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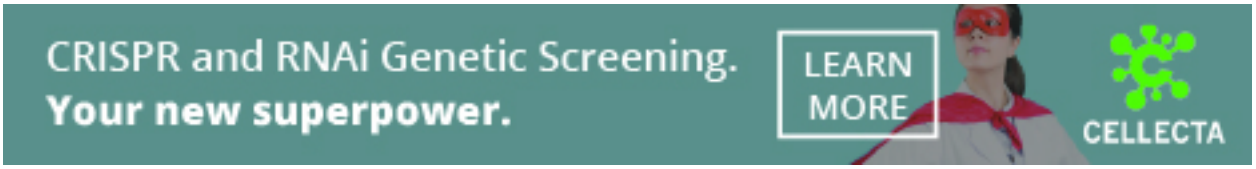

RESEARCH

The Organization of the Human Immunoglobulin λ Gene Locus

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To elucidate the complex structure of the human immunoglobulin λ gene locus, a 1020-kb contig was constructed using 184 cosmid clones and one bacterial artificial chromosome (BAC) clone. A high-resolution physical map of this contig revealed that the entire λ gene locus is 911 kb in length. It contains seven constant region (C_λ) gene segments and 69 unique *Eco*RI–*Hin*dIII segments that hybridize to variable region gene (V_λ) probes. The VpreB gene, BCRL4, and γ-glutamyl transpeptidase gene (GGT)-like sequences are also located within the λ gene locus. Hybridization analysis suggested that the λ gene locus has undergone extensive amplification events in evolution.

Immunoglobulin molecules are composed of light (L) and heavy (H) chains, each consisting of variable (*V*) and constant (*C*) regions. There are two types of light chains, κ and λ, each encoded by separate genes on different chromosomes (Tonegawa 1983; Lai et al. 1989). Although the human V_H locus (Matsuda et al. 1993; Cook et al. 1994) and the V_κ locus (Zachau 1993) have been studied extensively, little is known about the V_λ locus, located within the chromosome 22q11 region (Lai et al. 1989).

A 124-kb region, including the C_λ locus, has been precisely described in detail (Combriato and Klobeck 1991). It contains five V_λ and seven C_λ gene (pseudogene) segments, and each C_λ is preceded by a single joining region (J_λ; carboxyl portion of the V region) gene segment (Vasicek and Leder 1990). However, pulsed-field gel electrophoresis (PFGE) mapping suggested that the entire λ gene locus was >124 kb, and it appeared that the V_λ gene subgroups apparently cluster along the locus (McDermid et al. 1993). The extensive repertoire of V_λ gene segments (Kabat et al. 1991) suggests that the majority of the locus has yet to be described. The VpreB gene (Bauer et al. 1988a), BCRL4 (Croce et al. 1987), and a γ-glutamyl transpeptidase gene (GGT)-like sequence (Heisterkamp and Groffen 1988; Morris et al. 1993) have also been localized close to the V_λ locus (Kawasaki et al. 1994).

To precisely analyze these complex loci, we had previously employed the "shotgun PCR" method (Kawasaki et al. 1992a). A 1.4-Mb *Not*I fragment containing the λ gene locus was isolated from flow-sorted human chromosome 22 (Minoshima et al. 1990; Kawasaki et al. 1992b). Small DNA fragments (~300 bp) were then amplified from the *Not*I fragment by linker ligation PCR. Using these DNA fragments and other DNA probes, we isolated yeast artificial chromosomes (YACs) encompassing the region. Hybridization of these YACs to a chromosome 22-specific cosmid library, fingerprinting the obtained clones, and subsequent chromosome walking with cosmid clones and bacterial artificial chromosome (BAC; Shizuya et al. 1992) clones enabled us to construct a contig of 1020 kb in length. A high-resolution physical map of this contig elucidated the locations of 69 unique *Eco*RI–*Hin*dIII segments after probing with four distinct V_λ probes. The VpreB gene, BCRL4, and a GGT-like sequence were also localized within the contig. Southern analysis suggested the evolutionary history that the λ gene locus was involved in extensive amplification.

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RESULTS

A YAC Contig

Three probes, D22S10, 12D5, and HuCλ2, were used to screen YAC libraries. This resulted in the isolation of four YACs (yS10-8, yS10-1, yCλ-11, and yCλ-5) from a RIKEN library and two YACs (191A11 and 35B2) from a CEPH library. The YAC ends were then subcloned and used to determine overlap, resulting in contiguous YAC ordering (Fig. 1).

Southern hybridization using two V_λ probes, pHλ6 and Vλ3C4, confirmed the order of YAC clones (Fig. 2). Only one band, a 3.3-kb fragment in the genomic DNA (arrowhead in Fig. 2a), is missing from the YAC contig. This suggests that >95% of the V_λ gene segments detected by these probes are included in the YAC contig.

Generation of Cosmid Contigs

Some of the YAC clones (yS10-1 and yS10-8) were unstable and/or chimeric. Hence, a chromosome 22-specific cosmid library, consisting of 12,480 clones, was screened with four YACs and four distinct V_λ probes, pHλ6, Vλ3C4, 4A, and Vλ2.DS. The YAC probes yS10-1, 35B2, 191A11, and yCλ-11 detected 368, 338, 310, and 230 cosmid clones, respectively. The four V_λ probes detected a total of 196 clones. A large portion of these clones were positive for two or more probes and a total of 569 unique clones were identified.

Fingerprinting of these cosmids generated four contigs within the λ gene locus (Fig. 3, contigs I-1, II-1, II-2, and II-3) consisting of 148 clones. The overlapping of these 148 clones corroborates the size of this cosmid library (7.4× equivalent of chromosome 22). A large portion of the other cosmid clones (i.e., 421 clones; 569 YAC-positive clones minus 148 clones in the λ locus) formed several contigs of related sequences for the C_λ gene (McDermid et al. 1993), BCR (Croce et al. 1987) or GGT (Heisterkamp and Groffen 1988; Morris et al. 1993) (data not shown).

