGCACGCTGCTTAC	CCCCAAGAGAGATGAAACCA	rabbit	D13668	138
HAS3	TCTCGTTCCTGAGCAGTGTG	GGAATTGCTGGAGGAGACTG	rabbit	AB055979	132
ENO3	CCTGGACTTCATTCCTTTCG	ACTGAGCCGATCTGGTTGAC	rabbit	AF260259	142
TP53	CTGCCAGCTAGCAAAGACCT	ACAACTTCCGTCATGTGCTG	rabbit	X90592	117
NDEL1	GGACTCTGCGCGATATCAAT	CTTCTGCATCCAGTGACCAA	rabbit	AF015037	128
SREBF1	ATCCTGGCCACAGTACCACT	AACGGTAGCGCTTCTCAATG	rabbit	AF278696	142
NOS2A	CCATCCCTGCATCCTCATT	CCTTTGTACTCGGAGTCATGGAGT	rabbit	AF200351	90
CRYBA1	GGTTATGAGCACACCAGCTTC	GCTCAATATGATAGGCGTTGC	rabbit	AJ306649	103
KRT12	GACTCCTTGGCCGAAACTG	GCGGCGGTAGGTCTCTATCT	rabbit	X77665	183
STAT5A	AGCAACGAGCTTGTGTTCCAGGTG	GCAAAGGCATTGTCCCACAG	rabbit	(pers. commun. E. Devinoy)	113
ATP6V0A1	TCAAGAAGCCCCTGAACATC	CCGAAGTGTGTGCATCGTAG	rabbit	AF393370	123
ITGB3	ACCACTGATGCCAAGACTCA	ATGGTGGTGGAGGCAGAGTA	rabbit	AF184591	116
NPC1	GGCTGCGTCATCTTCTTCTC	AAGTGCGCGTCAAAGAACTC	rabbit	AF202730	146
DSC3	AGAAAGCCATCAAGTGGTCCT	ACTGATCTTTGAACCCACTGC	bovine	L33774	199
GALNT1	AAGGACTGACTGGGCTACCTC	CAGTGTGCACAGTCTCGCTAC	bovine	L07780	198
ICAM5	CCTCACATTGACCCTGCTTC	GAGAAATTGGCTCCATGGTC	rabbit	L13199	135
RYR1	CATGGACATCCTGGAGCTGT	GTGGATGCTGATGAGGAGGT	rabbit	X15750	217
PRNP	AGCAACCAGAACAGCTTCGT	CCACGATCAGGAAGATGAGG	rabbit	AF015603	250
TIAM1	AAGCAAGTCTCTTGGGAGGAG	TCTCCTTGATGTCATCCTGCT	bovine	AV592043	188
PHKA2	TCCTGAATCGGGTACCTCAG	CCACAGCGATGATACTTCCA	human	M64656	114
ACATE2	AGACCATGTGAAAGCAATGGA	CTCGTAATTCAGGCTGACTGC	bovine	AV593791	140
SAT	CGAAGAGGGATGGAGACTCTT	CAACGCCACTGGTAATAAAGC	bovine	BF043097	201
ZFX	ATAACCACCTGGAGAGCCACAAGCT	GCACTTCTTTGGTATCTGAGAAAGT	rabbit	X59739	446
SMCX	GCTAAGGGCACTGGAGTCTG	CTCCACCTCACTCAGGCAGT	human	NM_004653	108
MSN	AGCCCTCTGCTTTTCAAGTTC	AGCCAGACTTGTGGACTTCCT	bovine	BG689153	217

Table 2. List of genes mapped in this study, their localization on human chromosomes, expected localization in rabbit and FISH localization. Gene symbols follow the HUGO nomenclature (http://www.gene.ucl.ac.uk/nomenclature). For each human chromosome genes are ordered from the p telomere to the q telomere according to the Ensembl database (http://www.ensembl.org), exceptions[a] which are LocusLink localizations.

