Interspersed repetitive sequence (IRS)–PCR for typing of whole genome radiation hybrid panels

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ABSTRACT

The typing of a radiation hybrid (RH) panel is generally achieved using a unique primer pair for each marker. We here describe a complementing approach utilizing IRS–PCR. Advantages of this technology include the use of a single universal primer to specify any locus, the rapid typing of RH lines by hybridization, and the conservative use of hybrid DNA. The technology allows the mapping of a clone without the requirement for STS generation. To test the technique, we have mapped 48 BAC clones derived from mouse chromosome 12 which we mostly identified using complex probes. As mammalian genomes are repeat-rich, the technology can easily be adapted to species other than mouse.

INTRODUCTION

Radiation hybrid (RH) mapping has tremendously accelerated the pace at which chromosomal maps can be constructed. Conceived at a time when DNA technology was still in its infancy by Goss and Harris (1), the concept was revived 15 years later by Cox and co-workers (2) who irradiated somatic cell hybrid lines containing single human chromosomes before fusing them to HPRT-deficient hamster cells. Subsequently, RH maps of several human chromosomes based on the approach of Cox *et al.* were constructed, for instance of chromosome 16 and X (3,4). It was Walter *et al.* (5) who returned to the original Goss and Harris protocols and presented a panel of cell hybrids that they had constructed by fusion of irradiated diploid human fibroblasts with hamster cells. Since then, whole-genome RH panels have been constructed and characterized for a variety of mammalian species other than human, including pig (6,7), cow (8), rat (9,10), dog (11,12), cat (13) and mouse (14–17).

Once a panel has been characterized and a dense framework map is available, it ideally suits the requirements for large-scale high-resolution mapping. To place a new marker on an RH map, all that is needed is an assay to determine the presence of a particular sequence in the individual hybrids. Co-retention with markers previously assayed on the panel can be used to map new markers.

Using the mouse as our model system, we demonstrate here the usefulness of interspersed repetitive sequence (IRS)–PCR with respect to mapping on whole genome RH panels. IRS–PCR exploits the frequent occurrence of repeat elements in the genome of mammals. For instance, a single primer that anneals to the B1 repeat sequence is able to amplify several thousand different fragments from a mouse genomic DNA template. IRS–PCR was originally developed for the species-specific amplification of sequences from somatic cell hybrids (18), and subsequently served as the technological platform for high-throughput genetic and physical mapping in the mouse (19–24).

We here employ complex probes generated by IRS–PCR to identify BAC clones from one specific mouse chromosome. Subsequently, using IRS–PCR fragments derived from these clones, the mouse T31 RH panel is typed by hybridization and the clones thereafter integrated into the RH framework map.

MATERIALS AND METHODS

DNA

DNA from the T31 mouse whole genome radiation hybrid panel was purchased from Research Genetics (Huntsville, AL). DNA from hybrid N2C1 (25) that contained mouse chromosome 12 on a human background was a gift from Dr Phil Avner (Institute Pasteur, Paris, France).

IRS–PCR

PCR amplification from cell hybrid DNA (5 ng) or genomic mouse DNA (50 ng) was carried out under conditions described previously (23), using 1 μg of B1-repeat primer B1R (5′-AGTTCCAGGACAGCCAGGGCTAYACAGA-3′; ref. 23) in a 60 μl reaction. The amplification of IRS–PCR fragments from BAC colonies or purified BAC-DNA was carried out as described (23).

Filter generation and hybridization

For the preparation of Southern blots, 5 μl of complex IRS–PCR product from each RH line was run on 1% agarose gels. A distance of 1.5 cm between gel combs allowed us to assemble all 100 RH lines plus controls on a filter the size of 16 × 4.5 cm (Fig. 1). Alkaline blotting was carried out according to standard procedures (23). Colony filters from a mouse BAC library (B.Birren, unpublished) were produced on robotic equipment in-house (26). For the preparation of hybridization probes, BAC-derived IRS–PCR fragments were run and cut out from 1.2% SeaPlaque GTG low melt agarose (FMC BioProducts, Rockland, ME). For the preparation of complex probes, 20 ng of unpurified IRS–PCR reaction product prepared from DNA of cell hybrid N2C1 was used. Radioactive labeling

