SUPPORTING INFORMATION

Encoding Phenotype in Bacteria with an Alternative Genetic Set

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Synthesis and purification of oligonucleotides.

Synthesis of dxA, dxG, dxT, dxC phosphoramidites were carried out as previously reported.[1,2] Oligodeoxynucleotides were synthesized on an Applied Biosystems 394 DNA/RNA synthesizer on a 1 μmole scale and possessed a 3'-OH, and a 5'-phosphate group (Chemical Phosphorylation II reagent from Glen Research). 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, or desalting via PolyPak cartridges. 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 260 nm) to the calculated value for the natural DNA fragments. 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 $M^{-1} \cdot cm^{-1}$. Nonnatural oligomers were characterized by MALDI-TOF mass spectrometry. Their observed and expected masses are listed in Table S1.

Table S1. MALDI-TOF mass spectrometry data for unnatural oligodeoxynucleotides used in this study containing dxA, dxT, dxG, and dxC. Error with the instrument is within ±0.05%.

ID	Template Sequence	Calc.	Obs.
1A	5'-pGTA CAG GA**G** CGC AC**xG** AT**T** TCT TTC AAA GAT GAC GG**A** AAC TAC AAG A-3'	14354.30	14313.26
1T	3'-TC CT**C** GCG TGC TA**A** AGA AA**xG** TTT CTA CTG CC**T** TTG ATG TTC TGC GCp-5'	14203.70	14208.05
2A	5'-pGTA CAG GA**G** CGC ACT AT**T** TCT TTC AA**xA** GAT GAC GG**A** AAC TAC AAG A-3'	14329.39	14329.49
2T	3'-TC CT**C** GCG TGA TA**A** **xA**GA AAG TTT CTA CTG CC**T** TTG ATG TTC TGC GCp-5'	14227.19	14229.33
3A	5'-pGTA CAG GA**G** CGC ACT AT**T** TCT TT**xC** AAA GAT GAC GG**A** AAC TAC AAG A-3'	14344.39	14333.97
3T	3'-TC CT**C** GCG TGA TA**A** AGA AAG TTT CTA CTG **xC**C**T** TTG ATG TTC TGC GCp-5'	14242.19	14240.99
4A	5'-pGTA CAG GA**G** CGC ACT AT**T** TCT TTC AAA GA**xT** GAC GG**A** AAC TAC AAG A-3'	14329.39	14326.10
4T	3'-TC CT**C** GCG TGA **xT**A**A** AGA AAG TTT CTA CTG CC**T** TTG ATG TTC TGC GCp-5'	14227.19	14224.90
5A	5'-pGTA CAG GA**G** CGC ACT AT**T** TCT TT**xC** **xA**AA GAT GAC GG**A** AAC TAC AAG A-3'	14394.48	14389.27
5T	3'-TC CT**C** GCG TGA **xTxAA** AGA AAG TTT CTA CTG CC**T** TTG ATG TTC TGC GCp-5'	14277.28	14270.62
6A	5'-pGTA CAG GA**G** CGC ACT AT**T** TCT TT**xC** **xAxA**A GAT GAC GG**A** AAC TAC AAG A-3'	14444.57	14437.74
6T	3'-TC CT**C** GCG TGA **xTxAxA** AGA AAG TTT CTA CTG CC**T** TTG ATG TTC TGC GCp-5'	14327.37	14326.78
7A	5'-pGTA CAG GA**G** CGC ACT AT**T** TCT TT**xC** **xAxAxA** GAT GAC GG**A** AAC TAC AAG A-3'	14494.66	14471.05
7T	3'-TC CT**C** GCG TGA **xTxAxA** **xA**GA AAG TTT CTA CTG CC**T** TTG ATG TTC TGC GCp-5'	14377.46	14458.87
8A	5'-pGTA CAG GA**G** CG**xC** ACG AT**T** TCT TTC AAA GAT GA**xC** GG**A** AAC TAC AAG A-3'	14434.50	14421.30
8T	3'-TC CT**C** GCG TG**xC** TA**A** AGA AAG TTT **xC**TA CTG CC**T** TTG ATG TTC TGC GCp-5'	14283.30	14193.50
9A	5'-pGTA CAG GA**G** CGC **xA**CT **xA**T**T** TCT TTC AA**xA** GAT G**xA**C GG**A** AAC TAC AAG A-3'	14479.66	14480.80
9T	3'-TC CT**C** GCG TG**xA** T**xAA** AGA A**xA**G TTT CT**xA** CTG CC**T** TTG ATG TTC TGC GCp-5'	14377.46	14285.76
10A	5'-pGTA CAG GA**G** CGA ACA AT**xT** TC**xT** TTC AAA GA**xT** GAC GG**A** AAC TAC AAG A-3'	14462.50	14413.87
10T	3'-TC CT**C** GC**xT** TG**xT** TA**A** AGA AAG TTT CTA CTG CC**xT** TTG ATG TTC TGC GCp-5'	14293.30	14289.39
11A	5'-pGTA CAG GA**G** CGC ACT AT**T** TCT TT**xC** **xAxAxA** **xGxA**T GAC GG**A** AAC TAC AAG A-3'	14594.84	14588.74
11T	3'-TC CT**C** GCG TGA **xTxAxA** **xAxGxA** AAG TTT CTA CTG CC**T** TTG ATG TTC TGC GCp-5'	14477.64	14470.58
12A	5'-pGTA CAG GA**G** CGC ACT AT**T** TCT T**xTxC** **xAxAxA** **xGxAxT** GAC GG**A** AAC TAC AAG A-3'	14695.02	14680.69
12T	3'-TC CT**C** GCG TG**xA** **xTxAxA** **xAxGxA** **xA**AG TTT CTA CTG CC**T** TTG ATG TTC TGC GCp-5'	14577.82	14671.39

