Interspersed repetitive sequence (IRS)-PCR amplification of pulsed-field gel fractionated DNA to derive markers from the incontinentia pigmenti 1 (IP1) locus

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Positional cloning relies on closely spaced regional DNA markers. Here, we report on the combined use of preparative pulsed-field gel electrophoresis (PFGE) and interspersed repetitive sequence (IRS) polymerase chain reaction (PCR) amplification to isolate two DNA markers from a 660 kb *Sfi*I fragment that contains locus DXS14 and maps to a region between disease-specific incontinentia pigmenti type 1 (IP1) chromosome translocation breakpoints (1).

Genomic DNA from a somatic cell hybrid containing a human X chromosome (GM06318) and a radiation hybrid (RH 128) containing approximately 10% of a human X chromosome, including DNA markers spanning the IP1 X-chromosomal translocation breakpoints within Xp11.21 (2), was digested with *Sfi*I and fractionated by PFGE. By Southern blot analysis, probe p58.1 (DXS14) and probe 30C_E2.8 (DXS370) detected a 660 kb and a 400 kb fragment, respectively, in both hybrid DNA samples (Figure 1). Sequential hybridizations of the same membrane with human and hamster genomic DNA indicated that most human and hamster *Sfi*I fragments ranged from 250 kb to

600 kb in size. These results suggested that the 660 kb *Sfi*I restriction fragment within RH 128 (a) had not undergone rearrangement during hybrid construction and (b) could be separated from most human *Sfi*I fragments by PFGE. To recover the 660 kb *Sfi*I fragment containing locus DXS14, RH 128 DNA (30 µg) was digested with *Sfi*I and fractionated by PFGE. The preparative portion of the gel was sliced into 14 serial 5-mm sections (A through N; Figure 1); preparative slice F contained DNA fragments ranging from 600 kb to 670 kb in size and included the *Sfi*I restriction fragment containing DXS14. To obtain DNA markers from the 660 kb *Sfi*I fragment, DNA was electroeluted from preparative slice F, extracted, precipitated, and subjected to IRS-PCR (3). PCRs were 100 µl in volume and contained 5 ng of preparative slice F human DNA, 50 mM KCl, 10 mM Tris-HCl, 1.6 mM MgCl₂, 0.01% gelatin, 250 µM

Figure 1. Southern blot analysis of the flanking lanes of a preparative PFGE gel containing *Sfi*I-digested cell line GM06318 (lane 1) and RH 128 (lane 2) DNA; a single filter was sequentially hybridized with probes p58-1, 30C_E2.8, and total human and total hamster DNA. The preparative portion of the gel containing *Sfi*I-digested RH 128 DNA was cut into 5 mm gel slices (A–N). Probe p58.1 detected a similar 660 kb fragment in both lanes within a region corresponding to preparative gel slice F. Probe 30C_E2.8 recognized a similar 400 kb fragment in both lanes. Total human and total hamster DNA hybridized to a smear of restriction fragments; most of the fragments detected ranged from 250 to 600 kb in size. DNA was fractionated by CHEF electrophoresis in 1.2% LMP agarose (0.5×TBE) using 200 V at 14°C; switch times were 60 sec for 15 hours and 90 sec for 9 hours.

Figure 2. Comparison of A-TC65N primed PCR amplification products obtained from hamster, cell line GM06318, RH 128, and preparative gel slice F (RH 128 *Sfi*I-F) DNA electrophoresed in 1.2% agarose. RH 128 *Sfi*I-F DNA yielded at least 24 distinct products ranging from 0.5 to 4.3 kb in size; compared to the other samples containing human DNA, RH 128 *Sfi*I-F DNA yielded a reduced number of products; hamster DNA failed to yield products.

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each dNTP, 1 μ g RNase A, and 0.5 μ M primer A-TC65N (3, 4). PCR reactions were incubated at 37°C for 1 hr, 94°C for 9 min, and, after the addition of 5 U *AmpliTaq* DNA polymerase (Perkin-Elmer/Cetus), 36 cycles of 94°C denaturation (1 min), 55°C annealing (1 min), and 72°C extension (4 min) were performed as described (4). Amplification yielded at least 24 distinct products ranging from 0.5 to 4.3 kb in size (Figure 2). A portion of the amplification products was digested with *NorI* and cloned into a plasmid vector to yield 20,000 potentially recombinant independent clones; of 98 recombinants examined, 41 unique amplification products ranging from 0.8 to 4.0 kb in size (mean size = 2.4 kb) were identified. Of these, 19 products were found to contain low-copy DNA fragments. By hybridization to an IP1 somatic cell hybrid mapping panel (1), one fragment, probe p49A (DXS740), mapped to a region between IP1 X-chromosomal translocation breakpoints. A second fragment, probe p75A, was mapped to a region within 10 kb of DXS14 by hybridization to a yeast artificial chromosome (YAC) clone containing a portion of the targeted *SfiI* restriction fragment. To determine the origin of the cloned IRS-PCR products, amplification products were used as probes and hybridized to *SfiI* partially digested GM06318 DNA; all 7 products tested hybridized to a fragment between 600 and 670 kb in size and multiple smaller fragments (data not shown). These results suggested that most of the IRS-PCR products recovered from preparative slice F DNA were derived from partially digested restriction fragments of the expected size range.

This method provides a means of obtaining DNA markers distributed along the length of a specific restriction fragment. Compared to previous studies that used PFGE fractionated DNA to construct bacteriophage lambda libraries (5, 6), this method yields human-specific products and obviates the need to screen a large number (10^4) of recombinant clones. Additionally, in contrast to PCR-based strategies using non-specific, ligation mediated primers and fluorescent-activated cell-sorted chromosomal DNA (7), this method is applicable to somatic cell and radiation hybrid DNA. Furthermore, by isolating amplification products contained on partially overlapping restriction fragments, it may be possible to use repeated rounds of IRS-PCR amplification to march along contiguous restriction fragments.

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