Troubleshooting Guide for DNA Digestion: Unexpected Cleavage Pattern and Gel Shift

Possible Cause	Recommended Solution
Star activity (relaxed specificity)	● See Star Activity Table for more details. ● Reduce the amount of enzyme in the reaction mixture. ● Use the recommended reaction buffer. ● Ensure that the glycerol concentration in the reaction mixture doesn't exceed 5%. ● Ensure that the volume of the reaction mixture was not reduced due to evaporation. The resulting increase in glycerol concentration may result in star activity.
Contamination with another restriction enzyme	● The restriction enzyme or buffer may be contaminated with another restriction enzyme due to improper handling. Use a new tube of restriction enzyme and/or buffer.
Contamination with another substrate DNA	● The sample mixture may contain a mixture of more than two DNAs. Prepare a new sample of DNA. ● For PCR products, check the purity on an agarose gel. If necessary, purify the product prior to digestion.
Incomplete DNA digestion	● See Incomplete Digestion Troubleshooting Guide.
Unexpected recognition sites	● Newly generated target sites in constructed DNA may be overlooked. Re-check your DNA sequence and cloning strategy.
Gel shift	● Enzymes that remain bound to the substrate DNA will affect electrophoretic mobility of digested products. AarI, AloI, and Eco57I are particularly prone to remaining bound to the substrate DNA. This will result in a smear above the expected band. ● Heat the digested DNA for 10 min. at 65°C in the presence of 6X DNA loading dye & SDS solution.
Contaminated reagents	Any component of the reaction mixture may become contaminated with nucleases due to improper handling or storage. Nuclease contamination degrades DNA, which appears as diffused DNA bands on a gel. ● Perform four control reactions to check for nuclease contamination: (I) without the restriction enzyme, (II) with a new vial of buffer, (III) without the restriction enzyme, with a new vial of buffer, (IV) with commercially available nuclease-free water(BP2819) ● Contaminated sample DNA: Prepare new DNA or re-purify DNA. ● Contaminated enzyme: The enzyme may become contaminated due to improper handling or storage. Use a new vial of enzyme. ● Contaminated buffer: Bacterial contamination in the reaction buffer will cause DNA degradation. Use a new vial of buffer. Store all buffer at -20°C. ● Contaminated water: Bacterial or DNase contamination of water in improperly handled water will cause DNA degradation. Use commercially available nuclease-free, molecular biology-grade water (BP2819).