Supporting Information

Facile Preparation of Non-self-quenching Fluorescent DNA Strands with Degree of Labeling up to Theoretic Limit

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Experimental Section

General information. Aminoallyl-dUTP (aa-dUTP), dNTPs, DNase I (RNase-free), DNA Polymerase I, Klenow Fragment exo-, Random Hexamer Primer, Long PCR Enzyme Mix and GeneJET^{TM} PCR Purification Kit, GeneRuler^{TM} 100 bp Plus DNA Ladder(ready-to-use, 100-3000 bp), 6× DNA Loading Dye were purchased from Fermentas (Thermo Scientific). Deep Vent$_R$^{TM} exo- DNA

Polymerase was purchased from New England Biolabs (NEB). Synthesized oligonucleotides (primers) were purchased from Tech Drogon Limited. GelRed^{TM} Nucleic Acid Gel Stain (10000× in water) was purchased from Biotium. Other chemicals, reagents and solvents were all purchased from Aldrich or Invitrogen. NMR spectra were taken on a Bruker ARX 400 NMR spectrometer using $CDCl_3$ as solvent. HRMS spectra were recorded on a Finnigan TSQ 7000 triple quadrupole spectrometer operating in a MALDI-TOF mode. Photoluminescence (PL) spectra were recorded on a Perkin-Elmer LS 55 spectrofluorometer. UV spectra were measured on a Milton Roy Spectronic 3000 Array spectrophotometer. FL quantum yields (Φ_F) of amorphous powders of SITC was measured using C-701 Time-Resolved Spectrofluorometer with the integrating sphere as accessory.

Preparation of 4-bromobenzylazide (3). Into a flask equipped with a magnetic stirrer were added 4-bromobenzyl bromide (7.5 g, 30 mmol), sodium azide (7.8 g, 120 mmol), and 40 mL of DMSO. After stirred at 70 ^oC for 12 h, the solution was poured into 150 mL of water and extracted with CH_2Cl_2. The crude product was purified by silica-gel chromatography to give a colorless viscous liquid in 96.2% yield (6.12 g). ^{1}HNMR ($CDCl_3$, 400 MHz), δ (TMS, ppm): 7.47 (d, J = 8.2 Hz, 2H, Ar), 7.15 (d, J = 8.2 Hz, 2H, Ar), 4.26 (s, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 100 MHz), δ (TMS, ppm): 134.3, 131.8, 129.6, 122.1, 53.9. HRMS (MALDI-TOF): *m/z* 210.9640 (M^+, calcd 210.9745).

Preparation of 1,1-dimethyl-2,5-bis[4-(azidomethyl)phenyl]-3,4-diphenylsilole (2). A mixture of lithium (0.056 g, 8 mmol) and naphthalene (1.04 g, 8 mmol) in 8 mL of THF was stirred at room temperature under nitrogen for 3 h to form a deep dark green solution of LiNaph. The viscous solution was then added dropwise to a solution of **2** (0.52 g, 2 mmol) in 5 mL of THF over 4 min at room temperature. After stirring for 1 h, the mixture was cooled to 0 ^oC and then diluted with 25 mL THF. A black suspension was formed upon addition of $ZnCl_2$·TMEDA (2 g, 8 mmol). After stirring for an additional hour at room temperature, a solution of **3** (0.89 g, 4.2 mmol) and $PdCl_2(PPh_3)_2$ (0.08 g, 0.1 mmol) in 25 mL of THF was added. The mixture was refluxed overnight. After cooled to room

