Supporting Information

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SI Text

Materials. DNA oligonucleotides used to construct nanostructures were purchased from Integrated DNA Technology and purified via denaturing PAGE. Restriction enzymes PstI and SacI, T4 ligase, phagemid vector Litmus 28I, and helper phage M13KO7 were purchased from New England BioLabs. T4 polynucleotide kinase and T4 endonuclease VII were purchased from USB. Competent cell XL1-Blue was purchased from Stratagene. DNA ladders (10-bp and 25-bp) were purchased from Invitrogen. Alkaline phosphatase was purchased from Amersham Pharmacia Biotech). Plasmid Spin Miniprep kit was purchased from Qiagen. All other reagents were purchased from Sigma–Aldrich.

Detailed Methods for Characterization of Replicated Nanostructure.
Nondenaturing PAGE. One picomole each of the original strand (J1, J1-O, or PX), the purified replicated strand, and a random-sequence ssDNA with the same length as the original strand were annealed separately in 10 μl of 1× TAE-Mg^{2+} buffer from 94°C to 25°C in 1 h. The annealed DNA species were resolved by electrophoresis to compare their mobility on a 12% (for PX) or 20% (for junctions) nondenaturing polyacrylamide gel in 1× TAE-Mg^{2+} buffer. The gel was run at a constant voltage of 10 V/cm for 10 h, stained by Stains-All and scanned by a desktop scanner (HP Scanjet 4670).
Ferguson analysis. Preannealed sense PX, purified final product of sense PX, and a four-stranded PX structure without loops together with a 25-bp DNA ladder were assayed each by using 8%, 12%, 15%, and 18% nondenaturing PAGE. The four gels with different gel concentrations were simultaneously run for 3.6 h at a constant voltage of 10 V/cm. After staining, the mobility of bands was measured manually by using a millimeter-scaled ruler from the gel images.
Hydroxyl radical analysis. The cloned long single-stranded DNA (J1 cloverleaf design or PX) was dephosphorylated on its 5′ end in 50 μl of a solution containing 20 mM Tris·HCl (pH 8.0) and 10 mM $MgCl_2$ and mixed with 5 units of alkaline phosphatase for 1 h at 37°C. The reaction was stopped by phenol extraction, and the DNA was purified by ethanol precipitation. Dephosphorylated DNA (4 pmol) was dissolved in 20 μl of a solution containing 50 mM Tris·HCl (pH 7.6), 10 mM $MgCl_2$, and 10 mM 2-mercaptoethanol and mixed with 1 μl of 1.25 mM γ-[^{32}]ATP (10 mCi/ml) and 2 units of T4 polynucleotide kinase for 1 h at 37°C. The reaction was stopped by desiccation or ethanol precipitation of the DNA. The labeled DNA strand was additionally purified from a 10% denaturing polyacrylamide gel. The labeled strand (≈2 pmol in 10-μl solution containing TAE and 10.5 mM $MgCl_2$) was annealed to an excess of the unlabeled complementary strand, or it was annealed by itself to form the DNA (J1 cloverleaf or PX) motif, or it was treated with sequencing reagents (S3) for a sizing ladder. The samples were annealed by heating to 90°C for 5 min and then step-wise cooled down at 65°C for 10 min, 45°C for 10 min, 37°C for 10 min, room temperature for 10 min, and 4°C for 10 min. Hydroxyl radical cleavage of the double-stranded and branched DNA motif (J1 cloverleaf or PX) samples took place at 4°C for 2 min, as described by Tullius and Dombroski (1) with modifications noted by Churchill *et al.* (2). The reaction was stopped by the addition of thiourea. The samples were dried, dissolved in a formamide/dye mixture, and loaded directly onto a 12% polyacrylamide/8.3 M urea sequencing gel. Autoradiograms were quantitated by using a Storm 860 Gel and Blot Imaging System (Amersham Pharmacia Biotech).
Endonuclease VII cleavage. The 5′-labeled cloned long single-stranded J1 cloverleaf (≈4 pmol in 20 μl of a solution containing TAE and 10.5 mM $MgCl_2$)was annealed by itself to form the single-stranded J1 cloverleaf structure. The sample was annealed by heating to 90°C for 5 min and then step-wise cooled down at 65°C for 10 min, 45°C for 10 min, 37°C for 10 min, and room temperature for 10 min. A reaction solution (20 μl) containing 10 mM 2-mercaptoethanol, 100 ng/μl BSA, and 500 units of T4 Endonuclease VII was incubated at 37°C for 1 h. After digestion, DNA was phenol/chloroform extracted and ethanol precipitated. The samples were dried, dissolved in a formamide/dye mixture, and loaded directly on a 12% polyacrylamide/8.3M urea sequencing gel. The gel was scanned by using a Storm 860 Gel and Blot Imaging System. The positions cleaved by endo VII were located by comparison with an A–G sequencing ladder.