Chromosome Walking

To link these four contigs, chromosome walking was initiated from each terminus, generating two large cosmid contigs (Fig. 3, contigs I and II) consisting of 184 clones. A BAC library (29,952 clones with an average insert size of 110 kb) was then screened to fill the gap between these two contigs using the proximal end of contig I and the distal end of contig II as probes. One BAC clone, 288A10 (Fig. 3), was detected by both of these probes.

High-resolution Physical Map

A high-resolution restriction map was constructed by partial digestion analysis of the 69 favorably overlapping cosmid clones and the single BAC clone (Fig. 4). A previous report revealed variation in the number of C_λ gene segments (Taub et al. 1983). Comparing the obtained restriction map to a recent investigation (Vasicek and Leder 1990), seven J_λ and C_λ gene segments (Fig. 4, JC1-7) are localized. The four V_λ probes hybridized to a total of 69 unique *Eco*RI–*Hin*dIII segments with various signal intensities (Fig. 5). These segments have been tentatively named 1–69 (Figs. 4 and 5). Southern hybridization analysis revealed the locations of 12D5, D22S10, and D22S68 (Fig. 4). These three probes hybridized weakly to several other locations within the λ gene

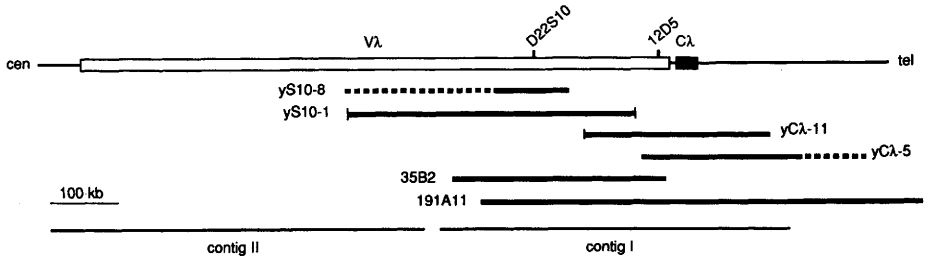

Figure 1 A YAC contig map within the λ gene locus. (*Top*) Locations of the V_λ (open box) and C_λ (solid box) loci and two probes, D22S10 and 12D5. Six YACs (35B2, yS10-1, yS10-8, 191A11, yCλ-11, and yCλ-5) are represented with solid lines and broken lines in which chimeric regions are found. Five YAC ends (both termini of yS10-1 and one terminal each of yS10-8, yCλ-11, and yCλ-5) were subcloned from λ phage libraries constructed from *Hin*dIII digests of total transformant DNAs. Both ends of yS10-1 and the proximal end of yCλ-11 (vertical bars) were mapped to chromosome 22 by using somatic cell hybrids or by fluorescent in situ hybridization analysis (FISH; Kawasaki et al. 1992b). Locations of cosmid contigs I and II (see Fig. 3) are also represented with horizontal lines.

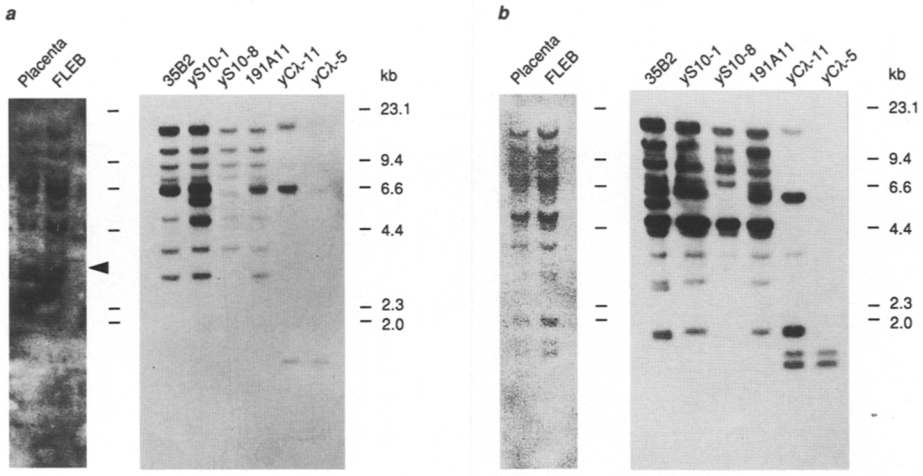

Figure 2 Southern hybridization analysis of YAC and genomic DNAs. DNA was prepared from placenta, a pre-pro-B cell line FLEB14-14, and six YAC clones (35B2, yS10-1, yS10-8, 191A11, yCλ-11, and yCλ-5). The DNA was then digested with *Eco*RI and analyzed using two V_λ probes, pHλ6 (*a*) and Vλ3C4 (*b*). From genomic DNA, at least 11 and 16 bands were detected using pHλ6 and Vλ3C4 probes, respectively. All of these bands except the 3.3-kb fragment detected by pHλ6 (arrowhead) are found in the six YAC clones.

locus (Fig. 5). The 3′-BCR probe also gave 1 strong and 12 weak signals in regions I and II (Fig. 5). From a published restriction map (Croce et al. 1987), the strongest signal was recognized as BCRL4. A GGT-like sequence was also found adjacent to the BCRL4 locus as described (Heisterkamp and Groffen 1988). The VpreB gene (Bauer et al. 1988a) was then localized between regions III and IV.

DISCUSSION

In this study a 1020-kb contig covering the entire λ gene locus was constructed using 184 cosmid clones and one BAC clone. A high-resolution restriction map for *Eco*RI, *Hin*dIII, and *Sfi*I sites was then generated, and seven J_λ–C_λ gene segments as well as 69 unique *Eco*RI–*Hin*dIII segments detected by V_λ probes were localized within a 911-kb region.

