Supplementary Information

Triazole linking for preparation of a next-generation sequencing library from single-stranded DNA

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Supplementary Figures
Supplementary Figure S1. TdT-mediated incorporation of an azido functional group to the 3’-end of ssDNA. A. Schematic overview for the experimental design. One of the azido-modified nucleotides shown in B was incubated with fluorescently labeled oligonucleotide and TdT. B. Samples were loaded on 10% Novex TBE-Urea Gel (Thermo Fisher Scientific, Waltham, MA). After electrophoresis, an image of the FAM-signal was taken using a ChemiDoc Touch Imaging System (Bio-Rad Laboratories, Hercules, CA).
Supplementary Figure S2. Incorporation efficiency of azido-modified nucleotide analogs showed dependencies on the nucleotide base of the 3′-terminal end of the target DNA. **A.** Schematic overview of the experimental design. Random 29-mers with or without specific nucleotide bases at their 3′-ends were incubated with Az-ddGTP and TdT (indicated as “z” in B). As controls for the fully extended product, reactions were also performed with 2′,3′-dideoxyguanosine-5′-triphosphate (ddGTP) instead of Az-ddGTP (indicated as “d” in B). **B.** Samples were loaded on 10% Novex TBE-Urea gel. After electrophoresis, the gel was stained with SYBR Gold Nucleic Acid Gel Stain (Thermo Fisher Scientific) and image was taken using a ChemiDoc Touch Imaging System (Bio-Rad Laboratories).
Supplementary Figure S3. Optimized reaction conditions for azido-incorporation at the 3′-end of the target DNA that almost reached completion. A. Scheme overview of the experimental design. B. Samples from three independent experiments were loaded on a gel (10% Novel TBE-Urea gel). Lanes indicated by “M” (size marker) at the top of image contained four chemically synthesized DNAs with sizes of 28, 30, 32, and 34 nucleotides. The minus (−) and plus (+) signs at the top of the image indicate samples without and with the addition of TdT, respectively.
Supplementary Figure S4. Double Sera-Mag purification of azido-labeled DNA after the TdT reaction. Yields of DNA (chemically synthesized random 60-mer; N60) and removal efficiency of azido-modified nucleotide analog were analyzed by denatured gel electrophoresis and high-performance liquid chromatography (HPLC), respectively. A. Electrophoretic analysis of model DNA amounts before and after solid-phase reversible immobilization (SPRI) purifications. Samples from three independent experiments were loaded on a gel (10% Novel TBE-Urea gel). The gel image was taken using a ChemiDoc Touch Imaging system after SYBR Gold staining, and the signal intensity for each band was calculated using Image Lab Software (Bio-Rad Laboratories). B. Analysis of Az-ddGTP in DNA solutions before and after SPRI purifications. Samples were analyzed on an Agilent 1260 Infinity II system (Agilent Technologies, Santa Clara, CA) with a COSMOSIL PBr column (2.0 × 150 mm, Nacalai Tesque, Kyoto, Japan). The analysis was performed with an isocratic eluent that contained 1x phosphate buffer solution (Nacalai Tesque) and 1% (v/v) acetonitrile (Nacalai Tesque) at a flow rate of 0.5 ml/min. Absorbance at 258 nm was detected with a 1260 Infinity II Variable Wavelength Detector, and signal intensities for peaks corresponding to Az-ddGTP were quantified. C. Relative yields for three independent SPRI purifications are summarized with the input taken as 100%.
Supplementary Figure S5. Organic solvents improved the efficiency of click ligation. A. Schematic overview of the experiment design. B. Electrophoresis gel images showing the efficiencies of ligation. After CuAAC ligation, DNA was recovered from the solvent by using SPRI beads as described in the Materials and Methods section, and then loaded on a 10% Novex TBE-Urea gel. The images were taken after staining with SYBR Gold gel stain.
Supplementary Figure S6. The pH dependency of the CuAAC reaction between two DNA molecules. **A.** Schematic overview of experimental design. **B.** Gel images showing the efficiencies of ligation. The procedure for recovery of DNA was the same as described for Supplementary Figure S4. After gel electrophoresis on a 10% Novex TBE-Urea gel, the FAM-signal was detected with ChemiDoc Touch Imaging System.
Supplementary Figure S7. Replication of triazole-containing DNA by 16 DNA polymerases. A. Schematic overview and DNA sequence for experimental design. B. Primer extension reactions using FAM-Primer (0). C. Primer extension reactions using FAM-Primer (−5). The reaction conditions for the polymerases are described in Supplementary Procedure S3.
Supplementary Figure S8. Library preparation from chemically synthesized model DNA. A. Schematic overview for the experimental design. B. The 12 steps of the experimental protocol. The molar amounts of starting DNA (N100) and final library are shown. C. Electrophoretic analysis of the library preparation. An equivalent amount of the reaction mixture was sampled from each step listed in panel B and loaded on a 6% Novex TBE-Urea gel. The lower-case character at the top of each lane corresponds to the sampling step in panel B. The yellow arrowheads indicate the major product in each step.
Supplementary Figure S9. Determination of length and nucleotide composition of a sequencing library synthesized by the phosphoramidite method. A. Schematic overview of the library preparation. A randomized 100-mer DNA with GT placed between two different adaptor sequences was synthesized solely by a phosphoramidite-based method (PEA1Tr4-N100GT-anti-PEA2tr5, Supplementary Table S1). After PCR amplification using Primer-3 and Index-1 to make the library structure readable, sequencing was performed on an Illumina MiSeq system. B. Length distribution of reads. C. The nucleotide composition was determined by summarizing the nucleotide base at each position in the read. Note that the first two positions are specific bases expected from the experimental principle.
Supplementary Figure S10. Analysis of reads derived from 100-mers with defined sequences. A. Five 100-mers with defined sequences used in this experiment. These 100-mers were spiked in $N_{100}$ prior to library preparation. B. Schematic representation of the ideal structure of library DNA. C. Summary of alignments between the reads and specific 100-mer sequences. We mapped the reads on the five reference sequences shown in panel A. Note that positions 1 and 2 in the reads correspond to the attached GT, whereas positions 3 and later correspond to the sequence of each specific 100-mer. If the microdeletions described by Shivalingam et al (22) occur at the 3′-adjacent position of the $Tz3$, the reference sequences will be aligned from position 2 of the reads. However, more than 98% of the alignments start from position 3 of the reads, which suggests that no microdeletion events occurred in our system. In contrast, the rates for alignments declined at the later positions of the reads, which indicates that deletions occurred at the opposite end to the adaptor tagging with TCS ligation.
Supplementary Figure S11. Preparation of sequencing libraries from micrococcal nuclease-treated chromatin. A. DNA purified from micrococcal nuclease-treated nuclei from *Saccharomyces cerevisiae* S288C. At 5, 10, 15, and 20 min after addition of the nuclease to the yeast nuclei, aliquots of reaction were transferred to new tubes and the reactions were stopped by chelation of Ca\(^{2+}\) ions with EDTA. After RNase treatment, silica column purification was performed. The samples treated for 10 min were used for library preparation. B. Electrophoretic analysis of library prepared using the TCS-ligation-based protocol after global amplification with 15 cycles of PCR. Two technical replicates using micrococcal nuclease-treated DNA and chemically synthesized random 100-mer DNA as positive controls were loaded. C. Electrophoretic analysis of the library prepared by using a ThruPlex kit from Rubicon genomics. All the analyses were performed using a 6% Novex TBE-Urea gel, and images were taken after staining with SYBR Gold.
Supplementary Table
**Supplementary Table S1. Oligonucleotides used in this study**

