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Genetics

A contiguous *Not* I restriction map of band q22.3 of human chromosome 21

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ABSTRACT **A contiguous high-resolution *Not* I restriction map of the distal region of the long arm of human chromosome 21 was constructed by three strategies: linking clones to identify adjacent pieces of DNA, partial digestion to identify neighboring fragments, and cell line polymorphisms to prove identity or adjacency of DNA fragments. Twenty-nine single-copy DNA probes and five linking clone probes were used to determine the order of 30 *Not* I fragments, covering 10 megabases of DNA in band q22.3. Smaller *Not* I fragments occur preferentially in this region, suggesting that band q22.3 is unusually rich in genes, since *Not* I sites occur almost exclusively in CpG islands. Comparison of the physical map and genetic maps in this region reveals a 10-fold higher than average recombination frequency.**

Human chromosome 21, the smallest human chromosome, has ≈50 megabases (Mb) of DNA. Less than 1% of the 2000 genes estimated to be on chromosome 21 are known, but two major neurologic disorders, Down syndrome and familial Alzheimer disease, map to this chromosome (1–6). Human genetic and physical maps are helpful for understanding chromosome structure and function and for localizing human genes. Many strategies have been used to construct partial physical maps of chromosome 21 (7–11). A genetic linkage map of 27 DNA markers on the long arm of chromosome 21 has also been constructed (12). However, the only contiguous physical map of the long arm is the *Not* I map of the proximal region, including the centromere (13). Mapping of this region was greatly facilitated because the *Not* I fragments were large, and numerous single-copy DNA probes were available. In contrast, the construction of a complete restriction map is difficult in region 21q22.3 because of the presence of numerous GpC islands. Here, several approaches will be combined to construct this map. Two *Not* I linking libraries of chromosome 21 provide especially useful mapping probes (13, 14). Partial digestion strategies reveal neighboring restriction fragments and fill gaps in the map. Restriction fragment polymorphism patterns in different cell lines identify unique *Not* I fragments and find adjacent fragments.

MATERIALS AND METHODS

Cell lines used are listed in Table 1. DNA was prepared from these cell lines in agarose (29). Cloned sequences used as hybridization probes are listed in Table 2. Three λ linking clones (LA79, LA171, LA58) and two plasmid linking clones (D13, HMG14) were used (14). Usually, the large (lg) or small (sm) half of each was hybridized separately. A 450-base-pair (bp) segment of HMG14lg was amplified by PCR using primers 5′-GCGGGGGCGGCGTTCTGGAA-3′ and 5′-CTGCCTTTTTCGGCTTCGCT-3′. DNA purified from various cell lines was digested with *Not* I, fractionated by pulsed-field gel electrophoresis (PFG), and hybridized (29).

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RESULTS

Regional Localization of Clones. A panel of hybrid cell lines (Table 1) was used to determine the location of each clone listed in Table 2. WAV17 and 29-1F-3a have human chromosome 21 as the only human material in a background of mouse and hamster, respectively. A9 (mouse fibroblast) and CHO (Chinese hamster ovary) were used as controls. The other cell lines contain chromosome 21 lacking different regions in a rodent background. These cell lines divide 21q22.3 into four subregions: from q telomere to breakpoint 7;21, from 7;21 to 21;22, from 21;22 to the ring breakpoint, and from the ring breakpoint to 21q+ (7). *Eco*RI digests of DNA from the cell panel were hybridized with each half-linking clone or single-copy clone. This assigned the known telomeric clone pYHT1 to the q telomere; 7 DNA markers including 1 linking clone are between breakpoints 7;21 and 21;22; 19 single copy probes and 3 linking clones are between breakpoints 21;22 and the ring; and 2 single copy probes and 1 linking clone are above the ring breakpoint (Table 2).

Polymorphism Analysis. In some cell lines, occasional *Not* I sites are missing or are methylated and resistant to enzyme cleavage. This leads to a pattern of polymorphism when fragment sizes from different cell lines are compared. Two probes located on the same *Not* I fragment must have an identical polymorphism pattern. In Table 2, pairs of probes with the same polymorphism pattern and the same *Not* I fragment sizes are bracketed. Polymorphism patterns can also identify two adjacent *Not* I fragments. For example, S3 and S55 are adjacent because they have the same polymorphism pattern and detect the same 2100-kilobase (kb) band in cell lines 310, LAN-5, WAV17, and 29-1F-3a, while in other cell lines they detect 680- and 1420-kb bands, respectively. Thus, the 680- and 1420-kb fragments are adjacent, and the *Not* I site between them is completely methylated in some cell lines and partly methylated in others (Fig. 1). Equivalent situations occur with S19 and S56 and with S25 and S141.