It is possible that some of the V_λ gene segments contain *Eco*RI or *Hin*dIII sites; hence, the V_λ probes would hybridize to two adjacent *Eco*RI–*Hin*dIII segments. It is also possible that several different V_λ gene segments reside in a single *Eco*RI–*Hin*dIII fragment. Considering that the V_λ gene segments are small (~300 bp) and that some of the V_λ-positive *Eco*RI–*Hin*dIII segments are relatively large (e.g., 15 is 7.7 kb), the latter case is more likely. Therefore, the total number of the V_λ gene and pseudogene segments is probably >69. This is approximately equal to the total number of V_κ gene segments (Zachau 1993) or one-half the total number of V_H gene segments (Matsuda et al. 1993; Cook et al. 1994).

While this paper was under review, Frippiat et al. (1995) reported a physical map of the λ gene locus using YAC deletion sets. Because some regions of their physical map do not possess sufficient resolution, the distribution of the V_λ gene segments does not seem to corroborate our study. For example, we have localized nine V_λ gene segments in region V (Fig. 4), whereas Frippiat et al. (1995) placed only five gene segments in the corresponding region. Our preliminary sequencing data on this region V validated the presence of nine V_λ gene segments. Interestingly, a gap appearing in their YAC contig coincides with the gap between our cosmid contigs I and II (Fig. 4). This gap was covered by a BAC clone in our study.

The 69 V_λ-positive fragments seem to be distributed into five clusters (Fig. 4, regions I–V) and the clusters are each separated by a ⩾40-kb. Unlike the V_H (Matsuda et al. 1993) and V_κ (Weichhold et al. 1993) loci, no apparent repeats of restriction sites are detected. However, hybridization analysis (Fig. 5) revealed a characteristic organization of the V_λ gene locus. Region I can be divided into two different portions, the upstream and downstream portions. The upstream portion of region I is rich in V_λ gene segments and contains more positive segments for D22S68 and 3′-BCR probes as compared to the downstream portion. The probe 12D5 hybridized to two distinct locations within the downstream portion of region I, suggesting that this portion can be divided further into two small units. Thus, multiple amplifications may have been involved in the generation of region I.

The D22S10 probe-positive segments are distributed only within region I, whereas 3′-BCR-

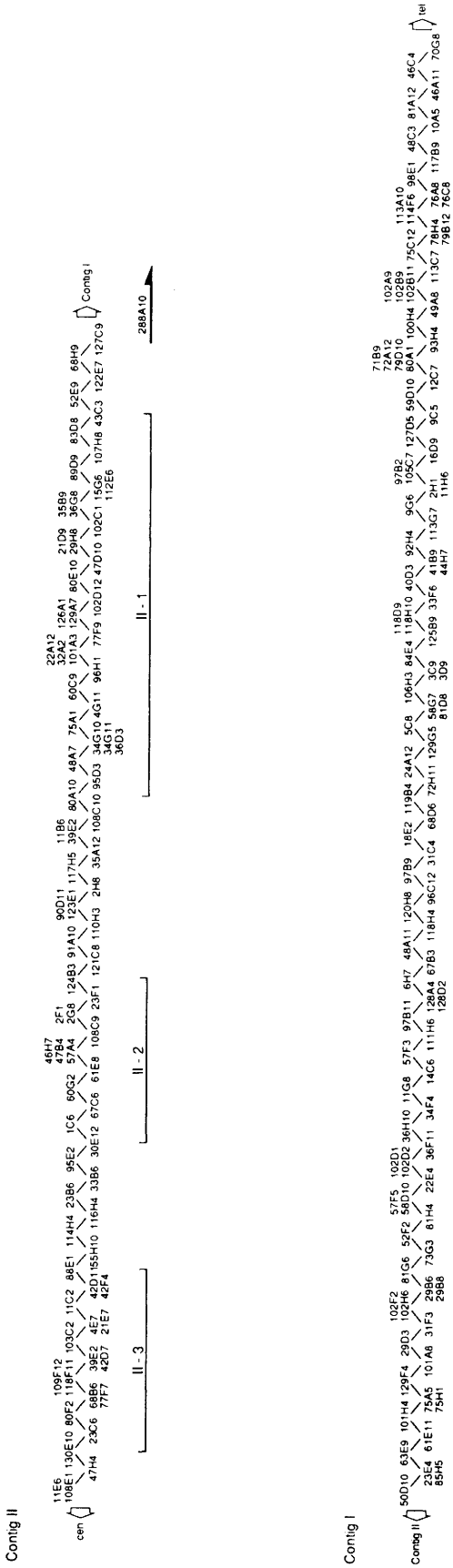

Figure 3 A large contig consisting of 184 cosmid clones and one BAC clone encompassing the λ gene locus. Cosmid clones extending upstream of this locus are placed to the *left*. Initially, four contigs (I-1, II-1, II-2, II-3) consisting of 148 cosmids were established by fingerprinting. Three contigs (II-1, II-2, II-3) were then linked by chromosome walking and two large contigs (I and II) of 184 clones were generated. These two cosmid contigs were joined eventually by the BAC clone 288A10.