| Name               | Sequence and modifications                                                                 |
|--------------------|-------------------------------------------------------------------------------------------|
| N{Number}*1        | 5’ 1N{Number}‘3’                                                                           |
| FAM-N{Number}*1    | 5’ -(6-FAM) N{Number}‘3’                                                                    |
| ET-anti-PEA2*2     | 5’ -(5’-Ethynyl T) TAG ATC GGA AGA GCG TCG TGT AGG GAA AGA GTG T-3’                         |
| PEA2               | 5’ -ACA CTC TTT CCC TAC ACG ACG CTC TTC CGA TCT-3’                                          |
| P-anti-PEA1-P      | 5’ -(phosphate) GAT CGG AAG AGC ACA CGT CTG AAC TCC AGT CAC (phosphate)-3’                  |
| PEA1T              | 5’ -GTG ACT GGA GTT CAG ACG TGT GCT CTT CCC ATC T-3’                                        |
| FAM-Primer (0)     | 5’ -(6-FAM) ACA CTC TTT CCC TAC ACG ACG CTC TTC CGA TCT-3’                                 |
| FAM-Primer (-5)    | 5’ -(6-FAM) ACA CTC TTT CCC TAC ACG ACG CTC TTC C-3’                                       |
| 9ATpT-anti-PEA2    | 5’ -AAA AAA AAA TTA GAT CGG AAG AGC GTC GTG TAG GG-3’                                       |
| 9ATzT-anti-PEA2*3,*4| 5’ -AAA AAA AAA (TzT)A GAT CGG AAG AGC GTC GTG TAG GG-3’                                    |
| 9ATzU-anti-PEA2*3,*5| 5’ -AAA AAA AAA (TzU)A GAT CGG AAG AGC GTC GTG TAG GG-3’                                   |
| PEA1Ttr4-N100GT-anti-PEA2tr5 | 5’ -CTG GAG TTC AGA CGT GTG CTC TTC CGA TCT (N100) GTA GAT CGG AAG AGC GTC GTG TAG GGA AAG-3’ |
| Primer 3           | 5’ -AAT GAT ACG GCG ACC ACC GAG ATC TAC ACT TCC CTA CAC GAC GCT CTT CCG ATC T-3’            |
| Index-1            | 5’ -CAA GCA GAA GAC GGC ATG GAT CGT GAT GTG ACT GGA GTT CAG ACG TGT GCT CTT CCG ATC-3’       |

*1 N denotes equimolar mixture of A, C, G, and T. “Number” indicates the length of nucleotides. In this study 28, 30, 32, 34, 40, 60, 80, and 100 are used.

*2 Structure of 5’-ethynyl T is shown in Figure 2; this is the same material as S8 in Supplementary Procedure S1.

*3 Structures of TzT and TzU are shown in Figure 3.

