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Quantitative Analysis of High-Resolution Trypsin-Giemsa Bands on Human Prometaphase Chromosomes

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Summary. We have constructed ideograms of human prometaphase chromosomes from synchronized and from standard 72-h lymphocyte cultures. G banding was achieved by a trypsin-Giemsa (or Wright's stain) method.

In addition to light (white) and dark (black) bands, we have distinguished three different shades of grey. This distinction is essential for proper identification of the increasing number of bands displayed by high-resolution chromosomes. The relative amount of chromatin in each category of staining intensity has been calculated and expressed as 'light value.'

The ideograms represent the maximal number of bands discernible with some consistency on prometaphase chromosomes, i.e., 721 euchromatic and 62 'variable' heterochromatic or heteromorphic bands.

The ideograms are based on measurements. On selected printed copies of each chromosome derived from different cells and different individuals, the relative width of each band was measured in relation to the length of the respective chromosome arm. The measurements per chromosome were averaged and used for construction of the ideograms. The distance of each border between bands or sub-bands from the centromere has been calculated on a relative scale, with positions 0 at the centromere and 1.0 at the p terminus or q terminus.

The numbering system for bands and sub-bands follows the Paris Conference (1971) recommendations.

Introduction

The development of differential staining methods for mammalian chromosomes has allowed intrachromosomal differentiation. Standardization of the major bands produced on human chromosomes by various banding methods as

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proposed by the Paris Conference 1971 nomenclature and ideogram (1972) has become internationally accepted. The initial ideograms were based on visual impressions of the banding patterns displayed by low-resolution metaphase chromosomes. Little or no attention was paid to the accurate number, relative width, or relative staining intensity of bands. Inadequacies of the initial ideograms became apparent when human chromosome banding patterns were compared with those displayed by primate chromosomes. For the purpose of comparative cytogenetics, minor modifications were then made in a subsequent publication (Paris Conference 1971, Supplement 1975).

On extended chromosomes from earlier stages of mitosis, several investigators have obtained significantly more than the 322 bands included in the Paris Conference ideogram, using G-banding as well as R-banding techniques. Schematic representations of these large numbers of bands have been published (Prieur et al., 1973; Bigger and Savage, 1975; Skovby, 1975; Yunis and Sanchez, 1975; Yunis, 1976). When high-resolution banding patterns produced by the R-banding and by the G-banding techniques are compared, it is apparent that they are not simply negative images of each other. The present study deals exclusively with trypsin-Giemsa(GTG)-banded chromosomes, while high-resolution R-banding patterns and ideograms derived from them will have to be considered separately.

We have found the previously published schematic representations of bands for high-resolution GTG-banded chromosomes of limited use, because they consist of a confusingly large number of equal-intensity bands, which are not based on measurements. Correlation of these ideograms with the actual banded chromosomes shown is often not convincing. It was the goal of this study to develop a more accurate representation of features displayed by high-resolution G-banded human chromosomes, using band measurements and shading to reflect relative staining intensity. An analogous approach has been used previously for the construction of ideograms for mouse chromosomes (Nesbitt and Francke, 1973).

The need for such accurate high-resolution ideograms of human chromosome bands has become apparent in different areas. The availability of simple synchronization methods for short-term human lymphocyte cultures (Yunis, 1976) enables clinical cytogenetic laboratories to use high-resolution chromosomes for routine cytogenetic analysis. Chromosomal breakpoints can be localized and rearrangements characterized with great precision when these high-resolution chromosomes are used, while the Paris Conference ideograms are not sufficient for the description of these findings. Furthermore, human chromosome mapping has proceeded from gene/chromosome assignments to regional, subregional, and even intraband mapping. Precise gene localization requires an accurate map of cytologically recognizable regions on human chromosomes. Such a cytological map could be related to linkage and chiasma maps (Morton et al., 1977).

Materials and Methods

Chromosome Preparations. Mitotic cells in prometaphase and late prophase were derived from phytohemagglutinin-stimulated lymphocytes by using either standard 72-h blood cultures or amethopterin synchronization (Yunis, 1976). Cells were exposed to colchicine (GIBCO) (0.2 μg/

ml final conc.) for 15 min before harvest. The synchronization method produces a lower mitotic index, but a higher percentage of cells in early stages of mitotis. Hypotonic treatment (0.075 M KCl) and fixation (3:1 methanol-glacial acetic acid) followed standard procedures.

The application of fixed mitotic cells to microscope slides is the most critical step for successful chromosome banding. Our current procedure uses glass slides cleaned with alcohol and covered with a thin even film of cold deionized water, obtained by dipping the clean slide into 95% ethanol and then swirling it in a beaker containing cold water. When pulled out of the water, the slide is immediately placed at a 45° angle and fixed cell suspension is dropped onto it. The slide is subsequently flooded with fresh fixative and allowed to air-dry. Depending on room temperature and relative humidity, a warming plate may be used to accelerate drying. Optimal spreading is indicated by the appearance of sharp chromosome images under 16× and 40× phase-contrast objectives. A light-refracting halo around the chromosomes indicates that the chromosomes will be difficult to band with a trypsin-Giemsa method (GTG), and that the conditions for drying will have to be modified.

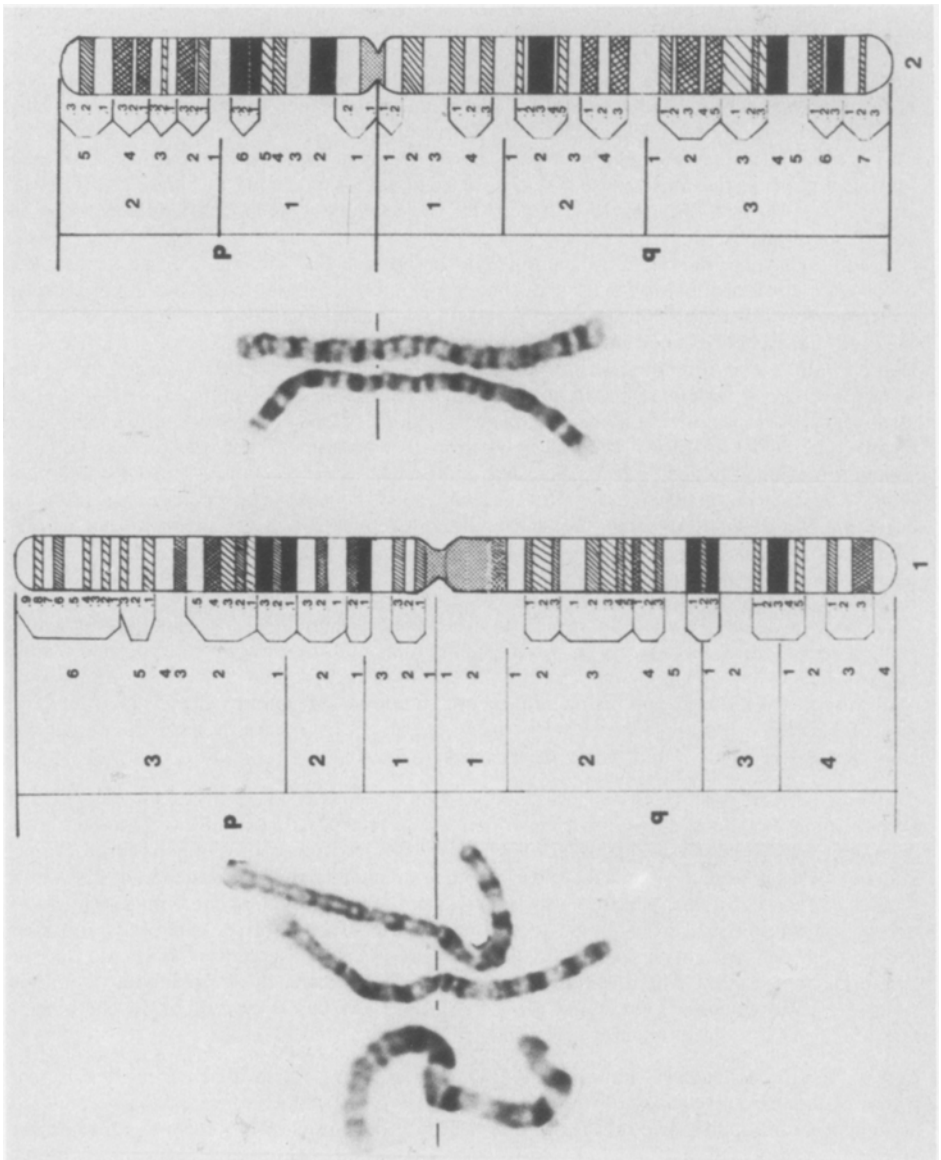
Chromosome Banding. Before chromosome preparations are subjected to the GTG-banding procedure, 'aging' of the slides is required. This temperature-dependent process is accomplished at room temperature for one week, at 55° C for three days, at 65° C overnight, or at 95° C for 10—20 min. The latter high-heat treatment allows slides to be banded soon after their preparation and gives the most consistent results with respect to quality of banding, while with storage at lower temperatures for a longer time the outcome is significantly influenced by the relative humidity of the environment.

