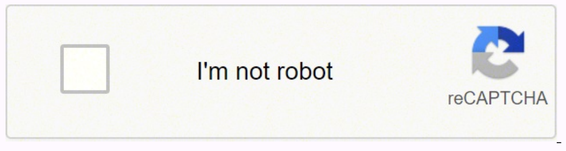

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Restriction enzyme digestion lab report answers key pdf free full

Since it is very difficult to assay for cutting near the end of DNA, the effectiveness of compensation with extra enzyme units or increased incubation time is difficult to determine. Substrate structural variations, concentration and special considerations are discussed below according to DNA type. Addition of BSA to restriction digests at a final concentration of 0.1mg/ml may also improve enzyme activity. DNA-RNA Hybrids: Digestion of DNA-RNA hybrid molecules has been described for several restriction enzymes (AluI, EcoRI, HaeIII, HhaI, HindIII, MspI, SalI, ThaI) (9). Genomic DNA purified by traditional techniques can contain double-stranded breaks due to mechanical shear forces. Restriction enzymes require varying amounts of flanking DNA around the recognition site, usually 1-3 bases but occasionally more (See Digestion of Sites Close to the End of Linear DNA). In lambda DNA, the cos ends, (12-base, complementary, single-stranded overhangs at the end of each molecule) may re-anneal during digestion. If the restriction site is built into the primer, primer dimers will contain a double-stranded version of the site, usually in vast molar excess over that of the desired target PCR fragment. Studies have shown, however, that several restriction enzymes that appear to cleave single-stranded DNA actually recognize folded-back duplex regions within the single-stranded genomes (e.g., M13, f1, single-stranded phiX174) (7) (8). Again, longer digestion times and/or more enzyme may be needed. Genomic DNA often digests more efficiently when it is diluted to a minimum concentration of 10µg per 50-200µl. If a supercoiled plasmid is first linearized with another restriction enzyme or relaxed with topoisomerase, less enzyme may be needed for digestion. Digestion near the end of a PCR product may also present problems. In these cases, the DNA strand of the hybrid was digested in the identical place as duplex DNA. Methylation: Methylation of nucleotides within restriction enzyme recognition sequences can affect digestion. Promega offers the Wizard® Genomic DNA Purification Kit (Cat.# A1120) for the isolation of genomic DNA from white blood cells (with reagents/protocol for removal of red cells), tissue cultured cells, animal tissue, plant tissue and Gram-positive and Gram-negative bacteria. When digesting other substrates, adjustments may be needed based on the amount of substrate, the number of recognition sites per molecule and the incubation time. Influence of Flanking Sequence: The sequences flanking the restriction enzyme recognition sequence can influence the cleavage rate of many restriction enzymes although the differences are usually less than 10-fold. These techniques can yield good quality DNA from small volumes of blood, but the DNA obtained after scale-up may be of poorer quality. To avoid this, mammalian, bacterial and yeast cells can be embedded in agarose strips and the cells lysed and treated with proteinase K in situ (11). Single-Stranded DNA: Cleavage of single-stranded DNA, although at a greatly reduced rate compared with double-stranded DNA, has been reported for a few restriction enzymes (6). The concentration of the DNA sample can influence the success of a restriction digestion. For further information, refer to Digestion of High Molecular Weight DNA. Viscous DNA solutions, resulting from large amounts of DNA in too small of a volume, can inhibit diffusion and can significantly reduce enzyme activity (1). For larger blood samples, a technique that separates white blood cells from red blood cells, such as pelleting red blood cells through a Ficoll® gradient, is recommended prior to DNA purification. A small number of enzymes (e.g., NaeI, HpaII, SacII, NarI, EcoRII) exhibit more pronounced site preferences and are designated Type IIe. See Site Preferences and Turbo™ Restriction Enzymes for further information. Longer incubation times may be required to ensure complete digestion. **Enzymes differ in their ability to digest supercoiled vs. DNA Substrate Base Pairs Picomoles in 1µg* Cut Sites (EcoRI) Picomoles Cut Sites Units Needed Unit definition (lambda) 48,502 0.0317 5 0.1585 1 plasmid 3,000 0.5 1 0.5 3** PCR fragment 700 2.2 1 2.2 14 oligonucleotide 25 62.5 1 62.5 394 *Based on 650 Daltons per base pair of DNA. Home Science Chemistry Substrates commonly used for restriction enzyme digestion include phage DNA, plasmid DNA, genomic DNA, PCR products and double-stranded oligonucleotides. This problem can be easily avoided by purifying the PCR fragment prior to restriction enzyme digestion using the Wizard® PCR Preps DNA Purification System (Cat.# A7170). Therefore, these enzymes are not digesting single-stranded DNA, rather individual sites that are in the duplex form. For further information see Digestion of High Molecular Weight DNA. Best results are obtained when the absorbance ratios at A260/A280 are at least 1.8. Spermidine can be added to a final concentration of 1mM and/or BSA to a final concentration of 0.1mg/ml to improve digestion of poor quality genomic DNA. Methylation may occur as 4-methylcytosine, 5-methylcytosine, 5-hydroxymethylcytosine or 6-methyladenine in DNA from bacteria (including plasmids), eukaryotes and their viruses. Recognition Site Density Restriction enzyme activity units are usually defined based on a one-hour digest of 1µg of lambda DNA. To avoid this problem, heat the DNA at 65°C for 5 minutes prior to electrophoresis to melt ends that have annealed. Consult the Promega Product Information sheet for the overdigestion value of the enzyme. A good rule of thumb is to use 5-10 units of enzyme per microgram of DNA and to avoid using restriction enzymes with low overdigestion values (

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