*4 The same material as 18TT in Supplementary Procedure S2.

*5 The same material with 18TU in Supplementary Procedure S2.
Supplementary Procedures
Supplementary Procedure S1
Chemical synthesis of 5'-ethynyl oligonucleotide

General
Compounds from solution-phase synthesis were purified by flash column chromatography with silica gel 60N (spherical and neutral gel, 40-50 µm). Elongation of chimeric oligonucleotides was performed on a Nihon Techno Service M-2-MX DNA/RNA synthesizer. Analysis of reaction mixtures with high pressure liquid chromatography (HPLC) were performed on HPLC systems equipped with ODS column (COSMOSIL C18-MS-II, 4.6 × 250 mm, Nacalai Tesque; column temperature 40 °C), and purification of products was performed with ODS column (COSMOSIL C18-MS-II, 20 × 250 mm, Nacalai Tesque). IR spectra were recorded on an FT-IR instrument and were reported as wavenumbers (ν) in cm⁻¹. NMR spectra were recorded at 400 or 600 MHz for ¹H, 100 or 150 MHz for ¹³C and 160 or 240 MHz for ³¹P and were reported as chemical shift values with respect to chloroform (δ 7.26: ¹H NMR; δ 77.16: ¹³C NMR) and 85% v/v aqueous H₃PO₄ (δ 0.0: ³¹P NMR, external standard). Methyl (CH₃), methylene (CH₂) and methyne (CH) signals in ¹³C NMR spectra were assigned by DEPT spectra. High resolution mass spectra were obtained on JEOL JMS-T100LC or SolariX 9.4T instrument (ESI-TOF MS). A molar amount of oligonucleotide or trityl cation at the final elongation step was quantified on a UV-visible spectrometer (JASCO, V-670) to determine the elongation efficiencies.

Materials
Anhydrous THF (stabilizer free) and DMF were purified by a solvent purification system equipped with columns of activated alumina and supported copper catalyst. Water was purified by a Milli-Q ultrapure water system. Other solvents were purified by distillation from calcium hydride and were dried over 4 Å molecular sieves. Reagents for the solid-phase oligonucleotide synthesis including dT-CPG 1000 S⁴, phosphoramidites S⁵ and UnySupport 500 S13 (Supplementary Information 2) were purchased from Glen Research.

Synthesis of ethynyl oligonucleotide
Figure S1-1. Synthesis of ethynyl oligonucleotide

Synthesis of 5'-deoxy-5'-trimethylsilylthynylthymidine 3'-[2-cyanoethyl \( N,N \)-bis(1-methylethyl)phosphoramidite] S3
To a solution of ester S1 (430 mg, 912 µmol) in dichloromethane/methanol (2:1 v/v, 60 mL) was added aqueous solution of potassium carbonate (0.72 M, 5 mL), and the mixture was stirred at ambient temperature for 1.5 h. After addition of aqueous solution of citric acid (0.21 M, 50 mL), the mixture was extracted with chloroform (3 × 50 mL). The combined organic layer was washed with brine (150 mL) and concentrated in vacuo. The crude material was purified by recrystallization from chloroform/hexane and by silica gel column chromatography (eluent: 50-67% gradient ethyl acetate/chloroform) to give alcohol S2 (278 mg). A part of S2 (237 mg) was then subjected to the subsequent phosphorylation. Thus, to a solution of S2 in acetonitrile (15 mL) were added 2-cyanoethyl N,N-diisopropyl chlorophosphoramidite (280 µL, 1.25 mmol) and N,N-diisopropylethylamine (510 µL, 2.94 mmol), and the mixture was stirred at ambient temperature for 40 min. After addition of saturated aqueous solution of sodium bicarbonate (30 mL) at 0 °C, the mixture was extracted with chloroform (3 × 30 mL). The combined organic layer was dried over magnesium sulfate and concentrated in vacuo. The crude material was purified by silica gel column chromatography using silica gel pretreated with 2% v/v triethylamine (eluent: 30-40% v/v ethyl acetate/hexane containing 2% v/v triethylamine) to give the title compound S3 (393 mg, 752 µmol, 97% as a racemic mixture for two-step transformations) as a white solid.

