Human-specific amplification of radiation hybrid DNA fractionated by pulsed-field gel electrophoresis

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Recently, a method named interspersed repetitive sequence polymerase chain reaction (IRS-PCR) was described by which, using primers directed against an IRS, human DNA sequences flanked by the IRS are selectively amplified from complex DNA mixtures such as somatic cell hybrid DNA (1,2). Hybrids containing human chromosomal fragments, such as radiation hybrids (RH) (3, 4), have been used to further reduce the complexity of the human DNA template and increase the specificity of IRS-PCR amplification (5). Here we describe a method which facilitates the isolation of specific DNA sequences from a subchromosomal region. We have developed a method to amplify human-specific DNA sequences from pulsed-field gel (PFGE) fractionated RH DNA; by amplifying a fraction of digested RH DNA containing an identifiable DNA restriction fragment, this method provides a means to selectively amplify and enrich for DNA sequences contained within a specific DNA restriction fragment.

Genomic DNA from RH 128, a RH harboring about 10% of a human X chromosome including region Xp11.22-Xp11.1 (P.N.Goodfellow and J.L.Gorski, unpublished data), was prepared in agarose blocks (6) and digested with *Sfi*I (NEB). DNA blocks were loaded into 1.2% LMP agarose gels (BRL) made with 0.5×TBE and electrophoresed using a contour-clamped homogeneous electric field (CHEF) gel system (CHEF-DRII; BioRad) at 200 V with pulse times of 60s (15 hrs) and 90s (9 hrs). Horizontal 5 mm gel slices were cut; DNA was recovered by electroelution, dialyzed against TE, sequentially extracted with phenol, chloroform, and ether, precipitated using ammonium acetate, ethanol, and carrier yeast tRNA (10 µg/ml), and resuspended in 50 µl of dH₂O. PCR reactions were 100 µl in volume and contained 400 ng of DNA, 50 mM KCl, 10 mM Tris-HCl, 1.6 mM MgCl₂, 0.01% gelatin, 250 µM each dNTP, 1 µg RNase A, and 0.5 µM primer; the primer, A-517N (5' AA-GTGCGGCCGCGATCTCGGCTCACTGCAA 3'), was directed against a consensus *Alu* repetitive sequence (1) modified to contain a 5' *Not*I restriction endonuclease recognition sequence. 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; amplification products were fractionated on a 1.2% agarose gel and stained with ethidium bromide. No products resulted from the amplification of hamster DNA indicating that the primer was

specific for human DNA (Fig. 1). Amplification of hybrid DNA containing only a human X chromosome yielded a large number of products while amplification of RH 128 DNA and RH 128 DNA digested with *Sfi*I yielded an identical subset of products (Fig. 1); *Sfi*I-digested RH 128 DNA isolated from LMP agarose (Fig. 1; RH 128 *Sfi*I gp) yielded a reduced but similar pattern of products. Compared to RH 128 DNA, amplification of a fraction of RH 128 DNA (450–500 kb) recovered from a preparative CHEF gel yielded relatively fewer products consistent with a further reduction of human template DNA complexity; the pattern of products observed upon the amplification of similar

Figure 1. Comparison of IRS-PCR products; similar PFGE fractions from 2 different preparative gels (450–500 kb and 470–540 kb) yield a nearly identical pattern of amplification products with slight variations of individual DNA fragment intensity. An adjacent fraction (400–470 kb) yields a different pattern of amplified products; a subset of the observed products are identical in size to those of the 470–540 kb fraction but vary in intensity. The amplification products of a nonadjacent fraction (50–120 kb) are distinctively different.

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fractions of 2 different preparative gels (450–500 kb and 470–540 kb), adjacent fractions (400–470 kb and 470–540 kb) and a widely separated fraction (50–120 kb) show that the amplifications are reproducible, distinctive, and specific (Fig. 1). Using this method, we have IRS-PCR amplified a variety of different preparative PFGE fractions of *Sac*II- or *Sfi*I-digested RH 128 DNA which contain DNA restriction fragments mapped to region Xp11.21; by isolating amplification products which identify partially overlapping DNA restriction fragments, it may be possible to use repeated rounds of IRS-PCR amplification to march along contiguous restriction fragments.

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