Human Molecular Genetics, 1996, Vol. 5, No. 3 339–346

A radiation hybrid map of the human genome

Gabor Gyapay[1,*], Karin Schmitt[2], Cécile Fizames[1], Hywel Jones[2], Nathalie Vega-Czarny[1], Dominique Spillett[2], Delphine Muselet[1], Jean-François Prud'Homme[1], Colette Dib[1], Charles Auffray[3], Jean Morissette[1,4], Jean Weissenbach[1] and Peter N. Goodfellow[2]

[1]CNRS URA 1922, Généthon, 1 rue de l'Internationale, 91000 Evry, France, [2]University of Cambridge, Department of Genetics, Downing Street, Cambridge, UK, [3]Genexpress, Généthon and CNRS UPR 420, 7 rue Guy Mocquet, 94801 Villejuif, France and [4]CHUL, 2705 Boulevard Laurier, Sainte-Foy, Québec, G1V 4G2, Canada

Received December 1, 1995; Revised and Accepted December 6, 1995

We have developed a panel of whole-genome radiation hybrids by fusing irradiated diploid human fibroblasts with recipient hamster cells. This panel of 168 cell lines has been typed with microsatellite markers of known genetic location. Of 711 AFM genetic markers 404 were selected to construct a robust framework map that spans all the autosomes and the X chromosome. To demonstrate the utility of the panel, 374 expressed sequence tags (ESTs) previously assigned to chromosomes 1, 2, 14 and 16 were localized on this map. All of these ESTs could be positioned by pairwise linkage to one of the framework markers with a LOD score of greater than 8. The whole genome radiation hybrid panel described here has been used as the starting material for the Genebridge4 panel that is being made widely available for genome mapping projects.

INTRODUCTION

Radiation hybrids, produced by fusing irradiated donor cells with recipient rodent cells, can be used for constructing genetic maps that are complementary to both recombination maps and physical maps based on contigs. Recombination maps are limited to polymorphic markers and are constrained by the recombination rate; in contrast, radiation hybrid methods exploit differences between species and can be used to map both polymorphic markers and non-polymorphic markers such as sequence tagged sites (STS) and expressed sequence tags (EST). The resolution of radiation hybrid mapping is a function of both fragment size and retention frequencies. The fragment size can be varied by altering the radiation dose and it is possible to construct panels designed either for map continuity with few markers or for high resolution with large numbers of markers. Information on localization using radiation hybrids is obtained by determining linkage to markers of the framework. Such a linkage is expressed by a distance measured in breakage frequency accompanied by a likelihood estimate expressed as a LOD score (1,2). Radiation hybrids allow robust mapping and provide an associated statistical assessment of reliability.

Physical mapping methods based on collections of ordered clones can also be used to integrate maps based on polymorphic and non-polymorphic markers. However, only parts of the genome are covered by YAC contigs that are sufficiently robust to allow high throughput mapping of randomly selected markers such as ESTs (e.g. 3–7). In addition, some regions such as terminal parts of chromosomes and other GC-rich regions are poorly covered by YACs (3) and such regions are likely to be particularly dense in genes. The construction of complete contigs of the genome using only YACs and unordered STS markers is proving difficult. An independent method for ordering STS markers and for integrating them with mapped polymorphic markers would facilitate the completion of a YAC contig of the human genome.

An initial drawback of radiation hybrid mapping was the need to produce chromosome specific hybrid panels which utilize somatic cell hybrids carrying single human chromosomes as donors (1). As it has been estimated that a panel of 100–200 clones is needed to construct a robust map, this would require the producton of over 4000 clones to cover the human genome. In previous work, we re-investigated the original protocols of Goss and Harris (8) and demonstrated that hybrid panels produced by using diploid human fibroblasts as donor cells are just as effective for chromosome mapping (9). A single set of whole genome hybrids produced in this way could be used for mapping the whole human genome.

In this report, we describe the production of a panel of 168 whole-genome radiation hybrids suitable for high throughput mapping of random markers. Donor human fibroblasts were irradiated at 3000 rads, a relatively low dose, to ensure continuity of the map. A framework map of 404 markers was constructed by typing the panel with polymorphic microsatellite markers and ordering them using standard methods (1,2). The utility of the panel has been demonstrated by mapping 374 ESTs.

Markers with identical retention patterns were not included in the framework maps. Other markers that were rejected included those that showed either unusually low or unusually high retention frequencies compared with flanking markers. From a total of 711 microsatellite markers tested, 404 were included in the framework map.