Methods for Hydroxyl Radical Analysis in the Presence of Psoralen. The cloned long single-strand J1 cloverleaf DNA molecule was labeled and additionally gel purified from a 10% denaturing polyacrylamide gel. The labeled strand (≈1 pmol in 10 μl of solution containing TAE and 10.5 mM $MgCl_2$) was annealed to the same amount of the unlabeled complementary strand, or it was annealed by itself to form the J1 cloverleaf motif, or it was treated with sequencing reagents for a sizing ladder. The samples were annealed by heating to 90°C for 5 min and then step-wise cooled down at 65°C for 10 min, 45°C for 10 min, 37°C for 10 min, room temperature for 10 min, and 4°C for 10 min. One microliter of 200 μg/ml TMP ethanol solution was added to the DNA solutions (the ratio between psoralen to DNA strand, which is 876:1, was kept same as the cross-linking reactions described elsewhere in this article). Hydroxyl radical cleavage of the double-stranded and J1 cloverleaf motif samples took place at 4°C for 2 min, as described by Tullius and Dombroski (1), with modifications noted by Churchill *et al.* (2). The reaction was stopped by the addition of thiourea. The samples were dried, dissolved in a formamide/dye mixture, and loaded directly on to a 12% polyacrylamide/8.3 M urea sequencing gel. Autoradiograms were quantitated by using a Storm 860 Gel and Blot Imaging System (Amersham Pharmacia Biotech)

Detailed Methods for Psoralen Cross-Linking Study. For *in vivo* cross-linking, 160 mg of cells (from ≈40-ml culture) containing nanostructure-inserted Litmus 28i were resuspended in 9.5 ml of lysis buffer [10 ml of Tris·HCl (pH 8) and 1 mM EDTA], and 0.5 ml of TMP ethanol solution (200 μg/ml) was added. The cells were incubated at room temperature with TMP for 10 min to allow adequate intercalation. Then, 1.5 ml of cell suspension was taken out and kept in the dark as a blank. The rest of the cell suspension was spread on a Petri dish to form a thin layer and irradiated by a 365-nm UV lamp (110 V, 0.16 A) at a 2-cm distance for 45 min. During the cross-linking reaction, the cells were kept on ice to avoid being overheated. DNA was extracted by using the Plasmid Spin Mini Prep kit, restriction digested by PstI and SacI as described above and filtered through a 100-kDa size-selection column. For *in vitro* cross-linking, 4 pmol of DNA was first annealed in 40 μl of 1× TAE-Mg buffer. Four microliters of 200 μg/ml TMP solution was added to the preannealed DNA solution. After 10 min of incubation, half of the TMP–DNA mixture was transferred into a UV-transparent cuvette and UV irradiated on ice as described above. The other half of the

mixture was kept in the dark as a blank. The DNA was recovered by ethanol precipitation. Cross-linked DNA samples, together with blank samples were loaded on a 14% denaturing polyacrylamide gel containing 8.3 M urea and resolved. Ferguson study was performed by processing cross-linking products on 8%, 12%, 14%, and 18% denaturing polyacrylamide gels simultaneously by using constant voltage of 15 V/cm at 43°C for 1.5 h.

Methods for Maxam-Gilber (A–G) Sequencing. DNA (4 pmol; synthetic and cell-cloned J1 and PX) was dissolved in 20 μl of a solution containing 50 mM Tris·HCl (pH 7.6), 10 mM $MgCl_2$, and 10 mM 2-mercaptoethanol and mixed with 1 μl of 1.25 mM γ-[^{32}]ATP (10 mCi/ml) and 2 units of T4 polynucleotide kinase for 1 h at 37°C. The reaction was stopped by desiccation or ethanol precipitation of the DNA. The labeled DNA strand was additionally purified from a 10% denaturing polyacrylamide gel. Twenty microliters of each labeled strand (≈2 pmol in water) was kept in 0°C for 1 h and then treated with 50 μl of formic acid (≈99%) at room temperature for 4 min. After treatment, 180 μl of HZ-stop solution (0.1 mg/ml tRNA, 0.1 mM EDTA, and 0.3M NaAc) was added to the above solution, and DNA samples were recovered by ethanol precipitation. After the samples were dried, each of them was incubated with 100 μl of peperidine solution (1 M) in 90°C for 20 min. After reaction, each sample was washed by water for several times before it was dried, dissolved in a formamide/dye mixture, and loaded directly onto a 12% polyacrylamide/8.3 M urea sequencing gel. The gel was scanned by using a Storm 860 Gel and Blot Imaging System (Amersham Pharmacia Biotech).

