Supplementary Data

Validation of experimental approach.

In the experiments reported here we examined pso-TFO mutagenesis in cells with varying repair competence. Different doses of AE-07 in the transfections were used depending on the cell line. Bioactive pso-TFOs containing 2'-AE residues are a recent development and it was important to show a direct relationship between the dose of AE-07 and deletion and base substitution frequencies. Accordingly, dose response studies were performed. We found that the frequencies of both deletions and base substitutions were proportional to the dose of TFO (**Supplementary Figure 1**). In previous work we showed that crosslinks were introduced at the target site by treatment of the cells with the pso-TFO (56). We interpret the dose response curves as indicating that, in this dose range, the mutation frequencies are proportional to the level of crosslinks, which is proportional to the TFO concentration.

The crosslink site is near the start of Exon 5 (Fig 1). Deletions that remove the splice acceptor or extend into the exon inactivate the gene. Consequently the classical thioguanine resistant colony assay gives an accurate measure of deletions. However, base substitutions at the T in the 5'-TpA crosslink sequence (the proximal T in Fig 1) are outside the exon and splice acceptor site, do not inactivate the gene, and are not scored by thioguanine selection. Since the crosslink lies in the *XbaI* site, genes with base substitutions can be identified by restriction enzyme resistance of PCR products of the target region. In recent work we determined the frequency of base substitutions by analysis of DNA from individual colonies chosen at random, without selection, following treatment of cells with AE-07. Resistant PCR products were then sequenced to confirm and identify the mutations (56). We have replaced this procedure with the small pool PCR strategy described in the Methods section. A comparison of the results with DNA isolated from colonies chosen at random vs the small pool PCR analysis of DNA from the same population of cells following treatment showed good agreement between the two methods **(Supplementary Figure 2)**.

Legends for Supplementary Figures

1. Dose response between deletion (A) and base substitution (B) frequencies and the concentration of pso-TFO in the electroporation cuvette.

2. Equivalency of Small Pool PCR and random colony isolation for measuring base substitution frequencies in cell treated with the pso-TFO. Cells were treated with the pso-TFO/UVA and divided in two aliquots. One aliquot was plated at low density to permit colony isolation. Isolated colonies were transferred to a 96 well plate and expanded. DNA was extracted and the frequency of base substitutions at the target site was determined by resistance to XbaI (A), followed by sequence analysis. The other aliquot of cells was cultured for 8 days after which time DNA was harvested from the entire population and assayed by small pool PCR, and sequence analysis of XbaI resistant PCR products (arrows). The pattern of XbaI resistant PCR products is shown in B. The frequency of base substitutions

determined by direct colony analysis was 3% (8 colonies with base substitutions in 270 total colonies). The frequency determined by small pool PCR analysis was 3.2% (22 base substitutions in 692 diploid genome equivalents).

SUPPLEMENTARY FIGURES