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Human *REG* family genes are tandemly ordered in a 95-kilobase region of chromosome 2p12

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Abstract ***Reg*, first isolated from a rat regenerating islet cDNA library, is expressed in regenerating islet β-cells. Recently, it has been revealed that *Reg* and *Reg*-related genes constitute a multigene family, the *Reg* family. In human, the four *REG* family genes, i.e., *REG Iα*, *REG Iβ*, *REG*-related sequence (*RS*) and *HIP/PAP*, have so far been isolated. In this study, we analyzed YAC clones containing the four genes and performed two-color FISH to determine the map order of the genes. The human *REG* family genes are tandemly ordered in the 95-kbp DNA region of chromosome 2p12 as follows: 2cen-*HIP/PAP-RS-REG Iα-REG Iβ*-ptel.**

Key words: *Reg* family; Gene cluster; Islet regeneration; Chromosomal mapping; Two-color FISH; YAC; (Human)

1. Introduction

Administration of poly(ADP-ribose) synthetase inhibitors such as nicotinamide to 90% depancreatized rats induces the regeneration of pancreatic islets [1–3]. In screening the regenerating islet-derived cDNA library, we identified a rat gene, *Reg* (i.e., regenerating gene), which is expressed in regenerating islets but not in normal islets [4,5]. Rat *Reg* encodes a 165-amino acid protein with a 21-amino acid signal peptide [4,5]. We have isolated several *Reg* and *Reg*-related genes from human [6,7], rat [8,9] and mouse [10,11], and revealed that they constitute a multigene family, the *Reg* family [11,12]. Based on the amino acid sequence homology among proteins encoded by *Reg* family genes, the members of the family can be grouped into three subclasses: type I, II and III [11,12]. In human, *REG Iα*, *REG Iβ*, *RS* (*REG*-related sequence) and *HIP/PAP* (gene expressed in hepatocellular carcinoma, intestine, pancreas/gene for pancreatitis associated protein) have so far been isolated. *REG Iα* and *REG Iβ* [6,7] belong to the type I subclass and each gene encodes a 166-amino acid protein. *RS* [6,13] shows a high degree of homology to *REG I* genes but has an in-frame stop codon in the protein coding region. *HIP/PAP* [14,15], which was isolated from a human hepatocellular carcinoma cDNA library, encodes a 175-amino acid protein exhibiting 49% amino acid identity with REG I proteins and belongs to the type III subclass [11,12]. Recently, *REG Iα* [16,17], *REG Iβ* [7,17] and *HIP/PAP* [18] have been reported to map to human chromosome 2p12.

In this study, we determined the chromosomal localization of *RS* in chromosome 2p12 and the order and polarity of the four human *REG* family genes in the chromosome by analysis of yeast artificial chromosome (YAC) clones and by two-color fluorescence in situ hybridization (FISH).

