Volume 11 Number 18 1983 Nucleic Acids Research

Chromosomal localization of the human c-*fms* oncogene

John Groffen[1], Nora Heisterkamp[1], Nigel Spurr[2], Sharon Dana[3], John J.Wasmuth[3] and John R.Stephenson[1]

[1]Lab. Viral Carcinogenesis, National Cancer Institute-FCRF, Frederick, MD 21701, USA, [2]Imperial Cancer Research Fund, P.O. Box 123, Lincoln's Inn Fields, London WC2A 3PX, UK, and [3]Dept. Biological Chemistry, California College of Medicine, Univ. California, Irvine, CA 92717, USA

Received 27 June 1983; Revised and Accepted 10 August 1983

ABSTRACT

A molecular probe was prepared with specificity for the human cellular homologue of transforming sequences represented within the McDonough strain of feline sarcoma virus (v-fms). By analysis of a series of mouse-human somatic cell hybrids containing variable complements of human chromosomes it was possible to assign this human oncogene, designated c-fms, to chromsome 5. Regional localization of c-fms to band q34 on chromosome 5 was accomplished by analysis of Chinese hamster-human cell hybrids containing as their only human components, terminal and interstitial deleted forms of chromosome 5. The localization of c-fms to chromosome 5 (q34) is of interest in view of reports of a specific, apparently interstitial, deletion involving approximately two thirds of the q arm of chromosome 5 in acute myelogenous leukemia cells.

INTRODUCTION

A number of well defined genetic sequences, designated "oncogenes," have been identified within the human genome (1,2). Such sequences were demonstrated by use of molecular probes corresponding to transforming sequences of oncogenic retrovirus isolates of various avian and mammalian species. In general, cellular oncogenes have remained highly conserved throughout vertebrate evolution although their functional significance is not known. The viral counterparts of many of the oncogenes studied to date are known to encode tyrosine specific protein kinases while enzymatic functions have not been ascribed to transforming proteins encoded by the others (3). Support for the possible involvement of cellular oncogenes in human cancer has recently been derived by the demonstraton that under certain conditions, members of the ras family, can transform cells in culture (4-7). Moreover, two other human cellular oncogenes, c-abl and c-myc, are involved in translocations associated with chronic myelogenous leukemia (8) and Burkitt's lymphoma (9,10), respectively.

The human c-fms oncogene represents a cellular sequence initially identified on the basis of homology to the transforming gene (v-fms) of the

McDonough strain of feline sarcoma virus (11,12). We have recently applied a cosmid vector system for the molecular cloning of this gene (12). Although the c-fms gene product has not been identified, the corresponding viral gene, v-fms, has been shown to encode a protein with transforming function (13,14) but lacking protein kinase activity (15). In the present study we have developed a specific probe for the human c-fms gene. Using this reagent, studies were performed to determine the chromosomal localization of c-fms and its relation to chromosomal deletions and/or rearrangements associated with specific forms of human cancers.

MATERIALS AND METHODS

Cells and Viruses: Cells were grown in Dulbecco's modification of Eagles medium supplemented with 10% calf serum and included NIH/3T3 mouse cells (15), A673 human cells (15) and the human-mouse somatic cell hybrids described in Table 1. A series of human-Chinese hamster cell hybrids, including HHW105 containing chromosome 5 as its only human component and segregants of HHW105, designated HHW207, HHW209, HHW212, HHW213, HHW224, characterized by a variety of terminal and interstitial deletions of chromosome 5, have been described (16,17).

Preparation of a Human c-fms Specific Probe: The isolation of a cosmid clone with a cellular insert of approximately 38 kb containing v-fms homologous sequences (cos 13) from a library of human lung carcinoma DNA has been described (12). For use as a probe, an 0.9 kb Kpn-1 restriction fragment, was isolated from cos 13 according to previously described methods (12).

Molecular Hybridization: Restriction enzymes were purchased from New England Biolabs and Bethesda Research Laboratories and were used according to the suppliers' specifications. DNAs were digested with restriction enzymes, subjected to electrophoresis through 0.75% agarose gels and transferred to nitrocellulose essentially as described by Southern (18). Nick translation of probes and filter hybridization were as described (19). Specific activity of the probes was $2\text{-}5\times10^8$ cpm/ug. After hybridization, filters were washed under high stringency conditions (0.1 xSSC,65^{o}C) and exposed to XAR-2 film (Kodak) for up to 5 days at -70^{o}C with Dupont Lightning Plus intensifying screens.

