Supplementary Information

for

Functional consequence of plasmid DNA modified site-specifically with 7-deaza-deoxyadenosine at a single, programmable site

Wee Han Ang, Stephen J. Lippard*

Department of Chemistry, Massachusetts Institute of Technology,

Cambridge, Massachusetts 02139.

*To whom correspondence should be addressed. E-mail: lippard@mit.edu

EXPERIMENTAL SECTION

Materials and Methods. BstAPI, Nt.BspQI, T4 polynucleotide kinase (T4 PNK), T4 DNA ligase and calf intestinal phosphatase (CIP) were obtained from New England Biolabs (NEB). Enzymatic reactions were carried out in the buffers supplied. Ethidium bromide (EtdBr) and nuclease S1 were purchased from Promega and competent DH5α *E. coli.* Cells were obtained from Invitrogen. Oligonucleotide synthesis was carried out on an Applied Biosystems 392 DNA/RNA synthesizer using reagents purchased from Glen Research. Mass spectra of the synthesized oligonucleotides were recorded on a Bruker OmniFlex instrument. The analyte was mixed with a matrix containing 10 mg/mL of 2,4,6-trihydroxyacetophenone with 25 mM ammonium citrate in 50% MeCN solution and applied on the target using the dried droplet method. External calibration was performed using ABI Biosystems Calibration Mixture 2.

Vector Construction and Preparation. A mammalian vector expressing *Gaussia* luciferase, pCMV-GLuc (NEB), was deleted between 1501-3279 bp and 3698-3900 bp by the polymerase chain reaction (PCR) to remove BspQI restriction sites and the SV40 origin of replication,

yielding pGLuc. pGLuc was treated sequentially with HindIII and BamHI to digest the vector between the *CMV* promoter and *GLuc* reporter genes. A synthetic insert designed for subsequent incorporation of the dG-Pt DNA lesion, prepared by annealing 5'-AGCTGGAAGAGCAGAGGATGCGAAGAGC with 5'-GATCGCTCTTCGCATCCTCTG-CTCTTCC, which left 5'-AGCT/GATC non-cohesive overhangs, was ligated to the HindIII/BamHI-digest of pGLuc. The ligation mixtures were transformed directly into DH5α cells on LB agar plates supplemented with 100 mg/L of ampicillin. Colonies were randomly picked and the plasmid was extracted (Qiagen miniprep kit). Incorporation of synthetic inserts was confirmed by restriction analysis and sequencing (MWG Operon, Huntsville, Alabama, USA) prior to large scale plasmid preparation. The modified vector was designated pGLuc6temG and has a plasmid length of 3.7 kbp.

Preparation of Insertion Strands. The 16-base insertion strand 5'-CATCCTCTG-CTCTTCC (16-is) and 16-base insertion strand containing 7-deaza-dA (highlighted by the underscore) 5'-C<u>A</u>TCCTCTGCTCTTCC (16-deaza-is) were synthesized using an Applied Biosystems 392 DNA/RNA synthesizer on 1 μmol scales by standard phosphoramidite protocols and purified with PolyPak II reverse-phase cartridges (Glen Research) in accord with the manufacturer's instructions. The strands were further purified using ion-exchange HPLC and a gradient of 25% B for 3 min followed by 25-40% B over 10 min, where solvent A contained 20 mM Tris·HCl pH 8.0, 20% acetonitrile and solvent B contained solvent A supplemented with 1 M NaCl. The insertion strands were characterized by MALDI-TOF MS to determine their molecular mass and analyzed for nucleotide composition by enzymatic digestion, using a reported procedure. Anal. (16-is) dC 8.2 (8), dG 1.0 (1), T 6.0 (6), dA 0.8 (1), m/z $[M-H]^-$ 4719

(4718); Anal. (16-deaza-is) dC 8.1 (8), dG 0.9 (1), T 6.1 (6), 7-deaza-dA 0.9 (1), m/z $[M-H]^-$ 4718 (4717); calculated values are given in parentheses.

Preparation of Site-Specifically Gapped Plasmid. A 100 μg portion of the plasmid was incubated with Nt.BspQI (100 U) at 50 °C for 1 h. The enzyme was deactivated by heating the reaction mixture at 80 °C for 20 min and removed by phenol/chloroform/isoamyl alcohol (25:24:1) treatment. A single-stranded gap was formed by trapping the nicked strand with its complement. Briefly, the nicked plasmid solution was concentrated with a diafiltration centrifugal device (Pall, MWCO 30 KDa), supplemented with synthetic cDNA (1:1000 molar ratio) in annealing buffer (10 mM Tris·HCl pH 8.0, 2 mM $MgCl_2$, 0.4 M NaCl), and heated at 80 °C for 5 min then cooled at 4 °C for 5 min for 8 cycles. The gapped plasmid was purified by repeated washing (6 times) through a diafiltration centrifugal device (Millipore, MWCO 30 KDa) and purified by preparative 0.8% w/v agarose gel electrophoresis containing 0.5 μg/mL EtdBr. Plasmid extraction from the gel was performed by using a commercial kit (Promega).

Incorporation of Insertion Strands into Gapped Plasmid. The insertion strands (1 nmol) were phosphorylated by T4 PNK (600 U) at 37 °C for 1 h. The phosphorylated insertion strands were treated with phenol/chloroform/isoamyl alcohol (25:24:1), purified by ethanol precipitation, and dried in vacuo. The gapped plasmid (5 μg) was added at 1:500 molar ratio (vs. the insertion strands) and the mixture was annealed in a thermocycler (80 °C for 10 min, then cooling to 4 °C at -1 °C/min). The reaction was supplemented with T4 DNA ligase (80 U) in buffer (50 mM Tris·HCl pH 7.6, 10 mM $MgCl_2$, 10 mM DTT, 1 mM ATP) and incubated at 16 °C for 12 h. The mixture was supplemented with 6X loading buffer (NEB), heated at 75 °C for 15 min, and separated using preparative 0.8% w/v agarose gel electrophoresis containing 0.5 μg/mL EtdBr. The DNA bands were revealed using a handheld UV lamp and the band with higher

electrophoretic mobility, corresponding to covalently closed circular product, was excised. Plasmid extraction from the gel was carried out using a commercial kit (Promega) and the eluted plasmid solution was dialyzed against TE buffer.

Restriction Analysis on Ligated Plasmids. A freshly prepared 2X buffer mixture containing BstAPI was distributed in equal volumes (10 μL) into solutions containing either the parent plasmid or ligated plasmids (50 ng), to ensure that equal amounts of enzyme (0.2U) were added to each digestion, and heated at 60 °C for 30 min. The digested plasmids were analyzed using 0.8% w/v agarose gel electrophoresis containing 0.5 μg/mL EtdBr against undigested controls and the gels were imaged with a BioRad Fluor-S MultiImager.