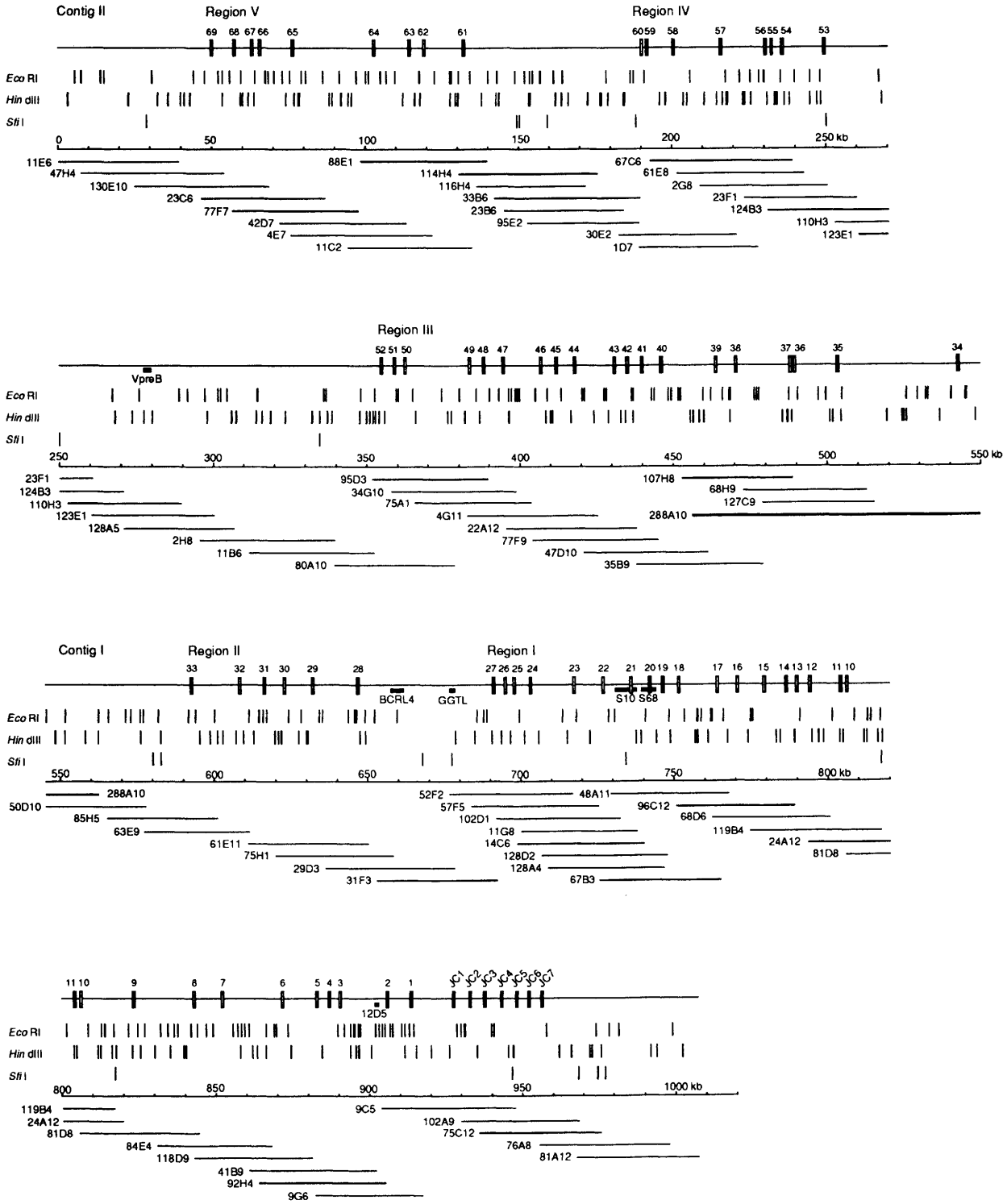

Figure 4 A high-resolution physical map of the λ gene locus. (*Top* lines) Locations of the DNA segments detected by the four V_λ probes (open boxes: 1–69) and the J_λ–C_λ gene segments (solid boxes; JC1–7). Open boxes are placed in the middle of *Eco*RI–*Hin*dIII segments that were positive for the V_λ probes. Locations of the VpreB gene, BCRL4, a GGT-like sequence (GGTL), D22S10 (S10), D22S68 (S68), and 12D5 are represented with horizontal bars. The locations of *Eco*RI, *Hin*dIII, and *Sfi*I sites were determined for 69 favorably overlapping cosmids and one BAC clone shown below the scale. Five V_λ clusters were defined as regions I–V.