Physical data of S3 (a racemic mixture): IR (powder) 2965, 1691, 1250, 1048, 844 cm⁻¹; ¹H NMR (600 MHz, CDCl₃) δ 0.16 (s, 9H), δ 0.16 (s, 9H), 1.18-1.22 (m, 12H), 1.18-1.22 (m, 12H), 1.94 (s, 3H), 1.94 (s, 3H), 2.15-2.22 (m, 1H), 2.15-2.22 (m, 1H), 2.45 (dd, J = 5.4, 14 Hz, 1H), 2.53 (dd, J = 5.4, 14 Hz, 1H), 2.60-2.77 (m, 4H), 2.60-2.77 (m, 4H), 3.58-3.67 (m, 2H), 3.58-3.67 (m, 2H), 3.73-3.81 (m, 1H), 3.73-3.81 (m, 1H), 3.83-3.91 (m, 1H), 3.83-3.91 (m, 1H), 4.13 (dd, J = 1.2, 1.2 Hz, 1H), 4.20 (dd, J = 1.2, 1.2 Hz, 1H), 4.48-4.53 (m, 1H), 4.48-4.53 (m, 1H), 6.26-6.30 (m, 1H), 6.26-6.30 (m, 1H), 7.49 (s, 1H), 7.53 (s, 1H); ¹³C NMR (150 MHz, CDCl₃) δ 0.1 (CH₃), 0.1 (CH₃), 12.9 (CH₃), 12.9 (CH₃), 20.6 (CH₂), 20.6 (CH₂), 24.6 (CH₂), 24.7 (CH₃), 24.7 (CH₃), 39.6 (CH₂), 39.8 (CH₂), 43.4 (CH), 43.5 (CH), 58.4 (CH₃), 58.5 (CH₂), 75.4 (CH), 75.6 (CH), 83.3 (CH), 83.7 (CH), 84.8 (CH), 84.9 (CH), 88.1, 88.2, 102.3, 102.7, 111.1, 111.2, 117.6, 117.7, 135.4 (CH), 135.4 (CH), 150.2, 150.2, 163.5, 163.6; ³¹P NMR (240 MHz, CDCl₃) δ 149.91, 149.99; HRMS (JMS-T100LC) calcd for C₂₄H₂₉N₄O₅PSiNa [M + Na]⁺ 545.2295, found 545.2320.

¹. Isobe, H.; Fujino, T.; Yamazaki, N.; Guillot-Nieckowski, M.; Nakamura, E. Org. Lett. 2008, 10, 3729-3732.
Synthesis of ethynyl oligonucleotide S8

Ethynyl oligonucleotide S8 was synthesized on an automated synthesizer by using thymidine-immobilized CPG S4 (dT-CPG 1000; 26.5 mg, 1.00 μmol, hydroxy end-loaded) as a solid support. The 5'-hydroxy terminus of CPG S4 was coupled with N-acetylguanine phosphoramidite S5 (Base = GAc, 70 mM, 144 μL, 10.1 μmol) in the presence of BTT activator (450 mM in acetonitrile, 440 μL, 198 μmol). After a 70 s coupling reaction, the residual 5'-hydroxy end of CPG was capped with acetic anhydride (1.06 M in THF, 500 μL, 530 μmol). After oxidation of the phosphorous linker with iodine (20 mM in THF, 700 μL, 14.0 μmol), the 5'-dimethoxytryrityl (DMTr) group on the extended nucleoside (G) was removed with trichloroacetic acid (300 mM in dichloromethane, 1.3 mL, 390 μmol). The DMTr cation was roughly quantified by an LED detector equipped with the synthesizer to assure a coupling efficiency >99%. The elongation procedure was performed using the corresponding phosphoramidite S5 possessing corresponding nucleobases to afford 33-mer 5'-AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT-3' S6 on CPG. The ethynyl nucleoside S3 was then loaded to assemble 33-mer S6 on CPG under the identical conditions for the phosphoramidite of natural deoxyribonucleosides except for a longer coupling time of 3600 s to afford the 34-mer S7 on CPG. Finally, S7 on CPG was treated with a 28% solution of ammonium hydroxide at 80 °C for 6 h to afford S8 via cleavage from the solid support. A portion (1.50 mL; 33.0% v/v) of the resulting solution of the chimeric oligonucleotide S8 (4.55 mL) was purified by reverse-phase HPLC, and the fraction containing the target material (19.2 mL) was freeze-dried. The total amount of S8 was determined as follows: After HPLC purification, a small portion (1.50 mL; 7.81% v/v) of the fraction containing S8 was freeze dried. The resulting material was dissolved in water (400 μL), and a portion of the solution (40 μL; 10% v/v) was mixed with 10 mM Na phosphate buffer (360 μL, pH 7.0 containing 100 mM NaCl and 0.10 mM EDTA) to record \( \text{Abs}_{260} = 0.654 \text{ a.u.} \). By using a molar coefficient at 260 nm, \( \varepsilon_{260} = 3.54 \times 10^5 \text{ M}^{-1} \cdot \text{cm}^{-1} \) the amount of S8 in this solution was determined as 739 pmol, which showed that the total amount of S8 from the synthesis was 287 nmol from 1.0 μmol.

The yield for the 133-step synthesis from S4 was thus 29%. The average yield per synthetic operation was 99%; and the average yield per elongation of one unit was 96%. The identity of the chimeric oligonucleotides was confirmed by ESI-TOF mass spectrometry analysis. Physical data of S8 (5'-ethynyl-TpApGpApTpCpGpGpApApGpApGpApGpApCpGpTpCpGpTpGpTpApGpGpApApGpApGpApGpTpGpTpGpTp-3', p: phosphodiester linkage): HRMS (SolariX 9.4T) calcd for C_{339}H_{398}N_{143}O_{198}P_{33} [M + 15H]^{15+}...
592.2606, found 592.2610. HPLC analysis of the isolated chimeric oligonucleotide S8 is shown in Figure S1-2.

