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Supporting Information

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Supporting data

Efficient Replication Bypass of Size-expanded DNA Base Pairs in Bacterial Cells

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Contents

Oligonucleotide Synthesis. Oligodeoxynucleotides were synthesized on an Applied Biosystems 394 DNA/RNA synthesizer on a 1 μmole scale and possessed a 3'-phosphate group. Coupling employed standard β-cyanoethyl phosphoramidite chemistry, but with extended coupling time (600 s) for nonnatural nucleotides. All oligomers were deprotected in concentrated ammonium hydroxide (55 °C, 16 h), purified by preparative 20% denaturing polyacrylamide gel electrophoresis, and isolated by excision and extraction from the gel, followed by dialysis against water. The recovered material was quantified by absorbance at 260 nm with molar extinction coefficients determined by the nearest neighbor method. Molar extinction coefficients for unnatural oligomers were estimated by adding the measured value of the molar extinction coefficient of the unnatural nucleoside (at 260nm) to the calculated value for the natural DNA fragments. Previous studies have shown that xDNA bases have very low hypochromicity in xDNA oligomers. Molar extinction coefficients for xDNA nucleosides used were as follows: dxA, ε_{260}=19,800 $M^{-1}\cdot cm^{-1}$; dxG, ε_{260}=8,100 $M^{-1}\cdot cm^{-1}$; dxT, ε_{260}=1,200 $M^{-1}\cdot cm^{-1}$; dxC, ε_{260}=5,800 $M^{-1}\cdot cm^{-1}$.

MALDI-TOF mass spectrometry was performed on the oligonucleotides that were to be ligated into the M13 viral genome, using a PerSeptive Biosystems Voyager-DE STR BioSpectrometry Workstation run in the negative ion linear mode, and conditions as described.[1] Masses for single, negatively charged 16mer DNA molecules of sequence 5' GAAGACCTXGGCGTCC 3' are as follows:

X = G 4,906.22 observed (4,906.18 calculated)

X = xG 4,956.19 observed (4,956.24 calculated)

X = xA 4,940.27 observed (4,940.24 calculated)

X = xT 4,931.19 observed (4,931.23 calculated)

X = xC 4,930.24 observed (4,930.24 calculated)

(1) Delaney, J. C., and Essigmann, J. M. (**2004**) Mutagenesis, genotoxicity, and repair of 1-methyladenine, 3-alkylcytosines, 1-methylguanine, and 3-methylthymine in *alkB Escherichia coli*. *Proc. Natl. Acad. Sci. U. S. A. 101*, 14051-14056.

Enzyme kinetics methods.
The 5'-terminus of the primer was labeled using [γ-^{32}P]ATP and T4 polynucleotide kinase. The labeled primer as annealed to the template in a buffer of 100 mM Tris•HCl (pH 7.5), 20 mM $MgCl_2$, 2 mM DTT, and 0.1 mg/mL acetylated BSA. Polymerase reactions were started by mixing equal volumes of solution A containing the DNA-enzyme complex and solution B containing dNTP substrates. Solution A was made by adding Klenow fragment (exo-) (Amersham) diluted in annealing buffer to the annealed duplex DNA and incubating for 2 min at 37 °C and terminated by adding 1.5 volumes of stop buffer (95% formamide, 20 mM EDTA, 0.05% xylene cyanol and bromophenol blue).

Steady state kinetics for standing start single nucleotide insertions was carried out as described.[2] The final DNA concentration was 5 μM. Final concentrations of triphosphates were 15, 25, 40, 60, 90, 120 μM. Amount of polymerase used (0.05-0.1 u/μL) and reaction time (2-60 min) were adjusted to give <20% conversion. Extents of reaction were determined by running quenched reaction samples on 20% denaturing polyacrylamide gel. Relative velocities were calculated as extent of reaction divided by reaction time and normalized to 0.005u/μL enzyme concentration.