Methods for *in Vivo* Replication of J1 by Using M13mp18 Vector. Replication form (RF) M13mp18 (New England BioLabs) was double restriction digested by PstI and SacI and purified by gel electrophoresis. J1 was hybridized with antisense J1 and ligated with double-digested RF M13mp18 vector. The ligated vector was then transformed into XL-1 Blue-competent cells (Stratagene). Transformed XL-1 Blue cells (50 μl) were mixed with 300 μl of freshly grown JM109 cells, 15 μl of 100 mM IPTG, 25 μl of 2% X-gal, and 3.5 ml of LB top agar (Sigma). The mixture was poured over a LB agar plate preheated to 37°C, incubated at room temperature until LB top agar solidified and then incubated overnight at 37°C. The developed plaques were resuspended in 2 ml of LB medium and incubated at room temperature for 1 h to allow bacteriophage particles to diffuse out from agar. The suspension was stored at −20°C if desired to make bacteriophage stock. Bacteriophage suspension (100 μl; either freshly made or from stock) was added to 200 μl of freshly grown JM109 cells and transferred into 6 ml of LB medium culture. The inoculated LB medium was incubated at 37°C for 6 h with constant shaking. Single-stranded M13 DNA was recovered by using Qiaprep Spin M13 kit (Qiagen), restriction digested in the presence of restriction helper strands, and resolved by 10% denaturing PAGE.

DNA Sequences Used in This Work. AT and TA segments on junction arms are italicized. /5Phos/ stands for 5′-phosphate.

J1 (79-nt): 5′-/5Phos/CCGCAGA*AT*CCTGCGGTTTTTC-CGCACCGAA*AT*GCTTTTTGC*AT*TTC*GGACGGCTTTT-TGCCGTGGAT*TCTGCGCTGCA-3′; anti-J1 (79-nt): 5′-/5Phos/GCGCAGA*AT*CCACGGCAAAAAGCCGTC-CGAA*AT*GCAAAAAGC*AT*TTCGGTGCGGAAAAACCG-CAGG*AT*TCTGCGGAGCT-3′; restriction helper-J1-SacI (20-nt): 5′-TTCTGCGGAGCTCTGGTACC-3′; restriction helper-J1-PstI (20-nt): 5′-GGAATTCCTGCAGCGCAGAA-3′; J1-R (79-nt): 5′-/5Phos/CCGCAGA*TA*CCTGCGGTTTTTCCG-CACCGAA*TA*GCTTTTTGC*TA*TTC*GGACGGCTTTTTG-CCGTGGTA*TCTGCGCTGCA-3′; J1-RN (79-nt): 5′-/5Phos/CCGCAGA*TA*CCTGCGGTTTTTCCGCACCGAAAGG-CTTTTTGCCTTTC*GGACGGCTTTTTGCCGTGGTA*T-CTGCGCTGCA-3′; J1-RS (79-nt): 5′-/5Phos/CCGCAGAA-GCCTGCGGTTTTTCCGCACCGAA*TA*GCTTTTTGC*TA*-TTCGGACGGCTTTTTGCCGTGGCTTCTGCGCTGCA-3′; J1-O (79-nt): 5′-/5Phos/CCGGAGAAGCCTGCGGTTTTTC-CGCACCGAAAGGCTTTTTGCCTTTCGGAGGGCTT-TTTGCCCTGGCTTCTCCGCTGCA-3′; anti-J1-O (79-nt): 5′-/Phos/GCGGAGAAGCCAGGGCAAAAAGCCCTCC-GAAAGGCAAAAAGCCTTTCGGTGCGGAAAAAC-CGCAGGCTTCTCCGGAGCT-3′; restriction helper-J1-O-SacI (20-nt): 5′-TTCTCCGGAGCTCTGGTACC-3′; restriction helper-J1-O-PstI (20-nt): 5′-GGAATTCCTGCAGCGGA-GAA-3′; J1-O no sticky ends (73-nt): 5′-CGGAGAAGCCT-GCGGTTTTTCCGCACCGAAAGGCTTTTTGCCTTT-CGGAGGGCTTTTTGCCCTGGCTTCTCCG-3′ random 79-mer: 5′-/5Phos/GCGGAGATCACGTGCACACAGACATTC-GAGTAGGTATAGCCCATGACAAGAGCATCATCAC-AATCGACTATCATACCAG-3′; sense-PX (146-nt): 5′-/5Phos/CCACCGCAGACGCAATCCCAGAATTATACACAGTT-TTCTGTGGAGGTGTCTGGCGACTGGTCTGGCATCTT-TTGATGCTGCCTCAGTCGGTACCCACCTCGCCGC-TTTTGCGGCTATAATGGTACGATTGCAGGCACGG-TGCTGCA-3′; antiense-PX (146-nt): 5′-/5Phos/GCACCGT-GCCTGCAATCGTACCATTATAGCCGCAAAAGCGG-CGAGGTGGGTACCGACTGAGGCAGCATCAAAAG-ATGCCAGACCAGTCGCCAGACACCTCCACAGAAA-ACTGTGTATAATTCTGGGATTGCGTCTGCGGTGG-AGCT-3′; restriction helper 28i-PX-SacI (20-nt): 5′-TGCGGT-GGAGCTCTGGTACC-3′; restriction helper 28i-PX-PstI (20-nt): 5′-GGAATTCCTGCAGCACCGTG-3′; random 146-mer: 5′-ACTCACTGTTGTAGCTCGGATTTCGAGGATGG-GTGAAGTAACCTCTTGGGACTCTCGCGCTGAACAA-ACGCTTTCTAGACCGGTAGGTCGTCATGGAACTA-ACAAGGAAATCGGGCGATACAAGACACTGCTAC-GGCTGCGGAATG-3′; R1 (79-nt): 5′-/5Phos/CCGGAGAT-CACGTGCACACAGACATTCGAGTAGGTATAGCCCA-TGACAAGAGCATCATCACAATCGACTATCCACTGCA-3′; antisense-R1 (79-nt): 5′-/5Phos/GTGGATAGTCGATT-GAGATGATGCTCTTGTCATGGGCTATACCTACTC-GAATGTCTGTGTGCACGTGATCTCCGGAGCT-3′; restriction helper-R1-SacI: 5′-ATCTCCGGAGCTCTGGTACC-3′; restriction helper-R1-PstI: 5′-GGAATTCCTGCAGTG-GATAG-3′; restriction helper M13-J1-SacI (20-nt): 5′-TTCTGCGGAGCTCGAATTCG-3′; restriction helper M13-J1-PstI (20-nt): 5′-TGCATGCCTGCAGCGCAGAA-3′.