*To whom correspondence should be addressed

Table 1. Quantitative characteristics of radiation hybrid maps

Chromosome	Number of markers	Maximum interval cR	Minimum interval cR	Mean distance cR	Total distance cR	Genetic length cM	cM/cR	Physical length kb	kb/cR	Mean retention frequency (range)
1	40	ND	10	33	1333	305	0.20	263	197	21.1 (14.3–50.6)
2	32	85	11	35	1133	271	0.21	255	225	23.8 (14.3–37.5)
3	25	ND	18	37	918	237	0.27	214	233	26.9 (14.3–41.1)
4	20	ND	17	40	794	244	0.28	203	256	24.3 (11.3–44.0)
5	16	ND	28	45	713	224	0.29	194	272	26.9 (17.3–49.4)
6	21	57	24	36	753	207	0.24	183	243	30.6 (21.4–49.4)
7	19	85	28	39	746	178	0.23	171	229	27.7 (19.0–44.6)
8	19	ND	17	30	572	172	0.26	155	271	31.0 (24.4–45.8)
9	14	ND	24	34	475	146	0.38	145	305	22.1 (14.3–27.4)
10	20	ND	17	28	569	181	0.26	144	253	28.5 (16.2–56.3)
11	15	96	21	36	533	150	0.30	144	270	25.7 (20.2–38.7)
12	15	101	21	41	610	160	0.21	143	234	32.1 (23.2–50.6)
13q	15	60	14	37	548	130	0.22	98	179	22.9 (16.7–36.3)
14q	13	56	9	34	447	122	0.34	93	208	32.0 (21.4–57.1)
15q	13	53	22	34	438	154	0.36	89	203	31.7 (24.4–45.2)
16	14	ND	14	35	488	157	0.43	98	201	30.5 (21.6–39.9)
17	17	99	27	37	626	208	0.23	92	147	61.6 (33.9–93.5)
18	14	90	21	35	494	143	0.30	85	172	34.3 (20.7–43.5)
19	16	86	13	38	611	148	0.20	67	110	32.2 (21.4–45.8)
20	13	76	16	29	377	122	0.30	72	191	31.0 (16.1–41.1)
21q	10	43	11	26	258	114	0.31	39	151	50.1 (39.3–71.3)
22q	7	56	11	33	232	81	0.95	43	185	36.6 (30.4–50.6)
X	16	102	30	44	709	220	0.31	164	231	18.4 (11.9–30.4)
Genome	404			37	14377	4074	0.28	3154	208	29.2 (11.3–93.5)

The chosen set of markers has a mean overall retention frequency of 0.29. The highest values are found with microsatellite markers which map in the pericentromeric region, as has been noted previously (10), and with markers on chromosome 17 (Table 1). Chromosome 17 carries the selected marker TK which is, by definition, retained at 100%; markers flanking TK show elevated retention frequencies as do markers from elsewhere on chromosome 17. The biological explanation of inter-chromosomal differences in mean retention frequencies remains unclear; however, there is a global trend towards increased retention for markers on smaller chromosomes versus larger ones (Table 1). This might imply that the centromere is more frequently utilized by fragments from smaller chromosomes. Retention frequencies for X-linked markers are as expected, about half of autosomal values, as the parental human cell line has a single X chromosome (46,XY).

RESULTS

The radiation hybrid panel

A total of 220 whole genome radiation hybrids were produced by fusing the human diploid fibroblast cell line HFL121 (karyotype, 46,XY) to hamster A23tk- cells (see Materials and Methods). The fibroblasts were irradiated with 3000 rads and the hybrids were selected in HAT media.

The hybrids were tested for human DNA content using an inter-Alu PCR assay. Most hybrids displayed a complex pattern of amplification consistent with a large number of human fragments suitable for radiation hybrid mapping; however, a small number of hybrids produced a limited number of inter-Alu PCR products and were discarded. Hybrids were also discarded if they lacked sequences corresponding to human TK, the human locus used for selecting the hybrids. A total of 199 hybrids survived these selection procedures; a subset of 168 hybrids were chosen at random for further experiments.

Construction of a framework map

The panel was intended primarily as a tool for mapping non-polymorphic markers including ESTs. In order to construct a framework map suitable for this purpose, the 168 hybrids were first tested by PCR for the presence or absence of microsatellite markers of known map position (see Materials and Methods). These markers were chosen to cover the genome at approximately 10 cM intervals. When adjacent markers showed pairwise linkage LOD scores of less than 9, the map was completed by adding additional markers until all chromosomal arms were covered by a connected set of markers in which adjacent markers were linked at LOD scores of greater than 9. This corresponds to maximal pairwise breakage distances of approximately 60 cRays between adjacent markers. Several intervals around the centromeres and four other intervals could not be covered using these conditions despite the inclusion of additional markers.

Table 2. Comparison of chromosome assignments using somatic and radiation hybrids

Chromosome	ESTs assigned (SH)	ESTs assigned (RH)	% confirmed	Discrepant assignments	% discrepant	Multiple assignments
1	104	92	88.5%	4	3.8%	8
2	167	157	94.0%	6	3.6%	4
14	36	33	91.7%	2	5.6%	1
16	67	61	91.0%	3	4.5%	3
Total	374	343	91.7%	15	4.0%	16

SH: somatic hybrids; RH: radiation hybrids.

Parameters of the human genome were taken from ref. (15). The radiation hybrid maps with distances between framework markers based on multipoint analysis (see Materials and Methods) are presented in Figure 1. The genetic linkage map of each chromosome is also presented; it can be be seen that although the order of the markers is the same, there are discrepancies in the distances. In nearly all cases (>98%), the orders obtained were determined with odds ratios greater than 1000:1. On some chromosomes, linkage gaps remained in the centromeric regions despite efforts to add additional markers from the centromeric regions (Table 1).