temperature, 100 mL of 1 M HCl solution was added and the mixture was extracted with DCM. The combined organic layer was washed with brine and water and then dried over magnesium sulfate. After solvent evaporation under reduced pressure, the residue was purified by a silica-gel column using hexane as eluent. The product was obtained as a yellow solid in 57.3% yield. ^{1}H NMR (400 MHz, $CDCl_3$), δ (TMS, ppm): 7.06 (d, J = 8.1Hz, 4H, Ar), 7.01 (m, 6H, Ar), 6.92 (d, J = 8.1Hz, 4H, Ar), 6.78 (m, 4H, Ar), 4.24 (s, 4H, CH_2), 0.47 (s, 6H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$), δ (TMS, ppm): 154.3, 141.3, 139.9, 138.5, 132.4, 129.9, 129.1, 127.9, 127.5, 126.3, 54.6, –3.9. HRMS (MALDI-TOF): m/z 524.2200 (M^+, calcd 524.2145).

Preparation of 1,1-dimethyl-2,5-bis[4-(isothiocyanatemethyl)phenyl]-3,4-diphenylsilole (SITC) To a solution of **2** (131 mg, 0.25 mmol) and CS_2 (0.24 ml, 4.0 mmol) in $CHCl_3$ (1.0 ml) was added PPh_3 (131.1 mg, 0.5 mmol) at room temperature. The mixture was stirred for 1.5 h. After solvent evaporation under reduced pressure, the crude product was purified by a silica-gel column using ethyl acetate/hexane (1:10 v/v) as eluent. The product was obtained as a yellow solid in 57.3% yield. ^{1}H NMR (400 MHz, $CDCl_3$), δ (TMS, ppm): 7.04 (d, J = 6.4 Hz, 4H, Ar), 7.00 (m, 6H, Ar), 6.93 (d, J = 8.4Hz, 4H, Ar), 6.79 (m, 4H, Ar), 4.62 (s, 4H, CH_2), 0.47 (s, 6H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$), δ (TMS, ppm): 154.5, 141.2, 139.9, 138.4, 131.2, 129.9, 129.2, 127.5, 126.5, 126.4, 48.4, –3.9. HRMS (MALDI-TOF): m/z 556.1462 (M^+, calcd 556.1463).

Preparation of SITC conjugated dUTP. To a water solution of 5-(3-aminoallyl)-2'-deoxyuridine-5'triphosphate (aa-dUTP, 10 mM, 10 μL) was added SITC (5 mM in DMSO, 25 μL). The mixture was incubated at room temperature for 6 hrs with agitation and used directly without purification. HRMS (MALDI-TOF), m/z: 1077.2860 ($[M+2H]^+$, calcd 1077.1465).

Labeling DNA by nick-translation. General nick-translation process was carried out as follows: DNase I (1 unit/mL) was firstly diluted to 1/150 with 1× DNA polymerase I reaction buffer (10× from Fermentas) containing 0.05 mg/mL BSA and kept on ice. 2 μg of DNA template was on ice mixed

with 1 μL of d A/G/CTP mixture (10 mM for each of them), 3.5 μL of SITC conjugated dUTP mixture (containing 1 μL of 10 mM conjugated dUTP), 10 μL of 10× DNA polymerase I reaction buffer (Fermentas), 10 μL of diluted DNase I and 4 μL of DNA polymerase I (10 units/ μL) and the adequate water was added to make a final volume of 100 μL. The reaction mixture was incubated at 15 ^{o}C for 2 hrs and then purified by GeneJET™ PCR Purification Kit.

Labeling DNA by random priming. General random priming process was carried out as follows: 2 μg of DNA template was denatured at 95 ^{o}C for 5 minutes and immediately cooled on ice. The denatured DNA template was on ice added with 1 μL of d A/G/CTP mixture (10 mM for each of them), 3.5 μL of SITC conjugated dUTP mixture (containing 1 μL of 10 mM conjugated dUTP), 10 μL of 10× random priming reaction buffer (Fermentas), 25 μL of random hexamer primer (0.2 μg/ μL), 2 μL of Klenow fragment exo- (5 units/ μL) and then adequate water was added to make a final volume of 100 μL. The reaction mixture was incubated at 37 ^{o}C for 4 hrs and then purified by GeneJET™ PCR Purification Kit.