**HPLC charts of isolated oligonucleotides**

![HPLC chart of the isolated ethynyl oligonucleotide S8](image)

**Figure S1-2.** HPLC chart of the isolated ethynyl oligonucleotide S8. Column: ODS column (COSMOSIL C18-MS-II, 4.6 × 250 mm); eluent: a linear gradient from 0% to 50% acetonitrile/H₂O (buffered as 100 mM CH₃CO₂NHEt₃, pH 7) during 30 min; flow rate: 1.0 mL/min; column temperature: 40 °C; analysis wavelength: 260 nm.
Supplementary Procedure S2

Chemical synthesis of oligonucleotides containing triazole linking

(a)

(b)

1) BTT activator, ODMTr, Base

2) CuBr, 8Me2Ph (25 mol%), EIN-P2 (0.25 eq.)

3) iBuOH/DMF (1:2, v/v), rt, 5 h

4) S9 (1.0 eq.)

5) S10T (Base = T, R' = H)

6) S10U (Base = U, R' = OSiMe2Bu)

7) S11T (Base = T, R' = H); 96%

8) S11U (Base = U, R' = OSiMe2Bu); 93%

9) S12T (Base = T, R' = H); 62%

10) S12U (Base = U, R' = OSiMe2Bu); 83%

11) S13

12) S14

13) S15TT

14) S15TU

UnySupport 500

(Glen Research)
Synthesis of triazole-linked dinucleotide alcohol S11TT

A solution of azide S9 (350 mg, 614 µmol), acetylene S10T (154 mg, 615 µmol), copper bromide dimethyl sulfide complex (31.6 mg, 154 µmol) and N,N-diisopropylethylamine (27.0 µL, 155 µmol) in t-butanol/DMF (1/2 v/v, 2.0 mL) was stirred at ambient temperature for 5 h. After addition of saturated aqueous solution of ammonium chloride (2 mL), the mixture was extracted with dichloromethane (10 × 2 mL). The combined organic layer was dried over sodium sulfate and concentrated in vacuo. The crude material was purified by silica gel column chromatography using silica gel pretreated with 0.5% v/v triethylamine (eluent: 0-5% v/v methanol/dichloromethane) to give the title compound S11TT (483 mg, 589 µmol, 96%) as a white solid. The physical data was identical to the reported data in a literature.2

Synthesis of triazole-linked dinucleotide alcohol S11TU

A solution of azide S9 (350 mg, 614 µmol), acetylene S10U (225 mg, 614 µmol), copper bromide dimethyl sulfide complex (31.6 mg, 154 µmol) and N,N-diisopropylethylamine (27.0 µL, 155 µmol) in t-butanol/DMF (1:2, v/v, 2.0 mL) was stirred at ambient temperature for 5 h. After addition of saturated aqueous solution of ammonium chloride (2 mL), the mixture was extracted with dichloromethane (10 × 2 mL). The combined organic layer was dried over sodium sulfate and concentrated in vacuo. The crude material was purified by silica gel column chromatography using silica gel pretreated with 0.5% v/v triethylamine (eluent: 0-5% v/v methanol/dichloromethane) to give the title compound S11TU (534 mg, 570 µmol, 93%) as a white solid. Physical data of S11TU: IR (powder) 2928, 1690, 1253, 1036, 835 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 0.06 (s, 3H), 0.07 (s, 3H), 0.89 (s, 9H), 1.51 (s, 3H), 2.62-2.75 (m, 2H), 2.97-3.02 (m, 1H), 3.14 (dd, J = 5.6, 9.6 Hz, 1H), 3.22 (dd, J = 4.0, 9.6 Hz, 1H), 3.37 (dd, J = 2.8, 11 Hz, 1H), 3.64 (dd, J = 3.0, 11 Hz, 1H), 3.79 (s, 6H), 4.01 (dddd, J = 4.0, 5.6, 8.0 Hz, 1H), 4.13 (ddd, J = 5.2, 8.0 Hz, 1H), 4.38 (dddd, J = 2.4, 2.8, 3.0 Hz, 1H), 4.43 (dd, J = 4.0, 5.6, 8.0 Hz, 1H), 5.03 (d, J = 4.0 Hz, 1H), 5.43 (dddd, J = 2.4, 4.0, 5.2 Hz, 1H), 5.71 (d, J = 8.2 Hz, 1H), 6.55 (dd, J = 6.8, 7.2 Hz, 1H), 6.84 (dd, J = 1.6, 8.8 Hz, 4H), 7.19 (d, J = 8.2 Hz, 1H), 7.23-7.33 (m, 7H), 7.36-7.40 (m, 2H), 7.53 (s, 1H), 7.64 (s, 1H); ¹³C NMR (100 MHz, CDCl₃) δ −4.9

2. Varizhuk, A.; Chizhov, A.; Smirnov, I.; Kaluzhny, D.; Florentiev, V. *Eur. J. Org. Chem.* 2012, 2173-2179.
(CH₃)₄ -4.6 (CH₃), 12.0 (CH₃), 18.1, 25.8 (CH₃), 28.5 (CH₂), 38.6 (CH₂), 46.2, 55.4 (CH₂), 60.5 (CH),
63.0 (CH₂), 71.7 (CH), 73.7 (CH), 82.3 (CH), 84.1 (CH), 85.3 (CH), 87.3, 94.3 (CH), 103.0 (CH),
112.1, 113.5 (CH), 121.9 (CH), 127.5 (CH), 128.2 (CH), 128.2 (CH), 130.2 (CH), 135.1, 135.2, 135.4
(CH), 142.3 (CH), 143.7, 144.2, 150.0, 150.5, 159.0, 163.1, 163.5; HRMS (JMS-T100LC) calcd for
C₄₈H₅₇N₇O₁₁SiNa [M + Na]⁺ 958.3783, found 958.3794.