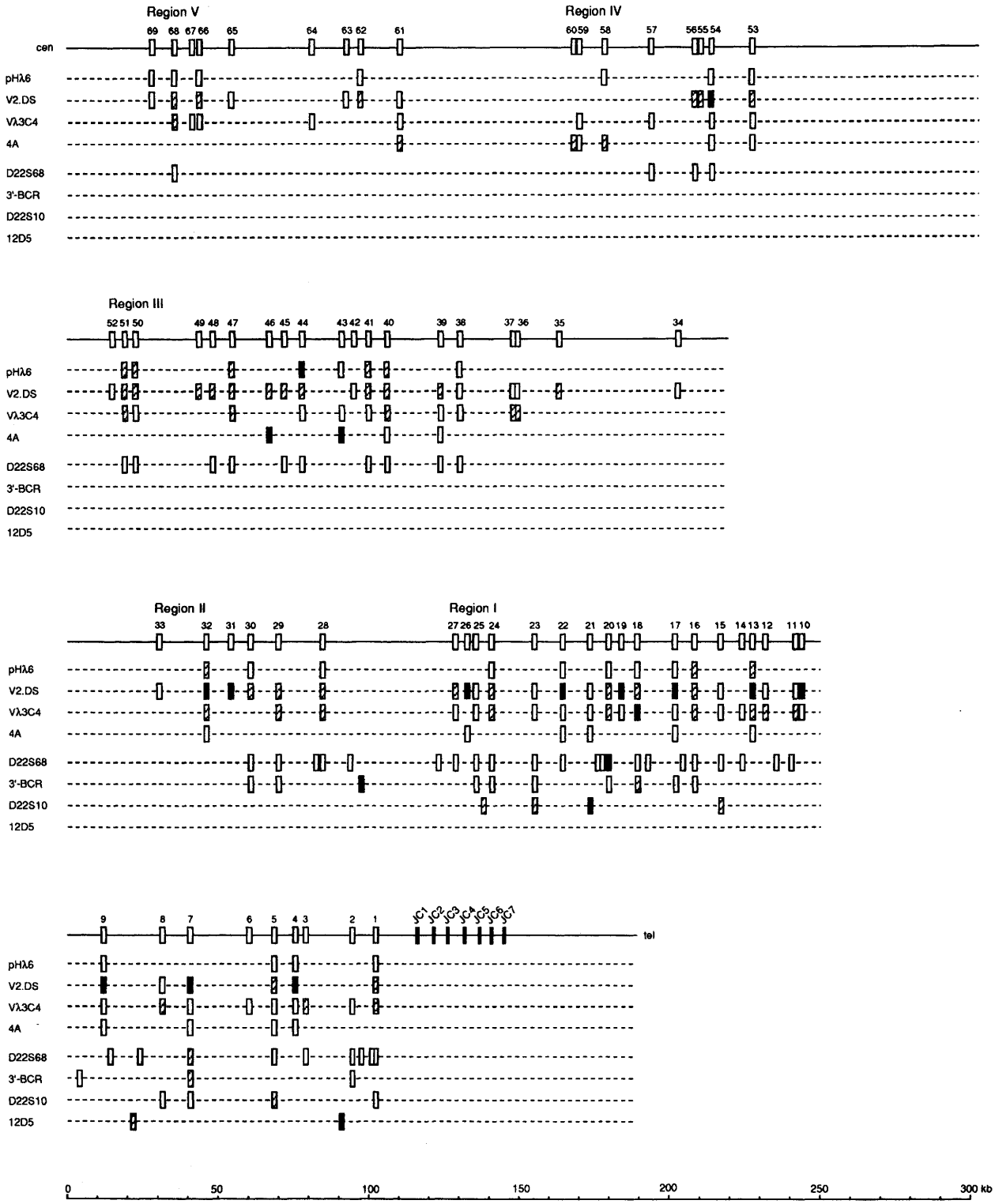

Figure 5 Hybridization profiles of eight probes. (*Top* lines) The locations of 69 V_λ probe positive DNA segments (1–69) and seven J_λ–C_λ gene segments (JC1–7). Locations of DNA segments detected by four V_λ probes (pHλ6, V2.DS, Vλ3C4, and 4A) as well as four other probes (D22S68, 3′-BCR, D22S10, and 12D5) are indicated separately *below*. Extremely strong, relatively strong, and weak hybridization signals are represented with solid, shaded, and open boxes, respectively. A scale is shown at the *bottom*.

positives are within regions I and II (Fig. 5). The D22S68 probe hybridizes more often to DNA segments in regions I, II, and III as compared to those in regions IV and V. Thus, the distributions of the D22S10, 3′-BCR, and D22S68 positives suggest that the sequence similarity of each region II, III, IV, and V to region I decreases as the distance from region I increases. The arrangement of D22S68-positive segments in region III is of particular interest. Five pairs of D22S68 positives are tandemly repeated and these positive signals are colocalized with V_λ positive segments (38 and 39, 40 and 41, 44 and 45, 47 and 48, and 50 and 51). The most proximal four pairs of these D22S68 positives are each accompanied by another V_λ positive segment in their upstream region (42, 46, 49, and 52). This suggests that the ancestral unit of this region may have contained three distinct V_λ gene segments.

Hybridization analysis using a specific V_λ probe can define V_λ gene subgroups (Kabat et al. 1991). Among the 69 V_λ-positive segments, 48 hybridized to two or more different probes (Fig. 5). As hybridization analysis using cosmid DNA is extremely sensitive, only strong signals (closed or shaded boxes in Fig. 5) are detectable in the genomic DNA analysis (Fig. 2). Highly positive segments for the V_λ probe pHλ6 are mainly distributed within region III and those for the Vλ3C4 probe are within region I (Fig. 5). This confirms the observation that >95% of the V_λ gene segments detected in genomic DNA by these two probes are included in the YAC contig (Fig. 2), even though the YAC contig (Fig. 1) covers regions I, II, and the downstream portion of region III (Fig. 4). The V_λ probe V2.DS gave extremely strong signals (closed boxes in Fig. 5) within regions I, II, and IV, whereas extremely strong signals using the 4A probe were detected only within region III. The distributions of V2.DS- and 4A-positive segments are significantly different, even though both belong to the same subgroup (Kabat et al. 1991). This suggests that a subgrouping based on protein sequences does not reflect DNA sequence homology. As discussed above, the highly positive signals for each of the V_λ probes are not distributed evenly along the V_λ locus. This could be interpreted as subgroup clustering within the V_λ gene locus (McDermid et al. 1993).

The VpreB protein is associated with the λ5 protein and plays an important role in early B-cell development (Kudo et al. 1987). Curiously, the VpreB gene has a sequence homology to the V_λ gene segments, whereas the λ5 gene has a homology to the J_λ and C_λ gene segments. In mice, the VpreB1 gene is localized 4.6-kb upstream from the λ5 gene on chromosome 16 (Kudo et al. 1987). The mouse λ gene is also located on chromosome 16. However, the λ gene locus has not yet been mapped precisely in conjunction with the VpreB1 and λ5 loci. In contrast, the human VpreB gene is located within the V_λ gene locus (Fig. 4) and the λ5 gene has been mapped ~750 kb distal to the C_λ locus (McDermid et al. 1993). This suggests that the λ and λ-related VpreB and λ5 genes were subjected to a shuffle through extensive chromosomal rearrangements. Joining of the human V_λ gene segments in regions IV or V (distal to the VpreB gene locus) to the J_λ gene segments could excise the VpreB gene from the genome. Interestingly, such excisions by *V–J* joining would occur only after the role of this gene was fulfilled.