Gene symbol	Gene name	Human localization Ensembl Sept 03	Expected rabbit localization	Rabbit localization
ENO1	enolase 1, (alpha)	1p36.23	13p/q	13q35
FLJ11838	hypothetical protein FLJ11838	1p34.2	13p/q	13p13
PGM1	phosphoglucomutase 1	1p31.3	13p/q	13q27
JAK1	Janus kinase 1, (a protein tyrosine kinase)	1p31.3	13p/q	13q27
FUBP1	far upstream element (FUSE) binding protein 1	1p31.1	13p/q	13q26
EDG7	endothelial differentiation, lysophosphatidic acid G-protein-coupled receptor, 7	1p22.3	13p/q	13q25dist
ADORA3	adenosine A3 receptor	1p13.2	13p/q	13q23
BCAS2	breast carcinoma amplified sequence 2	1p13.2	13p/q	13q22-q23
S100A4	S100 calcium binding protein A4	1q21.3	13p/q	13q21
CD1B	CD1B antigen, b polypeptide	1q23.1	13p/q	13q21
CRP	C-reactive protein, pentraxin-related	1q23.2	13p/q	13q21prox
MGST3	microsomal glutathione S-transferase 3	1q24.1	13p/q	13q11-q21
SIP	Siah-interacting protein	1q25.1	16q	13p13dist
GNPAT	glyceronephosphate O-acyltransferase	1q42.2	16q	16q13dist
HNRPU	heterogeneous nuclear ribonucleoprotein U	1q44	16q	16q13dist
FSHR	follicle stimulating hormone receptor	2p16.3	2q	2q22
PELI1	pellino homolog 1	2p14	2q	2q21
IL1B	interleukin 1, beta	2q13	2q	2q14dist
ACTR3	ARP3 actin-related protein 3 homolog	2q14.1	7q	7q14
TTN	titin	2q31.2	7q	7q21
ATIC	5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase	2q35	7q	7q25
UGT1A@	UDP glycosyltransferase 1 family, polypeptide A cluster	2q37.2	7q	7q27
GPX1	glutathione peroxidase 1	3p21.31	14p/q or 9p	9p13
ITIH3	pre-alpha (globulin) inhibitor, H3 polypeptide	3p21.1	14p/q or 9p	9p13prox
ITIH4	inter-alpha (globulin) inhibitor H4 (plasma Kallikrein-sensitive glycoprotein)	3p21.1	14p/q or 9p	9p13
GBE1	glucan (1,4-alpha-) branching enzyme 1	3p12.2	14p/q or 9p	14q23
RAB7	RAB7, member RAS oncogene family	3q21.3	14p/q	9p13
STAG1	stromal antigen 1	3q22.3	14p/q	14q11
MYNN	myoneurin	3q26.2	14p/q	14q15
FXR1	fragile X mental retardation, autosomal homolog 1	3q26.33	14p/q	14q17prox
HES1	hairy and enhancer of split 1	3q29	14p/q	14q21prox
APOD	apolipoprotein D	3q29[a]	14p/q	14q21prox
CSN3	casein kappa	4q13.3	15q	15q23dist
ALB	albumin	4q13.3	15q	15q23
ART3	ADP-ribosyltransferase 3	4q21.1	15q	15q23dist
TACR3	tachykinin receptor 3	4q24	15q	15q21
EDNRA	endothelin receptor type A	4q31.22	15q	15q11dist
F11	coagulation factor XI	4q35.2	2p	2p13
CDH10	cadherin 10, type 2 (T2-cadherin)	5p14.2	11p/q	11q12-q13
NNT	nicotinamide nucleotide transhydrogenase	5p12	11p/q	11q13-q14
PPAP2A	phosphatidic acid phosphatase type 2A	5q11.2	11p/q	11q15
RASA1	RAS p21 protein activator (GTPase activating protein) 1	5q14.3	11p/q	11p14prox
CD14	CD14 antigen	5q31.3	3p	3p21prox
HARS	histidyl-tRNA synthetase	5q31.3	3p	3p21prox
PRO1331	hypothetical protein PRO1331	5q33.3	3p	3p13prox
STK10	serine/threonine kinase 10	5q35.1	3p	3p11
SLC34A1	solute carrier family 34 (sodium phosphate), member 1	5q35.3	3p	3p11
DSP	desmoplakin	6p24.3	12p/q	12p15prox
LOC285849	similar to cytochrome c oxidase subunit VIa polypeptide 1 precursor	6p24.3	12p/q	12q15-q21
RNGTT	RNA guanylyltransferase and 5'-phosphatase	6q15	12p/q	12q15
ARG1	arginase	6q23.2	12p/q	12q23
FLJ20323	hypothetical protein FLJ20323	7p21.3	10q	10q15
AQP1	aquaporin 1 (channel-forming integral protein, 28kDa)	7p14.3	10q	10q14
STK17A	serine/threonine kinase 17a (apoptosis-inducing)	7p13	10q	10q15
PPIA	peptidylprolyl isomerase A(cyclophilin A)	7p13[a]	10q	10q16prox
PON1	paraoxonase 1	7q21.3	10q	10q15
DLX5	distal-less homeo box 5	7q21.3	10q	10q15
CFTR	cystic fibrosis transmembrane conductance regulator	7q31.2	7p	7p11
GPR37	G protein-coupled receptor 37(endothelin receptor type B-like)	7q31.33	7p	7p21-p12
PDGFRL	platelet-derived growth factor receptor-like	8p22	2p	2p14/2p11-p12
SNAI2	snail homolog 2	8q11.21	3q	3q12
ASPH	aspartate beta-hydroxylase	8q12.3	3q	3q16
BIG1	brefeldin A-inhibited guanine nucleotide-exchange protein 1	8q13.2	3q	3q14
JPH1	junctophilin 1	8q21.11	3q	3q16
HAS2	hyaluronan synthase 2	8q24.13	3q	3q23prox