Apart from the centromeric regions, no linkage gaps were detected. However, the pseudoautosomal marker DYS402 showed only very loose linkage to the rest of the X chromosome. This probably results from the presence of the Y-linked copy of this marker which cannot be distinguished from its X-linked counterpart. The double presence of this locus causes a doubling of the retention frequency, thereby inflating linkage distances.

Comparisons between distances on the radiation hybrid map and genetic distances revealed some apparent differences in map distances: some gaps (7–10 cM) on the linkage map corresponded to very short distances on the radiation map and conversely some clusters on the genetic linkage map are split on the radiation map.

Global ratios between physical and breakage distances also vary between chromosomes having a minimal value 110 kb/cR on chromosome 11 and a maximal value 305 kb/cR on chromosome 9 (Table 1).

EST mapping

A total of 374 ESTs developed as PCR markers by the Genexpress program were typed on the radiation hybrid panel. These ESTs had been previously assigned to specific chromosomes using somatic hybrid cell lines (11,12). We selected EST markers from chromosomes 1, 2, 14 and 16 to test the efficacy of our radiation hybrid panel. Pairwise linkage between each EST and the entire set of 404 framework markers was calculated. A pairwise linkage with a maximal LOD score >10 was found for each EST and a framework marker except for two ESTs exhibiting very high retention frequencies (>70%). One of these was located on chromosome 17 and the other one mapped in the centromeric region of chromosome 21. Data relative to these ESTs can be obtained at the following address: http://www.ebi.ac.uk.RHdb/index.html.

Their localization relative to the 404 framework markers is available at the web site: http://www.genethon.fr/RHmap.

Concordance with previous chromosomal assignments was >96% for the 374 markers tested (Table 2). Sixteen ESTs showed linkage to markers from more than one chromosome with a pairwise linkage LOD score above 6. These cases were considered as multiple localizations. In 10 cases the EST had also been assigned to more than one chromosome using the somatic hybrids. LOD scores of pairwise linkage for ESTs with double or multiple assignments were always lower than for ESTs linked to a single locus. As expected these ESTs also showed an increased retention frequency. These multiple localizations are presumably due to pseudogenes or gene families. Such situations also create problems with other mapping methods. Discrepancies between chromosome assignment using somatic hybrids and radiation hybrid mapping probably reflect a higher sensitivity and robustness of this latter method, which can be evaluated statistically.

Multipoint analyses were performed to determine the most likely position of the ESTs with respect to the framework map (Materials and Methods). As an example, the positions of ESTs from chromosome 2 are shown along the framework map in Figure 2. Some ESTs map outside the framework but are linked to the most distal markers. Similarly, markers mapping to the centromeric intervals are usually located close to a framework marker.

DISCUSSION

We have produced a panel of radiation hybrids and an associated framework map of the whole human genome constructed with markers from the genetic linkage map. This panel can be used for mapping nonpolymorphic markers such as ESTs and for integrating existing genetic and physical maps.

The panel has been tested with more than 711 microsatellite markers which showed retention frequencies on different chromosomes varying from 0.12 minimum on the haploid X chromosome to 0.94 in the selected TK region of chromosome 17 (Table 1). Significant variation in retention frequencies has also been detected for markers on the same chromosome. Abnormally elevated or reduced retention frequencies can result from differences in efficiencies of PCR for the different markers; this can result in overestimation of the number of breaks between markers and difficulties in constructing framework maps. To avoid this problem, it was necessary to remove markers which showed large differences in retention frequencies compared with the flanking markers from the framework map. Some form of normalization of PCR yields might help to obviate this problem but would be time consuming. In practical terms, unusual