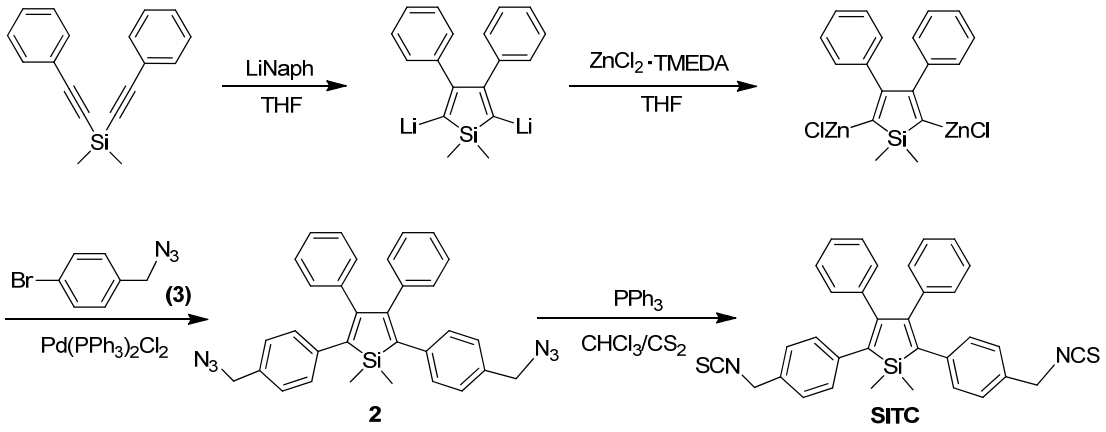
Labeling DNA by PCR. PCR reactions were carried out in a total of 100 μL mix containing 10 μL of 10× Long PCR buffer with 15 mM $MgCl_2$ (for Long PCR Enzyme Mix, Fermentas) or 10× Thermopol reaction buffer (for Deep $Vent_R$ exo- DNA Polymerase, NEB), 2 ng of the template DNA, 0.2 μM of primers, 2 units of Long PCR Enzyme Mix or Deep $Vent_R$™ exo- DNA Polymerase, and 0.2 mM of each of the dNTPs. In the control reaction, 0.2 mM of all the four normal dNTPs and no SITC-dUTP was used. In other cases, dTTP was substituted with different amounts of SITC-dUTP. PCR was performed in Veriti^{R} 96–Well Thermal Cycler (Applied Biosystems). After an initial denaturation at 94°C for 4 min, 35 cycles of PCR were carried out with denaturation at 94°C for 0.5 min, annealing at 55°C–58 °C (primer T_m-dependent) for 1 min and extension at 68 °C (Long PCR Enzyme Mix) or 72°C (Deep $Vent_R$ exo- DNA Polymerase) for 1.5 min. After a 10 min final extension, the labeled

DNA products were purified using GeneJET™ PCR Purification Kit. The DNA products were in 100 μL sterile water for spectroscopic measurement.

Gel electrophoresis. The DNA samples with equal volumes were mixed with 6× DNA loading dye and analyzed on 1.5% agarose gel. The running buffer contained 40 mM Tris acetate and 1 mM EDTA in water. All solutions were freshly prepared prior to use. Gel electrophoresis was carried out on a Thermo Scientific horizontal Owl B1A EasyCast Mini Gel system. Separation was performed at 100 V for 30 min. The gels were either prestained with 1×GelRed (10000× in water from Biotium) or poststained with 50 mL 3×GelRed water solution.

Detection of dsDNA. All stained gels were imaged with the Gel Doc XR+ documentation system (Bio-Rad). The images were analyzed by Quantity One gel image analysis software (Bio-Rad).

