Title
Rapid RFLP screening procedure identifies new polymorphisms at albumin and alcohol dehydrogenase loci.

Permalink
https://escholarship.org/uc/item/6191p80v

Journal
Human genetics, 76(3)

ISSN
0340-6717

Authors
Murray, JC
Shiang, R
Carlock, LR
et al.

Publication Date
1987-07-01

DOI
10.1007/bf00283622

License
https://creativecommons.org/licenses/by/4.0/ 4.0

Peer reviewed
Rapid RFLP screening procedure identifies new polymorphisms at albumin and alcohol dehydrogenase loci

Jeffrey C. Murray1, Rita Shiang1, Leon R. Carlock2, Moyra Smith2, and Kenneth H. Buetow3

1Department of Pediatrics, University of Iowa Hospitals and Clinics, Iowa City, IA 52242, USA
2Department of Pediatrics, University of California Irvine, Irvine, CA 92717, USA
3Fox Chase Cancer Research Center, Philadelphia, PA 19111, USA

Summary. A rapid screening procedure for restriction fragment length polymorphisms (RFLPs) is reported. DNA from ten individuals is pooled and compared to DNA isolated from a cell line containing a single chromosome 4. This single chromosome-containing line is an obligate hemizygote for chromosome 4 RFLPs so that only one band corresponding to a single allele will appear on a Southern blot. In the pooled DNA sample lane bands corresponding to both alleles will be seen. The technique allows for efficient detection of RFLPs with easier use of large numbers of enzymes. It provides estimates of allele frequencies and disequilibrium. New RFLPs for albumin and alcohol dehydrogenase detected with this technique are described.

Introduction

The use of restriction fragment length polymorphisms (RFLPs) has had a dramatic impact on the study of human genetics. RFLPs have wide use in the establishment of human linkage maps in both normal individuals (White et al. 1985) and in families with genetic disorders segregating such as Huntington disease (Gusella et al. 1983), polycystic kidney disease (Reeder et al. 1985), and cystic fibrosis (Tsui et al. 1985). Because of this widespread use, simplifications in the search strategies for RFLPs may provide significant benefits for researchers employing these markers. We report here a strategy for identifying RFLPs that is both rapid and cost effective. We have used it to identify new RFLPs at the human albumin and alcohol dehydrogenase loci on chromosome 4.

Materials and methods

DNA was prepared from whole blood by the method of Poncz et al. (1982). Five to 10 μg samples of DNA were digested with appropriate restriction enzymes (Bethesda Research Laboratories or New England Biolabs) and electrophoresed on 0.8%-1.0% agarose gels. Restriction enzymes used included AluI, ApaI, AvaI, AvaII, BamHI, BanII, BclI, BglI, BglII, BsmI, Bsp1286, BstEII, BstNI, BstXI, ClaI, DdeI, DraI, EcoRI, EcoRV, HaeIII, HincII, HindIII, HpaII, HphI, KpnI, MboI, MboII, MluI,MspI, NaeI, NciI, NdeI, NheI.

Results

Figure 1 shows the identification of polymorphisms at the human albumin locus on chromosome 4 (Harper and Dlugoszyk 1983) using pooled DNA samples to compare to DNA isolated from the cell line containing a single human chromosome 4. RFLPs shown in the pooled samples here detected with SacI have previously been shown to be present in high frequency in Caucasian, Black, and Asian populations (Murray et al. 1984). Figure 2 shows a new Sau96I RFLP identified at the albumin locus with probe F47 using this method. This
Fig. 1. Autoradiograph of a Southern blot hybridized with the albumin F47 probe as described in Materials and methods. Lane 1 is a SacI digest of the DNA from the HHW 416 cell hybrid containing a single human chromosome 4 and showing only the 20kb polymorphic allele. The asterisk indicates a crosshybridizing CHO DNA sequence. Pooled samples of DNA digested with SacI from 10 individuals from Caucasoid, Asian, and Black populations are shown in lanes 2, 3, and 4 respectively and demonstrate both the 20kb and 16kb alleles.

Fig. 2. Autoradiograph showing results of a Southern blot hybridization with the albumin F47 probe on Sau96I-digested DNA. DNA was run on a 1.0% agarose gel and transferred to Zetabind. Lane 2 is DNA from an individual heterozygous for the Sau96I polymorphism showing the 5.25, 3.25, and 2.0kb fragments. Lane 1 is DNA from an individual homozygous for the 5.25kb fragment and lane 3 is DNA from an individual homozygous for the 3.25 and 2.0kb fragments. This polymorphism was shown to segregate in a Mendelian fashion within families (data now shown).