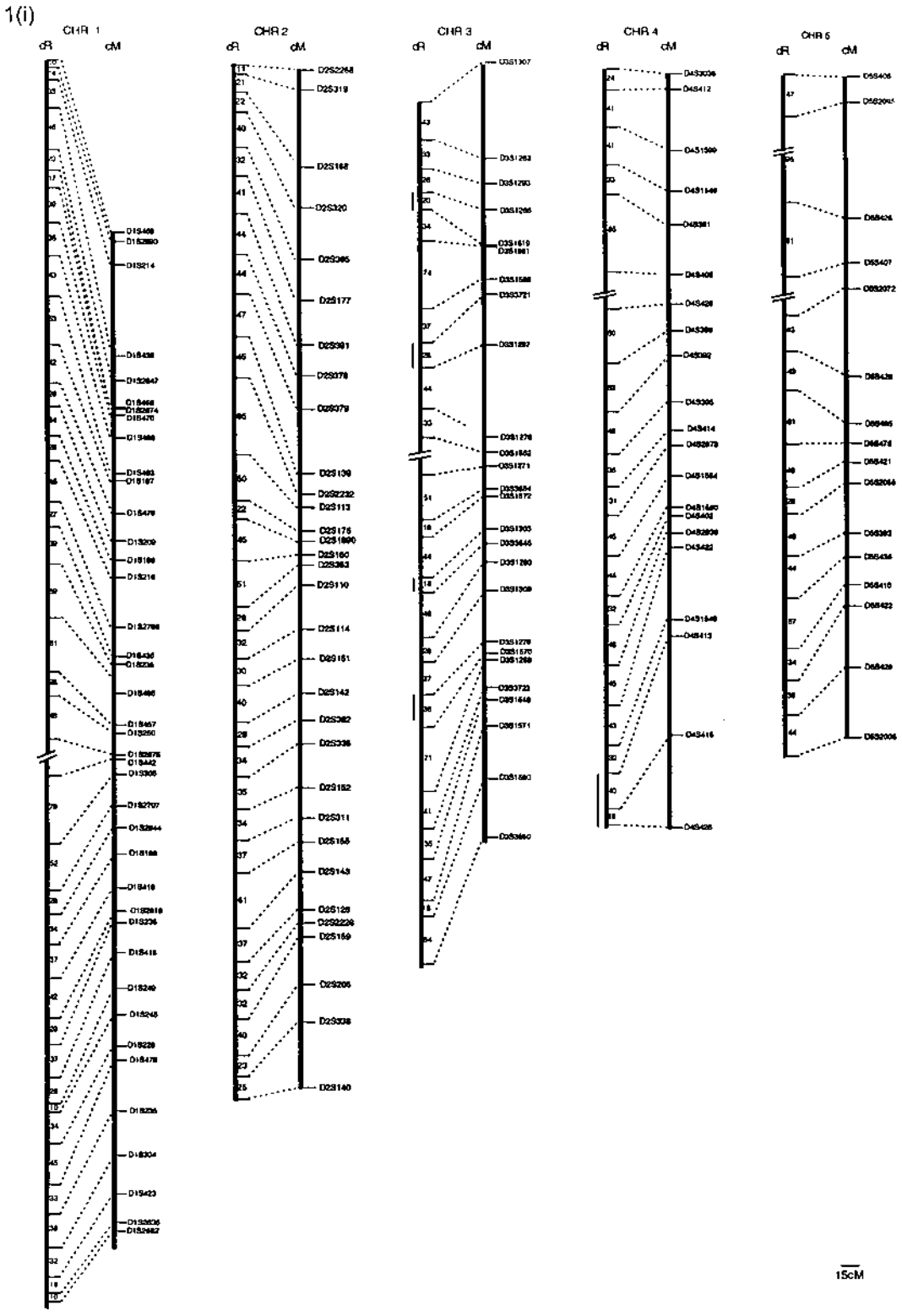
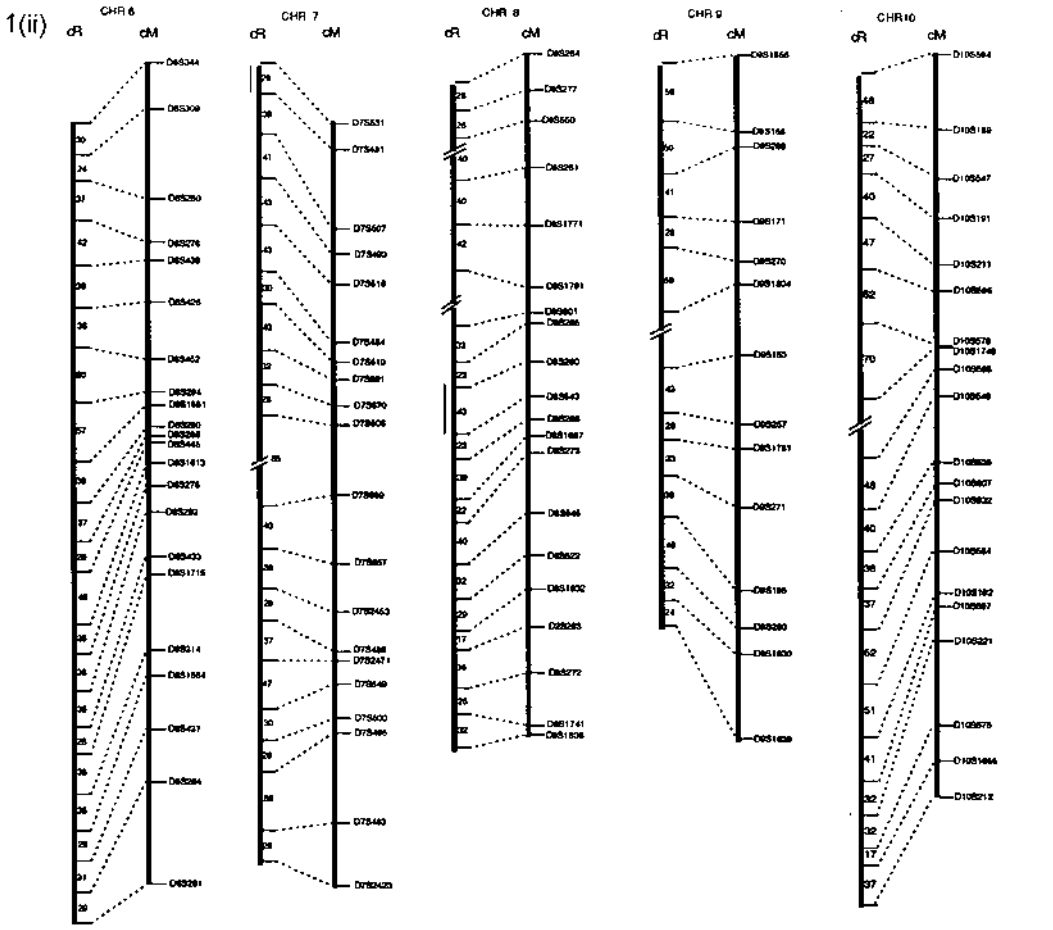
