Compatibility of Several HVR-Type Probes with Pvu II Restriction Endonuclease and Advantages of Pvu II Over Other Commonly Used Enzymes

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INTRODUCTION

One characteristic of chromosomal DNA loci exhibiting VNTR polymorphisms is the ability to study such polymorphisms using RFLP mapping with multiple restriction endonucleases. Three restriction enzymes, Pst 1, Hae III, and Hinf I, have been widely used in RFLP mapping for identity testing with a number of commonly used DNA probes.

There are several considerations in choosing a restriction enzyme for use with a collection of HVR probes for identity testing. One consideration is the activity of a particular enzyme and its susceptibility to inactivation by contaminants present in some samples of chromosomal DNA. A second consideration in choosing an enzyme is the size of restriction fragments produced at each locus being analysed. Ideally, one wants an enzyme that is highly active in digesting chromosomal DNA and produces allele sizes at each locus that are compatible with analysis on a single Southern blot.

We describe here the characteristics of a collection of commonly used, and readily available probes that can be used for identity testing in conjunction with Pvu II restriction enzyme. Pvu II has advantages over other restriction enzymes commonly used for identity testing in that Pvu II is highly active in digesting chromosomal DNA. In addition, Pvu II produces alleles at each of the loci that range in size from about 1-10 kb; a range of allele sizes easily separated during an overnight electrophoresis in a standard gel system employing 0.8% agarose.

MATERIALS AND METHODS.

The probes used in this study include: 3'HVR (Higgs et al 1981) detecting the D16S85 locus (kindly provided by Dr. David Werrett, Home Office Forensics Unit, England), pHRAS, a oligonucleotide probe detecting the HRAS1 locus (Krontiris et al 1985, Thein et al 1986), YNH24, an oligonucleotide probe detecting the D2S44 locus (Nakamura et al 1987), and pS194 (Collaborative Research, Bedford, MA) detecting the D7S107

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locus (Barker et al 1987). Standard conditions used for RFLP mapping included an overnight digestion of chromosomal DNA with a fivefold excess of restriction enzyme. Each sample of chromosomal DNA (5-10 ug) was mixed with 0.5-1.0 ug of lambda DNA prior to adding enzyme, and the extent of digestion was evaluated by electrophoresing 10% of the digest on an analytical agarose gel that was subsequently stained with ethidium bromide. Restriction fragments in samples digested to completion were recovered by ethanol precipitation and centrifugation and electrophoresed in 0.8% agarose gels in 89 mM Tris-borate pH 8.3 + 1 mM EDTA. Gels were blotted onto Zetabind membranes (AMF Cuno, Meriden, CT) in 20 mM NaOH (Fowler et al 1988) over a 4-6 hour period. Blots were hybridized to probes labeled with ^{32}P-dCTP by oligonucleotide primer mediated labeling (Feinberg and Vogelstein 1984). Filters were hybridized and washed under conditions of high stringency.

RESULTS

The purpose of this study was to evaluate RFLP mapping data generated with a collection of four HVR probes used in conjunction with different restriction enzymes. The probes used in this study have been shown to be informative for identity testing and are widely used in parentage testing and forensics. Although these probes are common to a number of laboratories, they are used in conjunction with different restriction enzymes; most notably Pst 1 and Hae III.

The activities of Pvu II, Hae III, and Pst 1 were compared using chromosomal DNA isolated from random blood donors. 1-2 ug aliquots of chromosomal DNA were mixed with 200-400 ng of lambda DNA and digested with a 5 fold excess of restriction enzyme overnight at 37^{o}C. The samples were then electrophoresed in an agarose gel which was subsequently stained with ethidium bromide to visualize the pattern of restriction fragments produced. Restriction enzymes were obtained from several commercial suppliers (BRL, New England Biolabs, Boehringer Mannheim, U.S. Biochemicals). If the expected pattern of restriction fragments from lambda DNA was observed in the gel along with a hazy smear of chromosomal fragments, the digestion was scored as complete. If the expected pattern of lambda fragments was not observed, a second aliquot of DNA was digested and analysed under the same conditions. If the second aliquot digested to completion, it was scored as complete. If neither the first nor the second digestion was complete, the sample was scored as incomplete. The results of this study indicated that Pvu II digested approximately 99% of the DNA samples followed by Hae III which