Linking Clones. *Not* I linking clones detect two adjacent *Not* I fragments by hybridization. Five linking clones were available in the distal region of the long arm. LA79, between breakpoints 7;21 and 21;22, detects two adjacent *Not* I fragments, 315 and 340 kb. LA58, between the ring breakpoint and 21q+, detects 2100- and 340-kb *Not* I fragments in WAV17. The 2100-kb band is also detected by S55 (Table 2 and Fig. 1). LA171, D13, and HMG14 are located between the 21;22 and the ring breakpoints. The order of these clones is LA171sm–LA171lg–D13lg–D13sm–HMG14sm–HMG14lg, because LA171lg detects the same 1500-kb fragment as D13lg; D13sm detects the same 290-kb fragment as HMG14sm. The three clones define four contiguous 800-, 1500-, 290-, and 75-kb fragments. In addition to a 1500-kb

Abbreviations: PFG, pulsed-field gel electrophoresis; Mb, megabase(s); cM, centimorgan(s).

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Table 1. Mammalian cell lines used in this work

Name	Cell line	Karyotype	Origin and description	Ref.
GM6167	Fibroblast	46,XY,normal	Lung biopsy	*†
GM3468	Fibroblast	46,XY,normal	Foreskin	15
310	Lymphoblast		Hemizygous HLA	16
LA-N-5	Neuroblast	46,XY,normal	Neuroblastoma	17
Gus3365A	Lymphoblast	46,XX,t(4;21)(p16;q21)		14
Gus4066	Lymphoblast	45,XX,-4,21,+der(4), t(4;21)(4qter–p16::21 q21–qter)		18
A9	Mouse fibroblast			19†
CHO-K1	CHO			20†
WAV17	Mouse with human chromosome 21			21†
HDm-15	Mouse with human chromosome 21 with *neo* gene			22
AHV1-17	Mouse with human chromosome 21 and others			23†
29-1F-3a	CHO-K1 with human chromosome 21			24†
72532X-6	CHO-K1 with human chromosome 21q22.3–p11.1			25
153E7bx	CHO-K1 with human chromosome 21q22.3–p11.2			26†
R2-10W	CHO-K1 with human chromosome 21q22.2–p11.2			27†
2Fur1	CHO-K1 with human chromosome 21q			27†
ACEM	CHO-K1 with human chromosome 21q22.22, 21p11.1–p11.2			28†
8q−	CHO-K1 with human chromosome 2122.2–22.3			27†
21q+	CHO-K1 with human chromosome 2122.1–pter			27†
9542C-5a(10;21)	CHO-K1 with human chromosome 21pter–portion of q22.2			7†
Raj5(21;22)	CHO-K1 with human chromosome 21pter–portion of q22.3			7†
643C-13(7;21)	CHO-K1 with human chromosome 21pter–portion of q22.3			7†

*National Institute of General Medical Sciences cell repository, Camden, NJ.
†Used for regional assignment.

band, D13lg detected a 70-kb *Not* I fragment in WAV17, but LA171lg did not. This reveals a partially methylated *Not* I site near the proximal terminus of the 1500-kb band. D13lg can reveal both the 70- and 1500-kb fragments, while LA171lg would detect two fragments of 1430 and 1500 kb, which would be indistinguishable at the low PFGE resolution above 1 Mb. Fragments mapped by linking clones were also detected with single-copy probes: SF13a(S39), SF93(S51), and 512-16P(S53) detect the same 800-kb band as LA171sm; JG373(S101) and pGSE8(S15) detect the same 1500-kb band as LA171lg or D13lg; and 6-40-3 (IFNBR) detects the same 290-kb band as D13sm or HMG14sm (Table 2).

Partial Digests. DNA from WAV17 was analyzed by *Not* I partial digests. When a single copy probe is hybridized to partially digested genomic DNA, several bands are detected. All the fragments detected must contain the probe. Partial digest products can potentially extend in both directions so there are many possible arrangements of *Not* I fragments around any single probe. Accurate map construction requires combining data obtained with more than one probe. A particularly simple case occurs when two probes lie on adjacent fragments. Linking clones are especially useful for partial digest analysis since two half-linking clones will lie on adjacent *Not* I fragments.