RESULTS

Identification of Human c-fms Specific Sequences: As a means of discriminating human c-fms sequences from related sequences in the mouse cellular genome, a series of probes were prepared from cos 13 (Fig. 1), a

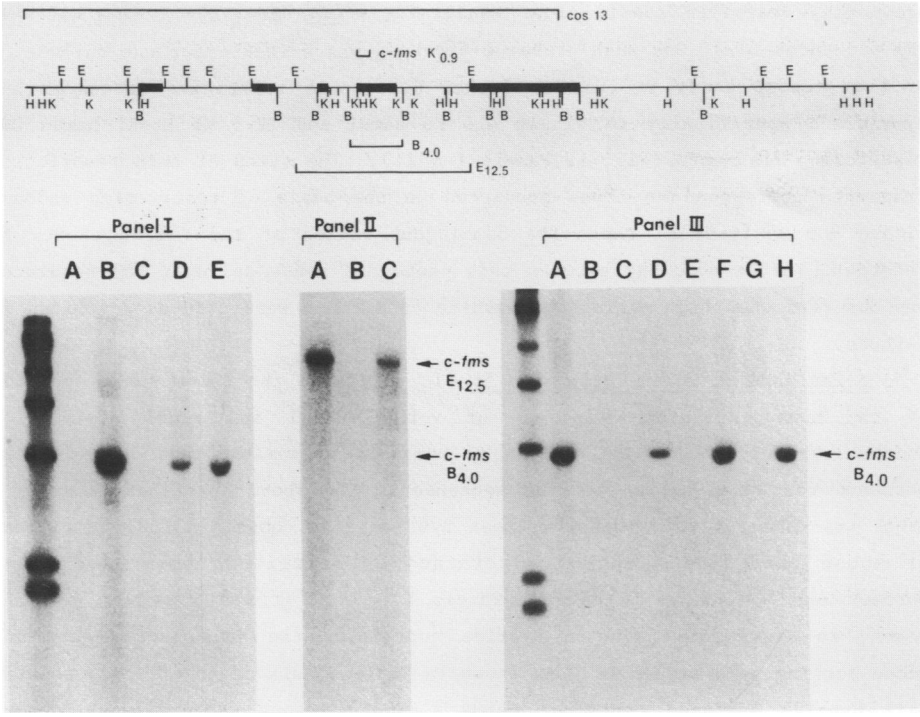

Figure 1 (Upper pannel) . Restriction endonuclease map of v-fms homologous human DNA sequences. The positions of v-fms homologous sequences, shown as solid boxes (▬) were determined as previously described (12). The position of sequences corresponding to the cellular insert in cos 13 and the 0.9 kb Kpn-1 restriction fragment used as a molecular probe for characterization of somatic cell hybrids are shown in upper portion of the figure. Single BamHl ($B_{4.0}$) and EcoRl ($E_{12.5}$) restriction fragments detected by the c-fms $K_{0.9}$ probe, are indicated below the restriction map. Restriction enzymes include: BamHl (B); HindIII (H); EcoRl (E);and Kpn-1 (K). Panels I & II: Analysis of mouse, human and representative mouse-human somatic cell hybrid cellular DNAs for human c-fms sequences. BamHl (I) and EcoRl (II) digested cellular DNAs (25 µg/lane) were electrophoresed on 0.7% gels, blotted to nitrocellulose and hybridized to the c-fms $K_{0.9}$ probe. Cell lines include: I: NIH/3T3 mouse (A); A673 human (B); and mouse-human somatic cell hybrids DT1.2R (C); Dur4R3 (D); and Dur4R4 (E); II: A673 human (A); NIH/3T3 mouse (B); and mouse-human hybrid, MOG-2E5 (C). The positions of single human DNA specific $K_{0.9}$ homologous BamHl ($B_{4.0}$) and EcoRl ($E_{12.5}$) restriction fragments are indicated to the right side of the figure. HindIII digested λDNA, included as a molecular weight marker is shown on the left. Panel III: Regional localization of c-fms on chromosome 5. BamHl digested DNAs of human A673 (A); Chinese hamster (B); and human-Chinese hamster somatic cell hybrids including HHW213 (C); HHW207 (D); HHW209 (E); HHW224 (F); HHW212 (G); HHW105 (H) were analyzed for human c-fms specific sequences as above.