Although some degree of G-banding can be obtained by directly staining heat-treated chromosome preparations, we get sharper bands and more consistent results by adding a trypsin pretreatment (Seabright, 1971). Heated slides are allowed to cool to room temperature before they are dipped for 15—60 s into a Coplin jar containing 0.05% trypsin solution (DIFCO) in isotonic saline. The optimal time in trypsin has to be determined for each batch of slides. The trypsin solution is rinsed off by two quick changes in isotonic saline or alcohol. Alcohol (95% ethanol) stops trypsin action more effectively than saline, allowing more accurate control over trypsin time. Slides are blown dry, placed horizontally on a staining rack, and covered with 5 ml staining solution (1 ml Wright's or Giemsa stain made up from powder and 4 ml 0.004 M Sörensen's phosphate buffer pH 7.0) for 90 s to 2 min. Slides covered with stain are then lifted off horizontally and rinsed in a large beaker containing deionized water. This minimizes the precipitation of stain components on the slide. The stained slides are air-dried and examined microscopically without coverslips.

Photomicroscopy and Printing. Suitable metaphases are photographed in a Zeiss Photoscope III using a green interference filter, a 63× planapochromat oil immersion objective, and Kodak High-Contrast Copy 35 mm film. The film is developed in D76 Kodak developer diluted 1:1 with water at 20° C for 11 min, with agitation once a minute. Metaphase spreads are printed on Kodak Ektamatic SC polycontrast paper using contrast 2 or 3 filters and are developed and stabilized in the Agfa Rapidoprint processing system.

Chromosome and Band Measurements. For band measurements 5—15 printed copies of each chromosome were selected from prometaphase and late prophase cells. Criteria for selection were high resolution and quality of banding, consistency in contraction along the chromosome, and lack of distortion by technical factors. We then determined the maximum number of bands that we could identify with certainty on several chromosomes, excluding bands only seen on one or two chromosomes. The bands to be measured were designated by a letter code and their widths were measured with calipers to the nearest 0.01 cm and expressed as fraction of the length of the respective chromosome arm. The relative distance of each band from the centromere was calculated, including the C-band heterochromatin region but not the heteromorphic 1qh, 9qh, and 16qh regions.

Construction of Ideograms. Band width/chromosome arm ratios derived from different copies of the respective chromosome were averaged for each band and these values were used for construction of the ideogram. The relative length of each chromosome and chromosome arm in relation to the total haploid set was based on measurement of all chromosomes in pro-


