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Genetics

Chromosome jumping from *D4S10* (G8) toward the Huntington disease gene

(molecular cloning/recombination/restriction fragment length polymorphism/pulsed field gel electrophoresis)

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ABSTRACT **The gene for Huntington disease (HD) has been localized to the distal portion of the short arm of human chromosome 4 by linkage analysis. Currently, the two closest DNA markers are *D4S10* (G8), located ≈3 centimorgans centromeric to HD, and *D4S43* (C4H), positioned 0–1.5 centimorgans from HD. In an effort to move closer to the HD gene, with the eventual goal of identifying the gene itself, we have applied the technique of chromosome jumping to this region. A 200-kilobase jumping library has been constructed, and a jump from *D4S10* has been obtained and its approximate distance verified by pulsed field gel electrophoresis. Two restriction fragment length polymorphisms have been identified at the jump locus, which is denoted *D4S81*. Linkage analysis of previously identified recombinants between *D4S10* and HD or *D4S10* and *D4S43* shows that in two of five events the jump has crossed the recombination points. This unequivocally orients *D4S10* and *D4S81* on the chromosome, provides additional markers for HD, and suggests that recombination frequency in this region of chromosome 4 may be increased, so that the physical distance from *D4S10* to HD may not be as large as originally suspected.**

A large number of human disorders are caused by mutations in single genes whose normal function, despite intensive investigation, is unknown. Recently, it has become possible to attempt the cloning of such genes by a process called "reverse genetics," in which the gene is identified primarily by its map position in the genome rather than by direct knowledge of its protein product (1, 2). Huntington disease (HD) is an autosomal dominant neurodegenerative condition with a wide range in age of onset (3, 4). The motor manifestations include incoordination and involuntary movements (chorea). Psychiatric features of forgetfulness, personality changes, depression, and eventual dementia are characteristic. Anatomically, the most pronounced changes are neuronal loss in the basal ganglia of the brain, especially the caudate nucleus; thus far, however, no specific protein abnormality has been found.

HD has been found to be tightly linked (5) to the DNA marker *D4S10* (G8), which has been mapped by *in situ* hybridization (6) and somatic cell genetics (7) to near the tip of the short arm of chromosome 4. Recent multipoint linkage analysis of markers on 4p has demonstrated that HD must lie distal to *D4S10* on 4p (8) and therefore is quite close to the telomere. This analysis also has allowed the *D4S10* sequence to be oriented on the chromosome by identifying an individual with a recombination within this locus. The genetic distance between *D4S10* and HD is estimated at 3 centimorgans (cM), with 95% confidence limits of 0.8–6 cM (8). On the average, 1 cM of genetic distance corresponds to ≈1000 kilobases (kb) of physical distance (9), but the rarity of DNA sequences in a chromosome 4 library that map distal to *D4S10* (10, 11) and the observed increase in chiasma formation near the telomeres of most autosomes (12) suggest that the physical distance between *D4S10* and HD might well be <3000 kb.

Very recently, another DNA marker, *D4S43*, has been identified that maps distal to *D4S10* and is therefore either closer to HD on the same side or is a flanking marker (11). Its distance from HD is estimated to be 0–1.5 cM.

We have undertaken chromosome jumping experiments to move from *D4S10* toward the HD gene. Chromosome jumping, as described by Collins and Weissman (13) and independently by Poutska and Lehrach (14), depends on the circularization of very large DNA fragments, followed by cloning of the junction fragments of these circles, which bring together DNA sequences that were originally located a considerable distance apart in the genome. We have previously demonstrated the successful application of this technique to a jump of 100 kb in the cystic fibrosis region (15). In this report, we describe the construction of a 200-kb jumping library and its successful utilization to move closer to the HD gene from *D4S10*.

MATERIALS AND METHODS

Cell Line. HHW661 is a somatic cell hybrid carrying a selectable human t(4;5) (p15.1;p15.1) translocation chromosome as its only human DNA (16).