1. Tullius TD, Dombroski BA (1985) Iron(II) EDTA used to measure the helical twist along any DNA molecule. *Science* 230:679–681.
2. Churchill MEA, Tullius TD, Kallenbach NR, Seeman NC (1988) A Holliday recombination intermediate is twofold symmetric. *Proc Natl Acad Sci USA* 85:4653–4656.
3. Maxam AM, Gilbert W (1977) A new method for sequencing DNA. *Proc Natl Acad Sci USA* 74:560–564.

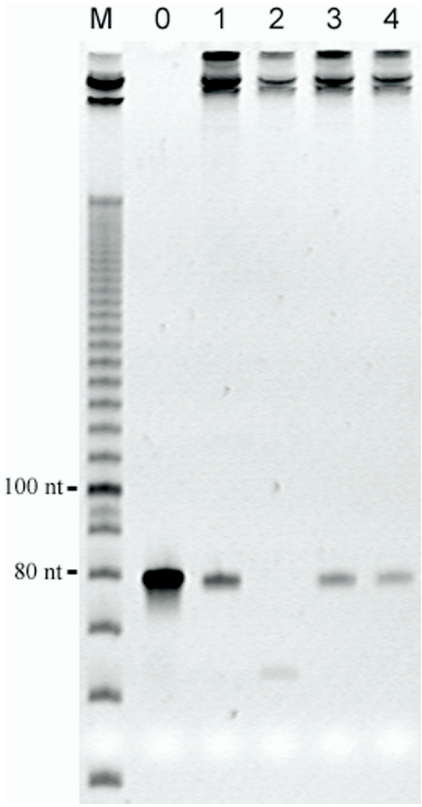

Fig. S1. Denaturing PAGE that confirms the correct DS insert inside the phagemid vectors. Lane M, 10-base DNA ladder; lane 0, J1 DS insert (79-bp); lane 1–4, each lane contains the restriction digested vectors extracted from cells amplified from a single colony. Note that among the four randomly picked colonies, three of them have the correct DS insert. The other one (in lane 2) has the wrong insert because of self-ligation.