Both the BCR- and GGT-like sequences are localized between regions I and II. A previous study using somatic cell hybrids showed that the 3′-BCR probe detected three BCR-like sequences (BCRL2–4) other than BCR on chromosome 22 (Croce et al. 1987). However, cosmid analysis revealed this probe hybridized to 12 locations within the V_λ locus other than BCRL4 (Fig. 5). These hybridization signals are considerably weaker as compared to those from BCR, BCRL2, and BCRL3 (data not shown) and detected only in cosmid DNAs but undetectable in genomic DNA. The GGT probe hybridizes to several locations along chromosome 22 (Morris et al. 1993); however, only one strong signal was detected from the λ gene locus.

The entire λ gene locus is covered with 31 cosmids (minimum overlapping clones) and one BAC clone. These clones, together with the high-resolution restriction map, would serve as excellent resources for sequencing of the entire germ line repertoire of the V_λ gene segments and the entire λ gene locus. These clones should also provide essential materials for producing human antibody molecules using either filamentous phages (Winter et al. 1994) or transgenic mice (Brüggemann et al. 1989).

METHODS

Library Screening

Both RIKEN YAC (Imai and Olson 1990) and CEPH YAC libraries (Albertsen et al. 1990) were screened as described (Green and Olson 1990). PCR primers and annealing tem-

Table 1. PCR primers and conditions

Name	Sequences (5′ → 3′)	Annealing temperature (°C)	Reference
Cλ[a]	AGGTGGTCAGGTGTCTAAGGTAAA CTAGGGAAGGCTAGGAATTATATG	65	Hollis et al. (1982)
D22S10	TTGACAACATGGTCCCTGTC AGATGATGCATCTTTGGTGT	65	Hofker et al. (1985)
12D5	GAGCAGGCATATTGGTGTCA CATGTATTGCTTTTCCAACC	65	Kawasaki et al. (1992a)
VpreB	CTGCAGTGGGTTCCATTTCT TGTGTACAGCGTCTACTGGT	60	Bauer et al. (1988b)
Vλ2.DS	ACAGTCGATCACCATCTCCT GCAGTAATAATCAGCCTCGT	55	R. Kannagi (pers. comm.)
4A	TCTCAGACTGTGGTGACTCA AGTAATACTCAGCCTCGTCC	60	Anderson et al. (1984)
GGT	TTCATCGCTGTGGTGCAAGC TCTGCTGCTCACAGGGGAAG	65	Collins et al. (1992)
Up	CCTAAGCTTGGGTCTCCCTA GCGCTCATTAAGCGGGCTAA	55	de Jong et al. (1989)
Dwn	GATCCTATGTATTCCCTTTA CTTAGCTTTCGCTAAGGATG	55	de Jong et al. (1989)
BACUP	CGCTTGCTGCAACTCTCTCA ATCATGGTCATAGCTGTTTC	55	S. Asakawa et al.[b] (in prep.)
BACBAC	CACTGGCCGTCGTTTTACAA GCTATCAGGTCATTGCCTGA	55	S. Asakawa et al.[b] (in prep.)

[a]Cλ primers are derived from $Ke^{-}Oz^{+}$ (*C*λ3).
[b]S. Asakawa, J. Kudoh, S. Minoshima, K. Kawasaki, and N. Shimizu.

peratures for each screening (C_λ, D22S10 and 12D5) are described in Table 1. A chromosome 22-specific cosmid library (LL22NC03; de Jong et al. 1989) was screened with gel-purified YAC, V_λ, and walking probes as described (Olsen et al. 1993). A BAC library of three genome equivalents (S. Asakawa, J. Kudoh, S. Minoshima, K. Kawasaki, and N. Shimizu, in prep.) was constructed from a human pre-pro-B cell line, FLEB14-14 (Katamine et al. 1984), using a BAC vector generated from the fosmid vector pFOS1 (Kim et al. 1992) and was screened as described (Olsen et al. 1993).

Fingerprinting

Cosmid DNA was isolated using a robot, PI-100 (Kurabo), digested with *Eco*RI and/or *Hin*dIII, and separated on an agarose gel (0.6% for single digestion and 0.8% for double digestion). Gel images were collected through a Fluorescence Analyzer System (Toyobo) and analyzed using Gel Reader software (National Center for Super-computing Applications). Those restriction fragments were also analyzed by Southern hybridization using nine probes: HuCλ2 (Hieter et al. 1981), pHλ6 (Tsujimito and Croce 1984), Vλ3C4 (Yamasaki et al. 1987), Vλ2.DS (R. Kannagi, pers. comm.), 4A (Anderson et al. 1984), 3′-BCR (Croce et al. 1987), D22S10 (Hofker et al. 1985), D22S68 (Budarf et al. 1991), and 12D5. Comparison of these restriction patterns together with comparison of fragments detected by these nine probes elucidated the overlapping cosmids.

Hybridization Analysis

Four YACs (yS10-1, 35B2 191A11, and yCλ-11) were used for screening the chromosome 22-specific cosmid library. Yeast chromosome and YAC DNAs were prepared as described (Anand and Southern 1990). YAC DNA was separated from yeast chromosomes using PFGE (CHEF DRII, Bio-Rad) and gel-purified from agarose with Geneclean II (BIO 101).

Four V_λ probes, pHλ6, Vλ3C4, Vλ2.DS, and 4A were used in this study. The V_λ gene portion (178-bp *Eco*RI–*Hae*III fragment) of pHλ6 and the entire insert of Vλ3C4

were used for probing. The probes Vλ2.DS and 4A, as well as the GGT probe, were amplified from the YAC yS10-1, whereas the VpreB probe was amplified from human genomic DNA (Table 1). The following probes derived from cosmid clones were used for chromosome walking and BAC screening: (1) The entire cosmid inserts from 76A8, 43C3, and 63E9. (2) terminal *Hin*dIII fragments from 30E12, 50D10, 61E11, 68H9, 85H5, 88E1, 124B3, and 130E10; (3) terminal *Eco*RI fragments from 80A10 and 127C9.