Methods for cellular assays.
The Competitive Replication of Adduct Bypass (CRAB) assay determines the replication blocking power of lesions, whereby a lesion-bearing genome is mixed with a nonlesion internal standard genome prior to transfection (Figure S1, left). Lesions that block replication cause an increased percentage of competitor signal in the output phage population, whereas the output from non-blocking lesions should maintain the original lesion:competitor genome input ratio. Quantification of the lesion and competitor output is performed on the output population as described,[2] using a specific set of PCR primers that amplifies both lesion and competitor signals equally. PCR products from the competitor signal are 3 bases longer than from the lesion signal, and can therefore be discriminated on a denaturing gel, and quantified by PhosphorImagery.

The Restriction Endonuclease And Postlabeling determination of mutation frequency (REAP) assay quantifies the type and amount of mutagenesis at the lesion site by obtaining the base composition at the lesion site after cellular replication (Figure S1, right). The methodology is similar to the CRAB assay; however, the primer set used will amplify only signal stemming from the lesion. PCR product from this 100% lesion signal is digested with *Bbs*I, cleaving a fixed number of bases outside of its recognition sequence to expose the lesion site as a 5'-overhang that is ultimately radiolabeled, trimmed with *Hae*III, and the products are separated on a denaturing gel. After the band containing the lesion site is excised, eluted, desalted, and dried, the residue is digested to 5'-dNMPs with nuclease P1, thus releasing the bases at the former lesion site as radioactive species, that are resolved by TLC and quantified by PhosphorImagery.

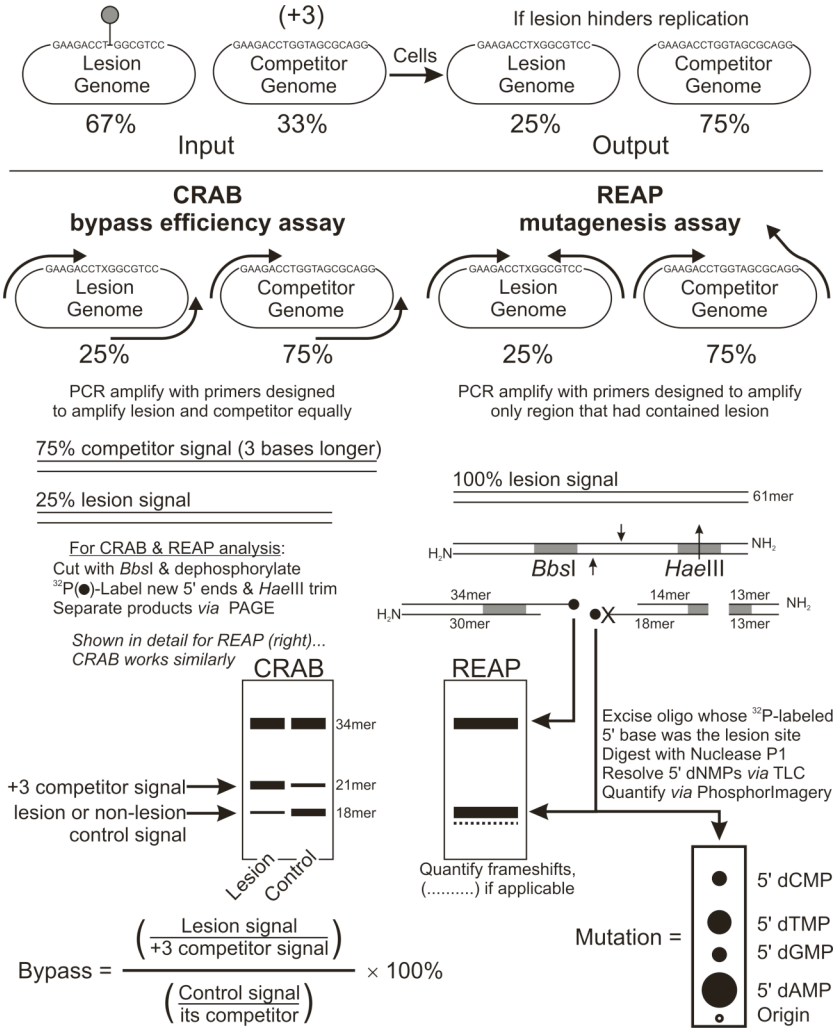

Figure S1. Schematic representation of CRAB bypass efficiency assay (left) and REAP mutagenesis assay (right), adapted from prior work.[3,4] The concentration of each genome construct (xDNA or G at lesion site reference control) was determined in triplicate and normalized. Each 2:1 formulation of lesion genome:competitor genome made for the xG, xA, xT, xC and G genomes was electroporated in the order shown, and this transfection process was repeated twice. Greater than 4×10^6 initial events were obtained per electroporation, with greater than 4×10^5 events stemming from the least bypassed lesion, providing for statistical robustness.

