

## **Development of a technique to use fluorescently labeled oligonucleotides to replace standard radioactive assays to study induction and repair of DNA damage – By Betsy Sutherland and Brigitte Paap**

Understanding the effects on humans of radiation in the environment, in the workplace and in radiotherapy requires insight into the induction and repair of damage to DNA. Most investigations of repair of clustered damages in synthetic oligonucleotides use small DNA molecules (known sequence oligonucleotides) labeled with <sup>32</sup>P or <sup>14</sup>C at one end of one strand. The intact <sup>32</sup>P- or <sup>14</sup>C-labelled strand migrates to a known position on an electrophoretic gel, and is detected by counting of the radioactivity (Figure 1). The researchers at Brookhaven National Laboratory's (BNL) Biology Department developed a technique that uses fluorescently labeled oligonucleotides to replace the use of radioactivity. The research team used two fluorescent tags of different color, and assembled them into an oligonucleotide containing two DNA damage sites. They tested the action of a DNA repair enzyme to cut the DNA at these sites, then denatured the DNA, and separated the individual strands by size. They can identify the cleavage products both by size and by color or the fluorescent tags (Figure 2). This technique avoids the use of radioactivity and all associated training, regulations and handling of waste. It provides better scientific insight into repair of complex DNA damages with increased efficiency and throughput.

Short, fluorescently tagged oligonucleotides are commercially available and inexpensive, and were used as constant end sequences, which the team linked to a central, variable oligonucleotide cassette containing different DNA damages for economy and flexibility. The research team analyzed repair reactions and developed and validated the use of the fluorescent tags. They also devised a method for accurate recording and quantitation of the fluorescence from the fluorophores. In addition, the team developed a method for multi-color true-fluorescence imaging of gels for complete recording and quantitation of the results.

The purchase cost of the fluorescently labeled oligos, including supplies for their use and labor, was \$24,6000. The waste reduction with this method included: 72 cubic feet of radioactive solid waste, 35 gallons of mixed liquid waste and 108 gallons of hazardous liquid waste all with a cost avoidance of \$67,600.

This pollution prevention project minimized waste generation, improved worker safety and risk reduction by avoiding the handling of radioactive material, improved compliance and increased productivity. This new fluorescent labeling technique can be distributed DOE wide for the Low Dose Initiative and to NASA for their Space Radiation Research program

This research is currently undergoing peer review for publication in the Journal of Nucleic Acids Research.

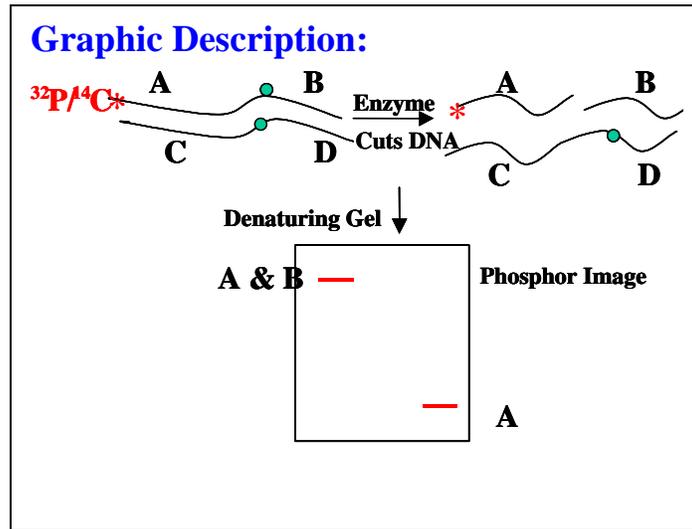


Figure 1 Radioactive Method

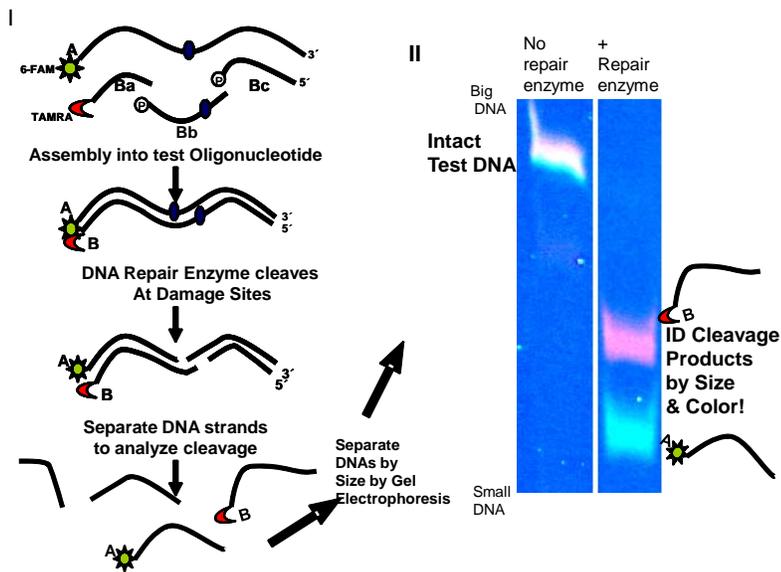


Figure 2. Fluorescent Method - showing the separated the individual strands