Cloning and expression experiments.

Expression of the pGFPuv plasmid.

The double stranded, 3.3 kb pGFPuv plasmid (Clontech, Cat No:632312), a high copy number plasmid yielding ~500 copies per cell, was expressed in BL21 (DE3) competent cells (Stratagene) by transforming 25-50 ng of the vector per the transformation protocol recommended by Stratagene. The transformed cells were plated on LB agar plates containing ampicillin and isopropyl thiogalactosidase (IPTG) and allowed to incubate at 37 °C overnight. On visualizing green colonies under long wavelength UV (365 nm) transilluminator, a green colony was picked and grown in LB media, and the plasmid DNA extracted with a MIDI prep kit (Qiagen #12923) and quantified by UV absorbance at 260 nm.

Plasmid digestion.

The pGFPuv vector (3 μL, ~ 2 μg) was then digested by addition of 3 μL each of the restriction enzymes BsrGI and MluI (New England Biolabs), 5 μL of NEB Buffer 2, 5 μL of 10X BSA, and 31 μL of H_2O (autoclaved) to an Eppendorf tube. The digest was allowed to proceed in a thermocycler at 37 °C for 6 h, followed by 65 °C for 20 min to denature the enzymes. After digestion, removal of the 5'-phosphate (to prevent self-ligation of the cut plasmid) was accomplished by addition (to the same Eppendorf tube) of 5 μL of antarctic phosphatase (or calf intestinal phosphatase (NEB) was used with the corresponding buffer) and 5 μL of 10X antarctic phosphatase buffer and then incubation for 1 h at 37 °C followed by denaturing at 65 °C for 20 min. The plasmid was purified on a 0.9% agarose (LMP) gel run at 20 volts for 12 h. The gel bands were visualized under a UV (365 nm) transilluminator, cut out (quickly, ~3 s), and the purified DNA was extracted with a plasmid gel extraction kit (Qiagen #28704). Concentration was determined by UV absorbance units at 260 nm.