Construction of 200-kb Jumping Library. Approximately 6 × 10^7 HHW661 cells were trypsinized, and high molecular weight DNA was purified in an agarose matrix (15, 17). A parallel flask of HHW661 was karyotyped and confirmed the presence of the human t(4;5) chromosome in virtually 100% of the cells. Construction of the 200-kb jumping library was carried out as described (15, 18), with the following exceptions. (*i*) Because the amount of DNA in the 100- to 300-kb region prior to partial *Mbo* I digestion was minimal as judged by pulsed field gel electrophoresis (PFGE), no pre-electrophoresis to eliminate small DNA fragments was performed. (*ii*) Optimum production of DNA in the 100- to 300-kb size range was obtained with 0.0074 unit of *Mbo* I per μg of agarose-embedded DNA. (*iii*) The DNA size range selected from the pulsed field gel was 160–240 kb. Confirmation of correct sizing and absence of degradation was carried out by analyzing a small amount of the electroeluted DNA by

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Abbreviations: HD, Huntington disease; PFGE, pulsed field gel electrophoresis; RFLP, restriction fragment length polymorphism.

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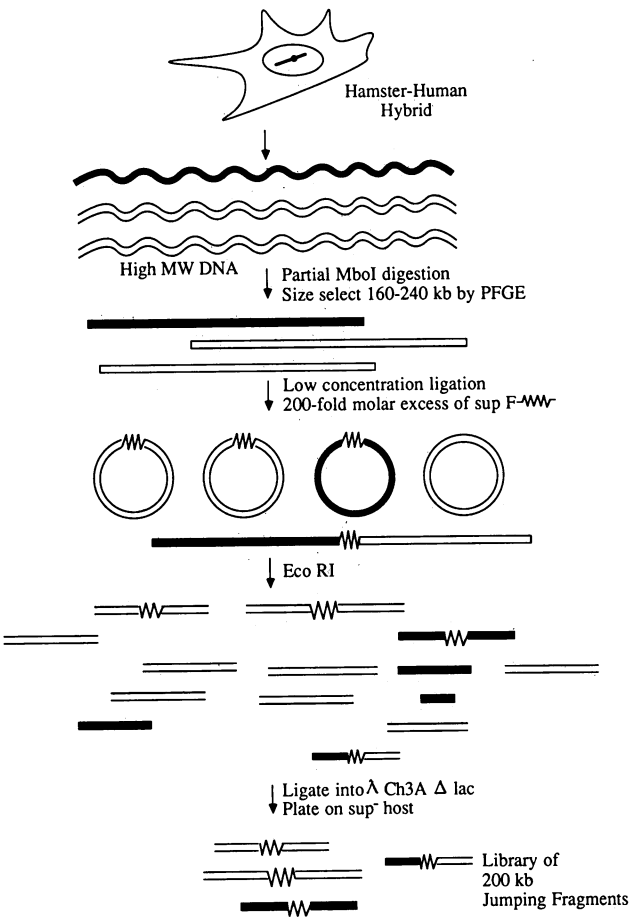

FIG. 1. Construction of a 200-kb jumping library from somatic cell hybrid (HHW661) containing a single human t(4;5) chromosome on a hamster background. Human sequences are represented as dark lines; hamster sequences are represented as open lines. Anomalous jumping fragments arising from the noncircular ligation of two different DNA molecules can be easily recognized since they connect human DNA to hamster DNA. *SupF* is a 219-bp suppressor tRNA gene that allows selective cloning of the junction fragments by cloning in an amber-mutated phage.

PFGE. (*iv*) The ligation reaction mixture contained 1.2 μg of size-selected DNA and 0.8 μg of *Bam*HI-ended supF (a suppressor tRNA gene) DNA, and was carried out in a volume of 7.5 ml for 24 hr at 4°C. T4 DNA ligase (12,000 units) (New England Biolabs) was added at the beginning and again 12 hr into the 18-hr ligation. (*v*) After *Eco*RI digestion of the circles, DNA was ligated into 70 μg of *Eco*RI-cut λCh3A Δlac, an amber-mutated phage vector that can accept *Eco*RI inserts from 0 to 12 kb (15, 18). (*vi*) A crude preparation of λ terminase (18, 19) was included in the final packaging reaction, since it improved efficiency by a factor of ≈3.

Library Screening. The primary library (2×10^6 jumping clones) was plated on bacterial host MC1061 ($supF^-$) at a density of 40,000 plaques per 150-mm plate and screened by standard techniques (15). Probes were labeled directly from LMT agarose (SeaPlaque, FMC, Rockland, ME) by the random hexamer priming method (20).

