ASSESSING THE ROLE OF DNA REPAIR AND WHOLE GENOME AMPLIFICATION IN FORENSICALLY RELEVANT SAMPLES

Angie Ambers
Institute of Applied Genetics, Dept of Forensic and Investigative Genetics, UNT Health Science Center, 3500 Camp Bowie Blvd., Fort Worth, Texas, USA

Previous research on DNA damage (and subsequent repair) has focused on exposing cell-line DNA to a variety of chemical agents in an effort to induce lesions similar to those that might occur in nature. In these studies, cell-line DNA typically is extracted and purified prior to being subjected to conditions in the laboratory that generate damage. In human cells, however, nuclear DNA is not a "naked" molecule. It is a supercoiled structure that is highly "packaged" into chromatin, and is always associated with a variety of other molecules (such as histone proteins, residual proteins, phosphoproteins, RNA species, & lipid species). Hence, the manner or degree in which damage occurs to DNA in its native complexed form is likely quite different than in its "naked" counterpart. Additionally, aside from the inherent limitations of repair investigations on naked cell-line moieties that arise and are stored in a controlled environment, previous studies often have involved inducing and repairing only a single type of lesion at a time in DNA. Authentic forensic samples, in contrast, usually contain a number of different lesions. The intent of this study was to evaluate the efficacy of DNA repair and WGA in samples that more realistically emulate those encountered in forensic laboratories: bleach-damaged blood, environmentally-damaged bloodstains, and skeletal remains. Extensive measures were taken to damage *native* DNA and hence generate samples that more closely mimic those encountered in casework.

Since many of the methods that have been used on "naked" DNA molecules to simulate *in situ* DNA damage (e.g. Fenton reaction, potassium permanganate treatment) have significantly less effect on DNA in its native state, we first identified methods that successfully cause damage in native DNA and then assessed the ability of this damage to be repaired. Results to date indicated that the PreCR™ Repair assay does hold promise as an additional tool for working with bleach-damaged DNA, although further studies are essential before its implementation into forensic casework could be considered. Conversely, the repair assay did not significantly improve DNA profiles from environmentally-damaged bloodstains or bone (and in some cases resulted in lower RFU values for STR alleles), leaving its utility with these types of samples in question.

Ultimately, forensic samples can experience destructive taphonomic conditions, and thus have often endured extensive microbial and environmental insults. Consequently, the DNA in these environmentally-damaged samples frequently contains multiple lesions and may be highly fragmented. The PreCR™ Repair Mix appeared to be challenged by the myriad of DNA damage states that may be encountered in forensically relevant samples. However, additional strategies do exist for potentially improving STR profiles of degraded and/or low-copy templates. A number of whole genome amplification (WGA) methods are presently being examined to assess their prospective utility in forensic casework. ⌘