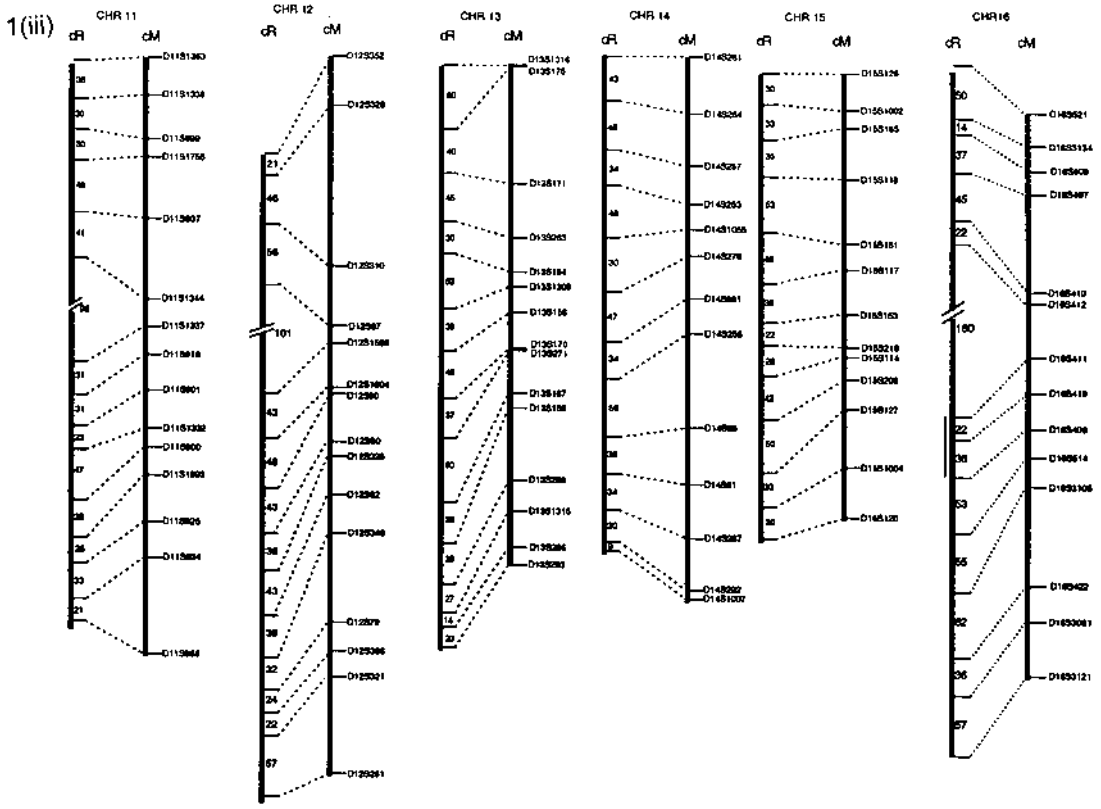
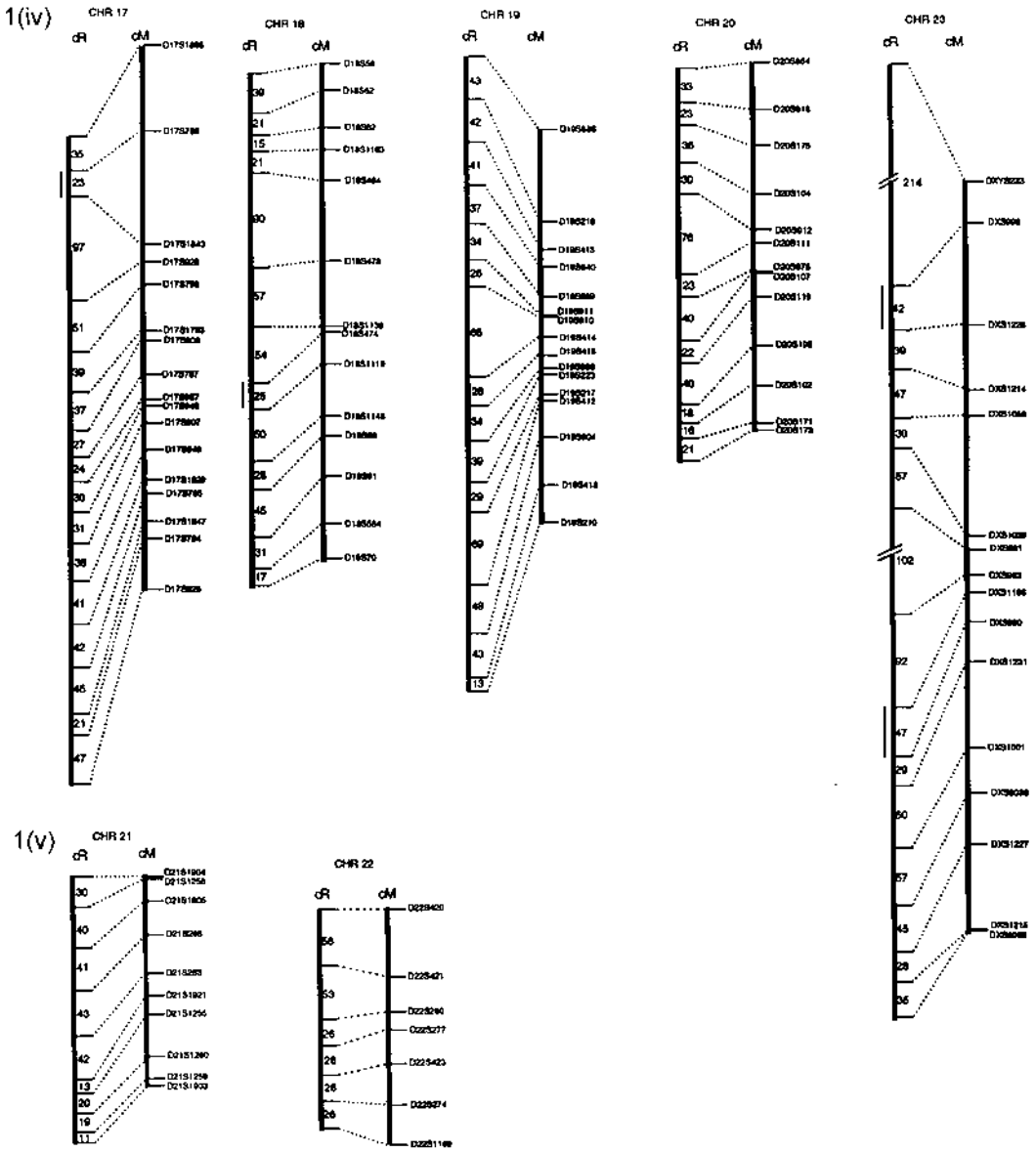