**Synthesis of triazole-linked dinucleotide phosphoramidite S12TT**

To a solution of alcohol S11TT (355 mg, 433 µmol) in dichloromethane (8.7 mL) were added 2-
cyanoethyl N,N-diisopropyl chlorophosphoramidite (490 µL, 2.20 mmol) and N,N-
diisopropylethylamine (760 µL, 4.36 mmol), and the mixture was stirred at ambient temperature for
15 h. After addition of saturated aqueous solution of sodium bicarbonate (10 mL) at 0 °C, the mixture
was extracted with dichloromethane (5 × 7 mL). The combined organic layer was dried over sodium
sulfate and concentrated in vacuo. The crude material was purified by silica gel column
chromatography using silica gel pretreated with 0.5% v/v triethylamine (eluent: 0-1% v/v methanol/dichloromethane containing 0.5% v/v triethylamine) to give the title compound S12TT (274
mg, 269 µmol, 62% as a racemic mixture) as a white solid. The physical data was identical to the
reported data in a literature.²

**Synthesis of triazole-linked dinucleotide phosphoramidite S12TU**

To a solution of alcohol S11TU (425 mg, 454 µmol) in dichloromethane (9.1 mL) were added 2-
cyanoethyl N,N-diisopropyl chlorophosphoramidite (510 µL, 2.29 mmol) and N,N-
diisopropylethylamine (790 µL, 4.54 mmol), and the mixture was stirred at ambient temperature for
15 h. After addition of saturated aqueous solution of sodium bicarbonate (10 mL) at 0 °C, the mixture
was extracted with dichloromethane (5 × 7 mL). The combined organic layer was dried over sodium
sulfate and concentrated in vacuo. The crude material was purified by silica gel column
chromatography using silica gel pretreated with 0.5% v/v triethylamine (eluent: 0-1% v/v methanol/dichloromethane containing 0.5% v/v triethylamine) to give the title compound S12TU (429
mg, 377 µmol, 83%) as a white solid. Physical data of S12TU (a racemic mixture): IR (powder) 2965,
1692, 1253, 1039, 834 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 0.01 (s, 3H), 0.03 (s, 3H), 0.04 (s, 3H),
0.07 (s, 3H), 0.85 (s, 9H), 0.86 (s, 9H), 1.10-1.25 (m, 24H), 1.51 (s, 3H), 1.52 (s, 3H), 2.45-2.60 (m,
2H), 2.61-2.75 (m, 6H), 2.90-3.08 (m, 3H), 3.09-3.17 (m, 1H), 3.17-3.25 (m, 1H), 3.25-3.33 (m, 1H),
3.33-3.40 (m, 2H), 3.55-3.75 (m, 8H), 3.79 (s, 12H), 3.80-3.98 (m, 2H), 4.12-4.22 (m, 2H), 4.30-4.50 (m, 4H), 5.35-5.46 (m, 2H), 5.48-5.52 (m, 2H), 5.66-5.72 (m, 2H), 6.50-6.60 (m, 2H), 6.78-6.90 (m, 8H), 7.20-7.41 (m, 20H), 7.52 (s, 1H), 7.60 (s, 1H), 7.64 (s, 2H); 31P NMR (160 MHz, CDCl3) δ 149.17, 150.2; HRMS (JMS-T100LC) calcd for C57H74O12N9PSiNa [M + Na]+ 1158.4862, found 1158.4886.

Synthesis of chimeric oligonucleotides S18TT and S19TU
Chimeric oligonucleotides were synthesized on an automated synthesizer by using linker-modified CPG S13 (Glen UnySupport 500) as a solid support (42.1 mg, 1.98 μmol hydroxy end-loaded). The 5’-hydroxy terminus of CPG S13 was coupled with N-acetylguanine phosphoramidite S5 (Base = GAc, 70 mM, 144 μL, 10.1 μmol) in the presence of BTT activator (450 mM in acetonitrile, 440 μL, 198 μmol). After a 70 s coupling reaction, the residual 5’-hydroxy end of CPG was capped with acetic anhydride (1.06 M in THF, 500 μL, 530 μmol). After oxidation of the phosphorous linker with iodine (20 mM in THF, 700 μL, 14.0 μmol), the 5’-dimethoxytrityl (DMTr) group on the extended nucleoside (G) was removed with trichloroacetic acid (300 mM in dichloromethane, 1.3 mL, 390 μmol). The DMTr cation was roughly quantified by an LED detector equipped with the synthesizer to assure a coupling efficiency >99%. This elongation procedure was performed using the corresponding phosphoramidite S5 possessing corresponding nucleobases to afford 24-mer 5’-AGATCGGAAGAGCGTCGTGAGGG-3’ S14 on CPG. The triazole-linked dinucleotide S12 was then loaded to assemble 26-mer S15 on CPG under the identical conditions for the phosphoramidite of natural deoxyribonucleosides except for a longer coupling time of 3600 s. Elongation procedure with phosphoramidite S5 was performed nine times on S15 to afford the 35-mer S16 on CPG. After the final elongation with A at the 5’-end, the amount of S16 on CPG was carefully determined by quantification of the DMTr cation using a UV-vis spectrometer in the following manner. The CPG loaded with S16 was treated with a solution of 4% v/v dichloroacetic acid in 1,2-dichloroethane (2.0 mL) for 30 s to release the DMTr cation. After separation of CPG, the solvent was removed in vacuo to afford an orange residue. The residue was dissolved in 0.1 M tosyl chloride solution in acetonitrile (10.0 mL), and 10% v/v of the solution was diluted with acetonitrile to 10 mL. The absorbance at 498 nm was 0.481 a.u. for S16TT and 0.392 a.u. for S16TU, and using a molar coefficient of the DMTr cation at 498 nm, ε498 = 72,000 mol−1•cm−1, the amount of DMTr at the 5’-end was determined as 670 nmol for S16TT and 546 nmol for S16TU. Based on these values, the total yields of detritylated