previously described cosmid clone containing v-fms homologous human cellular DNA sequences (12). One such probe, c-fms $K_{0.9}$ was suitable for the purpose of the present study in that it lacked detectable repetitive sequences and hybridized specifically to single 4.0 kb BamHl and 12.5 kb EcoRl human DNA restriction fragments (Fig. 1, Panels I & II). The sizes of both restriction fragments correspond to those predicted on the basis of restriction endonuclease map analysis of the v-fms homologous region of the human genome. No detectable cross-homology between this probe and mouse cellular DNA sequences was observed when high stringency washing conditions were used as described in Methods.

c-fms Maps on Human Chromosome 5: To determine the chromosomal location of the human cellular homologue of v-fms, EcoRl and BamHl restriction endonuclease-digested cellular DNAs of human-mouse somatic cell hybrids were screened for human c-fms specific sequences. The above described c-fms $K_{0.9}$ probe was used for this purpose. These hybrids each contain a full complement of mouse chromosomes but have retained only limited numbers of human chromosomes. As shown in Fig. 1, Panels I & II, it was possible, by this means, to distinguish between hybrids containing the human c-fms gene and those lacking such sequences. As shown in Table 1, six of the fifteen somatic cell hybrids analyzed contained c-fms $K_{0.9}$ homologous sequences. Only four chromosomes (5,12,14 and 17) are common to all six c-fms positive hybrids. These findings localize c-fms to human chromosomes 5,12,14 or 17. To further localize c-fms we examined the chromosomal content of the series of nine hybrids which were non-crossreactive with the c-fms $K_{0.9}$ probe. As summarized in the lower portion of Table 1, only human chromosome 5 was missing from each of these nine hybrids. This excludes the possibility of c-fms mapping on chromosomes 12,14 or 17, each of which were present in at least three of the c-fms negative hybrids, and thus localizes c-fms to human chromosome 5.

Regional Localization of c-fms to Chromosome 5 (q34): The recent isolation of a series of segregants of Chinese hamster-human somatic cell hybrids expressing variable numbers of genes mapping on chromosome 5 (16,17) provided a means of regionally localizing c-fms. The majority of these segregants are characterized by terminal deletions involving well defined regions of the long arm of chromosome 5; these were isolated by positive selection for leuS, which maps on the p arm or very near the centromere on the q arm of the chromosome, and negative selection directed against markers on the distal half of the q arm (emtB and chr). DNAs prepared from Chinese hamster-human hybrid cells were analyzed for c-fms $K_{0.9}$ homologous sequences

TABLE 1: ANALYSIS OF HUMAN-MOUSE SOMATIC CELL HYBRIDS FOR HUMAN c-fms SPECIFIC SEQUENCES[1]

Hybrid	Ref	Human Chromosomes																						c-fms
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	
$CTP_{34}B_4$	21	+	+	+	-	+	+	-	-	+	-	-	+	-	+	-	+	+	+	-	-	-	-	+	+
DUR_4R_3	22	-	-	+	-	+	-	-	-	-	-	+	+	+	+	-	-	+	+	-	+	+	+	-	+
DUR_4R_4	22	-	-	+	-	+	-	-	-	-	-	+	+	+	+	-	-	+	-	-	-	+	+	-	+
$MOG\text{-}2^4E5$	19	+	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
SIR-7 A2	19	+	+	-	-	+	-	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	-	+	+
MOG-2 G1	19	+	-	+	+	+	+	+	+	-	+	-	+	+	+	-	+	+	+	+	+	+	-	NT	+
DT1.2R	26	-	-	+	-	-	-	-	+	-	+	+	-	+	-	-	-	+	+	-	+	+	+	-	-
CTP41P1	21	-	-	+	-	-	+	+	-	-	-	-	-	-	+	-	+	+	-	-	+	-	-	+	-
$_3W_4C1_5$	25	-	-	-	-	-	-	-	-	-	+	+	+	-	+	+	-	+	-	-	-	+	-	+	-
$F4SC13C112$	10	+	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-
F1R5R3	23	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-
DUR 4.4	20	-	-	+	-	-	-	-	-	-	-	+	+	+	+	+	-	-	+	-	-	+	+	+	-
SIR-7 D1	19	+	+	-	+	-	-	+	-	-	-	-	+	+	+	+	-	+	+	+	+	+	-	+	-
SIR-7 G1	19	+	-	-	+	-	-	+	-	-	+	+	-	+	+	-	+	+	+	+	+	+	-	+	-
MOG 13/22	24	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-

[1]The origins and details of the initial characterizations of the somatic cell hybrids are described in the references indicated in the second column. The human chromosomal contents were deduced from a combination of karyotypic, antigenic and enzymatic analyses. Karyotypic analysis was done by a combination of G11 staining and quinacrine banding. Analysis of hybrids for c-fms sequences was performed as described in the legend to Fig. 1. Concordance beween the presence or absence of a particular chromosome from a hybrid and c-fms sequences is indicated by the open boxes.