2. Materials and methods

2.1. Isolation and analysis of YAC clones containing human REG family

The CEPH YAC human genome library [19,20] was kindly provided by Tsukuba Life Science Center, The Institute of Physical and Chemical Research (RIKEN). The CEPH YAC library was screened by PCR using the specific primers for *REG Iα*, *REG Iβ*, *HIP/PAP* or *RS*. Oligonucleotide primers specific for each gene were as follows: for *REG Iα*, 5′-TCTTTCGGGTCTCCCAG-3′ and 5′-TCAGCAGAGAAGAGAGTGTCCAGGTTGAGT-3′, corresponding to nucleotides 2618–2634 and 2812–2841, respectively, in Ref. 6; for *REG Iβ*, 5′-AGGTAAGCTCCTTATCTGGA-3′ and 5′-TGTTCATAAGCCTGACATAC-3′, corresponding to nucleotides 2587–2606 and 3093–3112, respectively, in Ref. 7; for *HIP/PAP*, 5′-TTAGTGACTCCTGATTGC-3′ and 5′-TCACATCACTGCTACTCC-3′, corresponding to nucleotides 311–328 and 2087–2104, respectively, in Ref. 15; and for *RS*, 5′-TCTCTGTACTTCTAGGGTAG-3′ and 5′-TGAGAAGATTCAGACTGAGG-3′, corresponding to nucleotides 605–624 and 1389–1408, respectively in Ref. 13. YAC DNA was prepared in agarose beads as described [21]. The YAC DNA was digested, either partially or completely, with several restriction enzymes and was separated by pulsed- field gel electrophoresis in a Beckman GeneLine II. Transfer and hybridization conditions were as previously described [22] using the YAC left and right arm sequences, *REG Iα* (0.7-kbp *Pst*I-*Xba*I fragment, nucleotides 1644–2349) [6], *REG Iβ* (0.6-kbp *Pst*I-*Xba*I fragment, nucleotides 1634–2229) [7], *HIP/PAP* (0.5-kbp fragment obtained by PCR, nucleotides 1589–2104) [15] or *RS* (0.55-kbp *Hin*dIII-*Xba*I fragment, nucleotides 553–1107) [13] DNA as probes. pBR322 was digested with *Bam*HI and *Pvu*II, and the resulting 2.7- and 1.7-kbp fragments were used for the YAC left- and right-side arm probes, respectively. We confirmed that cross-hybridization between *REG Iα*, *REG Iβ*, *HIP/PAP* and *RS* did not occur under the hybridization condition [22]. λDNA concatemers (Pharmacia Biotech, λDNA-PFGE markers) were used as molecular weight standards.

2.2. Fluorescence in situ hybridization (FISH)

The R-banding method for direct mapping with FISH was carried out as described [23]. DNA probes used for FISH were as follows: for *RS*, plasmid containing a 1.5-kbp *Eco*RV fragment of human *RS* [6]; for *REG Iα*, plasmid containing a 4.3-kbp DNA fragment of human *REG Iα* [6]; for *REG Iβ*, plasmid containing a 3.4-kbp *Hin*dIII fragment (5′ half of human *REG Iβ*) [7]; for *HIP/PAP*, plasmid containing a 2.1-kbp fragment (nucleotides 311-2422 in Ref. 15) obtained by PCR. These plasmid DNAs were labeled with biotin-16-dUTP using a nick translation kit (Boehringer Mannheim). The fragment size after nick translation was adjusted to approx. 300 bp as described [23]. The procedure for FISH was carried out as described [23] and the biotin signal was amplified according to the method of Lemieux et al. [24]. Two-color

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Abbreviations: bp, base pair(s); FISH, fluorescence in situ hybridization; *HIP/PAP*, gene expressed in hepatocellular carcinoma, intestine, pancreas/gene for pancreatitis associated protein; kbp, kilobase pair(s); PCR, polymerase chain reaction; R-banding, reverse-banding; *RS*, *REG*-related sequence; YAC, yeast artificial chromosome.