digested approximately 98%. The least active enzyme was Pst 1 which digested only 92% of the DNA samples.
A comparison of allele sizes produced with the different enzymes at each locus is shown in Table 1. The data indicate that Hae III can produce small alleles at the D16S85, D2S44, and HRAS1 loci. Small restriction fragments, which represent the most common alleles in the population at the D16S85 and HRAS1 loci (unpublished observations), can electrophorese out of the gel during extended electrophoresis, or, can diffuse within the gel during blotting, making accurate sizing of fragments difficult. Thus, Hae III is not the enzyme of choice for studying polymorphisms at the D16S85, D2S44, or HRAS1 loci.

Table 1. Characteristics of alleles produced at four HVR loci with different restriction enzymes.

Probe/Locus	Size Range of Alleles		
	Pvu II	Hae III	Pst 1
3'HVR/D16S85	1.5-10 kb	0.5-8.0 kb	0.6-8.0 kb
YNH24/D2S44	2.0-10.0 kb	<1-7.0 kb[1]	6.0-17 kb[2]
pHRAS/HRAS1	2.5-6.5 kb	0.3-4.0 kb	1.5-5.0 kb[3]
pS194/D7S107	1.3-8.0 kb	N.P.[4]	5.0-12.5 kb[5]

1. Michael Baird, Lifecodes, personal communication.
2. Balazs et al 1989.
3. Baird et al 1985.
4. Not polymorphic, Tim Keith, Collaborative Research, personal communication.
5. Dykes 1988.

Digestion of chromosomal DNA with Pst 1 produces relatively small alleles at the D16S85 locus, moderately sized alleles at the HRAS1 locus (Baird et al 1988 and unpublished observations), and large alleles at both the D2S44 and D7S107 loci (Table 1) (Balazs et al 1989, Dykes 1988). Adequate separation of alleles at the D2S44 and D7S107 loci therefore requires an extended period of electrophoresis (Balazs et al 1989, Baird et al 1985,1988, Dykes 1988) which is generally incompatible with visualization of small alleles at both the D16S85 and HRAS1 loci. Thus, Pst 1 is not the enzyme of choice for use with these four probes.

In contrast to Hae III and Pst 1, Pvu II digestion of chromosomal DNA produces alleles at all four loci that range

in size from about 1-10 kb (Table 1). The size of alleles produced at each locus with Pvu II is such that an overnight electrophoretic run of the digest in a 0.8% agarose gel of standard format (ie 15 X 20 cm) provides good separation of restriction fragments. Alleles at each locus exhibit an apparent continuous distribution of fragment sizes over the size range and frequencies of alleles generated with Pvu II are similar to those observed when Pst 1 or Hae III are used to digest the DNA as would be anticipated (results not shown).

The discriminatory capabilities of the four probes in parentage testing have been examined in a large number of paternity trios for which results of standard testing methods were known. The results (Table 2) indicate that this collection of probes used in conjunction with Pvu II will exclude approximately 99.90% of falsely accused men in paternity suits.

Table 2. Discriminatory capabilities of four HVR probes used with Pvu II for parentage testing.

Probe	PEX[1]
3'HVR	0.90
YNH24	0.88
pHRAS	0.60
pS194	0.80
Cumulative PEX= 0.9990	

1. PEX refers to the ability of a given probe (or collection of probes) to exclude a falsely accused man in a paternity dispute.

DISCUSSION

The results presented here indicate that a collection of four probes, commonly used for identity testing, are compatible with Pvu II restriction enzyme. Pvu II has several advantages over either Pst 1 or Hae III when used with this collection of probes. One advantage is the higher relative activity of Pvu II compared to the other enzymes which results in a lower number of chromosomal DNA samples that need further processing before complete digestion is obtained. The second advantage relates to the size range of alleles produced at each locus. Pvu II produces alleles at each locus that fall into a 1-10 kb

range. Thus, a single blot generated from a standard 0.8% agarose gel run overnight can be sequentially hybridized to the probe collection with the general assurance that alleles at each locus will be adequately resolved for subsequent analysis.

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