Determination of Degree of Labeling (DOL). The DOL of the labeled DNA, in number of dyes per 100 bases, was calculated using the Beer-Lambert law. DOL values were calculated based on background corrected absorption values at the absorption maximum wavelengths of the nucleic acid (260 nm) and the dye. For example, to first correct for the dye's contribution to the 260 nm absorption value, the product of the dye absorption value and its correction factor (CF, is a measure of the contribution of the dye to the 260 nm absorbance of the conjugate, since most dyes absorb light at 260 nm as well as at their excitation maximum, and is determined by the ratio of the dye absorbance at 260 nm to the dye absorbance at its maximal absorbance wavelength) is subtracted from the 260 nm absorption value [$A_{base} = A_{260} - (A_{dye} \times CF)$]. The number of dyes per 100 bases (DOL) is then derived using the following equation: $DOL = 100/[(A_{base} \times \varepsilon_{dye})/(A_{dye} \times \varepsilon_{base})]$, where ε_{dye} is the dye's extinction coefficient, ε_{base} is the average extinction coefficient for a base in double-stranded DNA (6600 $cm^{-1}M^{-1}$), A_{base} is the corrected nucleic acid absorption value, and A_{dye} is the dye absorption value.[9]



Scheme S1. Synthetic routes to functionalized silole derivative 1,1-dimethyl-2,5-bis[4-(isothiocyanatemethyl)phenyl]-3,4-diphenylsilole (SITC).

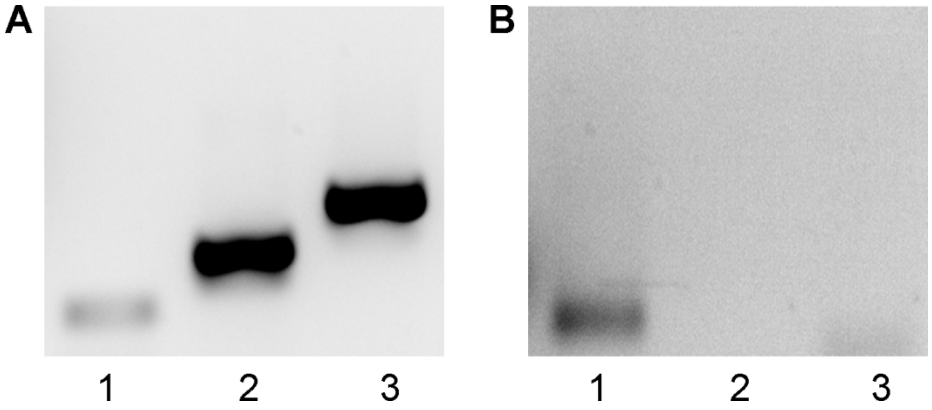


Fig. S1. DNA fragments with different bp numbers prepared by PCR. (A) DNA fragments prepared by PCR method with Long PCR Enzyme Mix and unlabeled dNTPs and (B) DNA fragments prepared by PCR method with Deep $Vent_R^{TM}$ (exo-) DNA polymerase and SITC-dUTP. Lanes 1-3 represent the DNA fragments with the base pair numbers of 94, 204 and 304. Both the agarose gels are prestained with GelRed^{TM}.