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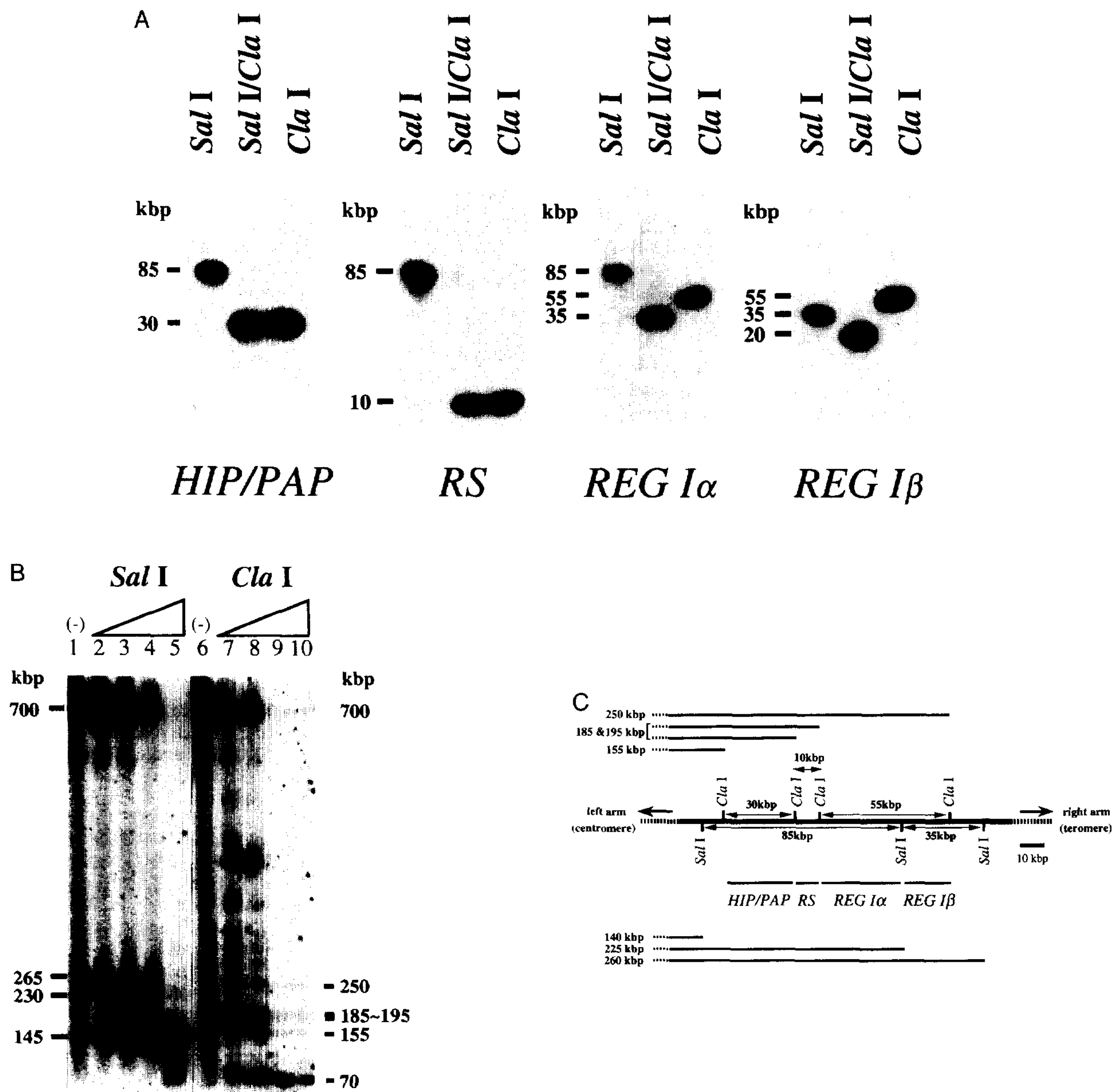

Fig. 1. Analysis of YAC clone containing human *REG* family. (A) Southern blot analysis of clone 642A1 DNA completely digested with *Sal*I, *Cla*I or *Sal*I/*Cla*I using *HIP/PAP*, *RS*, *REG Iα* or *REG Iβ*DNA as probe. (B) Southern blot analysis of partial *Sal*I digest (lanes 2–5) and partial *Cla*I digest (lanes 7–10) of clone 642A1 DNA using YAC left arm probe. Lanes of undigested YAC DNA (lanes 1 and 6) are indicated by (−). The size of restriction fragments, which are indicated by bars, is given in kbp. (C) *Sal*I and *Cla*I restriction map of the 120-kbp region of human chromosome 2p12 containing the *REG* family. Directions of left or right arm in the YAC clone 642A1, and centromere or telomere on chromosome 2 are indicated by arrows. DNA fragments produced by partial *Sal*I digestion (below) and partial *Cla*I digestion (above) are also indicated by bars. Restriction fragment length of complete *Cla*I or *Sal*I digestion are indicated by bi-directional arrows. Localization of *HIP/PAP*, *RS*, *REG Iα* and *REG Iβ* genes in a 95-kbp DNA region are indicated.