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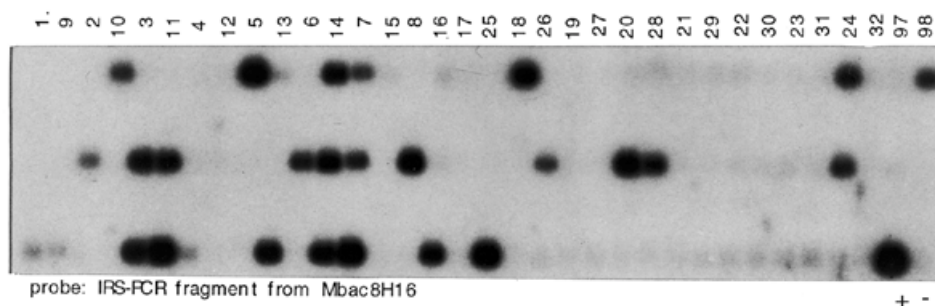
Figure 1. Typing of the mouse whole genome RH panel by hybridization. Inter B1 repeat PCR was carried out on the 100 cell lines of the mouse T31 panel, and mouse and hamster controls. Individual samples were loaded onto a gel with three comb rows at 1.5 cm spacing and used to prepare a Southern blot. Top row, hybrid lines 1–32, 97, 98; middle row, lines 33–64, 99, 100; bottom row, lines 63–96, positive control 129aa (indicated with +), negative control hamster A23 (indicated with –). Hybridization probe is an IRS–PCR-fragment from mouse BAC clone mbac8h16.

was carried out using the random priming method (27). Prior to hybridization, probes were pre-associated with an excess of sheared unlabeled genomic mouse DNA as described (23). Southern blots from the RH panel were hybridized inside 15 ml plastic tubes. Library filters (22 × 22 cm) were sealed into plastic bags. Hybridization was carried out in Church buffer (28) in a 65°C water-bath for 16 h. After hybridization, filters were washed at 65°C for 60 min each in 2× SSC/0.1% SDS and 0.1× SSC/0.1% SDS. Exposure against X-ray film was from 1 day (RH panel) up to 1 week (complex probes) at room temperature.

Data entry and evaluation

Autoradiograms from RH panel hybridizations were entered into our database using a scoring tool developed in-house. Data were matched against the T31 marker framework (17) using the RH Map server at the Whitehead Institute Center for Genome Research (www-genome.wi.mit.edu/mouse_rh/index.html).

RESULTS

Using DNA from the cell hybrid N2C1 that carried mouse chromosome 12 (MMU12) on a human background (25), IRS–PCR was carried out with the mouse B1-specific primer B1R. The complex reaction product was labeled radioactively and hybridized against a mouse BAC library. Out of 27 000 clones screened approximating one genome equivalent, 110 positive clones were identified and retrieved from a picking copy of the library. Thirty-two of the clones were found to contain a repeat element and were excluded from further analysis. Next, IRS–PCR was carried out on the remaining 78 clones to obtain probes for hybridization. Likewise, IRS–PCR was carried out on cell hybrid N2C1 plus suitable positive and negative controls for the preparation of Southern blots. Of 78 clones, 66 (85%) gave a positive signal with the N2C1 cell hybrid and could therefore be assumed to be correctly derived from MMU12 (data not shown). In order to place these 66 clones relative to the RH framework map of the mouse genome (17), DNA from each of the 100 hybrid lines that are comprised within the T31 mouse whole genome RH panel plus mouse and hamster controls were amplified by IRS–PCR and used to prepare Southern blots (Fig. 1). Of the 66 clones tested, 44 could be placed relative to the RH framework map of MMU12 at a LOD threshold of or

exceeding 10 (Table 1). Two markers mapped to MMU12 at a LOD threshold of 8. The remainder of IRS-fragments resulted in either weak hybridization signals or gave smeary hybridization results on the T31 panel (11 probes), mapped to multiple chromosomes (2 probes), or did not link to a framework marker at a LOD threshold as low as 7 (3 probes). Four markers out of 66 mapped to a chromosome other than MMU12 at a significant LOD threshold.

DISCUSSION

We have demonstrated here the feasibilty to type a whole genome RH panel by hybridization using IRS–PCR. The procedure that we describe offers some advantages in comparison to current STS-based approaches of RH mapping. First, a single primer is sufficient to map a number of markers on the panel. Second, one single hybridization is sufficient to generate the required typing data on a panel of 100 RH lines. Third, the technology is extremely conservative concerning the consumption of RH line DNA. Starting with 5 ng of hybrid DNA that is initially used for an IRS–PCR reaction, the resulting amplification product provides sufficient material to type (conservatively estimated) 100 markers.