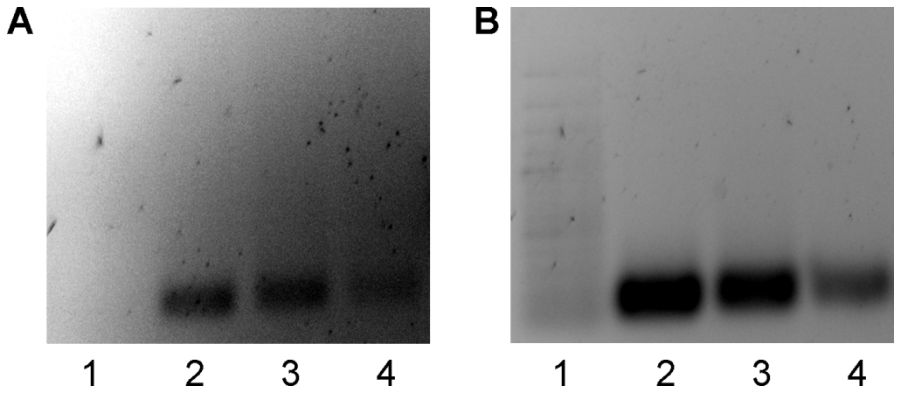


Fig. S2. Fluorescent DNA products with different sequences prepared by PCR method. (A) Fluorescent image of agarose gel of fluorescent DNA products with different sequences prepared by PCR method with Deep $Vent_R^{TM}$ (exo-) DNA polymerase and SITC-dUTP. (B) Fluorescent image of the same gel restained with GelRed™. Lane 1: DNA ladder, lanes 2-4 represent the SITC labeled DNA products with bp number 94, 94 and 103, respectively**.**

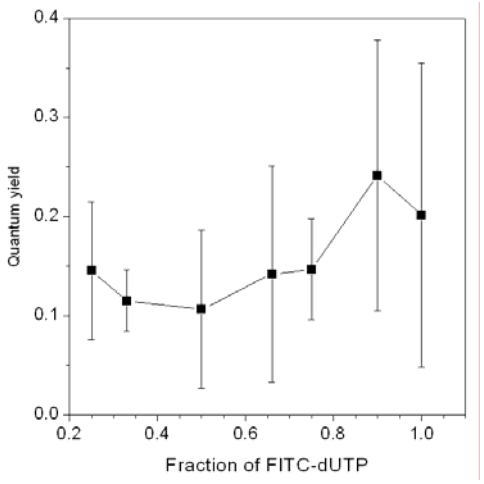


Fig. S3. Plot of quantum yield of the FITC labeled DNA versus Fraction of FITC-dUTP. The quantum yields were determined with fluorescein as the standard. 50 μM solution of fluorescein in 100 mM sodium borate buffer, pH 9.5; under these conditions, fluorescein exhibiting a quantum yield of 0.93.

Table S1. Sequence of DNA template used in nick translation and random priming.

5’-GGGCCCCTGCAGGCTAAGCTAAGTTAACTGAGCTCTACTCGAGAACAAGGACG
TCAACAGCTTCGACTTGGACGAACAAGATTTCGCTGACATTGCCAAGTTGGACAT
CAACTTGAGATTCAACGACCCATGGGACTGGGACAAGATTCCTATCTTCGTCTAA
AAGCTTGCGAATTTCTTATGATTTATGATTTTTATTATTAAATAAGTTATAAAAAA
AATAAGTGTATACAAATTTTAAAGTGACTCTTAGGTTTTAAAACGAAAATTCTTA
TTCTTGAGTAACTCTTTCCTGTAGGTCAGGTTGCTTTCTCAGGTATAGCATGAGGT
CGCTCTTATTGACCACACCTCTACCGGCATGCCGAGCAAATGCCTGCAAATCGCT
CCCCATTTCACCCAATTGTAGATATGCTAACTCCAGCAATGAGTTGATGAATCTC
GGTGTGTATTTTATGTCCTCAGAGGACAACACCTGTTGTAATCGTTCTTCCACACC
TCGACGCTTTTCAATTCATCTTTTTTTTTTTGTTCTTTTTTTTGATTCCGGTTTCTT
TGAAATTTTTTTGATTCGGTAATCTCCGAGCAGAAGGAAGAACGAAGGAAGGAG
CACAGACTTAGATTGGTATATATACGCATATGTGGTGTTGAAGAAACATGAAATT
GCCCAGTATTCTTAACCCAACTGCACAGAACAAAAACATGCAGGAAACGAAGAT
AAATCATGTCGAAAGCTACATATAAGGAACGTGCTGCTACTCATCCTAGTCCTGT
TGCTGCCAAGCTATTTAATATCATGCACGAAAAGCAAACAAACTTGTGTGCTTCA
TTGGATGTTCGTACCACCAAGGAATTACTGGAGTTAGTTGAAGCATTAGGTCCCA
AAATTTGTTTACTAAAAACACATGTGGATATCTTGACTGATTTTTCCATGGAGGGC
ACAGTTAAGCCGCTAAAGGCATTATCCGCCAAGTACAATTTTTTACTCTTCGAAG
ACAGAAAATTTGCTGACATTGGTAATACAGTCAAATTGCAGTACTCTGCGACTAG
TGAATTCGGGCCC-3’