in situ hybridization proceeded according to the method of Lebo et al. [25]. In brief, stretched chromosomes were hybridized to the combinations of the probes (see Table 1); one of the probes was labeled with biotin-16-dUTP and the other with digoxigenin-11-dUTP. In some cases, to clarify the polarity of the chromosome, digoxigenin-labeled chromosome 2 specific α-satellite DNA (Oncor, Inc.) was mixed in the hybridization solution. Then both biotin and digoxigenin signals were amplified simultaneously with avidin-rhodamine and mouse anti-digoxigenin. After washing, the biotin was further amplified with biotinylated goat anti-avidin, and digoxigenin was fluorescence labeled with rabbit anti-mouse FITC (fluorescein isothiocyanate). After washing, both signals were further amplified with avidin-rhodamine and goat anti-rabbit FITC. The slide was washed, stained with propidium iodide, and mounted with anti-fade solution containing *p*-phenylenediamine (pH 11.0).

3. Results and discussion

We and others have reported that *REG Iα* [16,17], *REG Iβ* [7,17] and *HIP/PAP* [18] are localized on chromosome 2p12. To order these human *REG* family genes, we screened the CEPH YAC library [19,20] by PCR as described in section 2

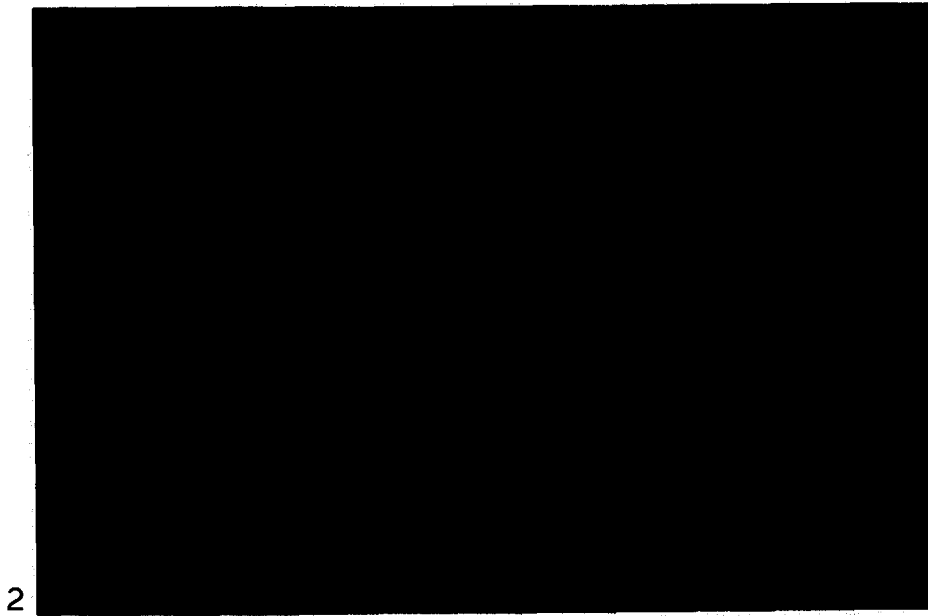

Fig. 2. Chromosomal localization of *RS* by FISH. (A) R-banded metaphase chromosome spread from human peripheral lymphocytes hybridized with the *RS*, showing the localization of spots on both chromatids of chromosome 2. Arrows indicate fluorescent signals on 2p12.