Fig. 3. Autoradiograph of StuI digested DNA on a Southern blot probed with pADH74 indicates a polymorphic band seen in lane 1 (indicated by arrow) that is not present in lane 2. Lane 1 contains DNA from a pooled sample of ten unrelated Caucasoid individuals digested with StuI. Lane 2 contains DNA from the HHW 416 cell line containing a single human chromosome 4 digested with StuI. Lanes 3 and 4 are DNA from homozygous individuals for the StuI polymorphism with fragments of 6.15kb and 9.55kb respectively. Lane 5 is DNA from a heterozygous individual with both the 9.55 kb and 6.15 kb fragments. Constant bands of 9.6kb and 5.0kb are also seen. The constant 9.6kb band is best seen in lane 3.

Table 1. Allele frequency and linkage disequilibrium of ADH3 RFLPs. StuI RFLP described here. XbaI RFLP described in Smith et al. (1986). Observed distribution of + and – alleles for each with respect to the other in a Caucasoid population. Numbers are the observed chromosomal haplotypes of a specific type (for example 25 chromosomes had both the StuI+ allele and the XbaI + allele). Fisher’s probability of P < 0.0001 for random association of StuI and XbaI alleles suggests marked linkage disequilibrium with Δ = 0.61. StuI+ allele has a frequency of 0.40 in this population.

|        | +    | –    |
|--------|------|------|
| XbaI   | 25   | 6    |
| StuI   | 9    | 38   |

RFLP is in tight linkage disequilibrium with previously described albumin RFLPs and thus does not provide any new information for linkage studies in the individuals studied to date in our laboratory (Ken Buetow, unpublished results).

Figure 3 shows a new polymorphism at the ADH3 locus on chromosome 4 (Smith et al. 1986) identified with the enzyme StuI on a screening Southern blot in lanes 1 and 2. Lanes 3, 4, and 5 show the alleles identified in the screening blot in single individuals. This polymorphism has a high frequency and is in linkage disequilibrium with a XbaI RFLP at the ADH3 locus reported elsewhere (Smith et al. 1986). Allele frequencies and disequilibrium values are shown in Table 1. Mendelian segregation has been observed in three families (data not shown).

For the albumin and ADH3 RFLPs, all enzymes used in the screening blots were also studied by screening 10 unrelated individuals in separate lanes. This included 30 different enzymes for albumin and 22 for ADH3. In no case was an RFLP not seen in the pooled samples identified in the individual lanes screened. The known RFLPs for albumin and the new RFLPs for albumin and ADH3 reported here were all identified in the pooled DNA screening procedure. Thus, no common RFLPs were missed by this technique.
Densitometry was performed using a Biorad 1650 densitometer on pooled samples of DNA containing from 2 to 40 chromosomes and digested with one or two enzymes known to be polymorphic at albumin or ADH3. The band intensities corresponded to the relative proportions of + or - alleles present in each sample. When a StuI/XbaI double digest was probed with ADH3 individual alleles did not have equal intensity of bands even when present in equimolar amounts (for example as in a single individual heterozygous for both enzymes). Thus, when calculating frequencies based on density it was necessary to normalize for the different signal strengths that arose from digests. A similar inequality of signal strengths was observed for the bands corresponding to the previously reported albumin Eco RV RFLP when double digests of SacI and Eco RV were performed.

**Discussion**

We have used DNA isolated from a cell line containing a single human chromosome 4 as a comparison lane against pooled samples of DNA from 10 unrelated individuals to search for RFLPs. Since the DNA isolated from a cell line retaining only a single human chromosome 4 is an obligate hemizygote, only a single allele can be present in this sample for any chromosome 4 RFLP. If a second allele is present in pooled DNA run in an adjacent lane, this will be seen as a band on a Southern blot not present in the DNA from the hemizygous cell line. This approach combines previous suggestions to use DNA from a male against a panel of DNA from females to enhance X-chromosome RFLP detection (Aldridge et al. 1984) and the use of pooled DNA samples to detect RFLPs in linkage disequilibrium in normal and insulin-dependent diabetic populations (Arnhem et al. 1985).