Ligation of 46 bp xDNA-containing segments into digested pGFPuv vector.

Ligations of xDNA-containing inserts were carried out at various vector:insert ratios (1:3, 1:5, 1:6, 1:8, 1:10). Solutions of xDNA inserts were prepared/annealed by adding 2.5 μL of each strand (100 ng/μL), 0.5 μL 1M $MgCl_2$, and 94.5 μL H_2O (autoclaved). This DNA solution was denatured in a thermocycler by heating to 72 °C for 3 min, then cooling slowly to room temperature. Appropriate volumes of the pGFPuv vector (~50 ng/μL), and annealed inserts (~2.5 ng/μL) were combined in an Eppendorf tube with 1 μL of 5X T4 DNA ligase buffer, 1 μL of T4 DNA ligase (New England

Biolabs), and H_2O (autoclaved) to reach a volume of 10 μL. The ligation reaction was carried out at 16 °C for 12 h in a thermocycler.

Transformation of modified vectors into *E. coli.*

Transformation of the ligated inserts into BL21 (DE3) competent cells (Stratagene) was carried out per the transformation protocol described by Stratagene. For each transformation, ~1 to 10 ng of xDNA-containing insert were transformed into the cells, and after transformation, ~100 to 150 μL of the cells were plated on LB agar plates containing ampicillin and isopropyl thiogalactosidase (IPTG), and allowed to incubate at 37 °C for 20 h. Colonies were briefly visualized under long wavelength UV (365 nm), and the number of white and green colonies were documented. Transformation efficienies were compared with that of a control ligation reaction under identical conditions using unmodified duplex DNA, which was otherwise identical in sequence to the xDNA-containing segment.

Colony picking and sequencing of plasmid DNA.

Green colonies (and some white colonies) from each transformation were picked, grown overnight at 37 °C in LB media, and the plasmid DNA was isolated using a mini prep kit (Qiagen #27104). The cloned region within the gene on the isolated DNA was sequenced (Sequetech, Mountain View, CA or Quintara Biosciences, Berkeley, CA) with the appropriate primers (GFPseq1.24).

Mutant *E. coli* strains.

Bacterial strains.

The bacterial strains used in this study are shown in Table S2. The bacterial strains were purchased from the *E. coli* Genetic Stock Center at Yale University. The *E. coli* cells were routinely grown in LB media containing 50 μg/mL kanamycin.

Table S2. Bacterial strain genotypes.