YAC probes as well as walking probes were reassociated with sonicated human DNA (10 mg/ml in 5× SSC at 67°C for 10 min) prior to hybridization (Sealey et al. 1985). Hybridization using YAC probes was carried out for 3 days. Final washing conditions were either 2× SSC, 0.1% SDS at 50°C or 0.1× SSC, 0.1% SDS at 65°C.

Construction of Restriction Map

The cosmid vector Lawrist16 has two *Sfi*I sites (one at each terminus) adjacent to the *Bam*HI cloning site. Cosmid DNA was digested completely with *Sfi*I, then partially digested with *Eco*RI or *Hin*dIII followed by Southern analysis using the two vector end probes, located between the cloning site and the *Sfi*I site upstream (Up) or downstream (Dwn). Up and Dwn probes were amplified from the vector by PCR (Table 1).

Similarly, the BAC vector has two *Not*I sites at each end. DNA from the BAC clone was digested with *Not*I, partially digested with *Eco*RI or *Hin*dIII, and analyzed by Southern hybridization using vector end probes as well as various *Eco*RI or *Hin*dIII fragments isolated from the BAC insert. The vector end probes (BACUP and BACBAC) were amplified from the vector (Table 1).

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REFERENCES

Albertsen, H.M., H. Abderrahim, H.M. Cann, J. Dausset, D. Le Paslier, and D. Cohen. 1990. Construction and characterization of a yeast artificial chromosome library containing seven haploid human genome equivalents. *Proc. Natl. Acad. Sci.* **87:** 4256–4260.

Anand, R. and E.M. Southern. 1990. Pulsed field gel electrophoresis. In *Gel electrophoresis of nucleic acids: a practical approach* (ed. D. Richwood and B.D. Hames), pp. 101–123. IRL Press, Oxford, UK.

Anderson, M.L.M., M.F. Szajnert, J.C. Kaplan, L. McColl, and B.D. Young. 1984. The isolation of a human Ig Vλ gene from a recombinant library of chromosome 22 and estimation of its copy number. *Nucleic Acids Res.* **12:** 6647–6661.

Bauer, S.R., K. Huebner, M. Budarf, J. Finan, J. Erikson, B.S. Emanuel, P.C. Nowell, C.M. Croce, and F. Melchers. 1988a. The human VpreB gene is located on chromosome 22 near a cluster of Vλ1 gene segments. *Immunogenetics* **28:** 328–333.

Bauer, S.R., A. Kudo, and F. Melchers. 1988b. Structure and pre-B lymphocyte restricted expression of the VpreB gene in humans and conservation of its structure in other mammalian species. *EMBO J.* **7:** 111–116.

Brüggemann, M., H.M. Caskey, C. Teale, H. Waldmann, G.T. Williams, M.A. Surani, and M.S. Neuberger. 1989. A repertoire of monoclonal antibodies with human heavy chains from transgenic mice. *Proc. Natl. Acad. Sci.* **86:** 6709–6713.

Budarf, M.L., H.E. McDermid, B. Sellinger, and B.S. Emanuel. 1991. Isolation and regional localization of 35 unique anonymous DNA markers for human chromosome 22. *Genomics* **10:** 996–1002.

Collins, J.E., L.A. Everett, D.R. Bentley, and I. Dunham. 1992. A panel of human chromosome 22-specific sequence tagged sites. *Genomics* **14:** 1098–1103.

Combriato, G. and H.-G. Klobeck. 1991. Vλ and Jλ–Cλ gene segments of the human immunoglobulin λ light chain locus are separated by 14 kb and rearranged by a deletion mechanism. *Eur. J. Immunol.* **21:** 1513–1522.

Cook, G.P., I.M. Tomlinson, G. Walter, H. Riethman, N.P. Carter, L. Buluwela, G. Winter, and T.H. Rabbitts. 1994. A map of the human immunoglobulin V_H locus completed by analysis of the telomeric region of chromosome 14q. *Nature Genet.* **7:** 162–168.

Croce, C.M., K. Huebner, M. Isobe, E. Fainstain, B. Lifshitz, E. Shtivelman, and E. Canaani. 1987. Mapping of four distinct *BCR*-related loci to chromosome region 22q11: Order of *BCR* loci relative to chronic myelogeneous leukemia and acute lymphoblastic leukemia breakpoints. *Proc. Natl. Acad. Sci.* **84:** 7174–7178.

de Jong, P.J., K. Yokobata, C. Chen, F. Lohman, L. Pederson, J. McNinch, and M. Van Dilla. 1989. Human chromosome-specific partial digest libraries in lambda and cosmid vectors. *Cytogenet. Cell Genet.* **51:** 985.

Frippiat, J.-P., S.C. Williams, I.M. Tomlinson, G.P. Cook, D. Cherif, D. Le Paslier, J.E. Collins, I. Dunham, G. Winter, and M.-P. Lefranc. 1995. Organization of the human immunoglobulin lambda light-chain locus on chromosome 22q11.2. *Hum. Mol. Genet.* **4:** 983–991.

Green, E.D. and M.V. Olson. 1990. Systematic screening of yeast artificial-chromosome libraries by use of the polymerase chain reaction. *Proc. Natl. Acad. Sci.* **87:** 1213–1217.

Heisterkamp, N. and J. Groffen. 1988. Duplication of the bcr and gamma-glutamyl transpeptidase genes. *Nucleic Acids Res.* **16:** 8045–8056.

Hieter, P.A., G.F. Hollis, S.J. Korsmeyer, T.A. Waldmann, and P. Leder. 1981. Clustered arrangement of immunoglobulin λ constant region genes in man. *Nature* **294:** 536–540.

Hofker, M.H., M.H. Breuning, E. Bakker, G.J.B. van Ommen, and P.L. Pearson. 1985. An anonymous single copy chromosome 22 clone, D22S10 (22cl-18) identifies an RFLP with PstI. *Nucleic Acids Res.* **13:** 7167.

