A 50 bp polymorphic insertion in the factor IX gene is readily detected by amplification and is in equilibrium with other polymorphic sites

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A factor IX polymorphism 5′ to exon 2 was described as 1.70 versus 1.75 kb bands on Southern blots of DdeI digests (1). By primer directed polymerase chain reaction (PCR) amplification, the two alleles are readily apparent on agarose electrophoresis of undigested fragments (430 or 380 bp); in heterozygous women or amplified mixtures of two hemizygous male types, an additional fragment (~480 bp) is seen (Fig. 1). On polyacrylamide gels, this fragment has very slow migration, suggesting an aberrant structure (perhaps a heteroduplex; not shown). In normal Caucasian and Black males, the frequency of the 50 bp insertion is 0.18 and 0.17, respectively which is quite different from relative frequencies of other polymorphisms (2, 3). It is absent in factor IX genes from Asian subjects (N = 56). In four families where other polymorphic analyses were not informative, the 50 bp insertion is heterozygous in carrier women; by calculation, ~30% of Black or Caucasian women will be heterozygous for the 50 bp insertion.

The previous report (1) suggested that the DdeI polymorphism was in linkage disequilibrium with a TaqI polymorphism. As shown in Table 1, however, equilibrium is found between the 50 bp insertion by PCR and other intragenic polymorphisms including TaqI and Thr/Ala-148 (2), HhaI (4) and BamHI (3). Equilibrium accounts for the enhanced utility of the 50 bp insert analysis in carrier detection of hemophilia B.

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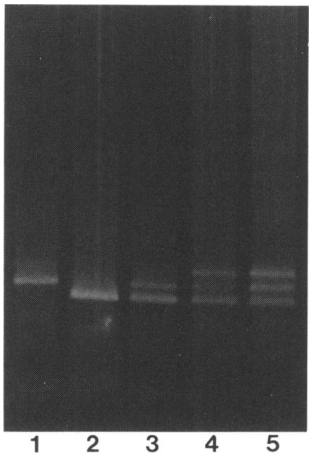

Figure 1. Shows amplified fragments electrophoresed directly on 1.6% agarose gels, stained with ethidium bromide and visualized by UV light. Lane 1, a hemizygous male with the insert corresponding to 430 bp from markers (not shown); lane 2, a hemizygous male without the insert corresponding to 380 bp; lane 3, a mixture of the two amplified separately; lane 4, a mixture of the two amplified together; lane 5, a heterozygous female. Conditions for amplification were essentially as described for BamHI (3), except primers were 5′-ATGTGG-TCCATCATTGACCA-3′ and 5′-ACCTAGTCTGAAGAGACACT-3′, and the $MgCl_2$ concentration in the PCR buffer was decreased to 0.34 M.

Table 1. 50 bp insert (DdeI) linkage analyses in Caucasians or, for BamHI, Blacks

Insert	Other	TaqI (N = 28) Obs	Exp	Thr/Ala-148-MnlI (N = 75) Obs	Exp	HhaI (N = 33) Obs	Exp	BamHI (N = 17) Obs	Exp
+	+	1	1.9	1	3.4	5	3.8	1	0.8
+	−	5	4.1	16	13.6	1	2.2	1	1.2
−	+	8	7.1	14	11.6	16	17.2	6	6.2
−	−	14	14.9	44	46.4	11	9.8	9	8.8
Chi Squared (all n.s.)			0.84		2.74		1.23		0.07