Strain	Deleted Gene	Function/Gene Product	Genotype
BW25113	--		F-, *Δ(araD-araB)567*, *ΔlacZ4787*(::rrnB-3), λ^-, *rph-1*, *Δ(rhaD-rhaB)568*, *hsdR514*
JW2703-2	*mutS*	methyl-mediated mismatch repair	F-, *Δ(araD-araB)567*, *ΔlacZ4787*(::rrnB-3), λ^-, *ΔmutS738::kan*, *rph-1*, *Δ(rhaD-rhaB)568*, *hsdR514*
JW0704-1	*nei*	endonuclease VII	F-, *Δ(araD-araB)567*, *ΔlacZ4787*(::rrnB-3), *Δnei-764::kan*, λ^-, *rph-1*, *Δ(rhaD-rhaB)568*, *hsdR514*
JW2928-1	*mutY*	adenine glycosylase: G-A repair	F-, *Δ(araD-araB)567*, *ΔlacZ4787*(::rrnB-3), λ^-, *ΔmutY736::kan*, *rph-1*, *Δ(rhaD-rhaB)568*, *hsdR514*
JW3610-2	*mutM (fpg)*	formamidopyrimidine-DNA glycosylase	F-, *Δ(araD-araB)567*, *ΔlacZ4787*(::rrnB-3), λ^-, *ΔmutM744::kan*, *rph-1*, *Δ(rhaD-rhaB)568*, *hsdR514*
JW4019-2	*uvrA*	excision nuclease: molecular matchmaker	F-, *Δ(araD-araB)567*, *ΔlacZ4787*(::rrnB-3), λ^-, *rph-1*, *Δ(rhaD-rhaB)568*, *ΔuvrA753::kan*, *hsdR514*
JW0221-1	*dinB*	Pol IV	F-, *Δ(araD-araB)567*, *ΔdinB749::kan*, *ΔlacZ4787*(::rrnB-3), λ^-, *rph-1*, *Δ(rhaD-rhaB)568*, *hsdR514*
JW1173-1	*umuC*	Pol V	F-, *Δ(araD-araB)567*, *ΔlacZ4787*(::rrnB-3), λ^-, *ΔumuC773::kan*, *rph-1*, *Δ(rhaD-rhaB)568*, *hsdR514*
JW0059-1	*polB (dinA)*	Pol II	F-, *ΔpolB770::kan*, *Δ(araD-araB)567*, *ΔlacZ4787*(::rrnB-3), λ^-, *rph-1*, *Δ(rhaD-rhaB)568*, *hsdR514*
JW0205-1	*dnaQ (mutD)*	Pol III ε subunit: 3'-> 5' proofreading	F-, *Δ(araD-araB)567*, *ΔdnaQ744::kan*, *ΔlacZ4787*(::rrnB-3), λ^-, *rph-1*, *Δ(rhaD-rhaB)568*, *hsdR514*

Preparation of chemically competent cells.

Competent cells were prepared using the Z-Competent *E. coli* transformation kit (Zymo Research). A starter culture was grown at 37 ºC for 8-12 h with vigorous shaking. An aliquot (100-500 µL) of the started culture was then added to 100 mL of Zymobroth (Zymo Research), and the culture was incubated with shaking at 18-22 ºC until the OD_{600} was 0.25-0.35. The cells were harvested, washed and resuspended in competent buffer per the manufacturer's instructions. The cell suspensions were aliquoted (200 µL) into microcentrifuge tubes and immediately frozen. The samples were stored at -80 ºC until needed.

Transformation of modified vectors into mutant *E. coli.*

Transformation of the ligated inserts into the competent cells was carried out per the transformation protocol described by Zymo Research. For each transformation, ~1 to 10 ng of xDNA-containing plasmid was transformed into the cells using a 90 s heat shock, and after transformation,

~250 µL of the cells was plated on LB agar plates containing ampicillin (100 µg/mL) and kanamycin (50 µg/mL), and allowed to incubate at 37 °C for 14 h. Colonies were treated as previously described.

Inducing an SOS response.

The SOS response was induced in *E. coli* cells (BW25113) using UV irradiation as described.[1] Briefly, cells were grown in 100 mL Zymobroth to an OD_{600} of 0.3, after which they were pelleted, resuspended in 50 mL of $MgSO_4$, and split. To induce the SOS response, half of the cells were irradiated with 254 nm light at 45 Joules/m^2 (UV Stratalinker 2400, Stratagene). Both the irradiated and uninduced cells were diluted 2-fold with LB media and incubated with shaking at 37 C for 20 min, and made chemically competent as previously described.

Table S3. Sequencing results for recovered plasmids. Modified GFP sequences and results with inserts containing one isolated xDNA base pair and two to four consecutive xDNA base pairs in the reading templates, showing successful reading and replacement of xDNA pairs. For each insert, between 10-15 colonies were picked for sequencing. All sequences containing silent mutations produced results shown in Table S3.