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oligonucleotides for the 136-step transformation were thus 34% for $S_{17TT}$ and 28% for $S_{17TU}$. Finally, $S_{17}$ on CPG was treated with a 28% solution of ammonium hydroxide at 80 °C for 10 h to afford $S_{18}$ via cleavage from the solid support. For $S_{18TU}$, the crude oligonucleotide was submitted to the subsequent desilylation reaction. Thus, $S_{18TU}$ was mixed with $n$-tetrabuthylammonium fluoride (1.0 M in THF, 0.500 mL, 500 µmol), and the mixture was stirred at ambient temperature for 24 h. After removal of volatile materials in vacuo, the residue was dissolved in water and desalted on a sep-pak column in accordance with the manufacturer’s instructions to afford desilylated $S_{19TU}$. The crude chimeric oligonucleotides ($S_{18TT}$, $S_{19TU}$) were purified by reverse-phase HPLC, and the fractions containing the target material were freeze-dried. The identity of the chimeric oligonucleotides was confirmed by ESI-TOF mass spectrometry analysis. Total yield of chimeric oligonucleotide $S_{18TT}$ was estimated as 34% (136 steps); the average yield per synthetic operation was 99%; and the average yield per elongation of one unit was 97%. Physical data of $S_{18TT}$ (5’-ApApApApApApApTpGpApTpCpGpApApGpApGpCpGpTpCpGpTpGpTpGpGpGpG-3’; $t$: triazole linkage): HRMS (SolariX 9.4T) calcd for C$_{349}$H$_{409}$N$_{154}$O$_{195}$P$_{33}$ [M + 15H]$^{18}$– 605.4348, found 605.4343. HPLC analysis of the isolated chimeric oligonucleotide $S_{18TT}$ is shown in Figure S2-2.

Total yield of chimeric oligonucleotide $S_{19TU}$ was estimated as 28% (136 steps); the average yield per synthetic operation was 99%; and the average yield per elongation of one unit was 96%. Physical data of $S_{19TU}$ (5’-ApApApApApApApTpUApGpApTpCpGpApGpApGpCpGpTpCpGpTpGpTpGpGpG-3’): HRMS (SolariX 9.4T) calcd for C$_{348}$H$_{407}$N$_{154}$O$_{196}$P$_{33}$ [M + 15H]$^{18}$– 605.5448, found 605.5449. HPLC analysis of the isolated chimeric oligonucleotide $S_{19TU}$ is shown in Supplementary Figure S2-3.

HPLC charts of isolated oligonucleotides
Figure S2-2. HPLC chart of the isolated chimeric oligonucleotide S18TT. Column: ODS column (COSMOSIL C18-MS-II, 4.6 x 250 mm); eluent: a linear gradient from 0% to 50% acetonitrile/H₂O (buffered as 100 mM CH₃CO₂NHEt₃, pH 7) during 30 min; flow rate: 1.0 mL/min; column temperature: 40 °C; analysis wavelength: 260 nm.

Figure S2-3. HPLC chart of the isolated chimeric oligonucleotide S19TU. Column: ODS column (COSMOSIL C18-MS-II, 4.6 x 250 mm); eluent: a linear gradient from 0% to 50% acetonitrile/H₂O (buffered as 100 mM CH₃CO₂NHEt₃, pH 7) during 30 min; flow rate: 1.0 mL/min; column temperature: 40 °C; analysis wavelength: 260 nm.
Supplementary Procedure S3

Reaction conditions for enzymatic synthesis of DNA complementary to natural and triazole-containing DNA templates shown in Figure 3 and Supplementary Figure 6

T4 DNA polymerase

A 20-µl reaction that contained 1× T4 DNA polymerase buffer (Takara Bio Inc.), 250 µM dNTPs (Takara Bio Inc.), 3.3 µM FAM-labeled primer, and 6.6 µM template oligonucleotide was incubated at 95 °C for 3 min, 45 °C for 5 min, and 37 °C for 5 min. To this reaction, 5 units of T4 DNA polymerase (Takara Bio Inc.) were added and further incubated at 37 °C for 15 min. The reaction was stopped with heating at 70 °C for 10 min.