Table 2 (continued)

Gene symbol	Gene name	Human localization Ensembl Sept 03	Expected rabbit localization	Rabbit localizatio
MYC	v-myc myelocytomatosis viral oncogene homolog	8q24.21	3q	3q23
IFN1@	interferon, type 1, cluster	9p21.3[a]	1p	1p23
TEK	TEK tyrosine kinase, endothelial	9p21.2	1p	1p24
ACO1	aconitase 1, soluble	9p21.1	1p	1p31prox
AQP7	aquaporin 7	9p13.3	1p	1p31prox
TPM2	tropomyosin 2 (beta)	9p13.3	1p	1p31
TJP2	tight junction protein 2	9q21.11	1p	1p21.1-21.3
DAPK1	death-associated protein kinase 1	9q21.33	1p	1p11dist
FBP1	fructose-1,6-bisphosphatase 1	9q22.32	1p	1p11
PTCH	patched homolog	9q22.32	1p	1p11
ITIH2	inter-alpha (globulin) inhibitor, H2 polypeptide	10p14	16p	16q11
KCNMA1	potassium large conductance calcium-activated channel, subfamily M, alpha member 1	10q22.3	18q	18q21
MINPP1	multiple inositol polyphosphate histidine phosphatase, 1	10q23.31	18q	18q23
TNFRSF6	receptor superfamily, member 6	10q23.31	18q	18q23
CYP2C18	cytochrome P450, family 2, subfamily C, polypeptide 18	10q23.33	18q	18q31
GSTTLp28	glutathione-S-transferase like; glutathione transferase omega	10q25.1	18q	18q31
FGFR2	fibroblast growth factor receptor 2	10q26.13	18q	18q33prox
ART1	ADP-ribosyltransferase 1	11p15.4	1q	1q21.1
PARVA	parvin, alpha	11p15.3	1q	1q21.3
SLC29A2	solute carrier family 29 (nucleoside transporters), member 2	11q13.2	1q	1q27
RBM4	RNA binding motif protein 4	11q13.2	1q	1q27
HTR3B	5-hydroxytryptamine (serotonin) receptor 3B	11q23.2	1q	1p11dist
SLC6A12	solute carrier family 6 (neurotransmitter transporter, betaine/GABA), member 12	12p13.33	8p	8p12
SSPN	sarcospan (Kras oncogene-associated gene)	12p12.1	8p	8p14.1
ERBB3	v-erb-b2 erythroblastic leukemia viral oncogene homolog 3	12q13.2	4q	4q11
TMEM4	transmembrane protein 4	12q13.3	4q	4q11dist
PMCH	pro-melanin-concentrating hormone	12q23.2	4q	4q15.1-15.2
DAO	D-amino-acid oxidase	12q24.11	21q	21q12dist
GCN1L1	GCN1 general control of amino-acid synthesis 1-like 1	12q24.23	21q	21q14prox
DACH	dachshund homolog	13q21.33	8q	8q22
C13ORF10	cutaneous T-cell lymphoma tumor antigen se70-2	13q31.1	8q	8q22
SLC15A1	solute carrier family 15 (oligopeptide transporter), member 1	13q32.2	8q	8q24
DHRS4	dehydrogenase/reductase (SDR family) member 4	14q11.2	17q	17q21prox