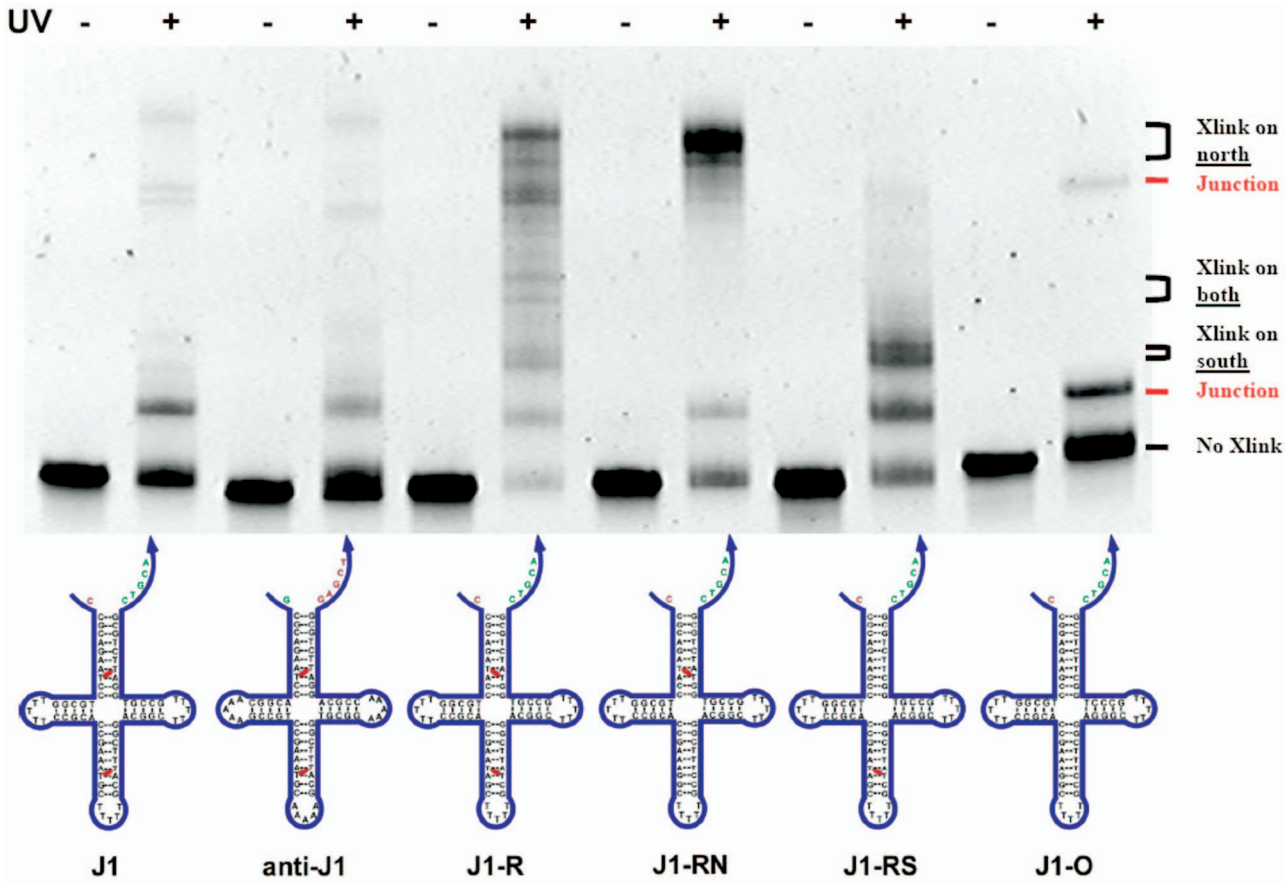

Fig. S2. Psoralen cross-linking on J1 control molecules *in vitro*. The schematic of J1 derivatives are present below corresponding lanes. Traditional ''hot-spot'' on the junction arms (i.e., 5′-AT or -TA segments) are highlighted by red dots on the molecule models. Bands are labeled on the right by the psoralen interaction site. ''Xlink,'' cross-link; ''north,'' ''south,'' and ''both'' refer to the cross-linking position on long arms. Two common bands (labeled by ''junction'' in red) are generated after UV treatment on all control molecules. Therefore they are attributed to the interaction between psoralen and the thymine bases flanking junction site. Note that the mobility of the band is inversely proportional to the size of the DNA circle formed by cross-linking. The lower common band, which ran faster than the cross-linked J1-RS, is thus suspected to be psoralen monoadduct. anti-J1, J1's full complement with different end-extensions; J1-R , J1 with all 5′-AT segment replaced by 5′-TA; J1-RN, J1-R with 5′-TA on south arm replaced by 5′-AG; J1-RS, J1-R with 5′-TA on north arm replaced by 5′-AG; J1-O, J1 without any 5′-AT or TA sequences.