Fig. 3. Two-color in situ hybridization on stretched chromosome with *REG Iβ*, *HIP/PAP* and chromosome 2 specific α-satellite DNA. *REG Iβ* and *HIP/PAP* were labeled with biotin-16-dUTP and digoxigenin-11-dUTP, respectively. A straight filled arrow, open arrows and filled curve arrows indicate yellow-green FITC-stained α-satellite DNA, yellow-green FITC-stained *HIP/PAP* and red rhodamine-stained *REG Iβ*, respectively. Therefore, the order of three signals was 2cen-α-satellite-*HIP-PAP-REG Iβ*-ptel.

and obtained four clones, 642A1, 814G7, 913H6 and 931A11, which contained not only *REG Iα*, *REG Iβ* and *HIP/PAP* but also *RS* (Fig. 1A). One of these clones, 642A1, which had an approx. 700-kbp insert, was analyzed by Southern blot analysis. As shown in Fig. 1A, in the complete *Sal*I digest of the 642A1 DNA, an 85-kbp fragment hybridized to *HIP/PAP*, *RS* and *REG Iα*, and a 35-kbp fragment hybridized to *REG Iβ* were detected. Complete *Cla*I digestion generated a 55-kbp fragment hybridized to both *REG Iα* and *REG Iβ*, a 10-kbp fragment hybridized to *RS*, and a 30-kbp fragment hybridized to *HIP/PAP*. Complete *Sal*I and *Cla*I digestion generated a 35-kbp fragment hybridized to *REG Iα* and a 20-kbp fragment hybridized to *REG Iβ*, indicating that the 55-kbp *Cla*I fragment contained a *Sal*I site and that *REG Iα* lies adjacent to *REG Iβ* (see Fig. 1C). Complete *Sal*I and *Cla*I digestion generated a 10-kbp fragment hybridized to *RS* and a 30-kbp fragment hybridized to *HIP/PAP* (Fig. 1A), indicating that the 10- and 30-kbp *Cla*I fragments lie in the 85-kbp *Sal*I fragment (see Fig. 1C). Essentially the same result was obtained from the analysis of the other three YAC clones (data not shown).

Then we determined the order of these DNA fragments by Southern blot analysis of the partial *Sal*I or *Cla*I digest of the YAC DNA using the YAC left arm probe (Fig. 1B). We detected 145-kbp, 230- and 265-kbp fragments in the partial *Sal*I digest (Fig. 1B), indicating that the *Sal*I sites were localized at 145, 230 and 265 kbp from the left arm flanking sequence (Fig. 1C). In the partial *Cla*I digest, we detected 70-kbp, 155-kbp, and 250-kbp bands, and a 185–195-kbp broad intense band (Fig. 1B), indicating that *Cla*I sites were localized at 70, 155, 250, and 185/195 kbp from the left arm. The 10-kbp *Cla*I fragment containing *RS* (Fig. 1A) appears to have derived from the complete digestion of adjacent *Cla*I sites at 185- and 195-kbp from the left arm (Fig. 1C): the 185–195-kbp broad band seen in Fig. 1B appears to contain two DNA fragments of 185 and 195 kbp. The presence of *Cla*I sites at 185-kbp and 195-kbp was confirmed by Southern analysis of the partial *Cla*I digest using *RS* as a probe: the *RS* probe was hybridized to the approx. 195-kbp band but not to the 155-kbp or 70-kbp band (data not shown), indicating that the 10-kbp fragment containing *RS* links to the 185-kbp fragment (Fig. 1C) but not to the 155-kbp or 70-kbp fragment. These results indicate that the *REG* family genes lie in a 95-kbp DNA region in the following order: *HIP/PAP-RS-REG Iα-REG Iβ* (Fig. 1C). These results also indicate that *RS* locates in the cluster of other *REG* family genes which had been assigned to chromosome 2p12 [7,16–18]. In fact, we assigned *RS* to 2p12 by FISH: Of 100 karyotypes analyzed, 57 of the chromosomes exhibited symmetrical double spots on 2p12, and 39 had single spots on one chromatid (Fig. 2).