Figure 1. Radiation hybrid framework map of the human genome. The radiation hybrid map is presented as a vertical bar on the left, and the corresponding genetic linkage map is shown on the right. This map contains data for 404 microsatellite markers spanning the human genome. Chromosome maps show the best supported order of markers. Gaps in the left-hand vertical bars indicate intervals with very loose linkage which did not allow unique ordering with support of 1000:1 odds. When pairwise linkage between markers framing these gaps was at LOD score >6, linkage distances are indicated. A vertical line to the left of the vertical bar indicates that the order is only supported at 100:1 odds. Numbers on the radiation hybrid map correspond to distances in cR proposed by multipoint calculations using RHMAXLIK (see Materials and Methods). In most cases (98%) the most likely order was supported at 1000:1 odds and identical to the genetic linkage map order.







retention frequencies for new markers will broaden the localization possible but will not lead to false localizations.

Microsatellite markers in the centromeric regions of chromosomes often showed the highest retention frequencies, varying from 0.27 (chromosome 9) to 0.71 (chromosome 21). This variation might indicate that human centromeres from different chromosomes have different probabilities of being retained on a hamster background or it may be a reflection of the physical distances separating the flanking markers from the functional part of the centromere on the different chromosomes.

The EST mapping results show that all the ESTs tested could be unambiguously linked to markers in the framework map with high statistical support. Although, the statistical support for localization decreases in cases of double or multiple localizations, the most likely localization still remains significant with LOD scores above 8 in the examples we have studied. Localization of some ESTs outside the most distal framework markers confirms that the coverage is extensive but that the framework needs to be further improved with additional telomeric markers as these become available.

The accuracy of the localizations has been verified by assigning ESTs to YACs which map to the same interval (unpublished results). In addition we have independently mapped all nine ESTs from the titin gene to the same position on chromosome 2 (between D2S382 and D2S335).

We conclude that this panel constitutes a robust mapping tool which can be used to reliably localize any STS or EST; it should prove a useful resource for positional cloning experiments and for improving framework maps prior to making a complete YAC contig of the genome. As a courtesy to the community, a subset of 93 hybrids from this panel showing the highest retention of human fragments has been regrown to obtain large quantities of DNA and is being made widely available for genome mapping projects under the panel name Genebridge4.