Using complex probes for clone identification, we have succeeded to place almost 50 BAC clones onto the RH framework map of MMU12 (Table 1). It is clear that this chromosome must contain many more IRS fragments that can be generated by PCR with primer B1R. However, the product that emerges as the outcome of a complex PCR reaction does not contain equal amounts of each individual amplified fragment. Rather, there is a strong bias towards some fragments that amplify preferentially and these will hybridize most strongly to the library filter.

When DNA from an RH line is amplified with primer B1R, the primer will not only amplify mouse sequences, but from the hamster background as well. This does not interfere with hybridization-based typing of the panel, because the positions of B1 elements in the mouse and hamster genomes, respectively, are not evolutionarily conserved. This is in contrast to STS-based RH panel typing, which frequently suffers from co-amplification of hamster background. In the context of another project, we have tested many hundreds of different mouse-derived IRS–PCR

Table 1. Placement map of mouse chromosome 12 (MMU12)

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cM      Name        cR    Type  Vector
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0.0     D12Mit145   0.0   F     00121000100010110010101010000100010011110000200010111100110011100010110001101000000010101101111
        mbac5h15    0.0   P>3.00 00101000000010200010111000010001000101111000000000100121001100010000000100011010000000010111100001
        mbac5l18    0.0   P>3.00 00101020101010100010101000010001000100110111000000001011001000100000001000100100000010101100101
        mbac65p7    16.8  P>3.00 00100000010101000000010000001000010011110000000010011100110011000001000100100000010101100101
1.0     D12Mit37    1.8   F     00100020100010110010101010100100010110111100101000101110000101110011001102110011000000002101011011
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2.0     D12Mit151   7.0   F     00001000100010111010101010110001101110001000100101200002001101010012010010010010100100111
        mbac50c1    28.1  P>3.00 00001000100010121010101210210210001101110000010001000101100000010101001000100120100010100100111
3.0     D12Mit241   9.3   F     0010001120001000000001000110001011110000200001001010000011101010010101011001001010100011110111
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6.0     D12Mit83    2.9   F     00001011100000000000010010100011011000010000100101010000100110111101010010010000010001012100111
        mbac67e5    2.1   P>3.00 00000011100000100000000100101001001010001000010000010020000000101101100101001000010000012110111
6.0     D12Mit219   3.3   F     00001111100000000000100101000101100000001001010000001001101101010010010000100000010121000111
        mbac19j17   3.7   P>3.00 000002110000010000000010010001010110001000010001000010000000001101120101001000000001110001
11.0    D12Mit185   0.2   F     00001011100001000000010010100010211000000000010010100000011011000101001001001100010111000111
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11.0    D12Mit283   0.6   F     00001111100000000000100101000111110000000000100101000010011011100101001000010001011100011
        mbac4j7     30.4  P>3.00 0000000020000000000000100010010100010001000010000100101010110120101001001000000010101101101
12.0    D12Mit106   3.5   F     01001000100000100000010110100010110000000001010110101000011000000121011000010001011000001
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13.0    D12Mit107   13.5  F     00001010100010100000010110100010011010000002020010201000111100101010010010010020010010000001
        mbac59k12   15.6  P>3.00 0000000010001000000000001101000100110100010021010001100110101010001001021000001011010101
14.0    D12Mit146   3.7   F     0100100010000010000001001020001001010000000101011010100000110000001210110000100010011010101
        mbac60n11   5.3   P>3.00 000001000000000101000000010010001200110000000101011010000000000000011012000011000010112201
15.0    D12Mit153   1.6   F     0100101010001000000001011000001001100000001010110100000010000011001101110000100010001010101
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22.0    D12Mit64    15.1  F     01001010000000000000000100000000000010001010011100002001011001001001100000110000010110001
        mbac7e21    0.4   P>3.00 01001000000000000000001000000010001100010002010111101000001011101001001100000000010000110000
        mbac55l8    0.0   P>3.00 0100000000000000000000010000000000011000100201011101110000010111010010011000000000010000110000
        mbac70m6    0.1   P>3.00 0100000000000000000001000000000001001001010111110100000101110100100110000000001000110000
24.0    D12Mit199   0.1   F     0100000000000000000001000000000011000000101001200121011001000001011101001001100012001002110001