Subcloning and Mapping of Inserts. Small amounts of DNA were prepared from positive phage clones and subcloned by ligating *Eco*RI-digested phage DNA to phosphatase-treated, *Eco*RI cut, pBRΔAva DNA. pBRΔAva was derived from pBR322 by removal of the single *Ava* I site (18). Transformation of the *lacZ*am host CARD-15 [a *recA* derivative of CA274, genotype rK^+, mK^+, *hfrC*, *lacZ* (amber), *trp* (amber), *recA*, tet^R; gift of R. Dunn] with this ligated DNA and plating on MacConkey's agar plus ampicillin (50 μg/ml) allowed immediate identification of the desired subclones by their purple color, indicating the presence of the *supF* gene (13). The inserts were then mapped by digestion with *Eco*RI, *Ava* I (which cuts in the middle of the *supF* gene), and *Eco*RI plus *Ava* I, followed by Southern blotting with *supF*, human DNA, and hamster DNA sequentially as probes.

PFGE Mapping. Single, double, and partial digests of high molecular weight DNA embedded in LMT agarose were carried out as described (15, 21). DNA was separated by field inversion gel electrophoresis (22) using a programmable time ramp (DNAStar, Madison, WI); most gels used a forward ramp from 6 to 210 sec and a reverse ramp from 2 to 70 sec, operating over 72 hr at 130 V and a temperature of 14°C. Gels were 1% agarose in 0.0445 M Tris borate/0.0445 M boric acid/0.001 M EDTA. After electrophoresis and ethidium bromide staining, DNA was nicked by exposure to 254 nm UV light for 1 min and then denatured and transferred to Hybond (Amersham) or GeneScreen (New England Nuclear) using 0.4 M NaCl/1.5 M NaOH. A few gels were run using an orthogonal field alternating gel electrophoresis (OFAGE) apparatus (23). Hybridization and washing were carried out as described (21). All restriction enzymes were from New England Biolabs.

Restriction Fragment Length Polymorphism (RFLP) Identification. High molecular weight DNAs isolated from lymphoblastoid lines of five unrelated individuals were digested with 30 restriction enzymes, fractionated on agarose gels, and subjected to Southern blot analysis with single copy subclones from the HDA29 clone as probes to identify RFLPs. Individual variations in the pattern of restriction fragments with *Bgl* II and *Sac* I were further characterized in 34

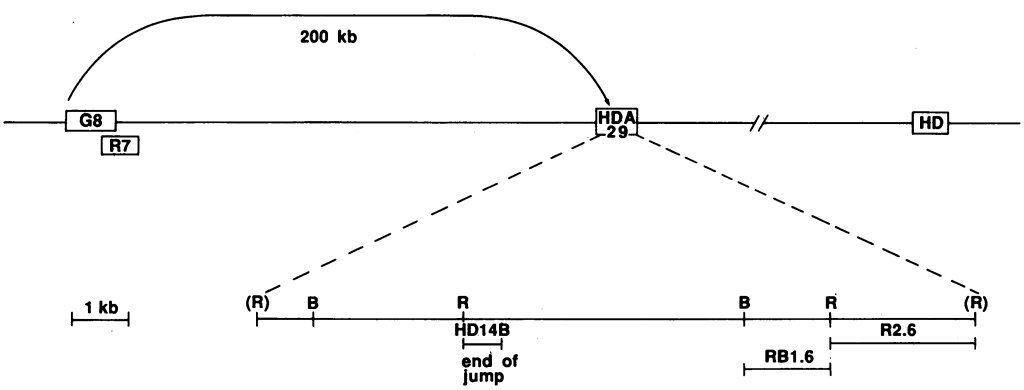

FIG. 2. Diagram of the 200-kb jump from G8. The start point of the jumping clone HD14B is in the pK083 subclone of G8, at its most centromeric end. The end of the jump was used as a probe to obtain a phage clone, HDA29, whose map is shown [B, *Bam*HI; R, *Eco*RI; (R), *Eco*RI site present in clone that is not derived from genomic DNA]. Probes RB1.6 and R2.6 were subsequently found to detect RFLPs, and the HDA29 locus has been designated *D4S81*.

unrelated North American individuals of northern European ancestry to determine allele frequencies and were typed in several nuclear families to establish that they displayed Mendelian segregation.

RESULTS

The protocol for generating a jumping library of 200-kb jump size from the human–hamster cell line HHW661 is illustrated in Fig. 1.