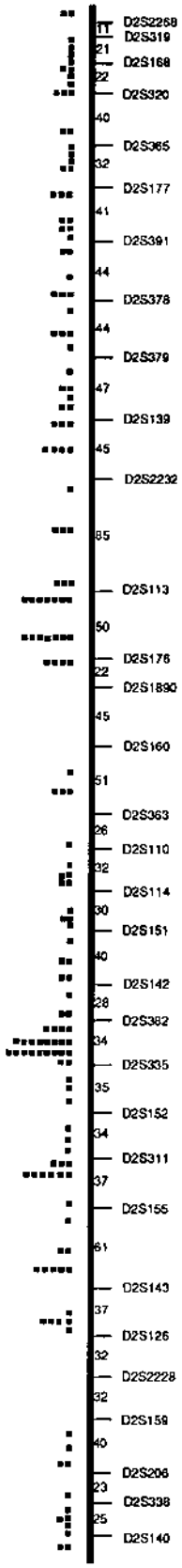


Figure 2. Positioning of ESTs on the RH map of chromosome 2. Each square on the left represents one EST. Three ESTs from the titin gene were mapped (between D2S382 and D2S335) in the same interval. Horizontal lines represent polymorphic AFM microsatellite markers. The genetic map of the chromosome 2 is shown on the right. The markers are in the same order as on Figure 1.

MATERIALS AND METHODS

Generation of whole genome radiation hybrids (WG-RH)

Five fusion experiments were carried out using HFL121 human fibroblasts (46,XY) which were irradiated with either 6000 rads (three experiments) or 3000 rads (two experiments) and fused with thymidine kinase-deficient A23 hamster cells as described (9).

PCR analysis

Alu-PCR was carried out using the TC-65 primer (5′-AAG TCG CGG CCG CTT GCA GTG AGC CGA GAT-3′) (13) and human thymidine kinase was detected using the TK1 primer couple (5′-ATC TGG CAC CCC TCT CCT TGACT-3′and 5′-TGA AAG ATG CTG TTG TTC CTG TGG-3′).

ESTs and AFM markers were analyzed by PCR using the hot start and touch down procedures as follows. Samples of 30 ng of DNA in 5 µl were distributed either with multichannel electronic pipettes or with a 96 needle Robbin's robot followed by addition of 4 µl of primers. The samples were then overlaid with heavy mineral oil.

After an initial denaturing step of 5 min at 96°C, the temperature was set at 94°C and 6 µl of the mixture containing dNTPs, amplification buffer and 0.25 units *Taq* poymerase was added manually on the thermal cycler, through the oil layer. The final concentrations in the PCR are: DNA 2 ng/µl, dNTP125 µM, primers (of each) 1.33 µM, KCl 50 mM, $MgCl_2$, 2 mM, Triton X-100 0.1%, Tris–HCl pH 9.0 (25°C), 10 mM, *Taq* polymerase 0.25 units/15 µl.

The first three cycles consisted of 30 s of annealing at 61°C and 40 s of denaturing at 94°C. The annealing temperature was then successively lowered by 2°C for each consecutive three cycles until 55°C, followed by 25 further cycles at an annealing temperature of 55°C. After completion of the PCR reaction 4 µl of loading mixture containing 0.1% (w/v) bromphenol blue and 50% (v/v) glycerol were added to each well. The PCR products were allowed to migrate on an agarose gel containing 1% SeaKem and 3% NuSieve agarose (FMC) in TBE buffer with 0.25 µg/ml ethidium bromide. Images of the gels were recorded with a high resolution CCD camera and scoring of the results was carried out semi-automatically. In case of discordance a third PCR was carried out.

Map construction

Maps were constructed using the RHMAP package (version 2.01) (14). Multipoint analysis was carried out in a stepwise manner to reduce the computing time.

Positioning of ESTs on framework maps

Validation of chromosome assignment was carried out by calculating pairwise linkage versus the entire set of framework markers. Positioning of ESTs on the relevant chromosome map was then first estimated using the RHMINBRK program. The first 20 best orders proposed by RHMINBRK were submitted to RHMAXLIK to estimate the most likely one.

ACKNOWLEDGMENTS

We thank Dr M. Walter for help at early stages of this work. PNG, HBJ and DS were supported by the MRC, KS was supported by an EMBO Fellowship. Collaborative work between PNG and JW was supported by European Union (Biomed1). Work at Généthon was supported by the Association Française contre les Myopathies and the Groupement de Recherches et d'Etudes sur les Génomes.