2
p
q
1
p
q

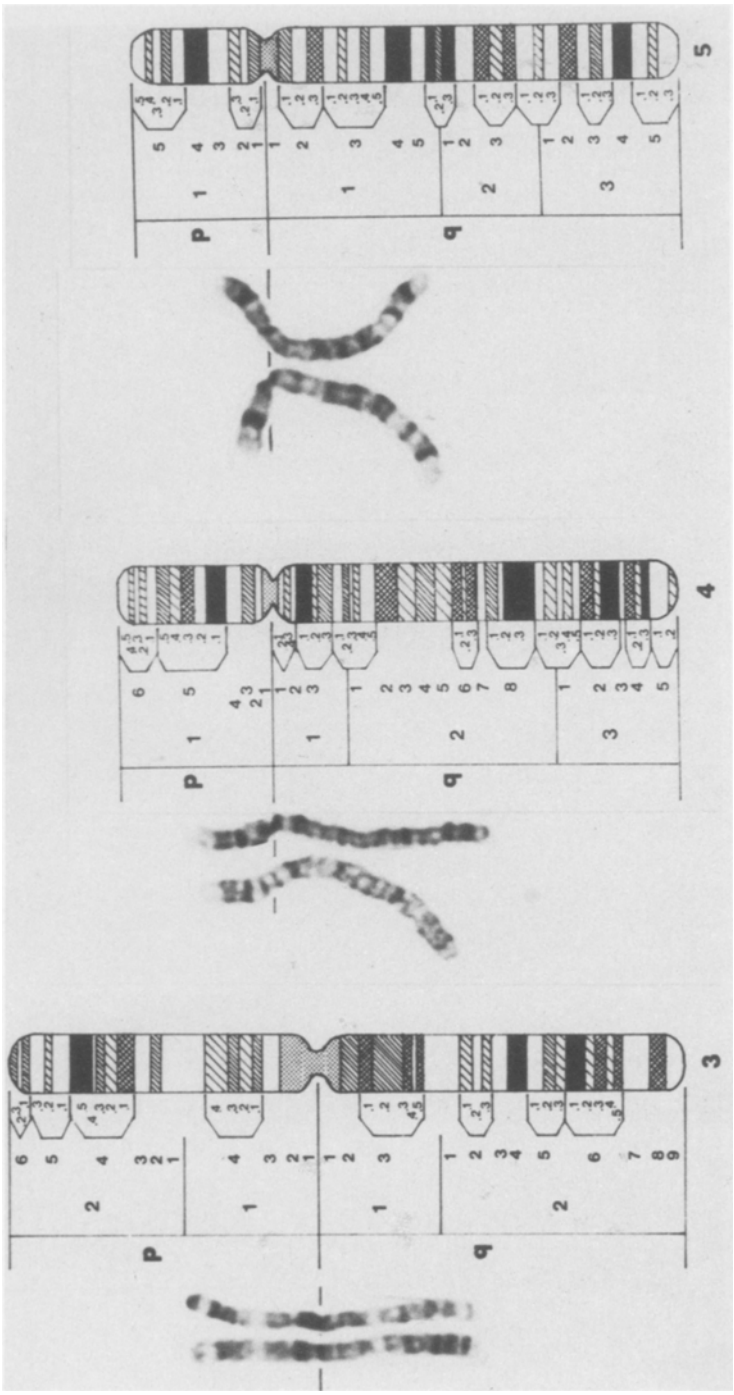
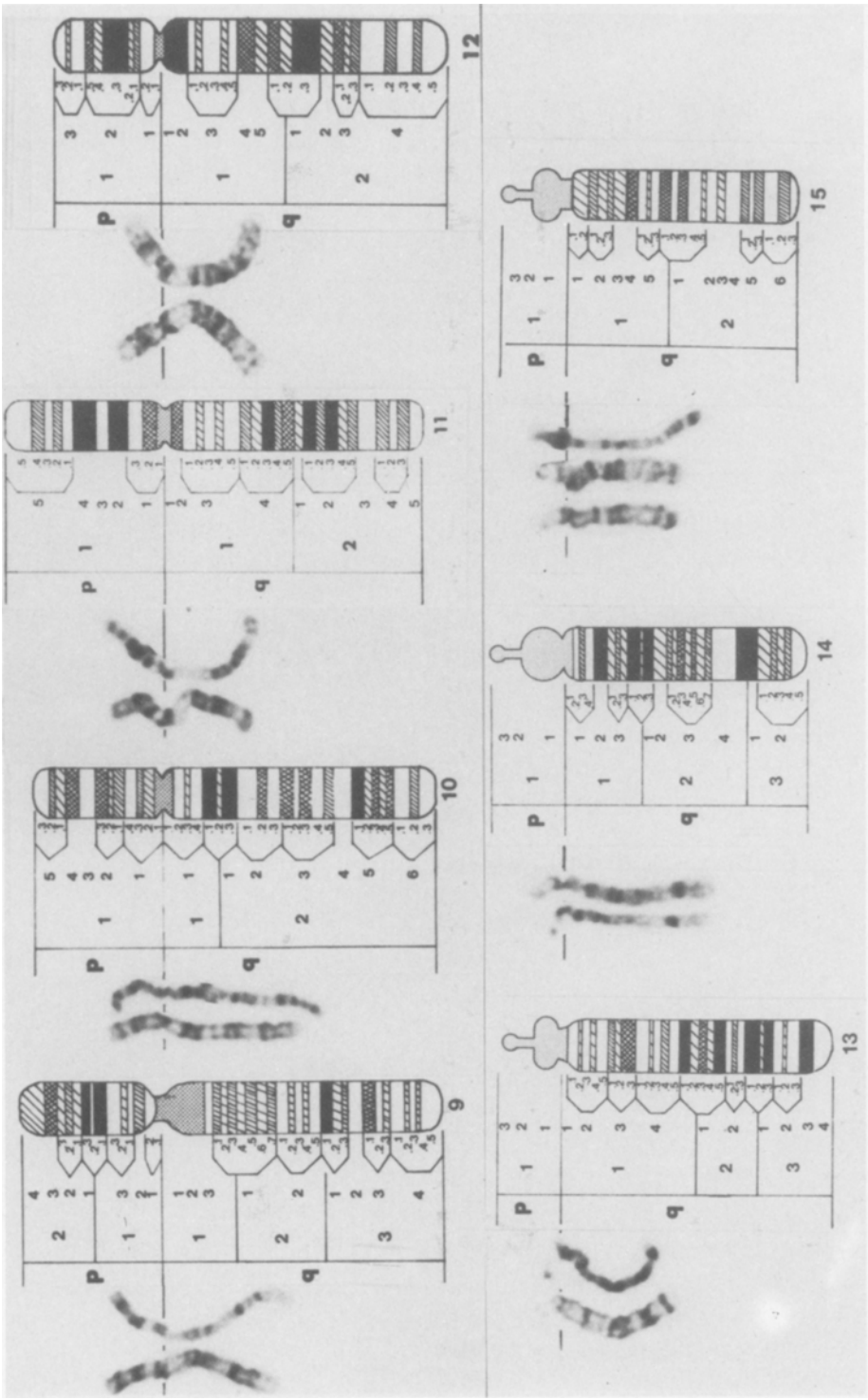


Fig. 1. Ideograms of GTG-banded human prometaphase chromosomes 1—5. Copies of representative chromosomes are placed to left of ideogram. See Materials and Methods for details of shading and numbering


12
11
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13
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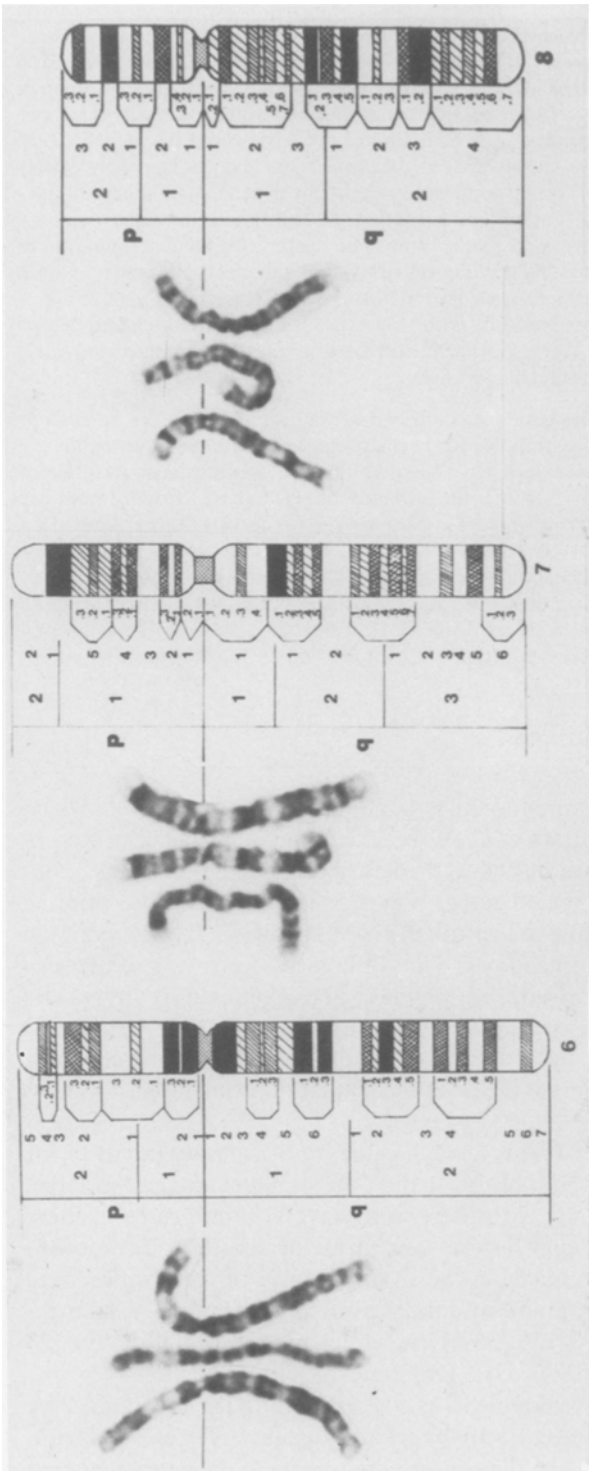

Fig. 2. Ideograms of GTG-banded human prometaphase chromosomes 6—15. Copies of representative chromosomes are placed to left of ideogram. See Materials and Methods for details of shading and numbering

metaphase spreads in representative states of contraction. Stalks and satellites on acrocentric chromosomes were excluded from measurements because of their variability.

To provide a more accurate representation of high-resolution G-banded chromosomes and to facilitate orientation among the large number of discernable bands, we have used a system of shading based on relative staining intensities of each band. Between white and black bands, we have distinguished three different shades of grey. Light grey is represented by widely spaced diagonal lines, intermediate grey by closely spaced diagonal lines, and dark grey by closely spaced cross-hatching. Realizing that staining intensities are highly dependent on the techniques used for staining as well as for photography and printing, we did not employ densitometric measurements, but rather visual comparisons of chromosome regions, for the determination of the shading for each particular band. The shading reflects staining intensity differences within each chromosome, but it is also consistent in the comparison of bands from different chromosomes. Chromosome regions that are variable with respect to size or staining characteristics (1qh, 9qh, 16qh, all C-band regions, Yqh, short arm, and satellite regions of acrocentrics), have been indicated by stippling rather than line-drawing.