Screening the library of 2×10^6 clones with plasmid subclones pK081, pK083, and pK084 of the original G8 phage (5), and also with the 14-kb *Eco*RI insert of R7, an additional clone that overlaps G8 to the HD side (8), produced a total of seven positive phage. No positive clones were obtained upon screening with the C4H clone from *D4S43* (11), but this is not surprising given the nature of the strategy shown in Fig. 1 and the fact that C4H resides near the middle of a large (≈10 kb) *Eco*RI fragment; jumping clones starting from C4H would thus be likely to exceed the vector cloning capacity.

Of the seven clones, three contained hamster sequences and represent DNA molecules that must have failed to properly circularize in the low concentration ligation. One clone contained a jumping fragment too small [<50 base pairs (bp)] to map. The remaining three jumps landed in human sequences; since only ≈3% of the DNA in HHW661 is human, such human–human junction fragments are unlikely to occur except by circular ligation.

From the anatomy of a jump clone, it is possible to determine which direction the jump has travelled. The previous determination that HD should lie on the R7 side of G8 (8) led us to focus on clone HD14B, which should represent a 200-kb jump toward the HD locus; the other two human jumps travelled back toward the centromere. The new DNA fragment in HD14B was 600 bp long and contained an *Alu* repeat sequence at one end; digestion with *Bgl* II, however, separated the repetitive from the nonrepetitive parts of this jump. The resulting 400-bp fragment mapped correctly to the distal part of chromosome 4 (data not shown). It did not detect any RFLPs, however, in a screen using 30 enzymes and 5 individuals. Therefore, this probe was used to screen a λ genomic library prepared from a hybrid containing only human 4p on a hamster background (16). A single positive clone (HDA29) was obtained. A partial restriction map of this clone is shown in Fig. 2. Fragments RB1.6 and R2.6 are single copy and were used for PFGE mapping and RFLP analysis. Mapping these fragments on a hybrid panel (16) confirmed that they reside on distal 4p (data not shown).

The distance of the jump from G8 was estimated by PFGE mapping. As shown in Fig. 3 (*Upper Left*), RB1.6 and R7 hybridize to the same *Bss*HII primary band of 240 kb, and the identity of the partial digest bands as well indicates that this is not due to coincidental migration of the fragments. Probing the same blot with pK083, however, which is located at the centromeric side of G8, gives a different *Bss*HII band of 40 kb. This indicates the presence of a *Bss*HII site within the G8/R7 region, which has been confirmed by digestion of the cloned DNA (data not shown).

The field inversion gel electrophoresis data in Fig. 3 (*Upper Right*) show that R7 and RB1.6 hybridize to different *Sfi* I and *Mlu* I fragments, but the same *Nru* I fragment. The partial *Mlu* I digest pattern suggests the R7 and RB1.6 *Mlu* I fragments are not adjacent. Analysis of additional controlled partial *Mlu* I digests, with or without *Bss*HII digestion (data not shown), leads to the map in Fig. 3 (*Lower*).

The pulsed field data indicate that HDA29 is between 170 kb and 230 kb away from G8, in good agreement with the size selection used in construction of the jumping library. The pulsed field gel blots have also been reprobed with DNA segments from the more distal *D4S43* locus (11), including