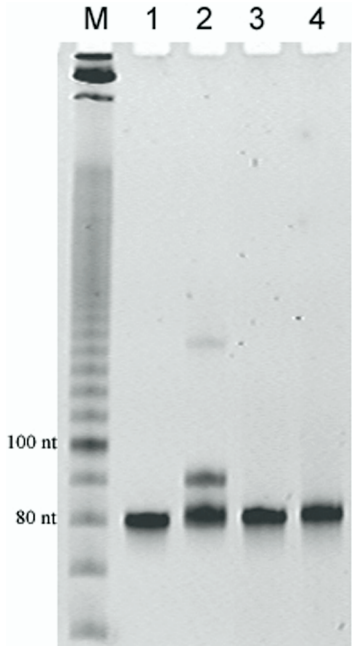

Fig. S3. Psoralen cross-linking on J1-O in Mg^{2+} and fomamide solutions. Lane M, 10-base DNA ladder; lanes 1 and 2, J1-O before and after UV treatment with psoralen in TAE-Mg^{2+} buffer; lanes 3 and 4, J1-O before and after UV treatment with psoralen in 40% formamide solution. Note that J1-O cannot interact with psoralen in denaturing condition.

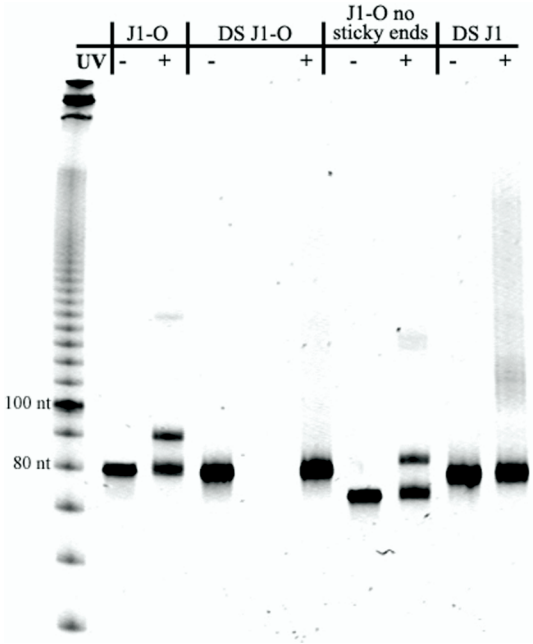

Fig. S4. Psoralen cross-linking on DS insert made from J1-O and J1. Samples and UV treatment are labeled above corresponding lanes. It is obvious that J1-O can only be cross-linked when it forms junctions, in contrast to J1, which can be cross-linked when hybridized with its complement (DS J1). In addition, J1-O with end extensions chopped off interacts with psoralen in the same fashion as intact J1-O, suggesting that the single-stranded overhangs are not essential for cross-linking.