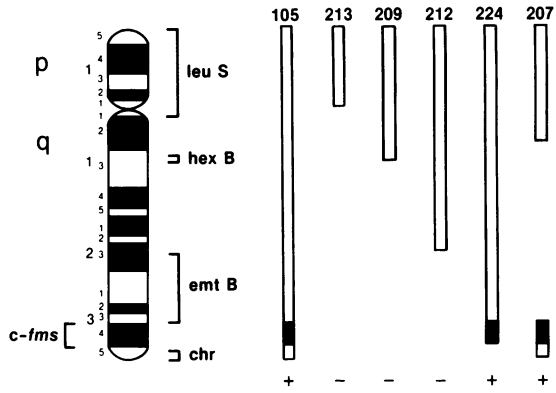

Figure 2. Schematic representation of human chromosome 5 indicating the localization of breakpoints of segregants utilized in the present study and previously reported map positions of leuS, hexB, emtB and chr (16,17). Localization of c-fms is based on the results of the present study. In the right panel of the figure the series of chromosome 5 deletion segregants are shown diagramatically and indicated as c-fms positive (+) or negative (-). Regions common to the three segregants containing human c-fms sequences are shown as solid bars.

following BamHl digestion (Fig. 1, Panel III). In contrast to human DNA, Chinese hamster cellular DNA lacked detectable homology with the c-fms $K_{0.9}$ probe. Hybrid HHW105, containing an intact chromosome 5 as its only human component, reacted strongly with the c-fms $K_{0.9}$ probe, thus confirming the localization of c-fms to human chromosome 5. In contrast, human c-fms sequences were absent from a segregant (HHW213) missing almost the complete q arm of chromosome 5 as well as from segregants HHW209 and HHW212 which have lost approximately 80% and 40% respectively, of the terminal portion of the q arm of chromosome 5. An additional segregant, HHW224, characterized by an apparent terminal deletion of chromosome 5 encompassing at most the q35 terminal band corresponding to the chr marker, was positive for c-fms, thus localizing c-fms between q23 and q34.

In a previous study (16), preliminary characterization of an additional segregant, HHW207, revealed a large interstitial deletion of chromosome 5 encompassing both the hexB and emtB markers. Upon hybridization analysis, this segregant was found to have retained c-fms (Fig. 1, Panel III). In order to more precisely localize c-fms, it was necessary to subject HHW207 to more detailed karyotypic analysis. Upon examination of chromosome preparations from HHW207 under conditions which gave much better banding resolution than was achieved previously we were able to unequivocally localize the interstitial

deletion as encompassing the region of chromosome 5 mapping from q12 to q33. As shown schematically in Fig. 2, these results establish the order of the individual markers on chromosome 5 as leuS, hexB, emtB, c-fms and chr and more specifically localize c-fms to band q34.

DISCUSSION:

In the present study we have utilized a panel of mouse-human somatic cell hybrids to map the human oncogene, c-fms, on chromosome 5. Additionally, the availability of a set of hybrids with well defined deletions in chromosome 5 (16,17) has allowed regional localization of c-fms to band q34. The generation of sets of deletion hybrids, such as those used in the present study, is possible for any human chromosome for which appropriate positive and negative selection markers can be developed. By use of such hybrids in combination with Southern hybridization the localization of specific genetic sequences can be achieved with much less ambiguity than is possible by use of alternative techniques such as in situ hybridization.

The localization of c-fms on chromosome 5, in addition to the previous assignment of other human oncogenes to specific chromosomes, establishes the distribution of the known oncogenes among human chromosomes to be relatively random. In fact, the eight human oncogenes which have been mapped to date are distributed among seven different chromosomes. These include c-fes (20,21), c-myb (21), c-abl (20), c-sis (22,23) and c-ras-H (24) which have been mapped to chromosomes 15, 6, 9, 22 and 11, respectively, c-fms localized in the present study on chromosome 5, and two human oncogenes, c-myc (9,10) and c-mos (25,26) both of which map on chromosome 8.