GMFB	glia maturation factor, beta	14q22.2	20q	17q23prox
RAD51	RAD51 homolog (RecA homolog, E. coli)	15q15.1	17p/q	17q15prox
SLC28A2	(nucleoside transporters), member 2	15q21.1	17p/q	17q15prox
CA12	carbonic anhydrase XII	15q22.2	17p/q	17q11
RHCG	Rhesus blood group, C glycoprotein	15q26.1	17p/q	17p11
MYH11	myosin, heavy polypeptide 11, smooth muscle	16p13.11	6p	6p12-p13
PRKCB1	protein kinase C, beta 1	16p12.2	6p	6p12-p13
EIF3S8	eukaryotic translation initiation factor 3, subunit 8, 110kDa	16p12.1	6p	6p12prox
SULT1A1	sulfotransferase family, cytosolic, 1A, phenol-preferring, member 1	16p12.1	6p	6p12prox
FTS	fused toes homolog	16q12.2	5q	5q12
LCAT	lecithin-cholesterol acyltransferase	16q22.1	5q	5q14
HAS3	hyaluronan synthase 3	16q22.1	5q	5q14
ENO3	enolase 3, (beta, muscle)	17p13.2	19q	19q12.3
TP53	tumor protein p53(Li-Fraumeni syndrome)	17p13.1	19q	19q12.3prox
NDEL1	LIS1-interacting protein NUDEL; endooligopeptidase A	17p13.1	19q	19q12.3
SREBF1	sterol regulatory element binding transcription factor 1	17p11.2	19q	19q12.1
NOS2A	nitric oxide synthase 2A	17q11.2	19q	19q12.3
CRYBA1	crystallin, beta A1	17q11.2	19q	19q12.3
KRT12	keratin 12	17q21.2	19q	19q21
STAT5A	signal transducer and activator of transcription 5A	17q21.2	19q	19q21
ATP6V0A1	ATPase, H+ transporting, lysosomal V0 subunit a isoform 1	17q21.2	19q	19q21
ITGB3	integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61)	17q21.32	19q	19q21
NPC1	Niemann-Pick disease, type C1	18q11.2	9q	9q13
DSC3	desmocollin 3	18q12.1	9q	9q14.2
GALNT1	UDP-N-acetyl-alpha-D-galactosamine: polypeptid N-acetylgalactosaminyltransferase 1	18q12.1	9q	9q14.2
ICAM5	intercellular adhesion molecule 5, telencephalin	19p13.2	10p	10p12
RYR1	ryanodine receptor 1	19q13.2	5p	5p12
PRNP	prion protein (p27-30)	20p13	4p	4p13
TIAM1	T-cell lymphoma invasion and metastasis 1	21q22.11	14q	14q25
PHKA2	phosphorylase kinase, alpha 2	Xp22.13	Xp/q	Xp15
ACATE2	likely ortholog of mouse acyl-Coenzyme A thioesterase 2, mitochondrial	Xp22.11	Xp/q	Xp15
SAT	spermidine/spermine N1-acetyltransferase	Xp22.11	Xp/q	Xp15
ZFX	zinc finger protein, X-linked	Xp22.11	Xp/q	Xp15
SMCX	Smcx homolog, X chromosome	Xp11.22	Xp/q	Xp11
MSN	moesin	Xq12	Xp/q	Xq12prox

[a] LocusLink human localization (LocusLink: http://www.ncbi.nlm.nih.gov/LocusLink).