Table 1
Ordering of human *REG* family using two-color FISH

Position of/relative to	Distal (n)[a]	Proximal (n)[a]	P^b
REG Iβ/REG Iα	32	3	<0.001
REG Iβ/RS	36	4	<0.001
REG Iβ/HIP/PAP	118	2	<0.001
REG Iα/RS	28	8	0.05–0.02
REG Iα/HIP/PAP	36	12	0.05–0.02
RS/HIP/PAP	27	30	NS

[a]Number of spots counted.
[b]Probability associated with χ^2-test comparing the number of distal versus proximal spots to that of a random distribution. NS, not significant.

To determine the polarity of the 95-kbp region on chromosome 2, we performed two-color FISH with combinations of the four *REG* family gene probes. A representative result is shown in Fig. 3. Fluorescent spots of *REG* family genes accumulated on chromosome 2p12 and the discriminated spots were counted (Table 1). Using this method, *REG Iβ* was found to lie distal to *REG Iα*, *RS* and *HIP/PAP* from the centromere. Similarly, *REG Iα* was distal to *RS* and *HIP/PAP* from the centromere. *RS* and *HIP/PAP* could not be ordered using this method. Based on these results, the following map order was obtained: 2cen-[*HIP/PAP*, *RS*]-*REG Iα-REG Iβ*-ptel.

All the results obtained from the YAC analysis and two-color FISH led to the conclusion that human *REG* family genes were tandemly ordered in a 95-kbp DNA region of chromosome 2p12 in the following order: 2cen-*HIP/PAP-RS-REG Iα-REG Iβ*-ptel. The fact that the human *REG* family genes are clustered in close proximity, having a common gene structure of six exons and five introns, suggests that these genes have evolved from a common ancestral gene by gene duplication; the common ancestral gene may have been duplicated and evolved into an ancestral type I gene and *HIP/PAP* (type III gene) and then, more recently, the ancestral type I gene may have been duplicated and evolved into *REG Iα*, REG I*β* and *RS*.

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References

[1] Yonemura, Y., Takashima, T., Miwa, K., Miyazaki, I., Yamamoto, H. and Okamoto, H. (1984) Diabetes 33, 401–404.

[2] Okamoto, H., Yamamoto, H. and Yonemura, Y. (1985) in: ADP-ribosylation of proteins (Althaus, F.R., Hilz, H. and Shall, S., eds), Springer-Verlag, Berlin, pp. 410–416.

[3] Okamoto, H. (1990) Molecular Biology of the Islets of Langerhans, Cambridge University Press, Cambridge, pp. 209–231.

[4] Terazono, K., Yamamoto, H., Takasawa, S., Shiga, K., Yonemura, Y., Tochino, Y. and Okamoto, H. (1988) J. Biol. Chem. 263, 2111–2114.

[5] Terazono, K., Watanabe, T. and Yonemura, Y. (1990) in: Molecular Biology of the Islets of Langerhans (Okamoto, H., ed.), Cambridge University Press, Cambridge, pp. 301–313.

[6] Watanabe, T., Yonekura, H., Terazono, K., Yamamoto, H. and Okamoto, H. (1990) J. Biol. Chem. 265, 7432–7439.

[7] Moriizumi, S., Watanabe, T., Unno, M., Nakagawara, K., Suzuki, Y., Miyashita, H., Yonekura, H. and Okamoto, H. (1994) Biochim. Biophys. Acta 1217, 199–202.

[8] Miyashita, H., Yonekura, H., Unno, M., Suzuki, Y., Watanabe, T., Moriizumi, S., Takasawa, S. and Okamoto, H. (1994) Biochim. Biophys. Acta 1219, 241–243.

[9] Suzuki, Y., Yonekura, H., Watanabe, T., Unno, M., Moriizumi, S., Miyashita, H. and Okamoto, H. (1994) Gene 144, 315–316.