        mbac43o14   4.4   P>3.00 0100100000000000000010000000000010010010101011110100000101110100100110100010010000110000
        mbac31a2    0.3   P>3.00 0100000000000000000010000000020110000000100111010000010101011101100000200000000100002110001
24.0    D12Mit54    2.0   F     010001000000000000001000000000011000000011000011020000111110100001000001000100012110001
        mbac18l23   5.7   P>3.00 00001000000000010000000100000010001011000100000001011100001010111010000000100000100112201
24.0    D12Mit87    4.2   F     01000000000000000000100000000000110000101010011011000001111101000001100021100010001110111
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25.0    D12Mit113   12.6  F     010011000000010000000100000000000110000101010011212100010101110100000010010110000001110121
        mbac4l8     0.3   P>3.00 000000000000010000010000000000001100010001010011100001010111010000001001001000000101110001
        mbac20a18   4.4   P>3.00 00000000000001000000010000000000001100010001010011100001010111010000000100100100000101110101
28.0    D12Mit191   12.9  F     000011001000001000000000000001001100010101001110011001112110010000001001011000010010101
        mbac27e6    1.0   P>3.00 000000001000010000010000000001001100010001010011100001011011010000010001000010001010101
28.0    D12Mit190   1.0   F     00000200100001010000100000001001100010201010011100000101101120000000010001010010001010001
        mbac4l10    1.2   P>3.00 00000000010000101000010000000010010001100010110111010120000000010010010000010010010100001
        mbac28o18   0.4   P>3.00 00000000100010000010000000000010010011000000001001011000010100110000001001001000010010001
        mbac60k7    5.6   P>3.00 00000001000100000010000000010000010011000000010011100010010010110100000010220000020001110101
29.0    D12Mit74    1.5   F     01001110200010001000000010010000010011011000001101011011011010120010020010010101001101
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31.0    D12Mit92    3.0   F     010000100001001000021000000010011010000201201001110001101101111010010000020000000011101
        mbac35f11   2.4   P>3.00 01000010000100010000100000000010102000020010010110010011201111010001000000000000011011
        mbac44a22   0.7   P>3.00 010000100001000010000100000000100012000001101001100010120111110001000002000000002001101
        mbac49e18   3.1   P>3.00 010000100001001000010000001001010100000000010120000101001111010001000000200000002001101
        mbac34c13   7.0   P>3.00 010000100001001000010000000100110000000120100112000021011110100010000000000000001101
        mbac28b18   3.7   P0.00  0102001000000200010000010000010011000010010100100101011101010011100100010010000000000011011
        mbac46n102  6.8   P>3.00 110000101000000010000000000010011000000100100120100010110111010000100010000000000110111
        mbac37b3    11.8  P>3.00 0100010001000100010000000100000120100100000010100010101000000001000000000000000011
32.0    D12Mit128   1.5   F     01001110000100210000010010000010011110000010100110000010112000012110000001010010100100111
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34.0    D12Mit4     3.8   F     01000110110000101000010010000000011011001101001000000111100001010000000000000101000001011
        mbac25o20   3.6   P>3.00 01000000010000000000100010000000100110100100100100000101000000010010001001000000100000101
        mbac38o18   0.0   P>3.00 010000000100010010000000010000011011001101001001000001110000010100000001001010000100000101
        mbac56f23   0.1   P>3.00 0100000001000100002100100000010001001100210010000001110000000021100000010010000000000100101
34.0    D12Mit156   1.1   F     010001000100011010000010010000010011010011010010000011110000201000000000010001010000101
        mbac34e4    1.0   P>3.00 01000010000100110100000101000001020100102100100010000101000000010000010000001000000100101
35.0    D12Mit143   3.5   F     010021100100010100000101000000010011011010101000000011110001010000000000100010010102101
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37.0    D12Mit288   1.1   F     0000011102100011010000210012000010011011011001100001000011110000121000010000010101010000101
        mbac5f5     2.0   P>3.00 00001000010011010000010010000020001101100220000100000211200001000010000010000101010000101
        mbac26g16   0.7   P>3.00 00001000010001101000000010000000010001101100001111000010000100010101010000101
        mbac71c24   0.3   P>3.00 010000000100010010000001000000010011002001001000000011000201010000000000020000010000101
37.0    D12Mit203   7.1   F     00001110110001101000001001000010011011001100010000001111000010001010101010100101
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38.0    D12Mit158   3.8   F     000011200100011001000001001000001001011011000100001100020011110001010000100001000100002001 01
        mbac37n20   2.5   P>3.00 20001000010010100000201001000001001011011000100001000000101100010100000100001000000000101