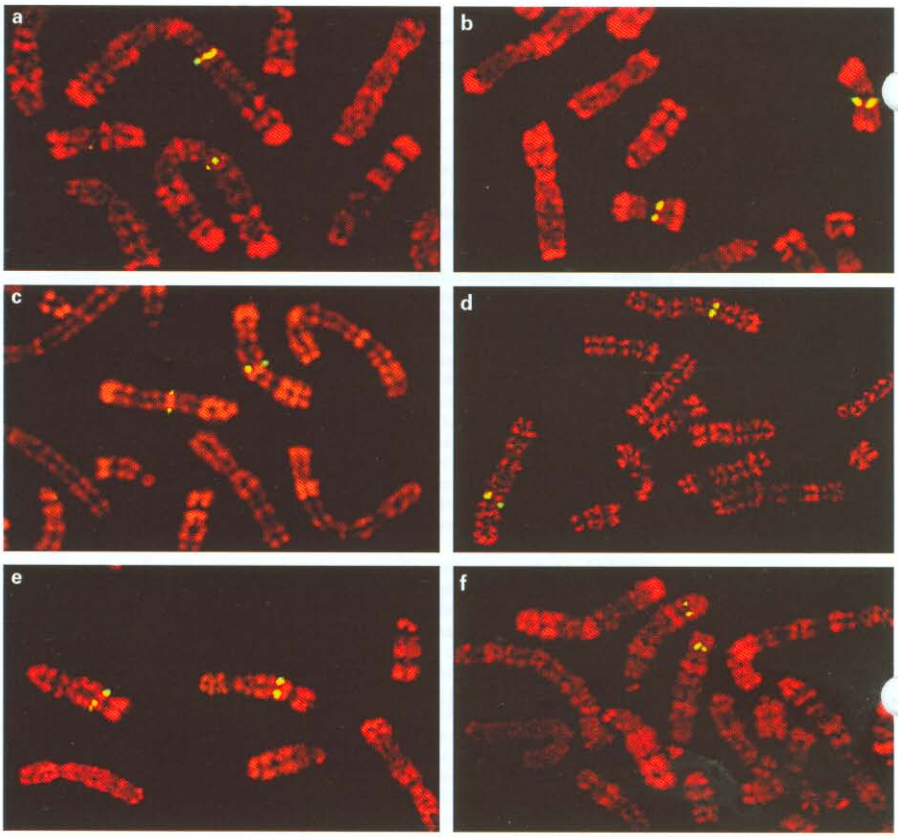


Fig. 1. Photographs of partial rabbit R-banded metaphase spreads hybridized with rabbit BAC clones containing respectively (**a**) PTCH, (**b**) EIF3S8, (**c**) ERBB3, (**d**) TTN, (**e**) CA12 and (**f**) FGFR2.