Mapping the q Telomere. The human telomeric yeast artificial chromosome clone yHT1 contains subtelomeric sequences that do not cross-hybridize with rodent DNA (48). This clone can identify telomeric *Not* I restriction fragments of chromosome 21 in human–rodent hybrid cell lines. Three groups of hybrid cell lines were used to determine which telomeres are detected by yHT1. In the first group, 153E7bx, 2Fur1, 72532X-6, and ACEM all have the chromosome 21q telomere but not the p telomere. In the second group, 21q+ and AHV1-17 have the chromosome 21p telomere but not the q telomere, while R2-10W has neither telomere. In the third group, WAV17 and 29-1F-3a contain human chromosome 21 as the sole human component. A subclone of yHT1, plasmid pYHT1, was hybridized to DNA from the three groups of cell lines, digested completely with *Not* I. The results revealed no hybridization to group 2, a single band in group 1, and two bands (580 and 750 kb) or one band (750 kb) in group 3, WAV17, and 29-1F-3a, respectively. This means that pYHT1 only detects the *Not* I fragments at the q telomere. The true telomeric 580-kb *Not* I fragment is followed by a 170-kb fragment, and the *Not* I site between them is partially or fully methylated in some cell lines. The same two *Not* I fragments

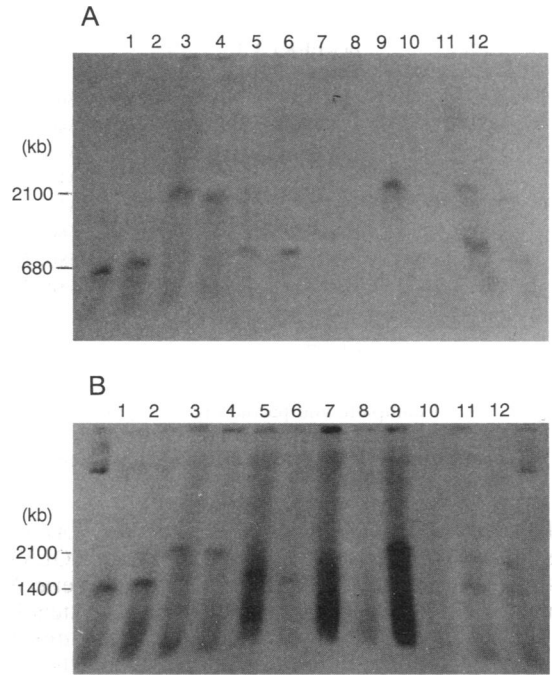

FIG. 1. Physical map construction using polymorphism analysis. Hybridization of DNA from different cell lines with probes S3 (*A*) and S55 (*B*). Unique 680- and 1400-kb DNA fragments were detected in cell lines GM6167 (lane 1), GM3468 (lane 2), HDm-15 (lane 5), 72532x-6 (lane 6), Gus3365A (lane 11), and Gus4066 (lane 12). A common 2100-kb fragment was detected in cell lines 310 (lane 4), LA-N-5 (lane 3), WAV17 (lane 9), and 29-1F-3a (lane 10). A9 (lane 7) and CHO-K1 (lane 8) are controls.