Hollis, G.F., P.A. Hieter, O.W. McBride, D. Swan, and P. Leder. 1982. Processed genes: A dispersed human immunoglobulin gene bearing evidence of RNA-type processing. *Nature* **296:** 321–325.

Imai, T. and M.V. Olson. 1990. Second-generation approach to the construction of yeast artificial-chromosome libraries. *Genomics* **8:** 297–303.

Kabat, E.A., T.T. Wu, H.M. Perry, K.S. Gottesman, and C. Foeller. 1991. "Sequences of proteins of immunological interest." NIH publication, Washington, D.C.

Katamine, S., M. Otsu, K. Tada, S. Tsuchiya, T. Sato, N. Ishida, T. Honjo, and Y. Ono. 1984. Epstein-Barr virus transforms precursor B cells even before immunoglobulin gene rearrangements. *Nature* **309:** 369–372.

Kawasaki, K., S. Minoshima, J. Kudoh, and N. Shimizu. 1992a. Shotgun polymerase chain reaction: Construction of clone libraries specific to a *Not*I fragment of flow-sorted human chromosome 22. *Genomics* **13:** 109–114.

Kawasaki, K., S. Minoshima, J. Kudoh, R. Fukuyama, and N. Shimizu. 1992b. Methylation status of ribosomal RNA gene clusters in the flow-sorted human acrocentric chromosomes. *Mamm. Genome* **3:** 173–178.

Kawasaki, K., S. Minoshima, K. Schooler, J. Kudoh, P. de Jong, T. Eki, E. Soeda, I. Chumakov, D. Cohen, and N. Shimizu. 1994. Cosmid contigs and fine physical map of the human immunoglobulin λ gene locus on chromosome 22. *Cytogenet. Cell Genet.* **67:** 288.

Kim, U.-J., H. Shizuya, P. de Jong, B. Birren, and M.I. Simon. 1992. Stable propagation of cosmid sized human DNA inserts in an F factor-based vector. *Nucleic Acids Res.* **20:** 1083–1085.

Kudo, A. and F. Melchers. 1987. A second gene, VpreB in the λ_5 locus of the mouse, which appears to be selectively expressed in pre-B lymphocytes. *EMBO J.* **6:** 2267–2272.

Lai, E., R.K. Wilson, and L.E. Hood. 1989. Physical maps of the mouse and human immunoglobulin-like loci. *Adv. Immunol.* **46:** 1–59.

Matsuda, F., E.K. Shin, H. Nagaoka, R. Matsumura, M. Haino, Y. Fukita, S. Takaishi, T. Imai, J.H. Riley, R. Anand, E. Soeda, and T. Honjo. 1993. Structure and physical map of 64 variable segments in the 3′ 0.8-megabase region of the human immunoglobulin heavy-chain locus. *Nature Genet.* **3:** 88–94.

McDermid, H.E., M.L. Budarf, and B.S. Emanuel. 1993. Long-range restriction map of human chromosome 22q11–22q12 between the lambda immunoglobulin locus and the Ewing sarcoma breakpoint. *Genomics* **18:** 308–318.

Minoshima, S., K. Kawasaki, R. Fukuyama, M. Maekawa, J. Kudoh, and N. Shimizu. 1990. Isolation of giant DNA fragments from flow-sorted human chromosomes. *Cytometry* **11:** 539–546.

Morris, C., C. Courtay, A.G. van Kessel, J. ten Hoeve, N. Heisterkamp, and J. Groffen. 1993. Localization of a gamma-glutamyl-transpeptidase-related gene family on chromosome 22. *Hum. Genet.* **91:** 31-36.

Olsen, A.S., J. Combs, E. Garcia, J. Elliot, C. Amemiya, P. de Jong, and G. Threadgill. 1993. Automated production of high density cosmid and YAC colony filters using a robotic workstation. *BioTechniques* **14:** 116–123.

Sealey, P.G., P.A. Whittaker, and E.M. Southern. 1985. Removal of repeated sequences from hybridisation probes. *Nucleic Acids Res.* **13:** 1905–1922.

Shizuya, H., B. Birren, U.-J. Kim, V. Mancino, T. Slepak, Y. Tachiiri, and M. Simon. 1992. Cloning and stable maintenance of 300-kilobase-pair fragments of human DNA in Escherichia coli using an F-factor-based vector. *Proc. Natl. Acad. Sci.* **89:** 8794–8797.

Taub, R.A., G.F. Hollis, P.A. Hieter, S. Korsmeyers, T.A. Waldmann, and P. Leder. 1983. Variable amplification of immunoglobulin λ light-chain genes in human populations. *Nature* **304:** 172–174.

Tonegawa, S. 1983. Somatic generation of antibody diversity. *Nature* **302:** 575–581.

Tsujimoto, Y. and C.M. Croce. 1984. Molecular cloning of a human immunoglobulin λ chain variable sequence. *Nucleic Acids Res.* **12:** 8407–8414.

Vasicek, T.J. and P. Leder. 1990. Structure and expression

of the human immunoglobulin λ genes. *J. Exp. Med.* **172:** 609–620.

Weichhold, G.M., R. Ohnheiser, and H.G. Zachau. 1993. The human immunoglobulin κ locus consists of two copies that are organized in opposite polarity. *Genomics* **16:** 503–511.

Winter, G., A.D. Griffiths, R. Hawkins, and H.R. Hoogenboom. 1994. Making antibodies by phage display technology. *Annu. Rev. Immunol.* **12:** 433-455.

Yamasaki, N., S. Komori, and T. Watanabe. 1987. Complementary DNA for a human subgroup IV immunoglobulin λ-chain. *Mol. Immunol.* **24:** 981–985.

Zachau, H.G. 1993. The immunoglobulin κ locus-or-what has been learned from looking closely at one-tenth of a percent of the human genome. *Gene* **135:** 167–173.

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