Numbering System. In numbering the bands and sub-bands, we have followed the Paris Conference (1971) recommendations. The numbers for chromosomal regions and major bands are consistent with the Paris Conference ideogram. When comparing chromosomes in different stages of contraction, one observes that the single bands on metaphase chromosomes are resolved into sub-bands (white, black, and grey) at earlier stages of mitosis. Rather than using different numbering systems for different stages of mitosis, we have determined the highest number of discernable sub-bands for each major band and have given them decimal numbers, counting outward from the centromere. In some instances arbitrary decisions had to be made as to which sub-band is derived from which major band. However, in most cases it was easy to determine which bands appeared to fuse together in the process of chromosome condensation.

Results

The ideograms as derived from individual band measurements are shown in Figures 1—3. Representative copies of GTG-banded human prometaphase chromosomes used for measuring are placed to the left of each ideogram. Thus, if not for the obscuring effect of repeated steps of reproduction during the printing process, the reader should be able to identify the bands shown in the ideogram on the copies of the actual chromosomes. No differences were noted between prometaphase chromosomes derived from standard PHA-stimulated cultures and those derived by synchronization with amethopterin (Yunis, 1976). Thus, arrest of the cell cycle at G1/S does not appear to have any effect on the pattern of chromosome condensation during mitosis, as evident from prometaphase GTG banding.

Table 1 lists the numbers of bands in each category of staining intensity for each ideogram. There are a total of 783 bands, 62 of them in the 'variable' category represented by stippling. No subdivisions have been indicated in these stippled variable regions, although on the short arms of acrocentrics morphological distinctions have been made between short arms proper, stalks, and satellites. When the heteromorphic variable regions are excluded, of the remaining 721 euchromatic bands 256 (36%) are white, 176 (24%) light grey, 130 (18%) intermediate grey, 90 (12%) dark grey, and 69 (10%) black.

On the basis of the band measurements used for drawing the ideograms, we have calculated for each chromosome arm the relative distance of each band from the centromere. On a scale from 0 at the centromere to 1.00 at the p terminal or q

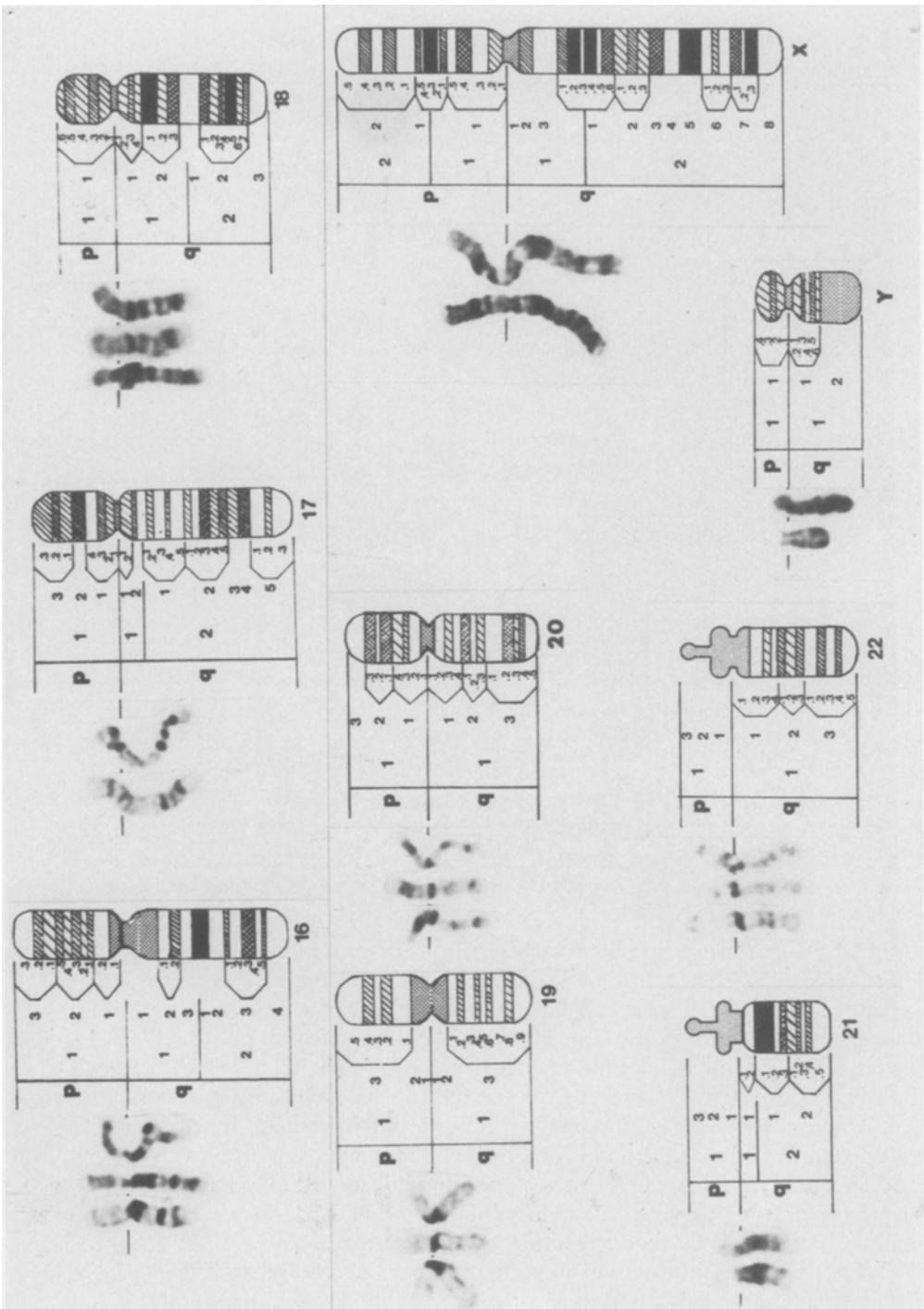

Fig. 3. Ideograms of GTG-banded human prometaphase chromosomes 16—22, X and Y. Copies of representative chromosomes are placed to left of ideogram. See Materials and Methods for details of shading and numbering

Table 1. Numbers of bands and sub-bands in each intensity category for each chromosome

Chromosome	White	Light grey	Intermediate grey	Dark grey	Black	Variable (stippled)	Total
1	21	13	12	6	6	3	61
2	24	7	9	8	6	2	56
3	14	9	9	7	3	3	45
4	18	15	5	6	5	2	51
5	17	6	6	4	5	2	40
6	13	8	5	6	6	2	40
7	11	8	5	10	2	2	38
8	10	11	6	6	4	2	39
9	14	12	7	2	3	3	41
10	12	7	5	7	3	2	36
11	11	5	8	3	5	2	34
12	9	9	4	4	3	2	31
13	11	8	3	2	5	4	33
14	4	9	7	1	4	4	29
15	9	6	5	3	0	4	27
16	8	3	7	1	1	2	22
17	8	9	4	3	0	0	26
18	2	9	5	2	2	2	22
19	8	6	0	0	0	2	16
20	8	4	2	4	0	2	20
21	4	1	2	1	1	4	13
22	5	2	4	0	0	4	15
X	15	4	7	4	5	2	37
Y	0	5	3	0	0	3	11
Total	256	176	130	90	69	62	783

terminal the relative position of each junction between bands has been determined (Appendix). The centromeric heterochromatin regions have been included, except for the highly variable 1qh, 9qh, 16qh and 19qh and ph regions. In these instances, the 0 position of the scale has been placed at the distal border of the qh region. Similarly, the heteromorphic distal Yqh region has been excluded. These linear scales of relative distances from the centromere should facilitate comparisons between genetic map, chiasma map, and chromosome map. The numbers in the appendix are derived from the original band measurements and may be more accurate than the ideograms, since some approximations may have occurred during the drawing process. The numbers listed in the appendix can be used for computerization of the high-resolution cytological map.