Recovery of the BAC clones

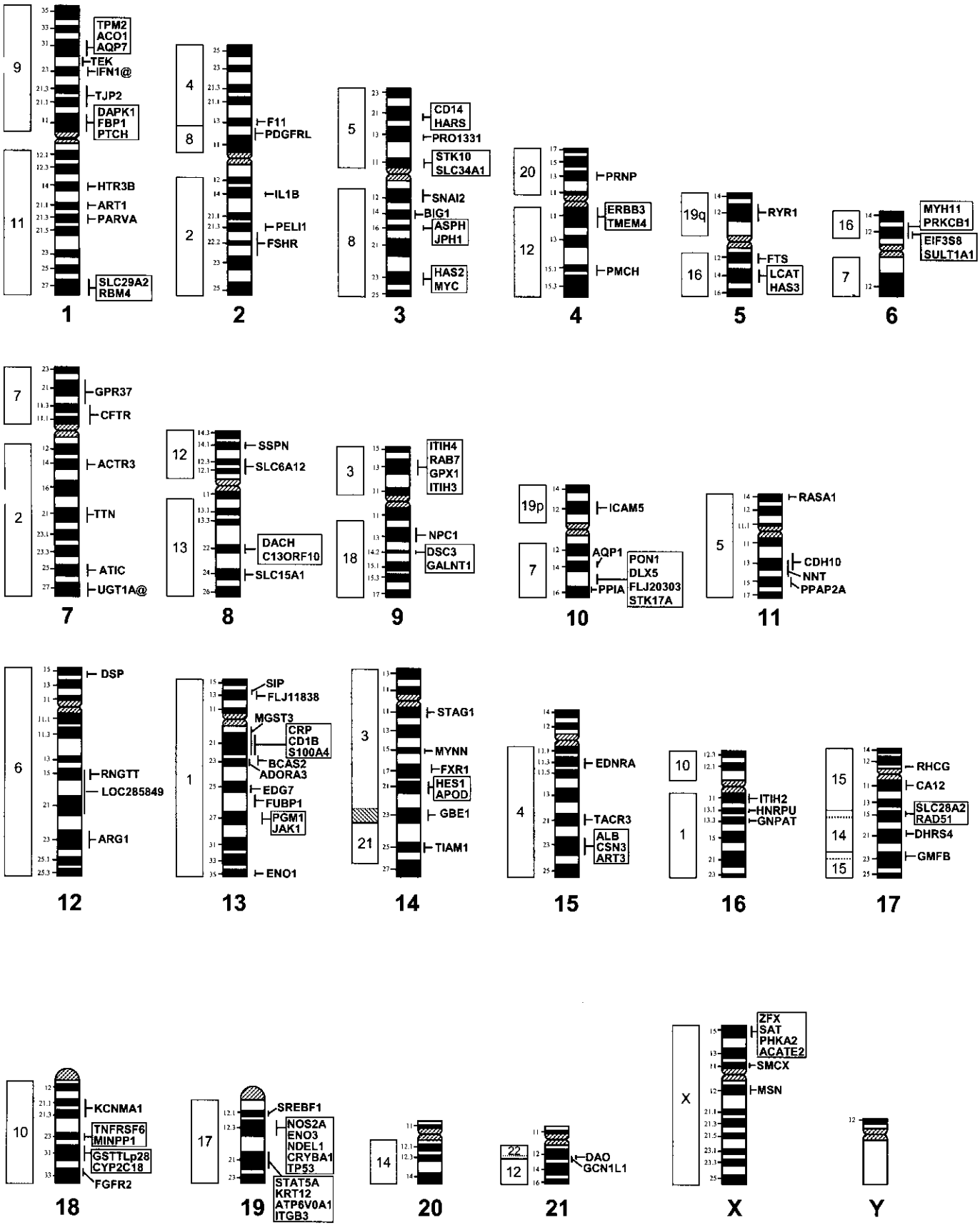
Different procedures were used to screen the rabbit BAC library. A standard PCR-based screening protocol permitted the isolation of clones for 25 genes with 23 rabbit and two human primer pairs. The overall efficiency of the PCR screening was about 70%. To diversify the screening and possibly increase the efficiency, we chose to hybridize high-density filters on which the whole collection of BAC clones was represented. A total of 73 rabbit probes and 149 bovine probes were hybridized to these rabbit membranes. BAC clones were identified for 58 genes with the rabbit probes and for 50 genes with the bovine probes. The efficiency of screening by hybridization was 33.4 % in heterologous conditions and reached 79.5% in homologous conditions. In this study, the use of high-density BAC DNA membranes was quite efficient to recover rapidly many BAC clones, as compared to the PCR-based screening method. As expected, hybridization with homologous probes was found to be much more powerful than hybridization with heterologous probes.

The gene content of each BAC clone was controlled first by checking the size of the PCR product on agarose gels and second by sequencing the PCR product and performing standard nucleotide-nucleotide BLASTN and nucleotide-protein BLASTX analyses to check the level of similarity with the original cDNA sequence in GenBank. We considered that a high level of sequence similarity combined with an expected cytogenetic localization of the corresponding BAC clone (i.e. concordant with the comparative human-rabbit map) strongly support that it contains the selected gene. 133 gene-specific BAC clones agreed with these two criteria. However, in a few cases, although sequences of PCR product and expected cDNAs matched well, non-concordant human/rabbit localization was obtained. These BAC clones were not included in this study but will be of interest for further detailed comparative mapping studies.