Evidence for an association of human oncogenes with translocations and deletions specific to various human cancers is accumulating. For instance, c-abl has been localized within a small terminal region of chromosome 9 (20) which is translocated to chromosome 22 in chronic myelogenous leukemia (9,10) while c-sis maps within the reciprocally translocated region of chromosome 22 (27). Similarly, c-myc has been identified near the breakpoint of the segment of chromosome 8 which translocates to chromosomes 2, 14 or 22 in Burkitt's lymphoma (9,10) and c-fes has been mapped within the region of chromosome 15 which is translocated to chromosome 17 in acute promyelocytic leukemia (28). In other studies, c-ras-H has been mapped on chromosome 11, a human chromosome with a characteristic deletion in Wilms tumor (24). The mapping of c-fms on chromosome 5 is of interest in that deletions within the long arm of chromosome 5 are also frequently observed in patients with refractory anemia

(29) and acute myelogenous leukemia (30,31). Such deletions have been reported to appear to be interstitial, involving approximately two thirds of the long arm of chromosome 5, but not extending to band q34 (29,31).

As one possible model, to account for their association with chromosomal rearrangements occurring in specific human cancers, cellular oncogenes, such as c-fms could map adjacent to chromosomal breakpoints possibly resulting in the juxtapositioning of potentially oncogenic sequences with cellular promotors. Such a model has been proposed to account for the translocations associated with chronic myelogenous leukemia and Burkitt's lymphoma (8-10); in fact, in Burkitt's lymphoma the cellular sequences in the chromosome to which c-myc is translocated appear to be involved in immunoglobulin rearrangements (9,10). It will thus be of interest to accurately map the proximity of c-fms to chromosome 5q- deletions associated with acute myelogenous leukemia and to analyze tumors with the 5q- deletion for expression of c-fms gene products. Independent of its potential involvement in neoplasia, the localization of c-fms provides a unique molecular marker for chromosome 5 which should be useful for identifying syntenic linkage associations among diverse mammalian species.

ACKNOWLEDGEMENT:

The authors thank G.T. Blennerhassett and P. Hansen for their excellent technical assistance. This work was supported under Contract No. NOI-CO-76380 from the National Cancer Institute, Bethesda, Maryland, and by Public Health Service Grant No. GM-25339 from the National Institute of General Medical Sciences, Bethesda, Maryland.

REFERENCES:

1. Varmus, H.E. (1982) Science 216, 812-820
2. Bishop, J.M. (1982) Retrovirus and Cancer Genes. In Adv. Cancer Res. 37, 1-32
3. Klein, G. (1982) Advances in Viral Oncology. New York, Raven Press.
4. Parada, L.F., Tabin, C.J., Shih, C. and Weinberg, R.A. (1982) Nature 297,474-478
5. Santos, E., Tronick, S.R., Aaronson, S.A., Pulciani, S., and Barbacid, M. (1982) Nature 298, 343-347
6. Der, C.J., Krontiris, T.G., and Cooper, G.M. (1982) Proc. Natl. Acad. Sci. USA 79, 3637-3640
7. Chang, E.H., Furth, M.E., Scolnick, E.M. and Lowy, D.R. (1982) Nature 297, 479-483
8. de Klein, A. Geurts van Kessel, A., Grosveld, G., Bartram, C.R., Hagemeijer, A., Bootsma, D., Spurr, N., Heisterkamp, N., Groffen, J. and Stephenson, J.R. (1982) Nature 300, 765-767
9. Dalla-Favera, R., Bregni, M., Erikson, J., Patterson, D., Gallo, R.C., Croce, C.M. (1982) Proc. Natl. Acad. Sci. U.S.A. 79, 7824-7827
10. Taub, R., Kirsch, I., Morton, C., Lenoir, G., Swan, D., Tronick, S., Aaronson, S., and Leder, P. (1982) Proc. Natl. Acad. Sci. USA 79, 7837-7841
11. Donner, L., Fedele, L.A., Garon, C.F., Anderson, S.J., and Sherr, C.J. (1982) J. Virol. 41, 489-500