The template is a PCR product of plasmid DNA containing 1061 bp

Table S2. Sequence of DNA templates used in PCR.

	Templates and primers
a (94 bp)	5’-TCGAGAACAAGGACGTCAACAGCTTCGACTTGGACGAACAA GATTTCGCTGACATTGCCAAGTTGGACATCAACTTGAGATTC AACGACCCATG-3’ Primers: 5’-TCGAGAACAAGGACGTCAACA-3’ (Forward), 5’-CATGGGTCGTTGAATCTCAA-3’ (Reverse)
b (204 bp)	5’-CACAGAACAAAAACATGCAGGAAACGAAGATAAATCATGT CGAAAGCTACATATAAGGAACGTGCTGCTACTCATCCTAG TCCTGTTGCTGCCAAGCTATTTAATATCATGCACGAAAAG CAAACAAACTTGTGTGCTTCATTGGATGTTCGTACCACCA AGGAATTACTGGAGTTAGTTGAAGCATTAGGTCCCAAAATTTGT-3’ Primers: 5’-CACAGAACAAAAACATGCAGG-3’ (Forward) 5’-ACAAATTTTGGGACCTAATGC-3’ (Reverse)
c (304 bp)	5’-TCGAGAACAAGGACGTCAACAGCTTCGACTTGGACGAACAA GATTTCGCTGACATTGCCAAGTTGGACATCAACTTGAGATTCAA CGACCCATGGGACTGGGACAAGATTCCTATCTTCGTCTAAAAGCT TGCGAATTTCTTATGATTTATGATTTTTATTATTAAATAAGTTATA AAAAAAATAAGTGTATACAAATTTTAAAGTGACTCTTAGGTTTT AAAACGAAAATTCTTATTCTTGAGTAACTCTTTCCTGTAGGTCAG GTTGCTTTCTCAGGTATAGCATGAGGTCGCTCTTATTGA-3’ Primers: 5’-TCGAGAACAAGGACGTCAACA-3’ (Forward) 5’-TCAATAAGAGCGACCTCATGC-3’ (Reverse)
d (94 bp)	5’-CACAGAACAAAAACATGCAGGAAACGAAGATAAATCATGTCGA AAGCTACATATAAGGAACGTGCTGCTACTCATCCTAGTCCTGTTG CTGCCA-3’ Primers: 5’- CACAGAACAAAAACATGCAGG-3’ (Forward) 5’- TGGCAGCAACAGGACTAGGAT-3’ (Reverse)
e (103 bp)	5’-GTTAAGCCGCTAAAGGCATTATCCGCCAAGTACAATTTTTTACT CTTCGAAGACAGAAAATTTGCTGACATTGGTAATACAGTCAAATT GCAGTACTCTGCGA-3’ Primers: 5’-GTTAAGCCGCTAAAGGCATT-3’ (Forward) 5’-TCGCAGAGTACTGCAATTTGA-3’ (Reverse)
f (103 bp)	5’-CAGGAAACAGCTATGACCATGATTACGAATTCGAGCTCGGTACC CGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTG GCCGTCGTTTTAC-3’ Primers: 5’-CAGGAAACAGCTATGAC-3’ (Forward) 5’- GTAAAACGACGGCCAG -3’ (Reverse)