Star Activity of Restriction Enzymes

The precise specificity of the approximately 3000 known restriction enzymes for their >200 different target sequences could be considered their most interesting characteristic. Although all restriction enzymes bind DNA nonspecifically, under optimal conditions the difference in cleavage rates at the cognate site and the next-best site (single base substitution) is very high. For example, the rate difference for EcoR I at its cognate site (5'-GAATTC-3') and next-best site (5'-TAATTC-3') is of the order of 105 (1). Similarly, for EcoR V, cleavage at its cognate site (5'-GATATC-3') is 106 times faster than at the next-best site (5'-GTTATC-3') (2).

However, under nonoptimal conditions, the differences in cleavage rates between cognate and next-best sites change dramatically for many enzymes. This loss of fidelity or increase in cleavage at sites similar to the cognate site is commonly referred to as star activity. A number of reaction parameters can increase the rate of cleavage at star sites relative to cognate sites. These include pH, type of ions present, ionic strength, metal cofactors other than Mg2+, high DNA:enzyme ratios and the presence of volume excluders (glycerol, ethylene glycol, etc.). In conjunction with this increase in star activity, cleavage rates at the cognate site generally decrease. For example, for EcoR I, the rate difference between cognate and star sites approaches zero as ethylene glycol concentration increases up to 4M (3) and for EcoR V, the rate difference drops to only 6-fold when Mn2+ is substituted for Mg2+ (2).

Several plausible explanations for star activity are based on the proposed mechanisms for target site identification and hydrolysis. During nonspecific binding, a large number of water molecules are present at the protein-DNA interface. When tighter binding and positioning of the catalytic site occur upon recognition of the target sequence, the number of these interface water molecules is significantly reduced. The higher osmotic pressure caused by volume excluders results in the same reduction in the amount of interface water molecules and allows easier active complex formation at star sites (3). At alkaline pH, higher OH- concentrations may reduce the need for an activated water molecule, which normally initiates nucleophilic attack on the scissile phosphorous. Mn2+ has a higher affinity for oxygen ligands than Mg2+ and may bind more easily to a catalytic site in a partially active conformation at a star site. Also, it is possible that Mn2+-bound water is better able to protonate the leaving group since it has a lower pKa than Mg2+-bound water (4).

Although all restriction enzymes probably exhibit some decrease in the cleavage rate difference between cognate and near-cognate sites under such extreme conditions as 4M ethylene glycol, most are not significantly affected under common usage conditions. Those that are susceptible to star activity are induced to different degrees by variations in reaction conditions or by combinations of the conditions listed above. The table lists the enzymes that may exhibit star activity, especially under reaction conditions that deviate from those recommended. In multiple enzyme digests or multiple step applications, it is advisable to stay at or near the optimal conditions for these enzymes whenever possible.

Part No.	OPTIZYME* Enzymes That May Exhibit Star Activity
BP8069	AarI
BP8053	BclI
BP8022	PvuII

Conditions that contribute to star activity	Steps to inhibit star activity
High glycerol concentration (≥5%)	● Restriction enzymes are stored in 50% glycerol. The amount of enzyme should not exceed 10% of the reaction volume
High concentration of enzyme/DNA	● Use the smallest number of units possible to achieve digestion (limits glycerol concentration)
Buffer is not optimal	● Set up the reaction in the optimal buffer whenever possible
Prolonged reaction time	● Use the minimim reaction time required for the restriction enzyme (prevents evaporation).
Solvents present in reaction mixture	● Ensure that the reaction mixture doesn't contain contaminants (i.e., DMSO, ethanol, etc.) from DNA purification applications.
Substitution of Mg^{2+} with other cations	● Use Mg^{2+} as the divalent cation, whenever required to ensure proper site recogniton.

References:

Lesser, D.R., Kurpiewski, M.R., and Jen-Jacobson, L. (1990) The energetic basis of specificity in the EcoR I endonuclease—DNA interaction. Science 250, 776.

Vermote, C.L. and Halford, S.E. (1992) EcoR V restriction endonuclease: communication between catalytic metal ions and DNA recognition. Biochem. 31, 6082.

Robinson, C.R. and Sligar, S.G. (1998) Changes in solvation during DNA binding and cleavage are critical to altered specificity of the EcoR I endonuclease.
Proc. Natl. Acad. Sci. USA 95, 2186.