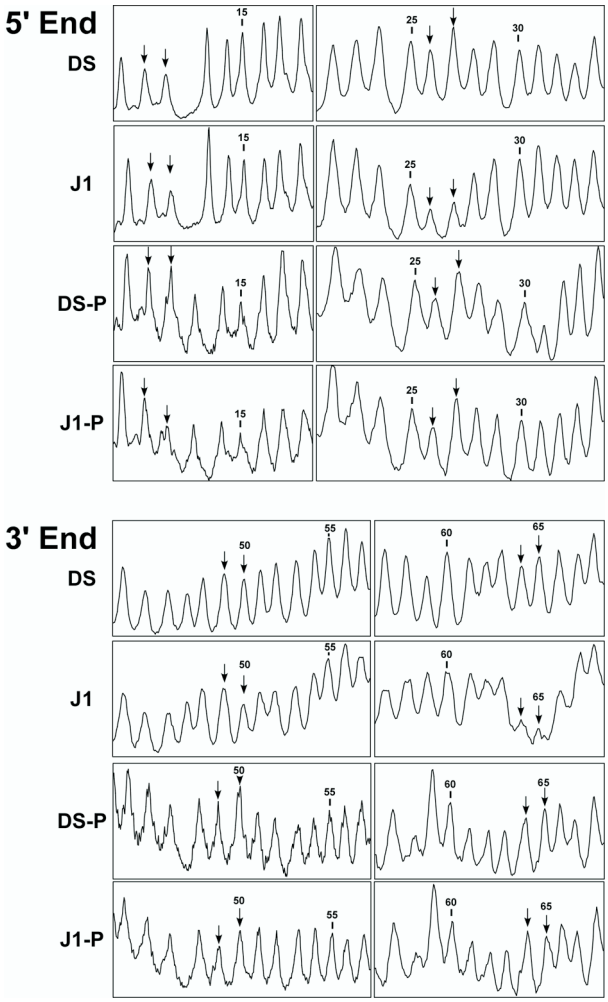

Fig. S5. The hydroxyl radical susceptibility of the cloned J1 junction in the presence of psoralen. The image is divided into two parts, indicating the 5′ and 3′ halves of the strands. Four images are shown for each half. DS is the strand paired with its Watson–Crick complement, J1 is the folded molecule by itself, DS-P is the DS molecule in the presence of psoralen, and J1-P is the folded molecule in the presence of psoralen. The nucleotide positions are labeled, and the positions of branching are indicated by downward pointing arrows. The key point is that the points exhibiting protection in the absence of psoralen (27, 28 and 64, 65) switch in the presence of psoralen (11, 12 and 49, 50).

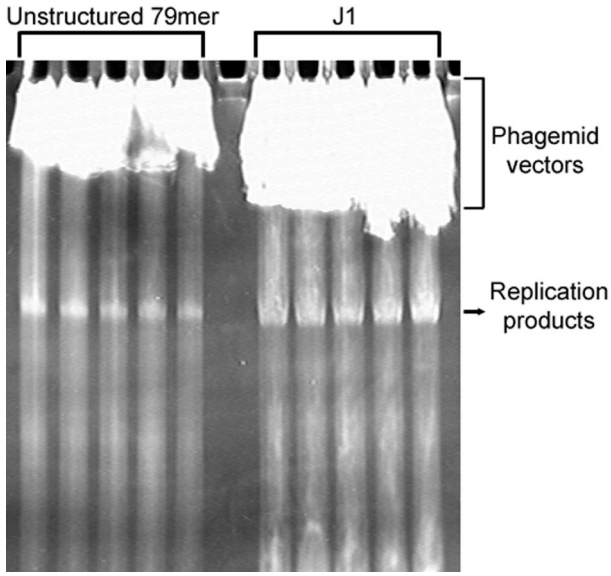

Fig. S6. Direct comparison between the replication efficiency of J1 (79-nt) and a 79-mer DNA with random sequence (R1). J1 and R1 were replicated by using the same *in vivo* cloning procedure, and the replication product after restriction digestion was resolved on a 10% denaturing polyacrylamide gel as shown. The replication yield of R1 is comparable with that of J1. Examination of the gel image by Image J software (National Institutes of Health, Bethesda) shows the relative band intensity of R1/J1 = 0.92:1.

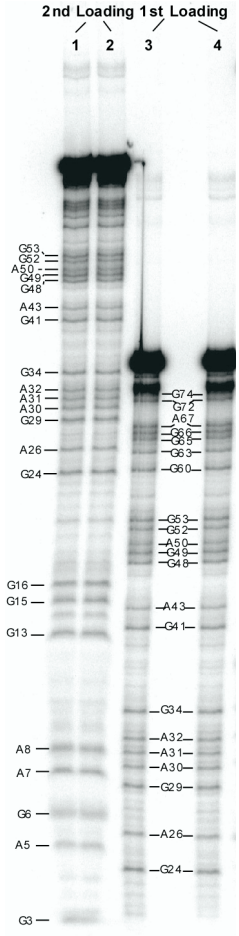

Fig. S7. Maxam-Gilbert (A–G) sequencing of synthetic and replicated J1. All of the As and Gs, numbered from the 5′ end, were labeled out beside the corresponding DNA bands. Lanes 1 and 3, sequencing of synthetic J1; lanes 2 and 4, sequencing of cell-replicated J1. The DNA sequences remained the same after *in vivo* cloning.