Synthetic insert	Sequence	Sequencing Results
xGFP.0 (control)	5′ GTA CAG GA**G** CGC ACT AT**T** TCT TTC AAA GAT GAC GG**A** AAC TAC AAG A 3′ TC CT**C** GCG TGA TA**A** AGA AAG TTT CTA CTG CC**T** TTG ATG TTC TGC GC	No change
xGFP.1 (one xG-C bp)	5′ GTA CAG GA**G** CGC AC**G** AT**T** TCT TTC AAA GAT GAC GG**A** AAC TAC AAG A 3′ TC CT**C** GCG TGC TA**A** AGA AA**G** TTT CTA CTG CC**T** TTG ATG TTC TGC GC	xG replaced by G
xGFP.2 (one xA-T bp)	5′ GTA CAG GA**G** CGC ACT AT**T** TCT TTC AA**A** GAT GAC GG**A** AAC TAC AAG A 3′ TC CT**C** GCG TGA TA**A** **A**GA AAG TTT CTA CTG CC**T** TTG ATG TTC TGC GC	xA replaced by A
xGFP.3 (one xC-G bp)	5′ GTA CAG GA**G** CGC ACT AT**T** TCT TT**C** AAA GAT GAC GG**A** AAC TAC AAG A 3′ TC CT**C** GCG TGA TA**A** AGA AAG TTT CTA CTG **C**C**T** TTG ATG TTC TGC GC	xC replaced by C
xGFP.4 (one xT-A bp)	5′ GTA CAG GA**G** CGC ACT AT**T** TCT TTC AAA GA**T** GAC GG**A** AAC TAC AAG A 3′ TC CT**C** GCG TGA **T**A**A** AGA AAG TTT CTA CTG CC**T** TTG ATG TTC TGC GC	xT replaced by T
xGFP.5 (two consec. xDNA bps)	5′ GTA CAG GA**G** CGC ACT AT**T** TCT TT**C** **A**AA GAT GAC GG**A** AAC TAC AAG A 3′ TC CT**C** GCG TGA **T**A**A** AGA AAG TTT CTA CTG CC**T** TTG ATG TTC TGC GC	xCxA replaced by CA
xGFP.6 (three consec. xDNA bps)	5′ GTA CAG GA**G** CGC ACT AT**T** TCT TT**C** **A**A**A** GAT GAC GG**A** AAC TAC AAG A 3′ TC CT**C** GCG TGA **T**A**A** AGA AAG TTT CTA CTG CC**T** TTG ATG TTC TGC GC	xCxAxA replaced by CAA
xGFP.7 (four consec. xDNA bps)	5′ GTA CAG GA**G** CGC ACT AT**T** TCT TT**C** **A**A**A** GAT GAC GG**A** AAC TAC AAG A 3′ TC CT**C** GCG TGA **T**A**A** **A**GA AAG TTT CTA CTG CC**T** TTG ATG TTC TGC GC	xCxAxAxA replaced by CAAA

xDNA bases in **red**, silent mutations in **blue**.

Table S4. Modified GFP sequences and results with inserts containing multiple isolated xDNA base pairs and six and eight consecutive xDNA base pairs in the reading templates, showing lack of colony growth in these cases.

Synthetic insert	Sequence	Sequencing Results
xGFP.8 (two xC-G bp)	5′ GTA CAG GA**G** CG**C** ACG AT**T** TCT TTC AAA GAT GA**C** GG**A** AAC TAC AAG A 3′ TC CT**C** GCG TG**C** TA**A** AGA AAG TTT **C**TA CTG CC**T** TTG ATG TTC TGC GC	No colonies observed
xGFP.9 (three xT-A bp)	5′ GTA CAG GA**G** CGA ACA AT**T** TC**T** TTC AAA GA**T** GAC GG**A** AAC TAC AAG A 3′ TC CT**C** GC**T** TG**T** TA**A** AGA AAG TTT CTA CTG CC**T** TTG ATG TTC TGC GC	No colonies observed
xGFP.10 (four xA-T bp)	5′ GTA CAG GA**G** CGC **A**CT **A**T**T** TCT TTC AA**A** GAT G**A**C GG**A** AAC TAC AAG A 3′ TC CT**C** GCG TG**A** T**AA** AGA A**A**G TTT CT**A** CTG CC**T** TTG ATG TTC TGC GC	No colonies observed
xGFP.11 (six cons. xDNA bps)	5′ GTA CAG GA**G** CGC ACT AT**T** TCT TT**C** **AAA** **GA**T GAC GG**A** AAC TAC AAG A 3′ TC CT**C** GCG TGA **TAA** **AGA** AAG TTT CTA CTG CC**T** TTG ATG TTC TGC GC	No colonies observed
xGFP.12 (eight cons. xDNA bps)	5′ GTA CAG GA**G** CGC ACT AT**T** TCT T**TC** **AAA** **GAT** GAC GG**A** AAC TAC AAG A 3′ TC CT**C** GCG TG**A** **TAA** **AGA** **A**AG TTT CTA CTG CC**T** TTG ATG TTC TGC GC	No colonies observed

