Establishment of a Fluorescence-based *In Vitro* Assay to Measure Base Excision Repair Activities

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Several studies showed an essential role for DNA repair in processes as distinct and important as mutagenesis, carcinogenesis, aging and evolution. The ability to measure DNA repair capacity and compare such activities in different biological conditions has been essential for these developments. Nonetheless, there are only few quantitative DNA repair assays, and most of them involve the use of radioactive-labeled probes. Here we describe a fluorescence-based *in vitro* assay to quantify base excision repair activities, one of the main DNA repair pathways. The substrates in these assays are oligonucleotides containing single, defined lesions. To measure the activity of OGG1 and UDG DNA glycosylases we employ substrates containing 8-oxodG or uracil, respectively. In order to assess the APE1 activity, the substrate used contains an abasic site analogue. The gap-filling activity performed by DNA polymerases utilizes an oligonucleotide substrate containing a single gap. The lesion-containing oligonucleotide are labeled with dCTP-AlexaFluor 647 at the 3’ end, gel-purified and annealed to the complementary strand. Using these substrates, we performed assays with commercially available enzymes, and generated incision products that were resolved in denaturing-polyacrylamide gels and visualized in a laser scanner. To test these substrates with complex biological samples we isolated mitochondrial and nuclear proteins from rat and human brains, which were used to optimize assay conditions such as the amount of protein and incubation time, for which linear ranges were established. We expect that the development of this technique will enable the quantitative detection of differences in the repair capacity in several experimental conditions.

Key-words: DNA repair, *in vitro* assay, base excision repair

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