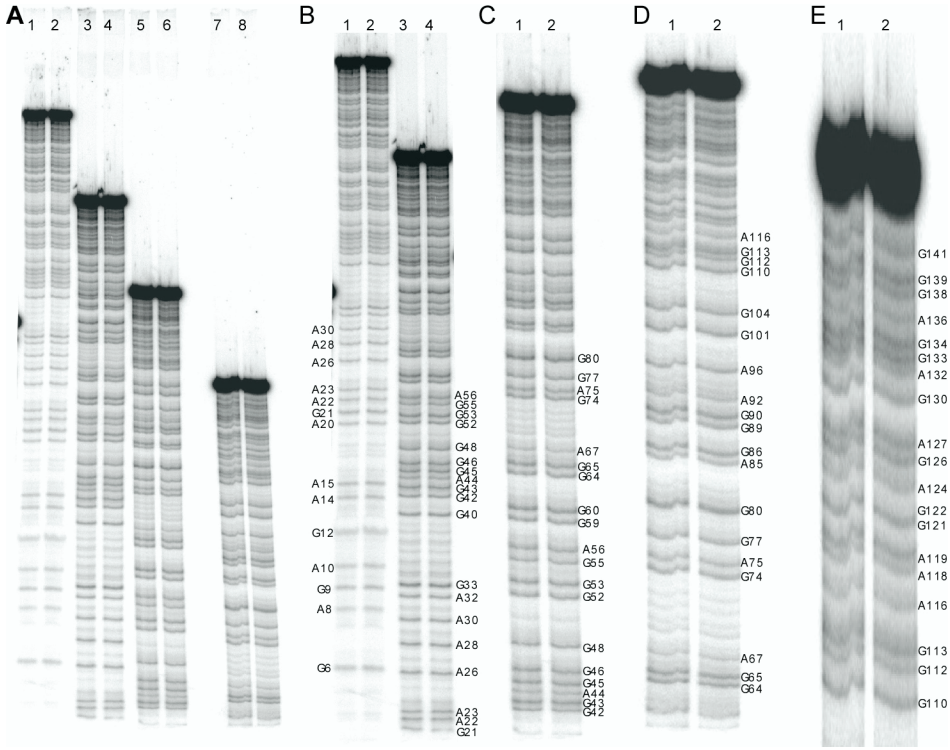

Fig. S8. Maxam-Gilbert (A–G) sequencing of synthetic and replicated PX. All of the As and Gs, numbered from the 5′ end, were labeled out beside the corresponding DNA bands. (*A*) Original scan of the gel image containing four-time loading; Lanes 1, 3, 5, and 7 for synthetic PX; lanes 2, 4, 6, and 8 for cell-replicated PX. (*B*) Enlarged image of fourth (lanes 1 and 2) and third loading (lanes 3 and 4); lanes 1 and 3 for synthetic PX; lanes 2 and 4 for cell-replicated PX. (*C*) Enlarged image of second loading; lane 1 for synthetic PX and lane 2 for cell-replicated PX. (*D*) Enlarged image of first loading; lane 1 for synthetic PX and lane 2 for cell-replicated PX. (*E*) Zoomed image of *D*; lane 1 for synthetic PX and lane 2 for cell-replicated PX. The DNA sequences remained the same after *in vivo* cloning.

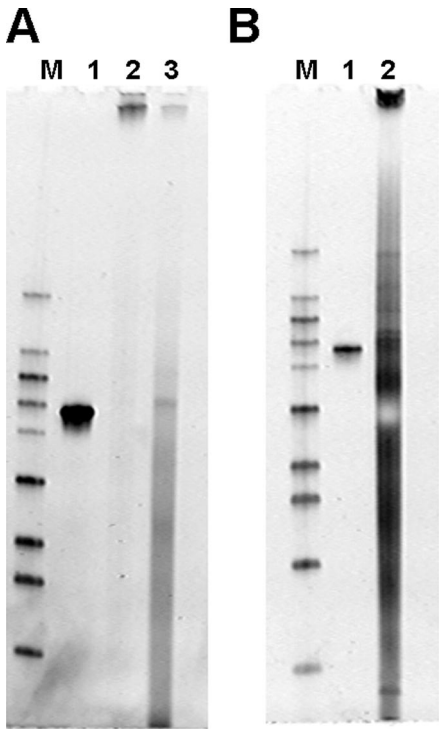

Fig. S9. *In vivo* cloning of J1 using M13mp18 (New England Biolabs) vectors. (*A*) Replication result from *E. coli* cells treated with freshly suspended bacteriophages. Lane M, 10-nt ssDNA ladder; lane 1, synthetic J1; lane 2, undigested M13 vector; lane 3, restriction-digested M13 vector. (*B*) Replication result from *E. coli* cells treated with bacteriophages from stock. Lane M, 10-nt ssDNA ladder; lane 1, synthetic J1; lane 2, restriction-digested M13 vector. Note that the use of M13mp18 vector can also generate some correctly replicated products but with much lower efficiency and reproducibility than the phagemid (Litmus 28i) system.