REFERENCES

1. Cox, D.R., Burmeister, M., Price, E.R., Kim, S. and Myers, R.M. (1990) Radiation hybrid mapping: a somatic cell genetics method for constructing high resolution maps of mammalian chromosomes. *Science* **250,** 245–250.
2. Boehnke, M., Lange, K. and Cox, D.R. (1991) Statistical methods for multipoint radiation hybrid mapping. *Am. J. Hum. Genet.* **49,** 1174–1188.
3. Chumakov, I., Rigault, P., Le Gall, I., Bellané-Chantelot, C., Billault, A., Guillou, S., Soularue, P., Guasconi, G., Poullier, E., Gros, I., Belova, M., Sambucy, J.L., Suzini, L., Gervy, P., Glibert, F., Beaufils, S., Bui, H., Massart, C., De Tand, M.F., F., D., Lecoulant, S., Ougen, P., Perrot, V., Saumier, M., Soravito, C., Bahouaylla, R., Cohen-Akenine, A., Barillot, E., Bertrand, S., Codani, J.J., Caterina, D., Georges, I., Lacroix, B., Lucotte, G., Sahbatou, M., Schmit, C., Sangouard, M., Tubacher, E., Dib, C., Fauré, S., Fizames, C., Gyapay, G., Millasseau, P., Nguyen, S., Muselet, D., Vignal, A., Morissette, J., Menninger, J., Jonathan, L., Desai, T., Banks, A., Bray-Ward, P., Ward, D., Hudson, T., Gerety, S., Foote, S., Stein, L., Page, D.C., Lander, E.S., Weissenbach, J., Le Paslier, D. and Cohen, D. (1995) A YAC contig map of the human genome. *Nature* **377,** 175–297S.
4. Gemmill, R.M., Chumakov, I., Scott, P., Waggoner, B., Rigault, P., Cypser, J., Chen, Q., Weissenbach, J., Gardiner, K., Wang, H., Pekarsky, Y., Le Gall, I., Le Paslier, D., Guillou, S., Li, E., Robinson, L., Hahner, L., Todd, S., Cohen, D. and Drabkin, H.A. (1995) A second-generation YAC contig map of human chromosome 3. *Nature* **377,** 299–319S.
5. Krauter, K., Montgomery, K., Yoon, S.J., LeBlanc-Straceski, J., Renault, B., Marondel, I., Herdman, V., Cupelli, L., Banks, A., Lieman, J., Menninger, J., Bray-Ward, P., Nadkarni, P., Weissenbach, J., Le Paslier, D., Rigault, P., Chumakov, I., Cohen, D., Miller, P., Ward, D. and Kucherlapati, R. (1995) A second-generation YAC contig map of human chromosome 12. *Nature* **377,** 321–334S.
6. Doggett, N.A., Goodwin, L.A., Tesmer, J.G., Meincke, L.J., Bruce, D.C., Clark, L.M., Altherr, M.R., Ford, A.A., Chi, H.C., Marrone, B.L., Longmire, J.L., Lane, S.A., Whitmore, S.A., Lowenstein, M.G., Sutherland, R.D., Mundt, M.O., Knill, E.H., Bruno, W.J., Madken, C.A., Torney, D.C., Wu, J.R., Griffith, J., Sutherland, G.R., Deaven, L.L., Callen, D.F. and Moyzis, R.K. (1995) An integrated physical map of human chromosome 16. *Nature* **377,** 335–365S.
7. Collins, J.E., Cole, C.G., Smink, L.J., Garrett, C.L., Leversha, M.A., Soderlund, C.A., Maslen, G.L., Everett, L.A., Rice, K.M., Coffey, A.J., Gregory, S.G., Gwilliam, R., Dunham, A., Davies, A.F., Hassock, S., Todd, C.M., Lehrach, H., Hulsebos, T.J.M., Weissenbach, J., Morrow, B., Kucherlapati, R.S., Wadey, R., Scambler, P.J., Kim, U.J., Simon, M.I., Peyrard, M., Xie, Y.G., Carter, N.P., Burbin, R., Dumanski, J.P., Bentley, D.R. and Dunham, I. (1995) A high-density YAC contig map of human chromosome 22. *Nature* **377,** 367–379S.
8. Goss, S.J. and Harris, H. (1975) New method for mapping genes in human chromosomes. *Nature* **255,** 680–684.
9. Walter, M.A., Spillett, D.J., Thomas, P., Weissenbach, J. and Goodfellow, P.N. (1994) A method for constructing radiation hybrid maps of whole genomes. *Nature Genet.* **7,** 22–28.
10. Benham, F., Hart, K., Crolla, J., Bobrow, M., Francavilla, M. and Goodfellow, P.N. (1989) A method for generating hybrids containing nonselected fragments of human chromosomes. *Genomics* **4,** 509–517.
11. Auffray, C., Béhar, G., Bois, F., Bouchier, C., Da Silva, C., Devignes, M.D., Duprat, S., Houlgatte, R., Jumeau, M.N., Lamy, B., Lorenzo, F., Mitchell, H., Mariage-Samson, R., Piétu, G., Pouliot, Y., Sébastiani-Kabaktchis, C. and Tessier, A. (1995) IMAGE: Integrated molecular analysis of the human genome and its expression. *C. R. Acad. Sci. Paris* **318,** 263–272.
12. Houlgatte, R., Mariage-Samson, R., Duprat, S., Tessier, A., Bentolila, S., Lamy, B. and Auffray, C. (1995) The genexpress index: a resource for gene discovery and the genic map of the human genome. *Genome Res.* **5**, 272–304.
13. Nelson, D.L., Ledbetter, S.A., Corbo, L., Victoria, M.F., Ramirez-Solis, R., Webster, T.D., Ledbetter, D.H. and Caskey, C.T. (1989) Alu polymerase chain reaction: a method for rapid isolation of human-specific sequences from complex DNA sources. *Proc. Natl Acad. Sci. USA* **86,** 6686–6690.
14. Lange, K., Boehnke, M., Cox, D.R. and Lunetta, K.L. (1995) Statistical methods for polyploid radiation hybrid mapping. *Genome Res.* **5,** 136–150.
15. Morton, N.E. (1991) Parameters of the human genome. *Proc. Natl Acad. Sci. USA* **88,** 7474–7476.