12. Heisterkamp, N., Groffen, J., and Stephenson, J.R. (1983) Virology 126, 248-258
13. Van de Ven, W.J.M., Reynolds, F.H., Nalewaik, R., and Stephenson, J.R. (1980) J. Virol. 35, 165-175
14. Barbacid, M., Lauver, A.V., and Devare, S.G. (1980) J. Virol. 33, 196-207
15. Reynolds, F.H. Jr., Van de Ven, W.J.M., Blomberg, J., and Stephenson, J.R. (1981) J. Vriol. 41, 1084-1089
16. Dana, S., and Wasmuth, J.J. (1982) Molec. & Cell Biol. 2, 1220-1228
17. Dana, S., and Wasmuth, J.J. (1982) Somatic Cell Gen. 8, 245-264
18. Southern, E.M. (1975) J. Mol. Biol. 98, 503-517
19. Groffen, J., Heisterkamp, N., Grosveld, F., Van de Ven, W., and Stephenson, J.R. (1982) Science 216, 1136-1138
20. Heisterkamp, N., Groffen, J., Stephenson,J.R., Spurr, N.K., Goodfellow, P.N., Solomon, E., Carritt, B., and Bodmer, W.F. (1982) Nature 299, 747-749
21. Dalla-Favera, R., Franchini, G., Martionotti, S., Wong-Staal, F., Gallo, R.C., and Croce, C.M. (1982) Proc. Natl.Acad. Sci. USA 79, 4714-4717
22. Swan, D.C., McBride, O.W., Robbins, K.C., Keithley, D.A., Reddy, E.P., and Aaronson, S.A. (1982) Proc. Natl.Acad. Sci. USA 79, 4691-4695
23. Dalla-Favera,R., Gallo,R.C., Giallongo,A., and Croce, C.M. (1982) Science 218, 686-688
24. McBride, O.W., Swan, D.C.,Santos, E., Barbacid, M., Tronick, S.R., and Aaronson,S.A. (1982) Nature 300, 773-774
25. Prakash, K., McBride, O.W., Swan, D.C., Devare, S.G., Tronick, S.R., and Aaronson, S.A. (1982) Proc. Natl. Acad.Sci. USA 79, 5210
26. Neel, B.G., Jhanwar, S.C., Chaganti, R.S.K.,and Hayward, W.S. (1982) Proc. Natl. Acad. Sci. USA 79, 7842-7846
27. Groffen, J., Heisterkamp, N., Stephenson, J.R., Geurts van Kessel, A., de Klein, A., Grosveld, G., and Bootsma, D. (1983) J. Expt. Med. 158, 9-15.
28. Sheer, D., Hiorns, L.R., Goodfellow, P.N., Trowsdale, J., Solomon, E., Swallow, D.A., Povey, S., Heisterkamp, N., Groffen, J., and Stephenson, J.R. (1983) Proc. Natl. Acad. Sci. USA (in press)
29. Van den Berghe, H., Cassiman, J.J., David, G., Fryns, J.P., Michaux, J.L., and Sokal, G. (1974) Nature 251, 437-438
30. Sokal, G., Michaux, J.L., Van den Berghe, H., Cordier, A., Rodhain, J., Ferrant, A., Moriau, M., de Bruyere, M., Sonnet, J. (1975) Blood 46, 519-553
31. Van den Berghe, H., David, G., Michaux, J.L., Sokal, G., and Verwilghen, R., (1976) Blood 48, 624-626
32. Whitehead, A.S., Solomon, E., Chambers, S., Bodmer, W.F., Povey, S., and Fey, G. (1982) Proc. Natl. Acad. Sci. USA 79, 5021-5025
33. Solomon, E., Bobrow, M., Goodfellow, P.N., Bodmer, W.F., Swallow, D.M., Povey, S., and Noel, B. (1976) Somat. Cell Genet. 2, 125-140
34. Jones, E.A., Goodfellow, P.N., Kennett, R.H., and Bodmer, W.F. (1976) Somat. Cell Genet. 2, 483-496
35. Solomon, E., Bobrow, M., Goodfellow, P.N., Bodmer, W.F., Swallow, D.M., Povey, S., and Noel, B. (1976) Somat. Cell Genet. 2, 125-140
36. Hobart, M.J., Rabbits, T.H., Goodfellow, P.N., Solomon, E., Chambers, S., Spurr, N., and Povey, S. (1981) Ann. Hum. Genet. 45, 331-335
37. Povey, S., Jeremiah, S.J., Barker, R.F., Hopkinson, D.A., Robson, E., Cook, P..J.L., Solomon, E., Bobrow, M., Carrot, B., and Buckton, K.E., (1980) Ann. Hum. Genet. 43, 241-248
38. Nabholz, M.,Miggiano, V., and Bodmer, W.F. (1969) Nature 223, 358-363
39. Swallow, D.M., Solomon, E., and Pajunen, L. (1977) Cytogenet. Cell Genet. 18, 136-148