Troubleshooting Guide for DNA Digestion: Incomplete Digestion or no Digestion

Possible Cause	Recommended Solution
Inactive Enzyme	If the enzyme doesn't cut the control DNA: ● Check the expiration date ● Verify that the enzyme has been stored at -20°C ● Check the temperature of your freezer. Do not allow the temperature to go below -20°C as the enzyme may freeze and multiple freeze thaw cycles may result in reduced enzyme activity.
Suboptimal Digestion Protocol	● Follow the digestion protocol specified for the restriction enzyme and the type of subtrate DNA. ● Use the recommended buffer supplied with the restriction enzyme. For double digests, use the "Relative Activity Buffer Table" to determine optimal buffer conditions. ● Use additives where required. Refer to package insert or "Relative Activity Buffer Table." ● Perform the reaction at the optimal temperature specified for the restriction enzyme. For double digestion reactions with enzymes requiring different incubation temperatures perform sequential DNA cleavage: complete the first digestion reaction at the lower temperature, add the second enzyme and increase the digestion temperature for the second enzyme cleavage. ● Ensure that the volume of the reaction mixture was not reduced due to evaporation during digestion: the increase in salt concentration may reduce enzyme activity.
Improper Dilution of the Enzyme	● Never dilute enzymes in water or 10X reaction buffer. ● Never dilute enzymes in 1X reaction enzyme buffer in the absence of DNA.
Improper Reaction Assembly	● The restriction enzyme should always be the last component that is added to the reaction mixture. ● The restriction enzyme may be inactivated if added directly to the 10X reaction buffer.
Excess Glycerol in the Reaction Mixture	● The glycerol concentration in the reaction mixture should not exceed 5%. ● Enzymes that are sensitive to high glycerol concentrations include: EcoRI, NcoI, HinfI, Eco9I, EcoRV, and BpiI.
Suboptimal DNA Concentration	● The optimal range of DNA concentration in the reaction mixture is 0.02-0.1 µg/µl.
Contaminants in the DNA Solution	● Template DNA may contain residual SDS, EDTA, proteins, salts or nucleases. Repurify the DNA using a spin column or by phenol/chloroform extraction (BP1752) and ethanol precipitation (BP2818). DNA $A_{260/280}$ should be 1.8-2.0. ● For digestion of unpurified PCR products, dilute DNA at least 3-fold in the recommended 1X restriction buffer. ● If the template DNA has been purified using silica or resin suspensions, remove all remaining particles by centrifugation for 10 min at 10,000 rpm and ensure that no resin is carried over while transferring the DNA solution into a new tube.
Substrate DNA doesn't contain a recognition sequence for the restriction enzyme	● Re-check the DNA sequence and the cloning strategy. ● Determine if the restriction enzyme selected requires more than one site per target DNA for 100% activity. ● If the restriction site was introduced using PCR, verify that the primer sequences contain the restriction site.
Methylaton Effects	● Refer to the package insert or methylation tables to identify which type of DNA methylation can occur on the recognition site and determine if the methylation impairs or blocks digestion with the restriction enzyme. ● If methylation blocks or impairs DNA cleavage, propagate your plasmid using an E.Coli *dam-* , *dcm-* strain. ● Use a restriction enzyme isochizomer not sensitive to DNA methylation.
Structure of Substrate DNA	● For some enzymes, additional units are required to completely digest supercoiled DNA. Check notes on the package insert. ● Some enzymes cleave poorly if the restriction site is close to the ends of PCR molecules. Refer to the table of cleavage efficiency close to the termini of PCR fragments.
Water contains impurities	● Check the pH and conductivity of water. The pH of high quality water should be 5.5-6.0 with a resistance of ≥18 MΩ. ● Determine if the water contains nucleases or bacterial contamination. ● Compare your results using commercially available nuclease free, molecular biology grade water (BP2819) ● Centrifuge (10min, 10,000 rpm) 1 ml of water and check to see if there is a visible pellet.