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REPAIRING DAMAGED DNA IMPROVES PCR YIELD AND ACCURACY

Tom Evans, Katherine Marks, Romas Vaisvila, Rebecca Kucera, Peter Hartline, Elizabeth Cantin, Jess Ingram, Barton Slatko and Lixin Chen
New England Biolabs, Inc., 240 County Road, Ipswich, MA 01938, USA

Identification of a species or individual using DNA can be compromised by the quality of the DNA sample. DNA degradation can prevent analysis entirely or yield incorrect results. Damage such as abasic sites and thymine dimers inhibit polymerases and therefore DNA amplification and sequencing. Furthermore, certain modified bases, eg 8-oxo-guanine, can mispair resulting in a PCR product, but one that is not a true representation of the original, undamaged source material. DNA damage can occur by environmental exposure, during extraction, or during storage. It is obvious that many forensic samples will contain degraded DNA, however, even fresh samples can yield damaged DNA if the extraction procedure is relatively harsh. We have sought to improve the range of degraded DNA samples accessible to analysis by creating a solution of DNA repair enzymes, termed PreCR, consisting of ligase, polymerase, apurinic/apyrimidinic endonuclease, uracil DNA glycosylase, formamidopyrimidine-DNA glycosylase, and pyrimidine dimer glycosylase activities. These enzyme activities should permit the repair of nicks, abasic sites, thymine dimers, oxidized guanines, deaminated cytosine, and gaps. Importantly, the ligase chosen seals nicked DNA effectively, but does not ligate double strand breaks consisting of blunt ends or small complementary sticky ends well. This should minimize chimeric gene formation. The spectrum of damages actually repaired by PreCR was investigated in three ways. First, lambda DNA was subjected to increasing amounts of heat, depurination, UV light, or oxidation. Second, mutagenic lesion repair was determined using the lacZ gene and blue/white selection coupled with DNA sequencing. Third, a repair time course for synthetic oligos that contained a lesion of known type and position was followed by urea gel electrophoresis. These investigations showed that PreCR increases the range of damage that a DNA template can possess and still permit accurate and robust amplification or analysis.