Table 1 provides information about the number and relative staining intensity of bands and sub-bands on each chromosome. More detailed quantitative data

Table 2. Composition of each chromosome arm determined by relative amounts of euchromatin in the five classes of staining intensity[a]

Chromosome arm	White	Light grey	Intermediate grey	Dark grey	Black	Light[b] value
1p	0.46	0.18	0.12	0.14	0.10	376
q	0.47	0.19	0.16	0.07	0.11	384
2p	0.46	0.05	0.14	0.18	0.17	345
q	0.47	0.12	0.13	0.17	0.11	367
3p	0.45	0.21	0.19	0.07	0.08	388
q	0.42	0.14	0.16	0.17	0.11	359
4p	0.45	0.15	0.17	0.09	0.14	368
q	0.30	0.27	0.10	0.16	0.17	337
5p	0.51	0.14	0.16	0.00	0.19	378
q	0.49	0.09	0.10	0.16	0.16	359
6p	0.49	0.13	0.11	0.09	0.18	356
q	0.36	0.14	0.11	0.18	0.21	326
7p	0.42	0.22	0.05	0.17	0.14	361
q	0.47	0.14	0.12	0.21	0.06	375
8p	0.61	0.02	0.04	0.20	0.13	378
q	0.21	0.37	0.13	0.14	0.15	335
9p	0.26	0.35	0.14	0.09	0.16	346
q	0.47	0.25	0.18	0.05	0.05	404
10p	0.35	0.23	0.19	0.23	0.00	370
q	0.51	0.09	0.06	0.18	0.16	361
11p	0.50	0.00	0.14	0.08	0.28	336
q	0.37	0.14	0.24	0.10	0.15	348
12p	0.42	0.20	0.05	0.09	0.24	347
q	0.37	0.21	0.10	0.14	0.18	345
13q	0.47	0.15	0.06	0.08	0.24	353
14q	0.24	0.33	0.15	0.04	0.24	329
15q	0.43	0.24	0.19	0.14	0.00	396
16p	0.38	0.28	0.34	0.00	0.00	404
q	0.63	0.00	0.15	0.10	0.12	392
17p	0.15	0.51	0.18	0.16	0.00	365
q	0.47	0.32	0.08	0.13	0.00	413
18p	0.00	0.62	0.38	0.00	0.00	362
q	0.28	0.29	0.10	0.14	0.19	333
19p	0.74	0.26	0.00	0.00	0.00	474
q	0.74	0.26	0.00	0.00	0.00	474
20p	0.46	0.15	0.08	0.31	0.00	376
q	0.55	0.21	0.04	0.20	0.00	411
21q	0.45	0.12	0.12	0.08	0.23	348
22q	0.56	0.16	0.27	0.00	0.00	425
Xp	0.49	0.08	0.25	0.09	0.09	361
q	0.37	0.12	0.14	0.15	0.22	327
Yp	0.00	0.80	0.20	0.00	0.00	380
q	0.00	0.70	0.30	0.00	0.00	370

[a] Heterochromatic and variable regions (stippled) have been excluded from calculations

[b] 'Light values' were derived by multiplying: white × 5, light grey × 4, intermediate grey × 3, dark grey × 2, and black regions × 1

are derived from the band measurements by calculating the relative *amounts* of white-, grey-, and black-staining chromatin regions for each chromosome arm. For these calculations only euchromatic regions have been included and all heterochromatic (stippled) regions have been excluded. In Table 2 the relative amounts of chromatin in each of the five categories of staining intensity are listed for each chromosome arm. Since staining intensity in some way reflects the underlying chromosome structure and/or DNA content, 'light values' have been calculated in order to compare the composition of different chromosome arms. The 'light values' were derived by multiplying the relative amounts of the white regions by 5, the light grey regions by 4, the intermediate grey regions by 3, the dark grey regions by 2, and the black regions by 1. Thus, chromosome arms with a relatively large amount of light-staining material should have higher 'light values' than those with a large amount of dark-staining chromatin. These values, as shown in Table 2, are consistent with visual impressions, e.g., 4q appears more darkly stained than 5q and has a lower 'light value.' The numbers span a rather narrow range for the larger chromosomes, indicating a balance in the relative contribution of light-staining, intermediate, and dark-staining chromatin to the composition of these chromosome arms. Among the smaller chromosomes, 17q, 19p, 19q, 20q, and 22q have the highest 'light values.'

These results may correlate with density of gene loci on specific chromosome arms, as well as with relative amounts of different classes of DNA in individual chromosomes, as more data on genetic content and molecular composition of human chromosomes become available. Evidence so far indicates significant correlations between intensity of G bands (or Q bands) and relative AT (dark) or GC (light) content (Weisblum and de Haseth, 1972), and between staining intensity and early (light) or late (dark) replication during the S phase (Grzeschik et al., 1975; Kim et al., 1975). Also, in situ hybridization studies indicate preferential hybridization of mRNA to light G-bands (Yunis et al., 1977). Duplication or deletion of chromosome regions with low 'light values' may be better tolerated and have less severe phenotypic effect than those of regions with high 'light values' (Hoehn, 1975).

Discussion

This quantitative analysis of features displayed by high-resolution trypsin Giemsa-banded human lymphocyte chromosomes was aimed at the construction of ideograms that accurately reflect these features. The results provide information about relative distances of individual bands from the centromere, about the quantitative composition of chromosome arms by chromatin of different staining intensities, and about patterns of chromosome condensation as manifested by fusion of particular bands during its course. The data and any conclusions derived from them have to be considered in the light of several limitations inherent in this kind of study.

Since mitotic (and meiotic) chromosome contraction is a continuous process, any subdivision into stages must be arbitrary. We have chosen, as objects of the present study, those elongated chromosomes displaying the maximal number of

bands discernable with some regularity by means of currently available lymphocyte culture and G-banding methods. We have observed that chromosome condensation does not progress uniformly over entire chromosomes or chromosome arms. Rather, in the transition from prophase to metaphase the rate of contraction is higher in light-staining than in dark-staining regions. Therefore, the data presented here on relative distances of individual bands from the centromere and on relative amounts of light, grey, and dark material per chromosome arm are only considered valid for that stage of contraction of the chromosomes used for measurements that were mostly taken from prometaphase cells.

These ideograms not only represent a short period within mitotic chromosome condensation, they also represent one stage of evolution of human cytogenetic technology. Currently, most cytogenetic laboratories detect more bands than those 322 represented in the Paris Conference (1971) ideogram, but may not have achieved the resolution shown in the ideograms presented here. We do not consider our ideograms definitive and recognize the need for ongoing refinement and improvement. Their publication at this time, together with some of the chromosomes they were derived from, is meant to stimulate cytogenetic laboratories to look for these high-resolution bands on their chromosome preparations.