E. coli DNA polymerase I, Klenow Fragment, and Klenow exo minus

E. coli DNA polymerase (10 unit/µl, Invitrogen), Klenow Fragment (5 unit/µl, Takara Bio Inc.), and Klenow Fragment (3′-5′ exo-) (50 units/µl, New England Biolabs) were used in the same reaction conditions as follows.

A 20-µl reaction that contained 1× NEBuffer 2 (New England Biolabs), 250 µM dNTPs, 3.3 µM FAM-labeled primer, and 6.6 µM template oligonucleotide was incubated at 95 °C for 3 min, 45 °C for 5 min, and 37 °C for 5 min. To this reaction, 1 µl of enzyme was added and further incubated at 37 °C for 15 min. The reaction was stopped with heating at 70 °C for 10 min.

Phi29 DNA polymerase

A 20-µl reaction that contained 1× Phi29 DNA Polymerase Reaction Buffer (New England Biolabs), 250 µM dNTPs, 3.3 µM FAM-labeled primer, and 6.6 µM template oligonucleotide was incubated at 95 °C for 3 min, 45 °C for 5 min, and 30 °C for 5 min. To this reaction, 10 units of Phi29 DNA polymerase (New England Biolabs) was added and further incubated at 30 °C for 15 min. The reaction was stopped with heating at 70 °C for 10 min.

SuperScript II and III reverse transcriptase

A 20-µl reaction that contained 1× 1st Buffer (Invitrogen), 500 µM dNTPs, 5 mM dithiothreitol, 3.3 µM FAM-labeled primer, and 6.6 µM template oligonucleotide was incubated at 95 °C for 3 min, 45 °C for 5 min, and 42 °C for 5 min. To this reaction, 200 units of SuperScript II or SuperScript III (Invitrogen) was added and further incubated at 42 °C for 15 min. The reaction was stopped with heating at 70 °C for 10 min.

SuperScript IV Reverse Transcriptase

A 20-µl reaction that contained 1× SSIV Buffer (Invitrogen), 500 µM dNTPs, 5 mM dithiothreitol,
3.3 µM FAM-labeled primer, and 6.6 µM template oligonucleotide was incubated at 95 °C for 3 min and 45 °C for 10 min. To this reaction, 200 units of SuperScript IV (Invitrogen) was added and further incubated at 45 °C for 15 min. The reaction was stopped with heating at 70 °C for 10 min.

**Sulfolobus DNA polymerase IV**

A 20-µl reaction that contained 1× ThermoPol Buffer (New England Biolabs), 250 µM dNTPs, 3.3 µM FAM-labeled primer, and 6.6 µM template oligonucleotide was incubated at 95 °C for 3 min and at 45 °C for 5 min. To the reaction, 2 units of Sulfolobus DNA polymerase IV (New England Biolabs) was added and further incubated at 55 °C for 15 min. The reaction was stopped with heating at 80 °C for 10 min.

**Bst DNA polymerase large fragment, Bst 2.0 DNA polymerase, and Bst 3.0 DNA polymerase**

Bst DNA polymerase LF, Bst 2.0 DNA polymerase, and Bst 3.0 DNA polymerase (all enzymes were from New England Biolabs) were used with their attached buffer, 10× ThermoPol Buffer, 10× isothermal amp buffer, and 10× Isothermal amp buffer II, respectively.

A 20-µl reaction that contained 1× buffer described above, 250 µM dNTPs, 3.3 µM FAM-labeled primer, and 6.6 µM template oligonucleotide was incubated at 95 °C for 3 min and 45 °C for 5 min. To this reaction, 8 units of enzyme was added and further incubated at 65 °C for 15 min. The reaction was stopped with heating at 80 °C for 10 min.

**Phusion DNA polymerase**

A 20-µl reaction that contained 1× Phusion HF Buffer (Thermo), 250 µM dNTPs, 3.3 µM FAM-labeled primer, 6.6 µM template oligonucleotide, and 2 units of Phusion Hot Start II DNA Polymerase (Thermo) was incubated at 95 °C for 3 min, 45 °C for 5 min, and 74 °C for 15 min.

**ExTaq**

A 20-µl reaction that contained 1× ExTaq Buffer (Takara Bio Inc), 250 µM dNTPs, 3.3 µM FAM-labeled primer, 6.6 µM template oligonucleotide, and 5 units of ExTaq HS (Takara Bio Inc.) was incubated at 95 °C for 3 min, 45 °C for 5 min, and 72 °C for 15 min.

**KOD DNA polymerase**

A 20-µl reaction that contained 1× Buffer for KOD -Plus- (Toyobo), 250 µM dNTPs, 3.3 µM FAM-labeled primer, 6.6 µM template oligonucleotide, and 1 unit of KOD -Plus- (Toyobo) was incubated at 95 °C for 3 min, 45 °C for 5 min, and 74 °C for 15 min.

**Tks Gflex DNA polymerase**
A 20-µl reaction that contained 1× Gflex Buffer (Takara Bio Inc), 250 µM dNTPs, 3.3 µM FAM-labeled primer, 6.6 µM template oligonucleotide, and 1.25 units of Tks Gflex DNA polymerase (Takara Bio Inc.) was incubated at 95 °C for 3 min, 45 °C for 5 min, and 68 °C for 15 min.