Several types of erroneous results may be produced by this method. First, the hemizygous cell hybrids must contain a single chromosome with a single clonal origin. A mixture of cells, containing chromosomes of more than one origin, are no more useful than analyzing DNA from a single individual. Even small amounts of contamination may seriously interfere with appropriate interpretation of screening blots. For analyzing our single chromosome hybrids we have overexposed our autoradiographs and detected no evidence of secondary bands, using probe and enzyme combinations that produce known polymorphic alleles. A second potential source of error would be partial digests of the pooled DNA samples. We only use highly purified DNAs that are known to completely digest with the appropriate restriction enzyme. In most cases digestions proceed for at least 12 hours with a two-fold enzyme excess. Since all potential RFLPs identified in screening need to be validated with family studies and Hardy-Weinberg calculations, this source of error is eventually revealed.

A third confounding variable is that approximately equal amounts of DNA from each individual should be present in the pooled sample. In our hands, signals of 5% intensity (representing one variant chromosome out of 20) can be detected. If by chance the DNA from a single variant individual in the pooled sample is present in disproportionately lower amounts, an RFLP in a single chromosome might be missed. Since RFLPs with a frequency of less than 5% are not as useful, we feel that 10 individuals is a reasonable number to use if looking for RFLPs to use in linkage studies (Skolnick and White 1983).

Using these screening blots we have also identified an epidermal growth factor RFLP with HincII that has allele frequencies of 0.40/0.6 (Murray et al. 1986). The same pooled DNA screening blots failed to identify a SacI RFLP with epidermal growth factor that has allele frequencies of 0.06/0.94, demonstrating that rare RFLPs can be missed by this as well as other screening procedures.

A fourth source of error is in using a probe that crosshybridizes with sequences from other chromosomes. These cross-hybridizing bands may appear as new alleles in comparing pooled samples to single chromosome samples. This will occur when the crosshybridizing sequence is found on a different chromosome not present in the cell line containing the single chromosome for which RFLPs are sought. These false RFLPs will be eliminated when one separates the pooled samples into individual lanes and sees that each has a constant band in a defined position. In addition, this band would not segregate with the particular chromosome under study in a panel of somatic cell hybrids.

There are significant benefits from using the procedure. First, it makes effective use of blot space and DNA. We formerly used 10 individuals in 10 lanes in our screening panels. We now use two lanes — one for hemizygous DNA and one for pooled DNA from 10 individuals. Thus, we use one-fifth as much blotting space and one-tenth as much DNA in the initial screen. It also uses one-fifth as much enzyme so that screening with expensive enzymes becomes more feasible.

It is not necessary to use a hybrid cell line containing a single chromosome of interest if one has access to cells from an individual monosomic for a portion of a chromosome that a particular probe has been localized to, and for which one wishes to identify polymorphisms. Thus, DNA from individuals who have partial monosomies of particular autosomes could be used to create a panel of DNAs useful in screening various subregions of chromosomes for RFLPs. An alternative would be to use DNA isolated from a complete hydatidiform mole. Such tumors have been shown to contain only a set of chromosomes of haploid paternal origin (Jacobs et al. 1980) and so would be hemizygous for all RFLP sites. Blots created using DNA from a hydatidiform mole when compared to pooled DNA samples could be used to identify RFLPs for any chromosome obviating the need for single chromosome-containing cell lines.

The use of pooled samples will also have utility in looking for rare RFLPs in individual families with specific disorders. Using pooled DNA from parental sets in a particular family, one could rapidly screen with a large number of restriction enzymes. Once an otherwise rare RFLP is found in such a family, that RFLP has great utility as a linkage marker in that particular pedigree. This will allow for screens for rare RFLPs in interesting families in a cost efficient and specific fashion.

Finally, we would propose that the use of pooled DNA samples from large numbers of individuals might be useful in providing estimates of both allele frequencies and linkage disequilibrium in such populations without having to examine each individual separately. Since signal strength of the bands is proportional to allele frequencies, the screen provides an estimate of frequencies so that apparently more common RFLPs may be pursued first. This requires densitometer determinations of band intensities in the pooled samples and so is subject to the limitations of densitometry but does provide useful approximations for many purposes. To determine the allele frequencies, only a restriction enzyme digest of the pooled...
DNA would be needed. To measure pairwise linkage disequilibrium, one would do a double digest using two polymorphic enzymes at a given locus. Four possible band sizes representing the four possible two allele/two enzyme haplotypes would then be studied by densitometer and the observed proportions compared to the expected proportions to determine if there is random association of alleles at the two loci. As noted above, it may first be necessary to normalize band intensities as equimolar amounts of different alleles may have different hybridization signals. The advantages here could be enormous in that even several thousand individuals (if mixed in equimolar amounts) could be studied in a single lane providing enormous savings of DNA, money, and time while providing reasonable assessment of frequencies and disequilibrium in very large population samples.