Correlation of Ideograms With Pachytene Chromomere Patterns and R-Bands. Chromosome condensation appears to follow a similar course during prophase in meiosis and in mitosis. Electron-microscopic preparations of synaptonemal complexes (SC) from the pachytene stage in Chinese hamster spermatocytes revealed a rate of contraction corresponding well with mitotic chromosomes, suggesting the possibility of 'SC karyotyping' (Moses et al., 1977). There is also a remarkable similarity between chromomere patterns observed on pachytene chromosomes derived from human male and female and Chinese hamster male meiotic cells and the high-resolution G-banding patterns on mitotic prometaphase chromosomes (Luciani et al., 1974, 1975; Pathak et al., 1976).

These observations suggest that G-banding patterns reveal a natural order of chromatin coiling, while high-resolution R-banding patterns are observed after distortion of the original structure by irreversible pretreatment steps, resulting in an opposite banding pattern. Comparison of our ideograms with high-resolution R-banding patterns, and with ideograms derived from them (Skovby, 1975), reveals significant differences beyond a mere negative image. Most obviously, dark-staining R-bands are more prominent than their light-staining counterparts on the G-banded chromosome. This suggests that the irreversible pretreatment leading to R-banding has in some way induced a structural change in the chromosomal proteins. In contrast, the similarities between pachytene chromomere patterns and G-banding patterns on mitotic prophase chromosomes suggest that our ideograms should be well suited for studies on the correlation between genetic and cytological maps.

Correlation of Ideograms With Linkage Map, Chiasma Map, and Breakage Map. Genetic map distances as established by family linkage studies reflect the distribution and frequencies of crossover events along pachytene chromosomes. In an attempt to correlate genetic map distances with cytological distances, we

have previously lined up ideogram with linkage map in a linear fashion (Francke and Pellegrino, 1976; Francke et al., 1977) and have found them to match well for the short arm of chromosome 1. The high-resolution ideograms and measured relative distances between individual bands and the centromere will be very useful when compared to recombinational distances between gene loci that have already been assigned to specific chromosome bands by somatic cell genetic methods. The availability of a reliable genetic map as well as an accurate cytological map will allow determination of the relationship between the two maps, the distribution of crossover events, and the density of structural gene loci along chromosome arms.

Morton and colleagues have constructed a 'chiasma map' based on Hultén's observations (1974) on the location of chiasmata in diakinesis cells from a single human male and the Paris Conference ideogram (Morton et al., 1977). The use of our detailed ideograms and measurements should make such an exercise a great deal more worthwhile.

Goss and Harris (1975) have devised an approach to human gene mapping based on expression of human genes in interspecific cell hybrids made with gamma-irradiated human cells. In the absence of karyotypic analyses, they assume that irradiation produces chromosomal rearrangements and use radiation-induced segregation of syntenic loci to infer linear order of, and spacing between, assigned gene loci. From these data they produced 'breakage maps' of human chromosomes. Good correlation is observed when our ideogram of chromosome 1 is lined up with their map of chromosome 1 (Goss and Harris, 1977).

Use of Ideograms for Clinical Cytogenetics. The availability of these ideograms is expected to stimulate clinical cytogenetic laboratories to use cell synchronization methods in order to achieve higher resolution of chromosome banding. The ideograms will assist cytogeneticists in the interpretation of the high-resolution chromosomes. Since we tried to be conservative and not to overinterpret, we did not include sub-bands that were only rarely detectable. We found that we can consistently observe the bands we described, now that we are more aware of their existence. One has to be familiar with the variability induced by technical factors, as well as with the patterns of consecutive fusions of sub-bands during chromosome condensation, to be able to distinguish between artefacts and small structural abnormalities. The use of high-resolution chromosomes will lead to the detection of small duplications and deletions, which may be associated with subtle clinical abnormalities and mild mental delay. High resolution also increases differentiation within a given chromosomal region. This should lead to the detection of paracentric inversions, and of reciprocal translocations involving equal-sized chromosome regions with similar banding patterns.

Furthermore, the use of high-resolution chromosomes and the availability of ideograms, reflecting their features will enable cytogeneticists to define the localization of points of chromosome breakage and rejoining more accurately. This will probably lead to the discovery that deletions are more often interstitial than terminal. It will also lead to the more accurate definition of deleted or duplicated chromosomal regions, which is relevant to phenotype/karyotype correlations and to regional gene mapping.

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Appendix

Relative distance of bands from centromere

Chromosome arm	Region	Band	Subband	Relative distance of borders between bands from centromere	Chromosome arm	Region	Band	Subband	Relative distance of borders between bands from centromere
1p	1	1		0.03	1q	1	1		
		2	.1	0.05			2		0.00
			.2	0.07		2	1		0.05
			.3	0.10			2	.1	0.07
		3		0.15				.2	0.12
	2	1	.1	0.18				.3	0.14
			.2	0.21			3	.1	0.21
		2	.1	0.26				.2	0.25
			.2	0.29				.3	0.29
			.3	0.33				.4	0.31
	3	1	.1	0.36				.5	0.33
			.2	0.38			4	.1	0.35
			.3	0.42				.2	0.39
		2	.1	0.44				.3	0.41
			.2	0.47			5		0.47
			.3	0.51		3	1	.1	0.50
			.4	0.55				.2	0.52
			.5	0.59				.3	0.55
		3		0.62			2		0.64
		4		0.67		4	1	.1	0.66
		5	.1	0.70				.2	0.68
			.2	0.74				.3	0.73
			.3	0.76				.4	0.75
		6	.1	0.78				.5	0.77
			.2	0.80			2		0.83
			.3	0.82			3	.1	0.85
			.4	0.84				.2	0.89
			.5	0.89				.3	0.94
			.6	0.91			4		1.00
			.7	0.93					
			.8	0.95					
			.9	1.00					

Appendix (continued)

Chromosome arm	Region	Band	Subband	Relative distance of borders between bands from centromere
2p	1	1	.1	0.05
			.2	0.13
		2		0.21
		3		0.29
		4		0.34
		5		0.38
		6	.1	0.41
			.2	0.42
			.3	0.47
	2	1		0.54
		2	.1	0.57
			.2	0.58
			.3	0.64
		3	.1	0.67
			.2	0.68
			.3	0.72
		4	.1	0.77
			.2	0.79
			.3	0.85
		5	.1	0.91
			.2	0.95
			.3	1.00
2q	1	1	.1	0.01
			.2	0.05
		2		0.09
		3		0.14
		4	.1	0.17
			.2	0.20
			.3	0.23
	2	1		0.28
		2	.1	0.29
			.2	0.30
			.3	0.34
			.4	0.35
			.5	0.37
		3		0.40
		4	.1	0.43
			.2	0.46
			.3	0.50
	3	1		0.56
		2	.1	0.58
			.2	0.59
			.3	0.63
			.4	0.64
			.5	0.67
		3	.1	0.73
			.2	0.74
			.3	0.76
		4		0.80
		5		0.84
		6	.1	0.87
			.2	0.88
			.3	0.91
		7	.1	0.94
			.2	0.95
			.3	1.00
3p	1	1		0.13
		2		0.19
		3		0.22
		4	.1	0.26
			.2	
3q	1	1		0.06
		2		0.10
		3	.1	0.13
			.2	0.21
			.3	0.23

Appendix (continued)