Table 2. Complete and partial digest fragment sizes analyzed by PFG

Locus	Probe	Ref.	*Not* I fragments		Breakpoint
			Complete	Partial	
	LA58sm	14	340	340, 530, 780	
	LA58lg		2100	1400, 1800, 2100, 2500, 3100	
D21S55	518-1R	30	2100		
ets2	H33ets2	31	2100	*440, 680, 780, 880, 1020, 1090, 2100, 2400, 2800*	
					Ring
D21S3	231C	32	2100	680, 2100, 2800	
HMG14	HMG14lg	33	75		
HMG14	HMG14sm		290	290, 365, 560, 655, 750, 800, 830	
IFNBR	6-40-3	34	290	290, 365, 560, 1000, 1200	
	D13sm	14	290	290, 2000, 2900	
	D13lg		70, 1500		
D21S101	[JG373	35	1500	1500, 1800, 2600	
D21S15	pGSE8]	36	1500	1500, 2300, 2700	
	LA171lg	14	1500		
	LA171sm		800	800, 880, 1100, 1400, 1500, 2300	
D21S51	[SF93	37	800	800, 850, 1400, 1900, 2300, 2600	
D21S53	512-16P	32	800		
D21S39	SF13a]	37	800	800, 1100, 2600	
D21S135	D22	38	340	340, 390, 460, 535, 580, 870, 1070, 1120, 1700, 2200, 2900	
D21S40	SF14	37	80, 390, 970	80, 120, 390, 440, 655, 855, 970, 1040	
BCE1	[pS2	39	390, 970		
D21S56	520-5B]	30	390, 970	390, 440, 510, 970, 1040, 1400, 1800, 2200, 3200	
D21S19	[pGSB3	40	580, 970	580, 970, 1040, 1400, 1700	
D21S42	SF43	37	580, 970	580, 775, 970, 1040	
	pSc7	40	580, 970	580, 775, 970, 1040	
D21S113	pMCT15]	41	580, 970	580, 775, 970, 1040, 1090	
CBS	[CBS	42	170	170, 680, 775, 1200, 1300, 1700	
CRYA1	pMαACr2]	43	170	170, 680, 725, 775, 1700	
D21S141	E73	38	510	510, 560, 610, 1100, 1400, 1700, 2400	
D21S25	10.2	44	510	145, 220, 510, 560, 725, 1100, 1400, 1700, 2400	
PFKL	pG-PFKL3.3	45	40	40, 220, 560, 610, 800	
					21;22
LFA-1	p3.1.1	46	80	80, 390, 580, 750, 1100, 1700	
	LA79lg	14	340	340, 390, 440, 490, 560, 655, 705, 755	
	LA79sm		315	315, 485, 1070, 1500, 2000, 2800, 3200	
D21S44	[SF50	37	315	315, 485, 1070, 1500, 2000, 2800, 3200	
D21S91	JG63]	35	315		
COL6A1	[p18COL6A1	47	580, 750		
COL6A2	p1COL6A2	47	580, 750	580, 750, 1070, 1500, 2000, 2800, 3200	
D21S123	B88]	38	580, 750		
					7;21
	pYHT1	48	580, 750	580, 750, 1070, 1500, 2000, 2800, 3200	
					q telomere

Not I complete and partial digest fragments were detected in hybrid cell line WAV17 except for italic numbers, which were detected in another cell line. Underlining means that multiple bands were seen. Horizontal lines indicate chromosomal breakpoints, which divide q22.3 into four subregions (9). All loci or probes, individual or clustered, are given in order, except for the bracketed items.

are detected by pYHT1 in partial digests (Fig. 2*A*) and by three distal DNA markers S123, COL6A1, and COL6A2.

Mapping Region q22.3. A probe from a telomeric *Not* I fragment will detect partial digestion products extending in only one direction. Such a partial digest provides, directly, a map starting from the chromosome end. When the q telomere-specific probe pYHT1 was hybridized to WAV17 DNA partially digested with *Not* I restriction enzyme, 7 bands were detected extending to ≈3 Mb (Table 2 and Fig. 2). Between the telomere and the 7;21 breakpoint, COL6A1, COL6A2, and S123 detected two *Not* I fragments (580 and 750 kb), the same as detected by pYHT1. Another three probes JG63, S44, and LA79sm detected a 315-kb *Not* I fragment, and LA79lg detected a 340-kb fragment. Partial digest data obtained with probes pYHT1, COL6A2, and LA79sm ordered the *Not* I fragments: 580, 170, 315, and 340 kb. Partial digestion data with probe LA79lg revealed three or four small fragments proximal to the 340-kb fragment. Their average size is ≈50 kb. The one closest to the 340-kb fragment is also detected by LFA-1, since partial digests probed with LFA-1 revealed an adjacent 340-kb *Not* I fragment.

Between the 21;22 and ring breakpoints, loci S25 and S141 were linked by their polymorphism pattern. Each detected several bands, but both detected a common largest 510-kb *Not* I fragment. The *Not* I cutting sites within this 510-kb fragment divide it into 50, 70, 145, and 245-kb pieces (Fig. 3). From partial digestion analysis, the distal neighbor of S25 is PFKL and the proximal neighbor of S141 is CRYA1. Thus,