Mapping of 133 genes on rabbit chromosomes by fluorescent in situ hybridization

The 133 rabbit gene-specific BAC clones reported here were hybridized to rabbit metaphase chromosome spreads. Clear and consistent hybridization signals were obtained in all cases as shown in Fig. 1. Results are summarized in Table 2 and on Fig. 2. As shown in Fig. 2, new localizations were obtained on

Fig. 2. Rabbit cytogenetic map with the 133 new genes. The regional positions of the 133 FISH mapped genes are indicated by bars on the right side of R-banded ideograms of rabbit chromosomes (Hayes et al., 2002). On the left side small numbers indicate numbers of R-positive bands and blocks indicate previously observed chromosome correspondences with human chromosomes (Korstanje et al., 1999). The hatched area in the block next to OCU14 indicates a segment for which no correspondence with the human chromosomes was previously reported, and dotted lines indicate refined positions of junctions between conserved human segments.


TPM2
ACO1
AQP7
TEK
IFN1@
TJP2
DAPK1
FBP1
PTCH
HTR3B
ART1
PARVA
SLC29A2
RBM4
1
F11
PDGFRL
IL1B
PELI1
FSHR
2
CD14
HARS
PRO1331
STK10
SLC34A1
SNAI2
BIG1
ASPH
JPH1
HAS2
MYC
3
PRNP
ERBB3
TMEM4
PMCH
4
RYR1
FTS
LCAT
HAS3
5
MYH11
PRKCB1
EIF3S8
SULT1A1
6
GPR37
CFTR
ACTR3
TTN
ATIC
UGT1A@
7
SSPN
SLC6A12
DACH
C13ORF10
SLC15A1
8
ITIH4
RAB7
GPX1
ITIH3
NPC1
DSC3
GALNT1
9
ICAM5
AQP1
PON1
DLX5
FLJ20303
STK17A
PPIA
10
RASA1
CDH10
NNT
PPAP2A
11
DSP
RNGTT
LOC285849
ARG1
12
SIP
FLJ11838
MGST3
CRP
CD1B
S100A4
BCAS2
ADORA3
EDG7
FUBP1
PGM1
JAK1
ENO1
13
STAG1
MYNN
FXR1
HES1
APOD
GBE1
TIAM1
14
EDNRA
TACR3
ALB
CSN3
ART3
15
ITIH2
HNRPU
GNPAT
16
RHCG
CA12
SLC28A2
RAD51
DHRS4
GMFB
17
KCNMA1
TNFRSF6
MINPP1
GSTTLp28
CYP2C18
FGFR2
18
SREBF1
NOS2A
ENO3
NDEL1
CRYBA1
TP53
STAT5A
KRT12
ATP6V0A1
ITGB3
19
20
DAO
GCN1L1
21
ZFX
SAT
PHKA2
ACATE2
SMCX
MSN
X
Y

all chromosomes except rabbit chromosomes OCU20 and OCUY. The number of genes per chromosome mapped in this study ranges from two on OCU21 (the smallest chromosome) to 14 on OCU1 (the largest chromosome). For the ten rabbit chromosomes OCU1, 3, 5, 7, 9, 10, 13, 17, 18 and 19, the genes are distributed fairly evenly although some bands still lack any mapped gene. To our knowledge, only the six chromosome arms OCU6q, 14p, 15p, 16p, 20p and 21p, corresponding to short chromosome arms, have no mapped genes. This can be partly explained by the fact that in a previous study of (Korstanje et al., 1999) they are either not painted by human chromosome probes (OCU15p, 20p and 21p) or are painted by large-sized human chromosomes conserved with two or three other rabbit chromosomes (OCU16p/HSA10, OCU6q/HSA7, OCU14p/HSA3). Thus, it was not possible to select genes putatively in these rabbit chromosome regions solely on the basis of the human/rabbit comparative map. As expected, most genes are located on the gene-rich R-positive bands.