Chromosome arm	Region	Band	Subband	Relative distance of borders between bands from centromere
3p (continued)			.3	0.30
			.4	0.38
	2	1		0.52
		2		0.55
		3		0.60
		4	.1	0.66
			.2	0.70
			.3	0.73
			.4	0.74
			.5	0.81
		5	.1	0.87
			.2	0.89
			.3	0.94
		6	.1	0.96
			.2	0.98
			.3	1.00
4p	1	1		0.05
		2		0.10
		3		0.18
		4		0.29
		5	.1	0.42
			.2	0.50
			.3	0.59
			.4	0.66
			.5	0.74
		6	.1	0.81
			.2	0.85
			.3	0.88
			.4	0.91
			.5	1.00
3q (continued)			.4	0.24
			.5	0.26
	2	1		0.38
		2	.1	0.41
			.2	0.44
			.3	0.46
		3		0.51
		4		0.56
		5	.1	0.61
			.2	0.64
			.3	0.67
		6	.1	0.72
			.2	0.75
			.3	0.78
			.4	0.80
			.5	0.82
		7		0.90
		8		0.94
		9		1.00
4q	1	1	.1	0.02
			.2	0.04
			.3	0.06
			.4	0.07
		2		0.11
		3	.1	0.12
			.2	0.16
			.3	0.19
	2	1	.1	0.205
			.2	0.215
			.3	0.23
			.4	0.27
			.5	0.32
		2		0.36
		3		0.40
		4		0.43
		5		0.455
		6	.1	0.465
			.2	0.49
		7	.3	0.52
		8	.1	0.55

Appendix (continued)

Chromosome arm	Region	Band	Subband	Relative distance of borders between bands from centromere
4q (continued)	3	1	.2	0.57
			.3	0.64
		2	.1	0.67
			.2	0.70
			.3	0.72
		3		0.74
		4	.1	0.76
			.2	0.79
			.3	0.81
		5	.1	0.85
			.2	0.86
				1.00
5p	1	1		0.06
		2	.1	0.13
			.2	0.19
			.3	0.27
		3		0.43
		4		0.61
		5	.1	0.70
			.2	0.77
			.3	0.84
			.4	0.89
			.5	1.00
5q	1	1		0.03
		2	.1	0.06
			.2	0.10
			.3	0.14
		3	.1	0.18
			.2	0.20
			.3	0.23
			.4	0.25
			.5	0.29
		4		0.35
		5		0.39
	2	1	.1	0.41
			.2	0.43
			.3	0.46
		2		0.51
		3	.1	0.55
			.2	0.58
			.3	0.61
	3	1	.1	0.66
			.2	0.68
			.3	0.72
		2		0.76
		3	.1	0.79
			.2	0.82

Appendix (continued)

Chromosome arm	Region	Band	Subband	Relative distance of borders between bands from centromere
5q (continued)			.3	0.85
		4		0.89
		5	.1	0.93
			.2	0.95
			.3	1.00
6p	1	1		0.01
		2	.1	0.10
			.2	0.11
			.3	0.20
	2	1	.1	0.33
			.2	0.39
			.3	0.56
		2	.1	0.62
			.2	0.66
			.3	0.75
		3		0.80
		4	.1	0.83
			.2	0.84
			.3	0.89
		5		1.00
6q	1	1		0.02
		2		0.09
		3		0.12
		4	.1	0.16
			.2	0.17
			.3	0.21
		5		0.26
		6	.1	0.31
			.2	0.33
			.3	0.37
	2	1		0.46
		2	.1	0.49
			.2	0.51
			.3	0.55
			.4	0.58
			.5	0.63
		3		0.68
		4	.1	0.72
			.2	0.74
			.3	0.77
			.4	0.82
			.5	0.85
		5		0.92
		6		0.95
		7		1.00
7p	1	1	.1	0.02
			.2	0.09
		2	.1	0.12
			.2	0.14
			.3	0.17
		3		0.30
		4	.1	0.36
			.2	0.39
			.3	0.44
7q	1	1	.1	0.01
			.2	0.08
			.3	0.11
			.4	0.18
	2	1	.1	0.24
			.2	0.27
			.3	0.30
			.4	0.33
			.5	0.36

Appendix (continued)

Chromosome arm	Region	Band	Subband	Relative distance of borders between bands from centromere
7p (continued)		5	.1	0.52
			.2	0.57
			.3	0.67
	2	1		0.81
		2		1.00
8p	1	1	.1	0.06
			.2	0.14
			.3	0.16
			.4	0.22
		2		0.33
	2	1	.1	0.44
			.2	0.48
			.3	0.59
		2		0.71
		3	.1	0.84
			.2	0.92
			.3	1.00
7q (continued)	3	2		0.46
		1	.1	0.49
			.2	0.52
			.3	0.55
			.4	0.58
			.5	0.62
			.6	0.64
			.7	0.67
		2		0.75
		3		0.79
		4		0.83
		5		0.88
		6	.1	0.92
			.2	0.94
			.3	1.00
8q	1	1	.1	0.01
			.2	0.05
		2	.1	0.09
			.2	0.15
			.3	0.18
			.4	0.20
			.5	0.23
			.6	0.26
			.7	0.28
		3		0.33
	2	1	.1	0.37
			.2	0.38
			.3	0.42
			.4	0.45
			.5	0.50
		2	.1	0.55
			.2	0.57
			.3	0.63
		3	.1	0.66
			.2	0.72
		4	.1	0.76
			.2	0.78
			.3	0.82
			.4	0.85
			.5	0.88

Appendix (continued)

Chromosome arm	Region	Band	Subband	Relative distance of borders between bands from centromere
8q (continued)			.6	0.91
			.7	1.00
9p	1	1	.1	0.03
			.2	0.10
		2		0.19
		3	.1	0.23
			.2	0.28
			.3	0.39
	2	1	.1	0.48
			.2	0.51
			.3	0.57
		2	.1	0.63
			.2	0.68
			.3	0.73
		3		0.82
		4		1.00
9q	1	1		0.00
		2		0.04
		3		0.07
	2	1	.1	0.10
			.2	0.14
			.3	0.18
			.4	0.23
			.5	0.28
			.6	0.32
			.7	0.38
		2	.1	0.40
			.2	0.44
			.3	0.46
			.4	0.52
			.5	0.57
	3	1	.1	0.61
			.2	0.63
			.3	0.69
		2		0.74
		3	.1	0.77
			.2	0.80
			.3	0.87
		4	.1	0.88
			.2	0.91
			.3	0.92
			.4	1.00
			.5	
10p	1	1	.1	0.07
			.2	0.16
			.3	0.21
			.4	0.31
		2	.1	0.40
			.2	0.44
			.3	0.55
		3		0.68
		4		0.78
		5	.1	0.86
10q	1	1	.1	0.04
			.2	0.09
			.3	0.10
			.4	0.15
	2	1	.1	0.21
			.2	0.23
			.3	0.28
		2	.1	0.36
			.2	0.39
			.3	0.44

Appendix (continued)

Chromosome arm	Region	Band	Subband	Relative distance of borders between bands from centromere
10p (continued)			.2	0.90
			.3	1.00
10q (continued)		3	.1	0.48
			.2	0.50
			.3	0.54
			.4	0.58
			.5	0.61
		4		0.68
		5	.1	0.72
			.2	0.75
			.3	0.78
			.4	0.81
			.5	0.84
		6	.1	0.90
			.2	0.93
			.3	1.00
11p	1	1	.1	0.05
			.2	0.13
			.3	0.23
		2		0.35
		3		0.44
		4		0.59
		5	.1	0.65
			.2	0.71
			.3	0.76
			.4	0.83
			.5	1.00
11q	1	1		0.02
		2		0.07
		3	.1	0.13
			.2	0.15
			.3	0.20
			.4	0.22
			.5	0.30
		4	.1	0.34
			.2	0.38
			.3	0.43
			.4	0.46
			.5	0.51
	2	1		0.54
		2	.1	0.59
			.2	0.62
			.3	0.67
			.4	0.70
			.5	0.73
		3		0.81
		4	.1	0.86
			.2	0.89
			.3	0.94
		5		1.00
12p	1	1	.1	0.06
			.2	0.20
12q	1	1		0.01
		2		0.09

