Altered specificity of restriction endonuclease *Hin*fl

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Some restriction endonucleases have been found to cleave sequences which are similar but not identical to their reported recognition sequences. This additional cleavage at other sites is known as star activity (1). It is reported here that *Hin*fl which recognizes 5' GATC 3' demonstrates an altered specificity under certain conditions.

*Hin*fl isolated in three different laboratories shows identical altered specificity (Fig. 1). Digestion of pBR322 DNA with 50–500 units of *Hin*fl for 16 hours results in the appearance of additional bands. All three sources of *Hin*fl demonstrate the identical banding pattern where at least two cleavage sites within the largest *Hin*fl fragment (1632 bp) have been cleaved. Further, the rate of cleavage per unit of *Hin*fl is similar for all three samples. This is particularly significant as two of the suppliers report their source as *Haemophilus influenzae* Rf and the other from a strain of *E. coli* carrying a plasmid encoding the gene which overproduces the *Hin*fl protein. Therefore, the possibility of this altered specificity being the result of another endonuclease copurifying seems unlikely.

Restriction endonucleases have been shown to exhibit star activity when high concentrations of endonuclease are used or when alterations in the standard assay conditions are made (1). High glycerol concentrations, 10% DMSO, and the substitution of manganese for magnesium are three such conditions under which *Hin*fl was examined. *Hin*fl appears in these situations to have increased proclivity for star sites. As little as 10% glycerol relaxes the specificity of *Hin*fl and a more marked effect is noted when the glycerol is in the presence of high concentrations of enzyme. One hundred units of enzyme for 16 hours at 10% glycerol exhibited greater star activity than 200 units for 16 hours at 1% glycerol. When 10% DMSO was added to the standard reaction buffer the star sites appeared at less than 50 units for 16 hours. The substitution of manganese for magnesium enhanced the star activity, but interestingly, inhibited the cognate activity by greater than 30-fold.

It has been suggested that star activity may be a general property of restriction endonucleases (2) and that any restriction enzyme given aberrant reaction conditions may exhibit relaxed specificities. *Hin*fl seems to be yet another restriction enzyme that falls in this category. It should be noted that no star activity could be detected with either 50 units of *Hin*fl for 16 hours in standard reaction conditions or 1000 units of *Hin*fl for 3 hours in standard reaction buffer containing 10% glycerol.

REFERENCES

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2. Nasari, M. and Thomas, D. (1986) *Nucl. Acids Res.* **14**, 811–821.

Figure 1. A 16 hour digestion of 1 μ g of pBR322 DNA in a 50 μ l standard reaction buffer containing 100 mM NaCl 10 mM Tris-HCl (pH 7.4), 10 mM $MgCl_2$, 5 mM 2-mercaptoethanol, 100 μ g/ml bovine serum albumin with 1-(50 units), 2-(100 units), 3-(150 units), 5-(250 units), and 10-(500 units) of *Hin*fl (50 U/ μ l in a 50% glycerol storage buffer). Source of *Hin*fl: A. New England Biolabs, B. Promega, C. Boehringer Mannheim. The size standard (M) is Φ X174 DNA digested with *Hae*III.

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