xDNA bases in **red**, silent mutations in **blue**.

Table S5. Colony counting data with xDNA-containing GFP inserts relative to an unmodified control insert (containing no xDNA bases), under varied conditions of vector:insert ratio during transfection.

GFP insert	Vector: Insert	# green colonies	# white colonies	# total colonies
None	NA	2	12	14
xGFP.0	1:2	83	16	99
(control)	1:3	116	11	127
	1:5[a]	44	12	56
xGFP.1	1:2	15	7	22
(one xG-C bp)	1:3	14	6	20
	1:5	20	9	29
	1:8[a]	18	6	24
xGFP.2	1:8[a]	7	5	12
(one xA-T bp)				
xGFP.3	1:8[a]	5	3	8
(one xC-G bp)				
xGFP.4	1:8[a]	5	5	10
(one xT-A bp)				
xGFP.5	1:8[a]	7	2	9
(two cons. xDNAs)				
xGFP.6	1:8[a]	6	4	10
(three cons. xDNAs)				
xGFP.7	1:2	0	6	6
(four cons. xDNAs)	1:3	2	0	2
	1:5[a]	10	4	14
	1:8[a]	19	7	26
xGFP.8[b]	1:2	0	0	0
(two xC-G bps)	1:3	0	2	2
	1:5	3	6	9
	1:8	4	1	5
xGFP.9[b]	1:2	2	9	11
(three xT-A bps)	1:3	1	4	5
	1:5	0	1	1
	1:8	1	0	1
xGFP.10[b]	1:2	1	1	2
(four xA-T bps)	1:3	0	4	4
	1:5	0	0	0
	1:8	6	0	6
xGFP.11[b]	1:8[a]	5	3	8
(six cons. xDNA bps)				
xGFP.12[b]	1:8[a]	0	2	2
(eight cons. xDNA bps)				

[a]average colonies for three trials. [b]no colonies contained silent mutations.

Table S6. Sequencing results of selected white colonies containing xDNA-modified inserts, showing that all mutations occurred at natural bases rather than xDNA bases.

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original    5′ GTA CAG GAG CGC ACT ATT TCT TTC AAA GAT GAC GGA AAC TAC AAG A
            3′      TC CTC GCG TGA TAA AGA AAG TTT CTA CTG CCT TTG ATG TTC TGC GC
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Synthetic insert	Observed Sequence	Sequencing Results
xGFP.1 (one xG-C bp)	5'-GTA CAG GAA GCG CAC GAT TTC TTT CAA AGA TGA CGG AAA CTA CAA GA-3'	xG replaced by G; G insertion
xGFP.2 (one xA-T bp)	5'-GTA CAG GAG CGC ACT ATT TCT TTC AAA GAT GAC AAC TAC AAG A-3'	xA replaced by A; GGA deleted
xGFP.3 (one xC-G bp)	5'-GTA CAG GAG CGC ACT ATT TCT TTA AAG ATG ACG GAA ACT ACA AGA C-3'	C deletion
xGFP.5 (two consec. xDNA bps)	5'-GTA CAG GAG CGC ACT ATT TCT TTC AAG ATG ACG GAA ACT ACA AGA C-3'	xCxA replaced by CA; A deletion
xGFP.7 (four consec. xDNA bps)	5'-GTA CAG GAG GCG CAC TAT TTC TTT CAA AGA TGA CGG AAA CTA CAA GA-3'	xCxAxAxA replaced by CAAA; G insertion

xDNA residues in **red**. Silent mutations in **blue**. Differences from expected sequence underlined.

