Digestion conditions resulting in altered cut site specificity for *HinfI*

Jodi Kriss, George Herrin, Lisa Forman and Robin Cotton*
Cellmark Diagnostics, Germantown, MD 20876, USA

Submitted May 8, 1990

Analysis of DNA samples for restriction fragment length polymorphisms requires correct recognition of the normal restriction sites. Some restriction endonucleases have been shown to exhibit altered cut site specificity (star activity) under certain reaction conditions (1, 2). We report here the results of experiments demonstrating *HinfI* star activity.

Two μ gs of pUC18 DNA or SV40 DNA were digested overnight with *HinfI* purchased from New England Biolabs (NEB), Beverly, MA; Bethesda Research Labs (BRL), Gaithersburg, MD; or Promega, Madison, WI in 40 μ l of 100 mM NaCl, 10 mM Tris-HCl (pH 7.4), 10 mM MgCl₂, 5 mM β -Mercaptoethanol (BME), and 100 μ g/ml bovine serum albumin (BSA). Four μ gs of human genomic DNA, extracted from blood according to standard procedures, were digested with *HinfI* in a final volume of 80 μ l under the conditions stated above. Final concentrations of *HinfI* storage buffer [50 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol (DTT), 200 μ g/ml BSA, and 50% glycerol], glycerol, BSA, NaCl, KCl, and DTT as well as digestion time were varied.

HinfI digested DNA samples were electrophoresed in agarose gels. Human DNA samples were transferred to a nylon membrane according to the procedure of Southern and hybridized with the four single-locus probes MS1, MS31, MS43, and g3 (3). Relaxed cut site specificity, as indicated by the appearance of additional bands on ethidium bromide stained gels (pUC18 and SV40 DNA) and autoradiographs (genomic DNA), is observed with *HinfI* from all manufacturers tested under certain digestion conditions.

Additional bands are faintly visible in *HinfI* digested pUC18 DNA using 80 or 160 units of enzyme with 2.5% glycerol or 320 units of enzyme and 1.25% glycerol. The effect of high enzyme and glycerol concentrations on relaxation of *HinfI* specificity is enhanced by increasing digestion times. When pUC18 is digested with 80 units of *HinfI* at 2.5% glycerol, additional bands appear at 16 hours of incubation and become more intense as digestion times are increased up to two days. At 200 units of enzyme and 25% glycerol, extra bands are visible at 4 hours of digestion and increase in intensity with longer incubation times. Varying the concentrations of KCl (10 mM to 200 mM), NaCl (10 mM to 200 mM), BSA (0.4 μ g or 4 μ g), and DTT (0.05 mM or 0.5 mM) in the reaction buffer did not affect *HinfI* specificity.

As shown in Figure 1, relaxed cut site specificity of *HinfI* can also be visualized in digests of human genomic DNA. Digestion with 40 units of enzyme in the presence of 5% to 30% glycerol gives no visible inappropriate digestion. At 80 units of enzyme,

faint extra bands become visible when storage buffer or glycerol alone is added to give final glycerol concentrations of 10–15% (Figure 1). These extra bands are clearly observed using 160 units of *HinfI* in 10% glycerol and become more intense as enzyme and glycerol concentrations are increased. With all DNA substrates tested, *HinfI* star activity is enhanced when the enzyme and glycerol concentrations are increased in concert (Figure 1). Banding patterns visualized using probes MS1, MS31, MS43, and g3 showed slightly different extents of altered enzyme activity as would be anticipated based on the presence or absence of star sites within the region recognized by each specific probe. Additionally, the extra bands resulting from star activity differ between the two individuals tested.

Our data indicate that *HinfI* exhibits star activity at high enzyme, glycerol or storage buffer concentrations, and prolonged incubation times. When enzyme is kept below 80 units with glycerol concentrations at or under 5%, no alterations in recognition site specificity are observed in *HinfI* digests of human genomic DNA.

REFERENCES

1. Chirikjian, J.G. and George, J. (1981) In: Chirikjian, J.G. (ed.), *Gene Amplification and Analysis*, Elsevier/North Holland, New York, Vol. I, pp. 73–99.
2. Roberts, R.J. (1983) *Nucl. Acids Res.* **11**, 135–167.
3. Wong, Z. et al. (1987) *Ann. Hum. Genet.* **51**, 269–288.

Figure 1. MS1 hybridization to *HinfI* digested human DNA. (U = units of *HinfI*, % = final % glycerol in digest). Lanes 1 and 9 (20 U, 1.25%), 2 and 10 (40 U, 2.5%), 3 (80 U, 5%), 4 (160 U, 10%), 5 (240 U, 15%), 6 (320 U, 20%), 7 (400 U, 25%), 8 (480 U, 30%), 11–15 (all 80 U with 5, 10, 15, 20, and 25%, respectively). Source of *HinfI*: NEB.

* To whom correspondence should be addressed