38.0    D12Mit176   0.0   F     000011201100011001100010010000100101011001100010000010000001011000010100001000010001000000101
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39.0    D12Mit254   2.0   F     00001110110001001000001001000010010101011011000000101100001010200100102000010010100201
        mbac14e14   1.9   P>3.00 00001000010001001000001001000000010010010110010000010000010000010000010000000000001
        mbac30i11   4.8   P>3.00 2000020001000100100200100100000000101001000100001001000010000010001001000000000000001
41.0    D12Mit228   1.5   F     0100111011200101000001001020001001010101110010101001000001011001010100000010010010010100001
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43.0    D12Mit76    2.0   F     0100100010010010100001000000010011201100010010010100011110011101011010100000001
        mbac39e8    3.2   P>3.00 01001000010001000100000010011110110010100101010001100011100111010110201000200002001000001
44.0    D12Mit239   3.9   F     0101111001000100100000100000001001112110010010011101000101100012101111001000000010110000221
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52.0    D12Mit261   9.7   F     01001110110100010000010011000010111110120000100100010010011100001010110010000100201001101
        mbac4d17    1.1   P>3.00 0120100010010001002020100000000100111011100001001010100010010010111011001011011010010011110
53.0    D12Mit181   0.0   F     010011101100010010000010011110111110000010010010001110101010111001000010000000101
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55.0    D12Mit141   2.2   F     0000211011010010010000100000001001111010100001001010100012110010101011100200001001020000101
        mbac15j3    3.2   P>3.00 000010101100010010000010000000100111101010000010010100010001010101011001000000000000100
        mbac44j13   0.7   P>3.00 00001010110001001000001000000001001111010100001001010100010001010101011001020000100000000100
        mbac29e4    1.2   P>3.00 0000101011002001001110000000010011101010100001001010100000100001001011110100000010000100
55.0    D12Mit280   1.5   F     0000101001001200100000010000000100111001010000100101010000110011001010001110100000010000100
        mbac8h16    0.4   P>3.00 000010100100100100000010000000100111001010000100101010001200101010101000000000000000100
        mbac36p17   0.3   P>3.00 0000101001000100100000010000000100111001010000100010120100001020101011001000010000000000100
        mbac11g5    1.4   P>3.00 0000101010001001200000010200000022000010001001010001000010101001010101101000000010000100
        mbac21k23   0.1   P>3.00 0000101000010010000001000000010011001001010010100010000001010101011001000010000010000000100
        mbac8i2     4.8   P>3.00 00001010010001001000010000000100011000010001001010100010000010010101101100000000000000100
58.0    D12Mit196   5.1   F     0000111001000100100000010000000100111001100101001010101001011101011101101000000000000000100
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59.0    D12Mit134   27.4  F     000011100100010010000010010000010010100110010100101010010011111000101110000010000000001101
        mbac8e6     0.0   P>3.00 0000100001000020100000100000001002110102010010010101000001000200010212200000010000000000000
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fragments on Southern blots that included IRS–products from hamster DNA, but observed cross-hybridization with hamster DNA only very rarely (<1% of cases).

While genetic mapping employing IRS–PCR requires the existence of highly inbred strains of a specific species, this is not a limiting constraint for RH mapping. Since a high content in dispersed repeat sequences has emerged to be a common feature of mammalian genomes, our method therefore makes IRS–PCR amenable to genome mapping in species other than rodents, such as farm animals (cow, pig), cat and dog.

For mapping markers on an RH panel following our strategy, it is necessary that the target contains repeat sequences located at short distance. Our method will therefore be most valuable if mapping of genomic clones is the primary focus. Projects in this context could be large-scale sequencing efforts, gene-mapping via genomic clone intermediates (23) or the construction of clone contigs, which require cost-effective protocols to rapidly either establish or confirm the location of large numbers of clones in the genome. In particular, mouse genomic sequencing has barely begun and a substantial amount of sequence-ready BAC clone contigs will be established from the YAC maps that are existing (29) or under development (L.C.Schalkwyk, H.Lehrach, H.Himmelbauer, in preparation). Similarly, other mammalian genomes that at least in part will be subjected to high-resolution physical mapping and genomic sequence determination (e.g. farm animal genomes) should greatly benefit from the procedure described.

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