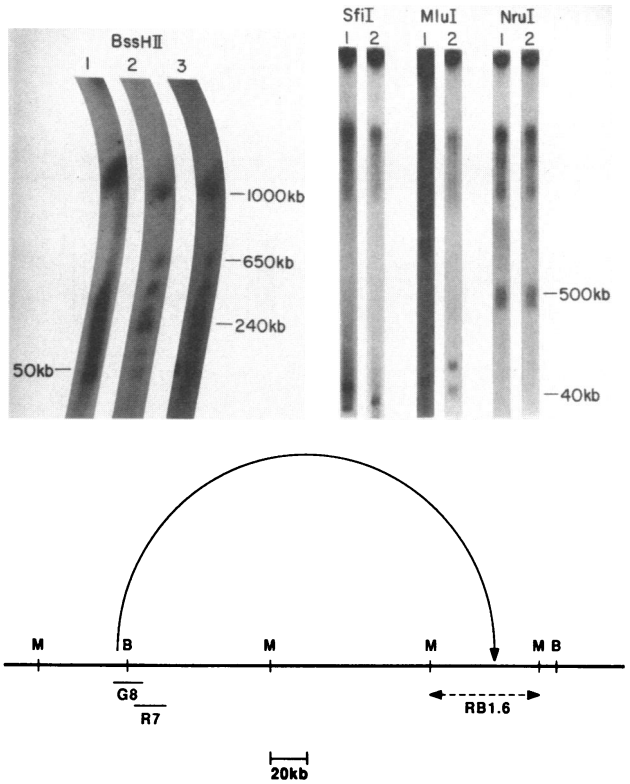

FIG. 3. PFGE mapping of the jump. (*Upper Left*) A partial *Bss*HII digest has been electrophoresed on an OFAGE gel and the blot was sequentially probed with pK083, R7-11 (a 7-kb *Not* I/*Eco*RI subclone of R7), and RB1.6. The blot was completely stripped between hybridizations. R7-11 and RB1.6 detect the same 240-kb fragment as well as the same partial digest pattern, indicating that they must be no more than 240 kb apart. The different pattern seen with pK083 indicates the presence of a restrictable *Bss*HII site in the G8 region. (*Upper Right*) *Mlu* I, *Sfi* I, and *Nru* I digests were separated by field inversion gel electrophoresis and probed with R7-11 and RB1.6. The *Mlu* I and *Sfi* I patterns are different, but the *Nru* I band is the same. (*Lower*) Partial restriction map of the region of the jump, using the *Bss*HII site within G8 to anchor the map. The distance of the jump (which begins in pK083) is seen to be 170–230 kb. M, *Mlu* I; B, *Bss*HII.

C4H and a probe (C42-RB1.8) obtained by walking 140 kb from C4H (kindly provided by H. Lehrach, A. M. Frischauf, and M. Zimmer). As yet, no overlapping fragments have been observed between HDA29 and C4H, indicating that the physical distance between them is at least 300–500 kb.

The RB1.6 and R2.6 probes from HDA29 were used to search for RFLPs in a panel of five unrelated individuals. RB1.6 detected a *Bgl* II polymorphism and R2.6 detected two uncommon *Sac* I polymorphisms (Table 1). These probes were used in linkage analysis on a set of reference pedigrees. Of 77 individuals informative for *D4S10*, HDA29, and *D4S43*, there were 3 with crossovers between *D4S10* and *D4S43*. In 2 of these the point of crossover could be identified as lying between *D4S10* and HDA29; in 1 the crossover point must lie between HDA29 and *D4S43*. Fig. 4 shows two of these

Table 1. RFLPs detected by HDA29 (*D4S81*)

Probe	Enzyme	Allele	Length, kb	Frequency
RB1.6	*Bgl* II	1	6.7	0.21
		2	6.5	0.79
R2.6	*Sac* I	1	7.5	0.06
		2	7.0	0.94
R2.6	*Sac* I	1	2.2	0.03
		2	2.0	0.97