Table S7. Colony counting data of selected xDNA-containing GFP inserts in various mutant strains of *E. coli* lacking DNA repair activities.

GFP insert	Deleted Gene	# green colonies[a]	# white colonies[a]	# total colonies[a]
xGFP.0	--	348.3	57.7	406.0
(control)	nei	202.5	29.5	232.0
	mutY	320.8	52.5	373.3
	mutM (fpg)	220.8	35.8	256.5
	uvrA	186.3	25.0	211.3
	mutS	92.7	12.3	105.0
	polB	788.0	75.3	863.3
	dinB	282.0	34.3	316.3
	umuC	662.7	86.0	748.7
	mutD	189.0	24.7	213.7
xGFP.2	--	60.7	53.0	113.7
(one xA-T bp)	nei	65.5	36.5	102.0
	mutY	189.3	113.5	302.8
	mutM (fpg)	163.5	92.0	255.5
	uvrA	32	25	57
	mutS	33.0	22.0	55.0
	polB	267.3	182.7	450.0
	dinB	36.0	32.7	68.7
	umuC	127.0	89.7	216.7
	mutD	41.0	29.0	70.0
xGFP.6	--	33.7	12.1	45.9
(three cons. xDNAs)	nei	18.3	6.5	24.8
	mutY	21	9	30
	mutM (fpg)	15.8	3.5	19.3
	uvrA	6.5	4.0	10.5
	mutS	14.3	4.7	19.0
	polB	139.3	44.0	183.3
	dinB	43.0	14.5	60.7
	umuC	55.0	19.3	74.3
	mutD	29.8	14.3	44.0
xGFP.7	--	7.8	3.5	11.2
(four cons. xDNAs)	nei	2	2	4
	mutY	3	3	6
	mutM (fpg)	4.3	4.8	9.0
	uvrA	8.8	4.8	13.5
	mutS	2.7	0.7	3.3
	polB	11.7	9.7	21.3
	dinB	12.3	7.7	20.0
	umuC	14.3	6.7	21.0
	mutD	4	4.7	8.7

[a]average colony numbers for at least three trials.

Table S8. SOS induction has little effect on GFP expression: Colony count data with selected xDNA-containing inserts relative to the unmodified control (GFP.0) in *E. coli* cells with and without SOS induction.

GFP insert		# green colonies	# white colonies	# total colonies	% green
GFP.0	-SOS	348.3	57.7	406.0	85.8
	+SOS	76.7	10.7	87.3	87.8
xGFP.2	-SOS	60.7	53.0	113.7	53.4
	+SOS	12.5	8.0	20.5	61.0
xGFP.6	-SOS	33.7	12.1	45.9	73.5
	+SOS	8.3	3.0	11.2	73.7
xGFP.7	-SOS	7.8	3.5	11.2	69.2
	+SOS	3.3	0.9	4.1	79.3