(2) Kim, T. W.; Delaney, J. C.; Essigmann, J. M.; Kool, E. T. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 15803-15808.
(3) Delaney, J. C., and Essigmann, J. M. (**2006**) Assays for determining lesion bypass efficiency and mutagenicity of site-specific DNA lesions *in vivo*. *Methods Enzymol. 408*, 1-15.
(4) Delaney, J. C., and Essigmann, J. M. (**2008**) Biological properties of single chemical-DNA adducts: A twenty year perspective. *Chem. Res. Toxicol. 21*, 232-252.

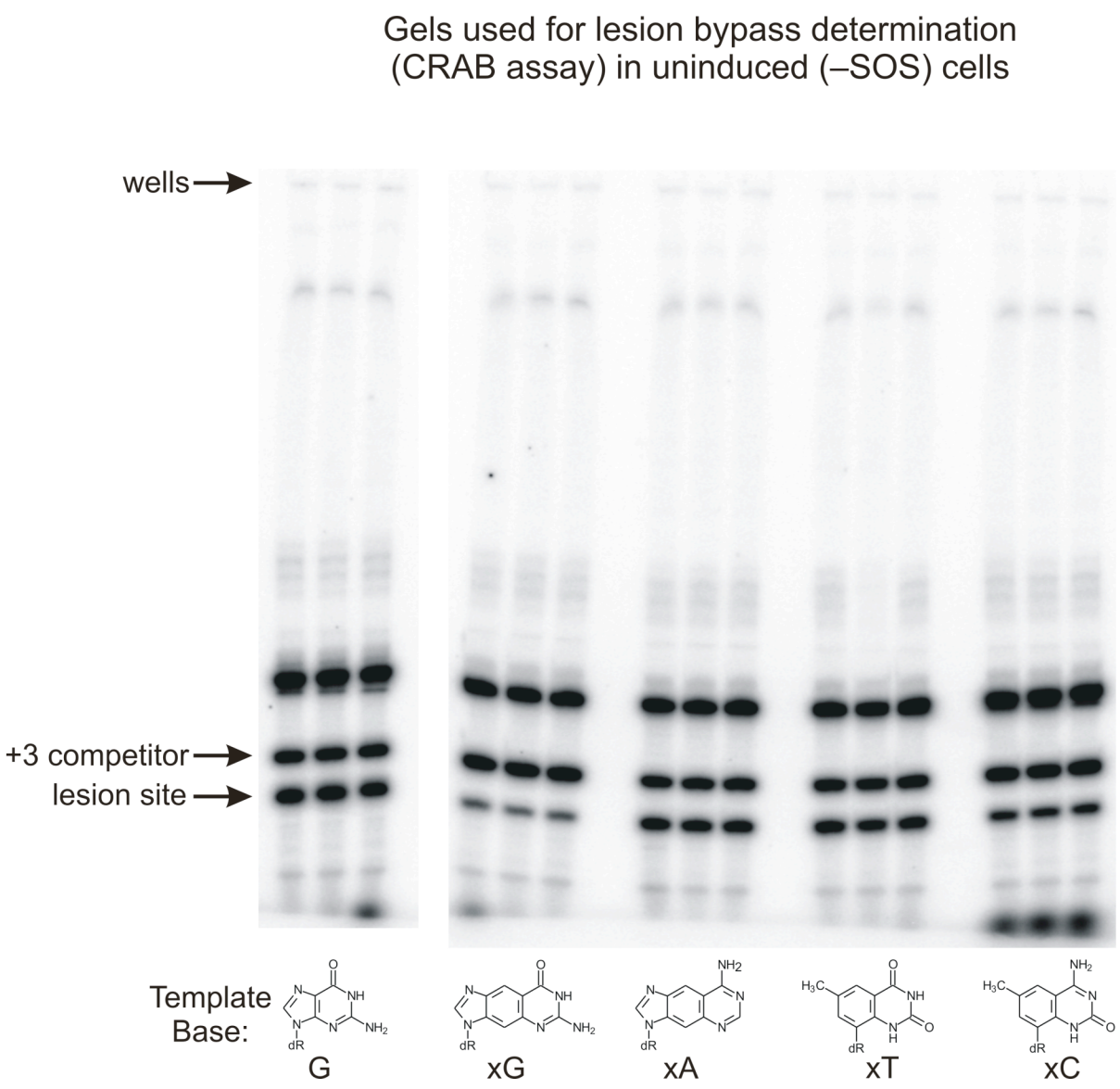

Figure S2. Gels used for lesion bypass determination (CRAB assay) in uninduced (–SOS) cells, showing the output after growth in liquid culture from each normalized genome construct mixed with competitor genome (2:1 lesion:competitor ratio) electroporated in triplicate. The xG lesion was the least bypassed, providing a lesion site signal of 10%, and a +3 competitor signal of 90%. The lower limit estimate of 4×10^5 for the number of initial events arising from xG is calculated by multiplying the lesion site signal by the number of total initial events (obtained by immediate plating of a portion of the transformed cells). The actual number of xG initial events may be even higher, since the lesion:competitor output from immediate plating, which can underestimate the genotoxicity of DNA lesions, is greater than that seen after growth in liquid culture.[1]