Appendix (continued)

Chromosome arm	Region	Band	Subband	Relative distance of borders between bands from centromere	Chromosome arm	Region	Band	Subband	Relative distance of borders between bands from centromere
		2	.1	0.25			3	.1	0.12
			.2	0.30				.2	0.15
			.3	0.53				.3	0.22
			.4	0.62				.4	0.25
			.5	0.70				.5	0.28
		3	.1	0.84			4		0.34
			.2	0.89			5		0.39
			.3	1.00		2	1	.1	0.43
								.2	0.47
								.3	0.57
							2		0.61
							3	.1	0.65
								.2	0.67
								.3	0.70
							4	.1	0.79
								.2	0.83
								.3	0.88
								.4	0.91
								.5	1.00
13p	1	1			13q	1	1		
		2					2	.1	0.02
		3						.2	0.06
								.3	0.07
								.4	0.10
								.5	0.12
							3	.1	0.16
								.2	0.18
								.3	0.20
							4	.1	0.25
								.2	0.31
								.3	0.32
								.4	0.35
								.5	0.38
						2	1	.1	0.42
								.2	0.46
								.3	0.50
								.4	0.53
								.5	0.56
							2	.1	0.60
								.2	0.63
									0.64

Appendix (continued)

Chromosome arm	Region	Band	Subband	Relative distance of borders between bands from centromere	Chromosome arm	Region	Band	Subband	Relative distance of borders between bands from centromere
					13q (continued)	3	1	.3	0.67
								.1	0.73
								.2	0.74
								.3	0.78
							2	.1	0.82
								.2	0.83
								.3	0.88
							3		0.93
							4		1.00
14p	1	1			14q	1	1	.1	0.05
		2						.2	0.07
		3						.3	0.09
								.4	0.12
							2		0.17
							3	.1	0.20
								.2	0.22
								.3	0.25
						2	1	.1	0.30
								.2	0.31
								.3	0.35
							2		0.41
							3	.1	0.43
								.2	0.45
								.3	0.49
								.4	0.52
								.5	0.54
								.6	0.58
								.7	0.60
							4		0.70
						3	1		0.79
							2	.1	0.85
								.2	0.87
								.3	0.90
								.4	0.92
								.5	1.00
15p	1	1			15q	1	1	.1	0.02
		2						.2	0.09
		3					2	.1	0.13
								.2	0.17
								.3	0.19

Appendix (continued)

Chromosome arm	Region	Band	Subband	Relative distance of borders between bands from centromere	Chromosome arm	Region	Band	Subband	Relative distance of borders between bands from centromere
					15q (continued)		3		
									0.25
							4		
									0.30
							5	.1	
									0.35
								.2	
									0.36
								.3	
									0.40
						2	1	.1	
									0.45
								.2	
									0.48
								.3	
									0.52
								.4	
									0.58
								.5	
									0.60
							2		
									0.65
							3		
									0.68
							4		
									0.75
							5	.1	
									0.79
								.2	
									0.81
								.3	
									0.85
							6	.1	
									0.91
								.2	
									0.96
								.3	
									1.00
16p	1	1	.1		16q	1	1		
				0.11					0.00
			.2				2	.1	
				0.26					0.09
		2	.1					.2	
				0.32					0.18
			.2				3		
				0.39					0.28
			.3			2	1		
				0.47					0.40
			.4				2		
				0.54					0.52
			.5				3	.1	
				0.61					0.55
		3	.1					.2	
				0.72					0.64
			.2					.3	
				0.81					0.74
			.3					.4	
				1.00					0.79
								.5	
									0.82
							4		
									1.00
17p	1	1	.1		17q	1	1	.1	
				0.02					0.01
			.2					.2	
				0.12					0.07
			.3				2		
				0.22					0.10
			.4			2	1	.1	
				0.37					0.16
		2						.2	
				0.53					0.20
		3	.1					.3	
				0.68					0.27
			.2					.4	
				0.75					0.30

Appendix (continued)

Chromosome arm	Region	Band	Subband	Relative distance of borders between bands from centromere
17p (continued)			.3	1.00
17q (continued)		2	.5	0.38
			.1	0.42
			.2	0.47
			.3	0.53
			.4	0.58
			.5	0.63
		3		0.70
		4		0.77
		5	.1	0.86
			.2	0.89
			.3	1.00
18p	1	1	.1	0.05
			.2	0.26
			.3	0.43
			.4	0.68
			.5	0.87
			.6	1.00
18q	1	1	.1	0.02
			.2	0.09
			.3	0.13
			.4	0.17
		2	.1	0.28
			.2	0.34
			.3	0.41
	2	1		0.55
		2	.1	0.62
			.2	0.68
			.3	0.70
			.4	0.71
			.5	0.79
			.6	0.83
			.7	0.87
		3		1.00
19p	1	1		0.00
		2		0.22
		3	.1	0.41
			.2	0.51
			.3	0.59
			.4	0.69
			.5	1.00
19q	1	1		0.00
		2		0.16
		3	.1	0.27
			.2	0.32
			.3	0.44
			.4	0.49
			.5	0.55
			.6	0.59
			.7	0.73
			.8	0.81
			.9	1.00

Appendix (continued)

Chromosome arm	Region	Band	Subband	Relative distance of borders between bands from centromere	Chromosome arm	Region	Band	Subband	Relative distance of borders between bands from centromere
20p	1	1	.1	0.06	20q	1	1	.1	0.04
			.2	0.19				.2	0.13
			.3	0.27				.3	0.21
			.4	0.41				.4	0.29
		2	.1	0.56			2	.1	0.38
			.2	0.61				.2	0.44
			.3	0.75				.3	0.52
		3		1.00			3	.1	0.69
								.2	0.79
								.3	0.83
								.4	0.87
								.5	1.00
21p	1	1			21q	1	1	.1	0.05
		2						.2	0.17
		3				2	1	.1	0.39
								.2	0.45
								.3	0.53
							2	.1	0.64
								.2	0.69
								.3	0.74
								.4	0.80
								.5	1.00
22p	1	1			22q	1	1	.1	0.16
		2						.2	0.28
		3						.3	0.34
								.4	0.39
							2	.1	0.45
								.2	0.53
								.3	0.60
							3	.1	0.71
								.2	0.77
								.3	0.84
								.4	0.88
								.5	1.00
Xp	1	1	.1	0.03	Xq	1	1		0.04
			.2	0.11			2		0.09
			.3	0.20			3		0.18
			.4	0.29		2	1	.1	0.22
			.5	0.34				.2	0.27

Appendix (continued)

Chromosome arm	Region	Band	Subband	Relative distance of borders between bands from centromere	Chromosome arm	Region	Band	Subband	Relative distance of borders between bands from centromere
Xp (continued)	2	1	.1	0.38	Xq (continued)			.3	0.28
			.2	0.39				.4	0.32
			.3	0.48				.5	0.34
			.4	0.49				.6	0.39
			.5	0.53			2	.1	0.44
		2	.1	0.63				.2	0.48
			.2	0.71				.3	0.52
			.3	0.78			3		0.57
			.4	0.86			4		0.62
			.5	1.00			5		0.70
							6	.1	0.74
								.2	0.77
								.3	0.81
							7	.1	0.85
								.2	0.87
								.3	0.91
							8		1.00
Yp	1	1	.1	0.10	Yq	1	1	.1	0.08
			.2	0.36				.2	0.35
			.3	0.54				.3	0.47
			.4	1.00				.4	0.66
								.5	0.82
								.6	1.00
							2		

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