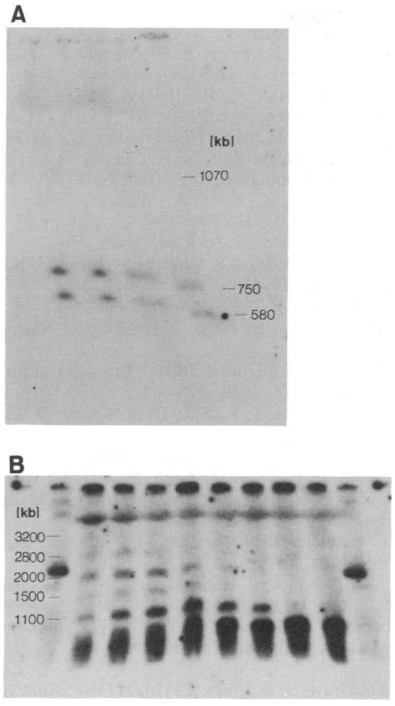

FIG. 2. Analysis of the structure of the q terminus of chromosome 21. Telomere-specific clone pYHT1 was hybridized to *Not* I partially digested WAV17 genomic DNA (increasing enzyme from left to right), fractionated by PFG in the size range 50–1000 kb (*A*), or 1000–3000 kb (*B*). The two outer lanes are *Pichia* size standards, which show some cross-hybridization to the probe. The partial digest products seen extend from the q telomere inward.

the locus order is PFKL–S25–S141–CRYA1. This was confirmed by partial digests probed with LA79lg and LFA-1.

Probes CRYA1 and CBS detected the same 170-kb *Not* I fragment. Both reveal partial bands of 680 and 775 kb. Thus, the 510-kb fragment detected by S141 is distal to them, and the 600-kb fragment is proximal. A cluster of loci—S113, S42, pSc7, and S19—detected the 580-kb band and also the 775-kb partial band, so their order is CRYA1–CBS–(S113, S42, pSc7, S19).

S19 and S56 have the same polymorphism pattern. They span a common 970-kb fragment with a partially methylated *Not* I cutting site between the 580- and 390-kb fragments in WAV17. Two small fragments, 80 and 50 kb, are next to the 390-kb fragment, since in partial digests S56 detects 390-, 440-, 510-, 970-, and 1040-kb bands, and S19 detects 580-, 970-, and 1040-kb bands. The 80-kb *Not* I fragment seen by S56 is likely to be the same fragment seen by S40 since the polymorphism pattern is the same.

Partial digests probed with S19 and S56 revealed an additional 350-kb *Not* I fragment proximal to them. This fragment is also detected by S135. Adjacent to the 350-kb fragment, 200- and 75-kb *Not* I fragments were revealed by partial digests probed with S135. The 75-kb partial product is also seen with LA171sm. Single copy probes S39, S51, and S53 detected the same 800-kb fragment detected by LA171sm. Combining this information with prior D13 and HMG14 results, the locus order is (S113, S42, pSc7, S19)–S56–S40–S135–(S39, S51, S53)–LA171sm–LA171lg–(S15, S101)–D13lg–D13sm–IFNBR–HMG14sm–HMG14lg. The order of adjacent *Not* I fragments is 580, 390, 80, 50, 350, 200, 75, 800, 1500, 70, 290, and 75 kb.

Between HMG14 and S3 there are no known markers. S3 detected a 2100-kb band in WAV17 and two bands, 680 and 2100 kb, in other cell lines. Since it is easier to see small partial bands next to 680 kb than next to 2100 kb, we analyzed partial digests probed with S3 using Gus4066. Three partial fragments—100, 100, and 190 kb—detected in Gus4066 have to be distal to S3, since S3 is linked with S55 proximally (Fig. 1). HMG14sm detects proximal partial digest fragments of 190, 100, and 100 kb. Thus, these three small fragments fill the gap between HMG14 and S3.

Between the ring breakpoint and $21q^+$, probe ets2 detected the same 680- and 2100-kb bands as did S3 in some cell lines. S55 detected the same 2100-kb band as S3 in WAV17 and a 1420-kb band in other cell lines (Fig. 1). The 340-kb fragment detected by LA58sm is proximal to the 2100-kb fragment, because LA58lg has the same polymorphism pattern and detects the same 2100-kb band as S55 in WAV17. Thus, the locus order is S3–ets2–S55–LA58lg–LA58sm (Fig. 3).

DISCUSSION

Comparison of Physical and Genetic Maps. The genetic linkage map of the long arm of human chromosome 21 contains 27 DNA markers (12). We used 11 of these. Except for PFKL and LFA-1, S113 and CRYA1, the order of the others is consistent. The genetic map puts PFKL more distal than LFA-1. Our regional assignments showed that LFA-1 is more distal. This ordering is consistent with other work (7, 9). The genetic map places CRYA1 proximal to S113, while our results reveal that S113 is proximal to CRYA1.