In summary, we report a rapid screening procedure for RFLPs using pooled DNA samples and DNA prepared from a hybrid cell line hemizygous for a human autosome. Two new RFLPs are reported and the utility of the procedure for several other applications discussed.

Acknowledgements. We gratefully acknowledge the technical assistance of Catherine DeHaven. IIHW 416 was generously supplied by John Wasmuth. Jeffrey C. Murray was supported by a Basil O'Connor Starter Research Award, National Foundation-March of Dimes.

References

Aldridge J, Kunkel L, Bruns G, Tantravahi U, Lalande M, Brewster T, Moreau E, Wilson M, Bromley W, Roderick T, Latt SA (1984) A strategy to reveal high-frequency RFLPs along the human X chromosome. Am J Hum Genet 36:546-564

Arntzen N, Strange C, Erlich H (1985) Use of pooled DNA samples to detect linkage disequilibrium of polymorphic restriction fragments and human disease: studies of the HLA class II loci. Proc Natl Acad Sci USA 82:6970-6974

Carlock L, Smith D, Wasmuth J (1986) Genetic counterselective procedure to isolate interspecific cell hybrids containing single human chromosomes: construction of cell hybrids and recombinant DNA libraries specific for human chromosomes 3 and 4. Somatic Cell Mol Genet 12:163-174

Dana S, Wasmuth J (1982) Linkage of the leuS, emtB and chr genes on chromosome 5 in humans and expression of human genes encoding protein synthetic components in human–Chinese hamster hybrids. Somatic Cell Mol Genet 8:242-264

Feinberg AP, Vogelstein B (1983) A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132:6-13

Feinberg AP, Vogelstein B (1984) A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Addendum. Anal Biochem 137:266-267

Gussel JS, Wexler NS, Connelly PM, Naylor SL, Anderson MA, Tantzi RE, Watkins PC, Ottina K, Wallace MR, Sakaguchi AY, Young AB, Shoulson I, Bonilla E, Martin JB (1983) A polymorphic DNA marker genetically linked to Huntington's disease. Nature 306:234-238

Harper ME, Dugaiczyk A (1983) Linkage of the evolutionarily related serum albumin and alpha fetoprotein genes within q11-22 of human chromosome 4. Am J Hum Genet 35:565-572

Jacobs PA, Wilson CM, Spenkle JA (1980) Mechanism of origin of complete hydatidiform moles. Nature 286:714-716

Murray JC, Demopoulos M, Lawn RM, Motulsky AG (1983) Molecular genetics of human serum albumin: restriction enzyme fragment length polymorphisms and analbuminemia. Proc Natl Acad Sci USA 80:5951-5955

Murray JC, Mills KA, Demopoulos CM, Hornung S, Motulsky AG (1984) Linkage disequilibrium and evolutionary relationships of DNA variants (restriction enzyme fragment length polymorphisms) at the serum albumin locus. Proc Natl Acad Sci USA 81:3486-3490

Murray JC, DeHaven CR, Bell GI (1986) RFLPs for epidermal growth factor (EGF), a single copy sequence at 4q25-4q27. Nucleic Acids Res 14:5117

Poncz M, Sowińscyzyk D, Harpel B, Mory Y, Schwarz E, Surrey S (1982) Construction of human gene libraries from small amounts of peripheral blood: analysis of β-like globin genes. Hemoglobin 6:27-36

Reeder ST, Breuning MH, Davies KE, Nicholls RD, Jarman AP, Higgs DR, Pearson PL, Weatherall DJ (1985) A highly polymorphic DNA marker linked to adult polycystic kidney disease on chromosome 16. Nature 317:542-544

Skolnick MH, White R (1983) Strategies for detecting and characterizing restriction fragment length polymorphisms (RFLP’s). Cytogenet Cell Genet 32:58-67

Smith M (1986) Genetics of human alcohol and aldehyde dehydrogenases. Adv Human Genet 15:249-286

Tsui L, Buchwald M, Barker D, Braman JC, Knowlton R, Schumm JW, Eiberg H, Mohr J, Kennedy D, Plavsic N, Zsiga M, Markiewicz D, Akots G, Brown V, Helms C, Gravius T, Parker C, Rediker K, Donis-Keller H (1985) Cystic fibrosis locus defined by a genetically linked polymorphic DNA marker. Science 230:1054-1057

White R, Leppert M, Bishop DT, Barker D, Berkowitz J, Brown C, Callahan P, Holm T, Jerominski L (1985) Construction of linkage maps with DNA markers for human chromosomes. Nature 313:101-105

Received April 14, 1986 / Revised December 1, 1986