[10] Unno, M., Itoh, T., Watanabe, T., Miyashita, H., Moriizumi, S., Teraoka, H., Yonekura, H. and Okamoto, H. (1992) in: Advances in Experimental Medicine and Biology, Vol. 321: Pancreatic Islet Cell Regeneration and Growth (Vinik, A.I., ed.), Plenum, New York, pp. 61–66.

[11] Unno, M., Yonekura, H., Nakagawara, K., Watanabe, T., Miyashita, H., Moriizumi, S., Okamoto, H., Itoh, T. and Teraoka, H. (1993) J. Biol. Chem. 268, 15974–15982.

[12] Yonekura, H., Unno, M., Watanabe, T., Moriizumi, S., Suzuki, Y., Miyashita, H., Yonemura, Y., Sugiyama, K. and Okamoto, H. (1995) in: Frontiers of Insulin Secretion and Pancreatic B-Cell Research (Flatt, P. R. and Lenzan S., eds), Smith-Gordon, London, pp. 581–588.
[13] DDBJ, EMBL and GenBank nucleotide sequence accession number D56494.
[14] Lasserre, C., Christa, L., Simon, M.-T., Vernier, P. and Bréchot, C. (1992) Cancer Res. 52, 5089–5095.
[15] Lasserre, C., Simon, M.-T., Ishikawa, H., Diriong, S., Nguyen, V.C., Christa, L., Vernier, P. and Bréchot, C. (1994) Eur. J. Biochem. 224, 29–38.
[16] Perfetti, R., Hawkins, A.L., Griffin, C.A., Egan, J.M., Zenilman, M.E. and Shuldiner, A.R. (1994) Genomics 20, 305–307.
[17] Gharib, B., Fox, M.F., Bartoli, C., Giorgi, D., Sansonetti, A., Swallow, D.M., Dagon, J.C. and Berge-Lefranc, J.L. (1993) Ann. Hum. Genet. 57, 9–16.
[18] Dusetti, N.J., Frigerio, J.-M., Fox, M.F., Swallow, D.M., Dagon, J.C. and Iovanna, J.L. (1994) Genomics 19, 108–114.
[19] Chumakov, I., Rigault, P., Guillou, S., Ougen, P., Billaut, A., Guasconi, G., Gervy, P., LeGall, I., Soularue, P., Grinas, L., Bougueleret, L., Bellanné-Chantelot, C., Lacroix, B., Barillot, E., Gesnouin, P., Pook, S., Vaysseix, G., Frelat, G., Schmitz, A., Sambucy, J.-L., Bosch, A., Estivill, X., Weissenbach, J., Vignal, A., Riethman, H., Cox, D., Patterson, D., Gardiner, K., Hattori, M., Sakaki, Y., Ichikawa, H., Ohki, M., Le Paslier, D., Heilig, R., Antonarakis, S. and Cohen, D. (1992) Nature 359, 380–386.
[20] Cohen, D., Chumakov, I. and Weissenbach, J. (1993) Nature 366, 698–701.
[21] D'Urso, M. and Schlessinger, D. (1991) in: EMBO Practical Course: Cloning into YAC Vectors, International Institute of Genetics and Biophysics Press, Naples, pp. 38–39.
[22] Takasawa, S., Tohgo, A., Unno, M., Yonekura, H. and Okamoto, H. (1992) FEBS. Lett. 307, 318–323.
[23] Nakagawara, K., Mori, M., Takasawa, S., Nata, K., Takamura, T., Berlova, A., Tohgo, A., Karasawa, T., Yonekura, H., Takeuchi, T. and Okamoto, H. (1995) Cytogenet. Cell Genet. 69, 38–39.
[24] Lemieux, N., Dutrillaux, B. and Viegas-Péquignot, E. (1992) Cytogenet. Cell Genet. 59, 311–312.
[25] Lebo, R.V., Lynch, E.D., Bird, T.D., Golbus, M.S., Barker, D.F., O'Connell, P. and Chance, P.F. (1992) Am. J. Hum. Genet. 50, 42–55.