The length of the sex-averaged genetic linkage map from the subtelomeric marker COL6A1 to S55 is 80 centimorgans (cM) (12), while the physical distance from COL6A1 to S55 is ≈8 Mb (Table 3). On average, 1 Mb in the human genome corresponds to ≈1 cM. In the q22.3 region of chromosome 21, 100 kb corresponds to 1 cM, a 10-fold higher meiotic recombination frequency than expected. Table 3 reveals an especially high frequency of crossovers between COL6A1 and PFKL, consistent with genetic analysis (49, 50).

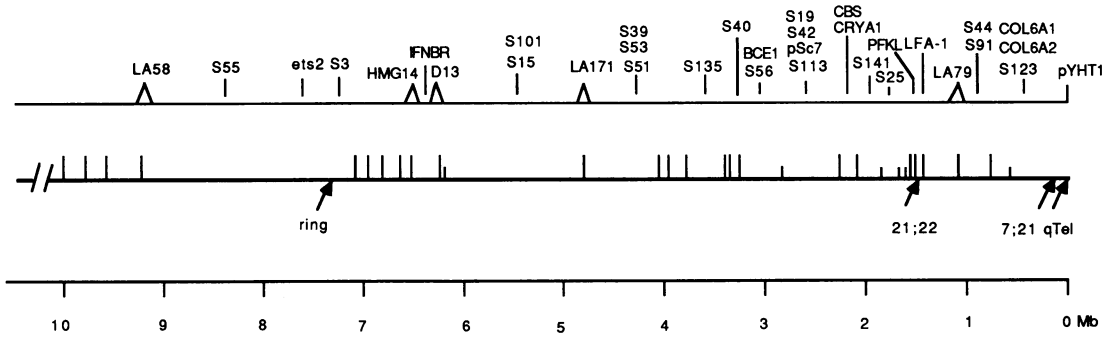

FIG. 3. *Not* I restriction map of chromosome 21 band q22.3. Top line is all markers used. Middle line is the physical map in WAV17; long and short vertical lines completely and partially cleaved *Not* I sites. Bottom line is the size scale.

Table 3. Comparison of genetic map with the physical map

DNA marker	Genetic length, cM	Physical length, kb	Ratio cM/Mb
D21S55			
	8	920	8.7
ets2			
	2	125	16.0
D21S3			
	6	750	8.0
HMG14			
	8	1040	7.7
D21S15			
	16	2475	6.5
BCE1			
	2	485	4.1
D21S113			
	5	375	13.3
CYRA1			
	8	615	13.0
PFKL			
	1	50	20.0
LFA-1			
	24	1150	20.9
COL6A1			
Total	80	7985	

Eleven DNA markers were used to compare the genetic linkage map with the physical map. Meiotic recombination values assume no sex differences and no interference. Physical distances assume that the locus is halfway between two adjacent *Not* I sites except for the linking clone HMG14, which is located at the *Not* I site.

Giemsa-negative bands are believed to be rich in G+C and rich in genes. This is consistent with our work, since band q22.3 contains many *Not* I sites, which occur almost exclusively in CpG islands, typically located near genes.

Previous Maps of Chromosome 21q22.3. Using more than 60 unique sequence probes, Gardiner *et al.* (7) identified 33 independent *Not* I fragments and assigned these to 14 regions on the long arm of chromosome 21 with somatic cell hybrids. Except for two markers, S123 and S91, their work is consistent with our results. We located S123 and S91 between the 7;21 and 21;22 breakpoints, while Gardiner *et al.* (7) mapped S91 between the 21;22 and ring breakpoints, and mapped S123 in 21q22.1. Subsequently, a more distal localization was reported for S123 when the correct B88 probe was used (9).

Burmeister *et al.* (9) constructed a map of the distal region of the long arm of human chromosome 21, spanning ≈8 Mb from the q telomere to DNA marker S3. This map emphasized locus order rather than contiguity. They (9) did not order S141 and CRYA1, S25 and PFKL, but we were able to order these markers as follows: PFKL–S25–S141–CRYA1. What they report as a putative contiguous 4.9-Mb *Not* I map (9) spanning S42, S51, S15, S101, and S3 we find is missing 1.8 Mb of DNA contained in 10 additional *Not* I fragments.

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