The BAC clones localized on unexpected rabbit chromosomes (data not shown) need further investigation to determine whether they reveal unknown conserved synteny groups between man and rabbit or whether they constitute experimental artifacts. Indeed, heterologous chromosome painting cannot detect conserved segments of less than about 10 Mb and thus small rearrangements between the rabbit and human genomes may not be as yet identified. To confirm the existence of a small novel synteny group it is necessary to have two or more orthologous gene pairs in the segment. At present, we are looking for BAC clones containing genes located in the human genome very close to the genes in question. Another possibility is that the DNA sequence of the BAC clone is very similar to that of another sequence situated elsewhere in the genome and that it hybridizes preferentially with the latter. This is the case with large gene families with members scattered on different chromosomes. For example, for the gene GALTN1 we obtained seven different BAC clones, which hybridized to three different rabbit chromosomes: OCU9, 13 and 15, and only two of these seven clones mapped to the expected rabbit chromosome OCU9q14.2. Finally, a BAC clone determined for a specific gene may in fact contain a pseudogene sharing a high level of sequence similarity with the gene, situated elsewhere in the genome but not mapped in man.

Rabbit/human comparative map

Although comparative mapping data between man and rabbit is scarce and given the criteria we used in this study, our results agree with the human/rabbit heterologous chromosome painting data reported (Korstanje et al., 1999) except in two cases, RAB7 and SIP. The gene RAB7, located on HSA3q21.3 was expected to be on OCU14 and was mapped to OCU9p13. HSA3 is composed of a succession of conserved segments with OCU14 and 9. Therefore, we decided to retain this localization because we hypothesize that the segment containing RAB7 was not detected previously (Korstanje et al., 1999) due to its probable small size below the level of detection of the painting technique. In the case of SIP, localized on HSA 1q25.1, it was expected on OCU16, but was mapped to OCU13p13distal. HSA1 is composed of two conserved segments with OCU13 and 16, their junction site being at the limit between bands q24/q25 on HSA1 (Korstanje et al., 1999). Our localization of SIP (HSA1q25.1) suggests that the junction site between OCU13 and OCU16 is slightly more telomeric than previously found. This has to be further confirmed and refined by the localization of other genes. In addition, we made a few other observations, also undetectable at the resolution level of heterologous chromosome painting, concerning the conservation of gene order and the position of synteny junctions. Indeed, we found that the order of the genes localized in this study was similar on most human and rabbit chromosomes with a few exceptions such as FLJ11838 on OCU13p13 or genes on OCU10q (see Table 2). Of course, this remains a very preliminary remark considering the small number of genes mapped in rabbit and does not give any indication on gene order conservation at the genome level. Moreover, four of the localizations obtained here modify slightly the position of synteny junctions on OCU21, OCU17 and OCU14 (see Fig. 2) as compared to a previous study (Korstanje et al., 1999). On OCU21, they reported that segments q11→13 and q14→16 are conserved respectively with parts of HSA22 and HSA12. We have localized two new HSA12 genes on OCU21: DAO and GCN1L1, positioned respectively on bands q12dist and q12dist→q13, which suggest that the OCU21 chromosomal segment conserved with HSA12 begins in band q12 nearer to the centromere than previously reported (Korstanje et al., 1999). Similarly on OCU17q, positions of RAD51 and SLC28A2 (OCU17q15prox/HSA15q15.1 and HSA15q21.1) and GMFB (OCU17q23prox/HSA14q22.2) suggest that the corresponding synteny junctions are slightly more telomeric than previously shown (see Fig. 2). Finally, on OCU14q23 (hatched box in Fig. 2), apparently Korstanje et al. (1999) did not detect any hybridization signal with either HSA3 or HSA21. However, we have mapped GBE1 (HSA3p12.2) precisely on this band (OCU14q23), which indicates that the segment conserved with HSA3 is longer than previously shown.

Conclusions

There is a need to map many more genes on the rabbit cytogenetic map to have at least one gene per chromosomal band, to refine the human-rabbit comparative map and to facilitate the construction of a high-density genetic map in rabbit. In addition, the availability of a detailed rabbit cytogenetic map will contribute to the identification of genes involved in economically important traits and disease susceptibility and resistance. It will make it possible to link genomic and phenotypic information between species with sequenced genomes i.e. man, mouse, rat and species with genomes that are not likely to be sequenced in the near future such as rabbit. The results reported here represent a first contribution increasing more than three-fold the number of genes precisely localized in rabbit.

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