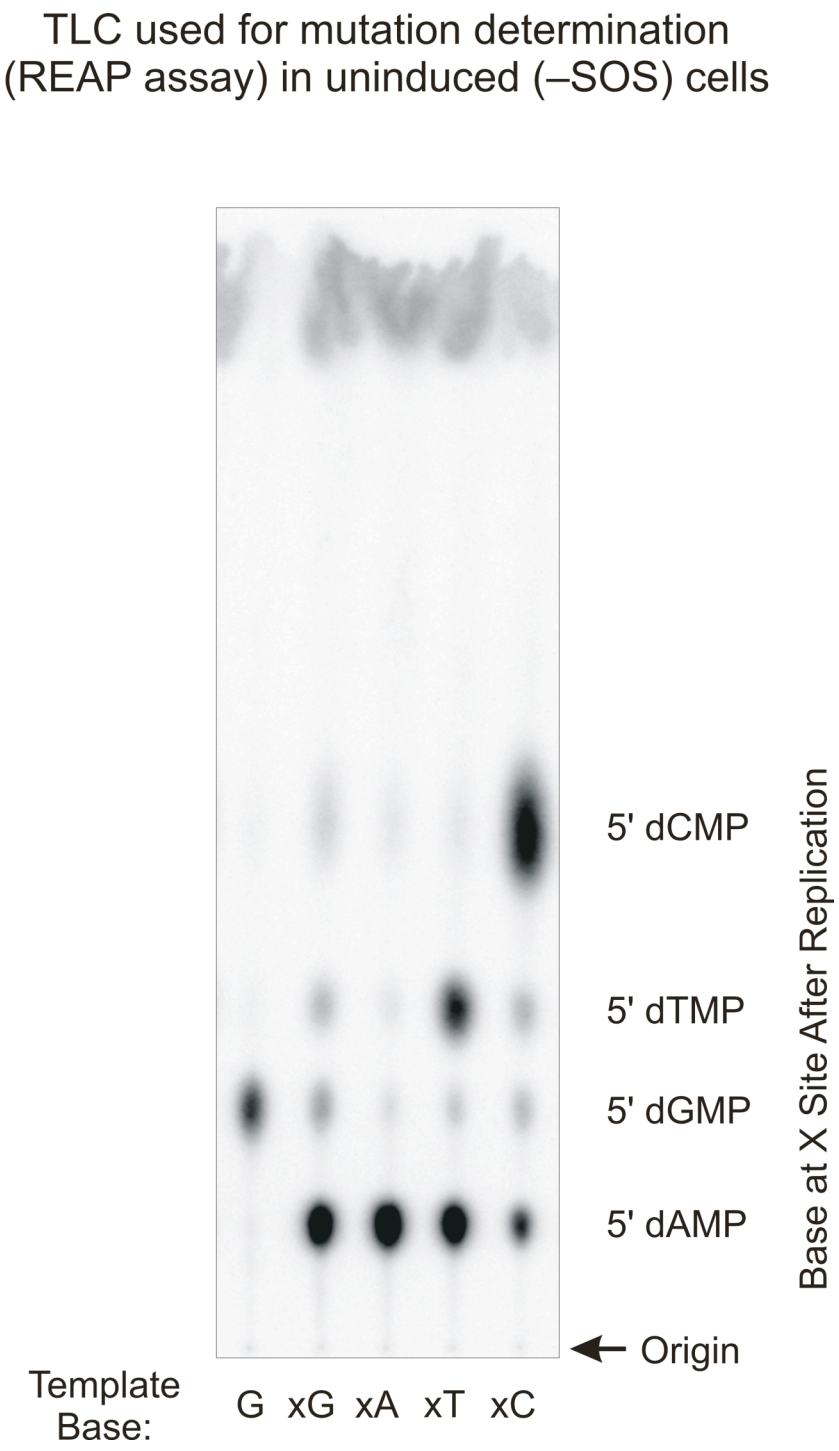

Figure S3. TLC used for mutation determination (REAP assay) in uninduced (–SOS) cells (one of three replicates shown).