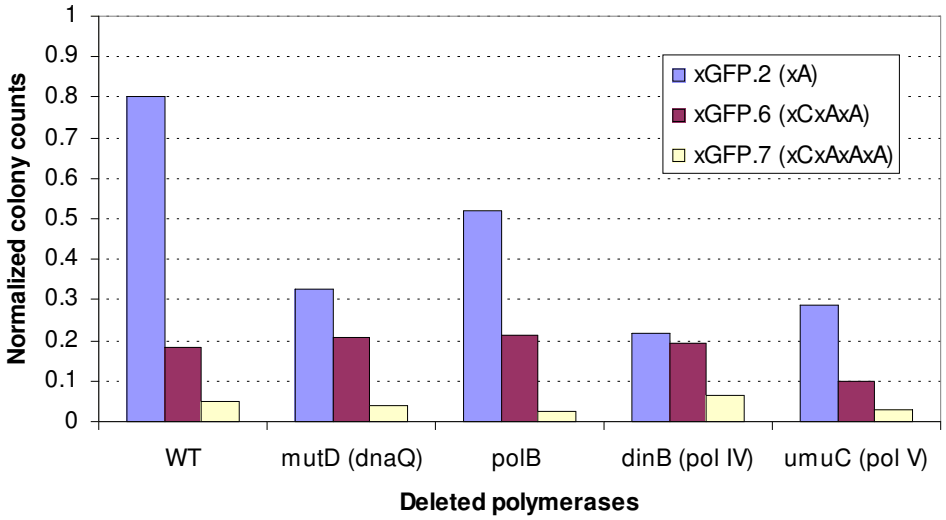

Figure S1. Normalized total colony counts for xDNA-containing inserts xGFP.2, xGFP.6 and xGFP.7 in *E. coli* strains with deleted SOS polymerases. Note that single xDNA substitutions (xGFP.2) are bypassed somewhat less efficiently with deletion of individual repair polymerases, but multiple substitutions (xGFP.6 and .7) are relatively unaffected.

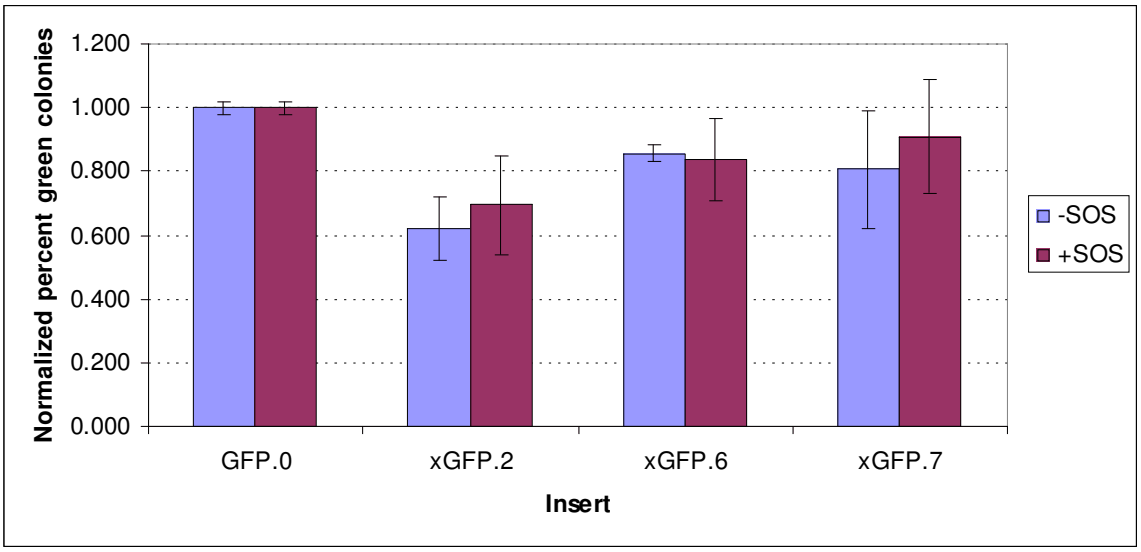

Figure S2. Normalized percent green colonies for xDNA-containing inserts relative to the unmodified control (GFP.0).

References

1. Delaney, J.C.; Henderson, P.T.; Helquist, S.A.; Morales, J.C.; Essigmann, J.M. & Kool, E.T. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 4469-4473.