Oligonucleotide-directed restriction endonuclease digestion

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The following is a simple procedure for efficiently targeting restriction endonuclease digestion to virtually any restriction endonuclease site in cloned single-strand DNA. A synthetic oligonucleotide complementary to the site of interest is hybridized to the single-strand DNA to form a duplex substrate for the restriction endonuclease. Cleavage is limited to only a single copy of a restriction endonuclease recognition sequence which may be present in multiple copies in the single-strand DNA because the sequences flanking the restriction endonuclease recognition sequence in the oligonucleotide target the oligonucleotide to the proper site. Nonspecific endonuclease digestion is blocked by the addition of *E. coli* single-strand DNA binding protein [SBP]. The cleaved single-strand DNA can be easily used for the preparation of site-specific inframe deletions and insertions by priming DNA synthesis on the cleaved DNA with an appropriately designed oligonucleotide complementary to the 3' end of the DNA, followed by ligation and transformation. Single-strand DNA at a ratio of 1 µg of DNA to 1 µg of oligonucleotide, 7.5 µg of SBP, and 2-5 units of restriction endonuclease is digested in a 20 µl to 100 µl volume of Buffer A [10mM Tris-HCl, pH 7.5, 10mM MgCl₂, 1mM DTT, and 1 µg/ml bovine serum albumin] depending upon the amount of single-strand DNA used for 1 to 2 hr at 37°C. The oligonucleotide and single-strand DNA are first incubated in Buffer A at 55°C for 30 min to anneal the oligonucleotide followed by a 15 min incubation at 37°C to adjust the temperature to the digestion conditions. The SBP is added and incubation is continued for 10 min to coat the single-strand regions of the DNA prior to the addition of the restriction endonuclease. The extent of digestion with each combination of oligonucleotide, SBP, and restriction endonuclease should be monitored by submerged agarose gel electrophoresis on 0.8% agarose gels buffered with Tris-Borate-EDTA [1]. The optimal conditions for targeting digestion with a particular oligonucleotide may have to be experimentally modified by varying the digestion conditions. Cleaved single-strand DNA stripped of accompanying cleaved oligonucleotide can be prepared by denaturing the digestion products with 0.1M NaOH and immediately purifying the DNA by agarose gel electrophoresis on 0.9% low melting temperature agarose gels buffered with Tris-Borate-EDTA [1] followed by electroelution. Cleaved single-strand DNA purified by phenol/chloroform extraction and precipitated with ethanol [1] can be used as a template for DNA synthesis or other procedures.

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[1] Maniatis, T. *et al.*, [1982] Molecular Cloning, CSH, N.Y.