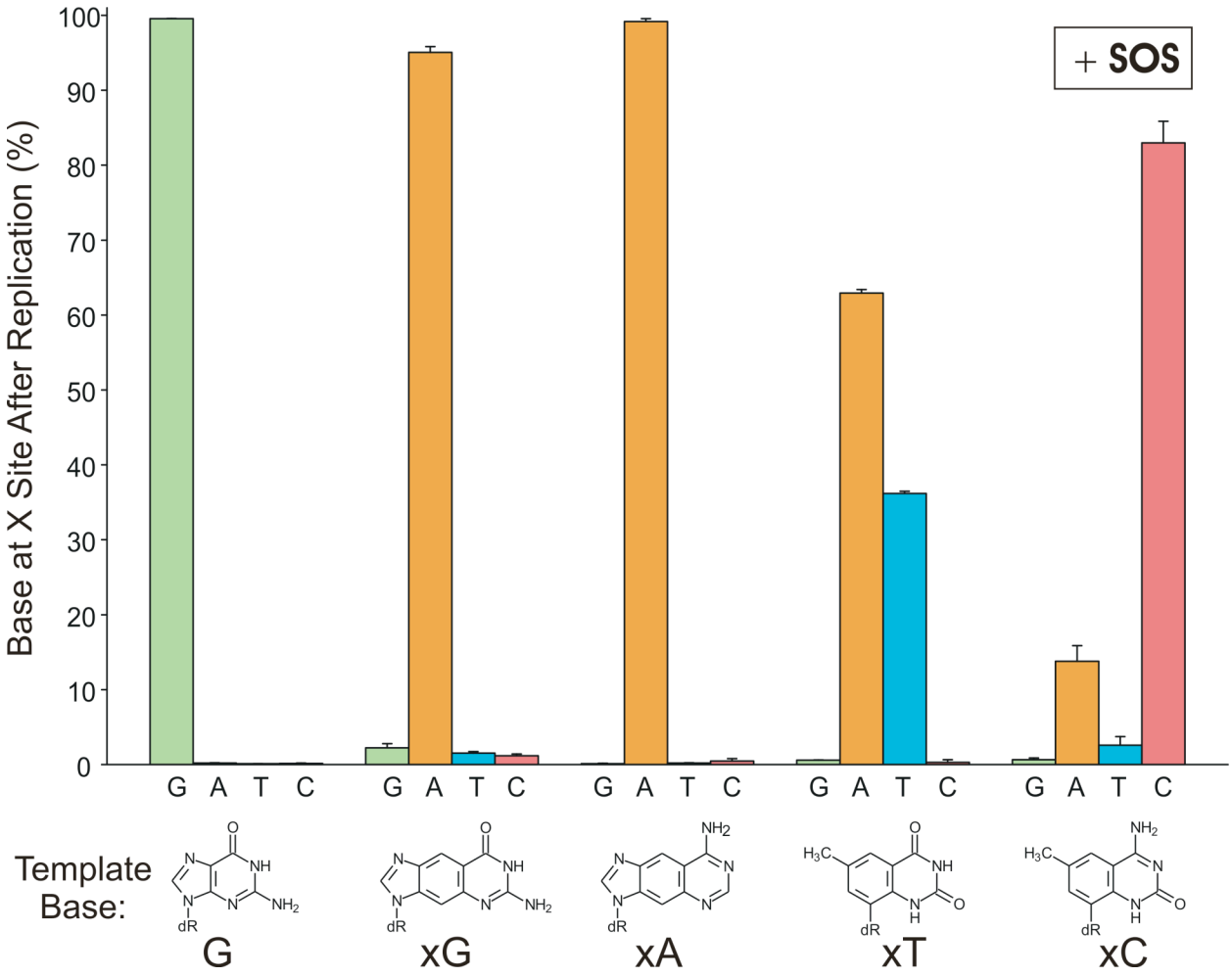

Figure S4. The fidelity of replication of single xDNA bases in *Escherichia coli*. Shown are the relative amounts of each natural base that replaced the xDNA base shown after phage replication occurred in *E. coli* cells that had the SOS response induced with UV light. The largest change with respect to uninduced cells was for xT, in which the value for T increased from 26% to 36%, with a decrease in A from 73% to 63% (compare to Fig. 3 in main text).

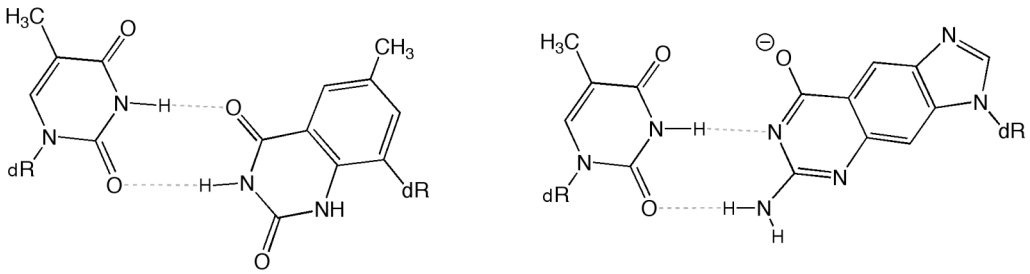

Figure S5. Hypothesized structures that may explain mispairing of T with xT and xG in the cellular experiments.

Table S1. Steady-state kinetics data for extension of xDNA pairs and mispairs by the Klenow fragment of DNA Pol I (exo-). Data are for addition of dCTP opposite a natural template G at the primer-template terminus beyond the xDNA pairs shown.[a]

template base	primer base	V_{max} (% min^{-1})[b]	K_m (μM)	efficiency (V_{max} / K_m)	relative efficiency
xA	A	0.0011 (0.0001)	141 (6)	7.7×10^{0}	1.4×10^{-1}
xA	C	ND	ND	$< 10^{0}$ [c]	$<1.8 \times 10^{-2}$
xA	G	0.00092 (0.00003)	131 (13)	7.2×10^{0}	1.3×10^{-1}
xA	T	0.011 (0.002)	248 (87)	5.5×10^{1}	1

[a]Conditions: 200 nM 23mer/28mer primer-template duplex and varied polymerase concentrations in a buffer containing 50 mM Tris•HCl (pH 7.5), 10 mM $MgCl_2$, 50 ug/mL BSA and 1 mM dithiothreitol, incubated at 37°C in a reaction volume of 10 μL. Standard deviations are given in parentheses.

[b]Normalized for the lowest enzyme concentration used.

[c]Value is an upper limit; extremely poor efficeincy (virtually no dCTP insertion) was observed.