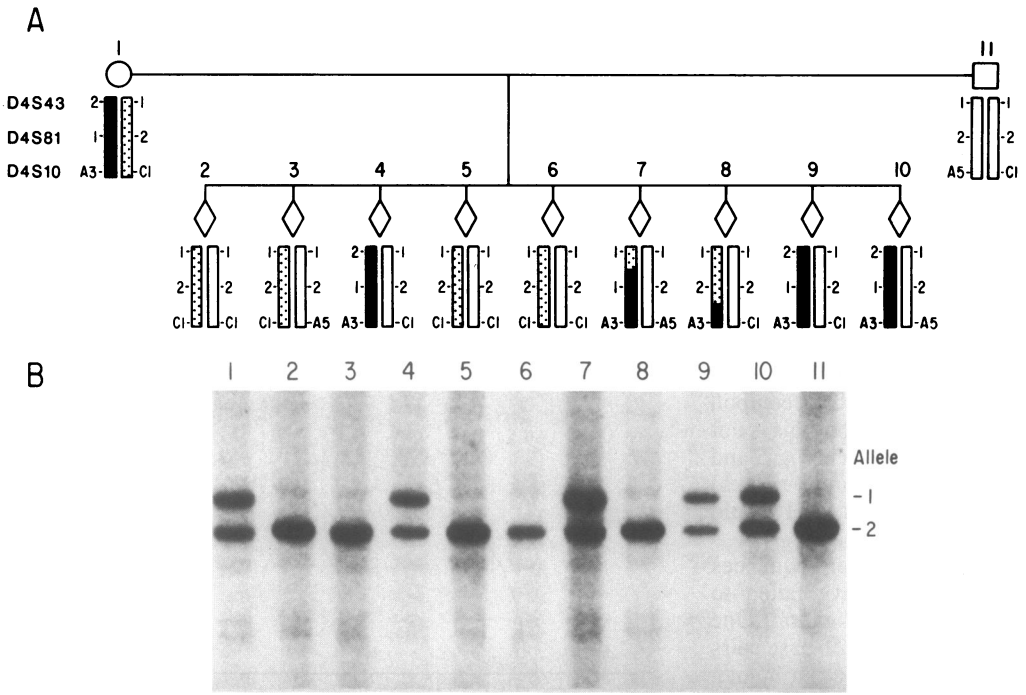

FIG. 4. Genetic localization of *D4S81* between *D4S10* and *D4S43*. *D4S81* was typed in a sibship of the extended Venezuela reference pedigree already known (12) to reveal two recombination events between *D4S10* and the more distal marker *D4S43* (individuals 7 and 8). The progeny of this mating are denoted as diamonds to protect the confidentiality of the family. (*A*) Typing results for all three marker loci in this sibship, with different shading provided to distinguish the two maternal chromosomes. (*B*) The data for the *D4S81 Bgl* II RFLP indicate that the recombination event detected in individual 7 occurred distal to *D4S81*, while that detected in individual 8 occurred somewhere in the ≈200 kb between *D4S81* and *D4S10*.

informative events from a single family. A total of 15 individuals with previously defined *D4S10*–HD crossovers were also studied. Of these, only 2 were informative for HDA29, and in both instances the recombination point mapped between HDA29 and HD. The HDA29 region has been designated *D4S81* by Human Gene Mapping Workshop 9 (24).

DISCUSSION

Until recently, the idea of moving along a chromosome from a tightly linked DNA marker to identify a gene causing human disease was impractical, largely because of the "resolution gap" problem. The gap exists because distances on the order of 1 cM, which are small in linkage terms, are still very large (on the order of 1000 kb) in molecular terms. Chromosome walking (25), which uses the end of one clone (a phage or a cosmid) to rescreen a library and obtain a new clone, can be expedited by several techniques (26–28) but is still tedious if more than a few steps must be taken and can be blocked if regions of recombinogenic or highly repetitive sequence are encountered. The recent development of PFGE has opened a window of size resolution (up to 10,000 kb) for physical mapping experiments (9, 17), but cloning specific fragments from PFGE gels is difficult (29). In some instances, the problem can be approached by construction of somatic cell hybrids bearing a small chromosomal fragment around the area of interest, but unless a selectable gene is fortuitously close by, this can require a great deal of labor. An exciting recent development is the successful cloning of 50- to 500-kb fragments of human DNA as artificial chromosomes in a yeast system (30). As yet, however, no complete genomic libraries of this sort have been reported, and it is not clear whether some regions of the human genome will be unclonable or prone to rearrangement.

Chromosome jumping (13–15) offers another alternative for crossing large molecular distances. The scheme shown in Fig. 1 can in theory be applied to any desired jump size, although the difficulty in constructing a library goes up roughly as the 3/2 power of the length of the jump (18). Since the size-selected DNA is generated by partial *Mbo* I digestion, the library should be accessible from nearly any starting point. Note that this approach differs from that of circularizing the products of a complete digest of DNA by enzymes such as *Not* I, which cut only rarely in human DNA (31); this latter sort of jumping library requires far fewer clones but is only usable when beginning from a region containing such a rare site. Although the scheme in Fig. 1 requires high efficiency of all the steps to obtain a deep enough library, in practice the library can be subsequently amplified and reused many times. Jumping has the additional potential advantage of allowing one to cross over otherwise unclonable sequences.

We have previously reported construction of a 100-kb human jumping library and shown that it could be used to move toward the cystic fibrosis locus from the closely linked *met* oncogene on chromosome 7 (15). Subsequent use of this 100-kb library for several other applications has demonstrated that ≈95% of the clones connect syntenic DNA fragments, as expected if the circular ligation was successful (F.S.C., J.L.C., M.L.D., and M. Iannuzzi, unpublished data).

In this report, we have described extension of the approach to larger jumps and have successfully derived a clone that jumps from the *D4S10* locus ≈200 kb toward the HD gene. This library was purposefully constructed from a somatic cell hybrid to provide a rapid means of checking the validity of the jumping clones. A major risk of the protocol in Fig. 1 is the possibility that genomic DNA sequences that do not reside at

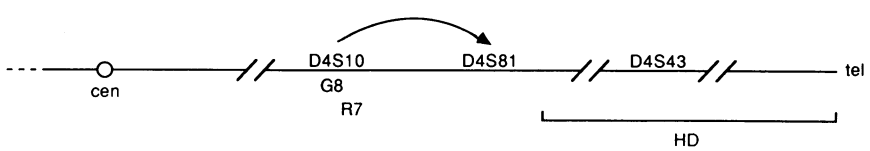

FIG. 5. Summary of the map of the tip of chromosome 4p.

the opposite ends of the same fragment will ligate together with a *supF* gene in between them; such junction fragments will appear in the library but will connect unrelated sequences. Three of seven jumping clones studied connected human and hamster sequences and must be examples of this phenomenon. Such junctions can arise in three ways: (*i*) failure to ligate at a low enough DNA concentration; (*ii*) the presence of molecules with one sticky end and one sheared end, which cannot circularize, but can form dimers with other molecules like themselves; (*iii*) failure of the circularization ligation to go to completion. In this last event, the subsequent ligation to the vector, carried out at high DNA concentration, can allow remaining unligated *Mbo* I/*Bam*HI ends to come together. The first problem can be directly controlled, and at the ligation concentration used here >90% of the ligations should have been circular. The second problem is difficult to assess, and can only be avoided by careful handling of the DNA. The third problem, however, was probably responsible for the background of anomalous junction fragments in this library; retrospective analysis indicated that the ligation, as assessed by multimerization of the *supF* gene, had only proceeded to ≈50% completion at this step (data not shown). The problem can be avoided in the future by including a phosphatase or Klenow fill-in step after the circularization to inactivate any unligated ends.

The HD14B clone, however, arose from a valid circularization and jumps from the *D4S10* marker a distance of ≈200 kb in the direction previously predicted on the basis of an intra-*D4S10* recombinant to be toward HD. The identification of two RFLPs detected by probes from the region of this jump has allowed further unequivocal verification that this is the correct orientation of *D4S10* on the chromosome. To our knowledge, this is the first crossing of recombination sites by chromosome jumping. The fact that the jump has traversed the crossover points in two of five informative *D4S10–D4S43* or *D4S10*–HD recombinants indicates that the gene order must be cen-G8-R7-HDA29-HD-pter, as shown in Fig. 5. That a jump of 200 kb in physical terms has crossed a substantial fraction of the 3-cM genetic distance between *D4S10* and HD is indicative that this region around *D4S10* is relatively recombination-rich. If not a statistical fluke, it is possible that this is due to a recombination "hot spot" (or hot spots) in this region. If, on the other hand, it reflects a general tendency for recombination to occur more frequently near the telomere of 4p, then the HD gene may lie less than a million bases away. Intense further efforts to cross this distance by using jumps from *D4S81*, *D4S43*, and other probes being generated in this region (32) are therefore highly in order.

Note Added in Proof. An additional DNA marker, *D4S95*, has recently been reported that is distal to *D4S10* and closely linked to HD (33).

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1. Ruddle, F. (1984) *Am. J. Hum. Genet.* **36**, 944–953.
2. Orkin, S. H. (1986) *Cell* **47**, 845–850.
3. Conneally, P. M. (1984) *Am. J. Hum. Genet.* **36**, 506–526.
4. Martin, J. B. & Gusella, J. F. (1986) *N. Engl. J. Med.* **315**, 1267–1276.
5. Gusella, J. F., Wexler, N. S., Conneally, P. M., Naylor, S. L., Anderson, M. A., Tanzi, R. E., Watkins, P. C., Ottina, K., Wallace, M. R., Sakaguchi, A. Y., Young, A. B., Shoulson, I., Bonilla, E. & Martin, J. B. (1983) *Nature (London)* **306**, 234–238.
6. Magenis, R. E., Gusella, J., Weliky, K., Olson, S., Haight, G., Toth-Fejel, S. & Sheehy, R. (1986) *Am. J. Hum. Genet.* **39**, 383–391.
7. Gusella, J. F., Tanzi, R. E., Bader, P. I., Phelan, M. C., Stevenson, R., Hayden, M. R., Hofman, K. J., Faryniarz, A. G. & Gibbons, K. (1985) *Nature (London)* **318**, 75–78.
8. Gilliam, T. C., Tanzi, R. E., Haines, J. L., Bonner, T. I., Faryniarz, A. G., Hobbs, W. J., MacDonald, M. E., Cheng, S. V., Folstein, S. E., Conneally, P. M., Wexler, N. S. & Gusella, J. F. (1987) *Cell* **50**, 565–571.
9. Smith, C. L., Lawrance, S. K., Gillespie, G. A., Cantor, C. R., Weissman, S. M. & Collins, F. S. (1987) *Methods Enzymol.* **151**, 461–489.
10. Gilliam, T. C., Healey, S. T., MacDonald, M. E., Stewart, G. D., Wasmuth, J. J., Tanzi, R. E., Roy, J. C. & Gusella, J. F. (1987) *Nucleic Acids Res.* **15**, 1445–1458.
11. Gilliam, T. C., Bucan, M., MacDonald, M. E., Zimmer, M., Haines, J. L., Cheng, S. V., Pohl, T. M., Meyers, R. H., Whaley, W. L., Allitto, B. A., Faryniarz, A., Wasmuth, J. J., Frischauf, A. M., Conneally, P. M., Lehrach, H. & Gusella, J. F. (1987) *Science* **238**, 950–952.
12. Laurie, D. A. & Hulten, M. A. (1985) *Ann. Hum. Genet.* **49**, 189–201.
13. Collins, F. S. & Weissman, S. M. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 6812–6816.
14. Poutska, A. & Lehrach, H. (1986) *Trends Genet.* **2**, 174–179.
15. Collins, F. S., Drumm, M. L., Cole, J. L., Lockwood, W. K., Vande Woude, G. F. & Iannuzzi, M. C. (1987) *Science* **235**, 1046–1049.
16. Wasmuth, J. J., Carlock, L. R., Smith, B. & Immken, L. L. (1986) *Am. J. Hum. Genet.* **39**, 397–403.
17. Smith, C. L., Warburton, P. E., Gaal, A. & Cantor, C. R. (1986) in *Genetic Engineering*, eds. Setlow, J. K. & Hollaender, A. (Plenum, New York), Vol. 8, pp. 45–70.
18. Collins, F. S. (1988) in *Gene Technology: A Practical Approach*, ed. Davies, K. (IRL, London), in press.
19. Rackwitz, H. R., Zehetner, G., Murialdo, H., Delius, H., Chai, J. H., Poustka, A., Frischauf, A. & Lehrach, H. (1985) *Gene* **40**, 259–270.
20. Feinberg, A. P. & Vogelstein, B. (1984) *Anal. Biochem.* **137**, 266–267.
21. Drumm, M. L., Smith, C. L., Dean, M., Cole, J. L., Iannuzzi, M. C. & Collins, F. S. (1988) *Genomics*, in press.
22. Carle, G. F., Frank, M. & Olson, M. V. (1986) *Science* **232**, 65–68.
23. Carle, G. F. & Olson, M. V. (1984) *Nucleic Acids Res.* **12**, 5647–5664.
24. Report of Human Gene Mapping Workshop 9 (1987) *Cytogenet. Cell Genet.*
25. Bender, W., Spierer, P. & Hogness, D. S. (1983) *J. Mol. Biol.* **168**, 17–33.
26. Poustka, A., Rackwitz, H. R., Frischauf, A. M., Hohn, B. & Lehrach, H. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 4129–4133.
27. Little, P. F. R. & Cross, S. H. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 3159–3163.
28. Wahl, G. M., Lewis, K. A., Ruiz, J. C., Rothenberg, B., Zhao, J. & Evans, G. A. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 2160–2164.
29. Michiels, F., Burmeister, M. & Lehrach, H. (1987) *Science* **236**, 1305–1308.
30. Burke, D. T., Carle, G. F. & Olson, M. V. (1987) *Science* **236**, 806–810.
31. Poustka, A., Pohl, T. M., Barlow, D. P., Frischauf, A. M. & Lehrach, H. (1987) *Nature (London)* **322**, 32–38.
32. Smith, B., Skarecky, D., Bengtsson, U., Magenis, R. E., Carpenter, N. & Wasmuth, J. J. (1988) *Am. J. Hum. Genet.* **42**, 335–344.
33. Wasmuth, J. J., Hewitt, J., Smith, B., Allard, D., Haines, J. L., Skarecky, D., Partlow, E. & Hayden, M. R. (1988) *Nature (London)* **332**, 734–736.