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Supporting Information

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Generation and Enzymatic Amplification of High-Density Functionalized DNA Double-Strands**

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Contents

1. Oligodeoxyribonucleotides

Primers and template employed for primer extension and PCR experiments:

P5': 5'-CAC TCA CGT CAG TGA CAT GC-3'
P3': 5'-GTG GTG CGA AAT TTC TGA C-3'
BP5': 5'-biotine-CAC TCA CGT CAG TGA CAT GC-3'
BP3': 5'-biotine-GTG GTG CGA AAT TTC TGA C-3'
M79: 5'-biotine-CAC TCA CGT CAG TGA CAT GCA TGC CGA TGA CTA GTC GTC ACT AGT GCA CGT AAC GTG CTA GTC AGA AAT TTC GCA CCA C-3'
M59: 5'- biotine- CAC TCA CGT CAG TGA CAT GCA TGC CGA TGA CTA GTC GTC AGT CAG AAA TTT CGC ACC AC-3'

Standard oligonucleotides and 5'-biotinylated oligonucleotides were purchased from MWG, Ebersberg, Germany. Labeling of Primer **P5'** at its 5'-terminus with [γ-^{32}P]ATP (NEN) was performed using T4 polynucleotide kinase (Stratagene).

2. Polymerases and PCR additives

Pwo DNA polymerase was purchased from Roche Diagnostics. The 10X reaction buffer used with Pwo DNA polymerase (PCR buffer with $MgSO_4$ from Roche Diagnostics) contained 100 mM Tris-HCl (pH 8.9), 20 mM $MgSO_4$, 250 mM KCl and 50 mM $(NH_4)_2SO_4$. Vent®(exo-) DNA polymerase was from New England Biolabs. The 10X reaction buffer (ThermoPol reaction buffer from New England Biolabs) contained 200 mM Tris-HCl (pH 8.8), 20 mM $MgSO_4$, 100 mM KCl, 100 mM $(NH_4)_2SO_4$ and 1% Triton X-100.

Betaine (BioChemika, anhydrous), formamide (MicroSelect for molecular biology), tetramethylammonium chloride (Ultra for molecular biology), dimethyl sulfoxide (Ultra for molecular biology) were purchased from Fluka. Single-strand binding protein (SSB) from *Escherichia coli* was from Sigma.

3. Synthesis of the single stranded modified template fM79

Single stranded functionalized DNA template **fM79** was synthesized by primer extension. Therefore 0.6 nmol of primer **P3'** was annealed to 1.2 nmol 5'-biotinylated template **M79** in 1 ml of 1X ThermoPol reaction buffer by heating the mixture to 95°C for 5 min and subsequently allowing the solution to cool over 1 h to room temperature. To this mixture 1 ml 1X ThermoPol reaction buffer was added containing the triphosphates **1**-**4** (100 nmol each, final concentration of 50 μM) and 100 U of Vent(exo-) DNA polymerase to a final volume of 2 ml. The reaction mixture was incubated at 72°C for 60 minutes. The primer extension was stopped by cooling to 4°C.

To separate the biotinylated template from the newly synthesized functionalized DNA-strand 0.5 ml of immobilization buffer (750 mM NaCl, 0.5 mM EDTA, 250 mM HEPES, pH 7.0) was added to the reaction, and the mix was immobilized on 500 μL streptavidin agarose (Pierce, UltraLink™), pre-washed with five column volumes of wash buffer (150 mM NaCl, 0.1 mM EDTA, 50 mM HEPES, pH 7.0), followed by washing with 15 column volumes of wash buffer at room temperature. The desired modified oligonucleotides were received by basic elution with 3X 500 μL elution buffer (0.1 m NaOH, 150 mM NaCl). After neutralization with 5% acetic acid the single stranded DNA was standard ethanol-precipitated and dissolved in 0.5X ThermoPol reaction buffer.

4. Primer extension experiments

An annealing mix containing 7,5 pmol primer **P5'** 5'-[^{32}P]-labelled and 22 pmol template **fM79** in 25 µL reaction buffer (1X) provided with the Pwo DNA polymerase was heated to 99°C for 8 min and cooled to room temperature over 1 h. To 5 µL of the annealing mix 5 µl of a reaction mix containing 200 µM of each of the appropriate modified deoxynucleotides in the same reaction buffer and 0.1 µL Pwo DNA polymerase (5 U/µl) were added, incubated at 72°C for 30 minutes and then cooled to 4°C. Reactions were quenched by adding 30 µl formamide/water (4:1), containing 20 mM EDTA, followed by heating to 99°C for 10 min and immediate cooling to 0°C. Aliquots of 5 µl of each reaction were analyzed by 8% denaturing PAGE (8M urea, 50-60°C). Gels were transferred to filter paper, dried and exposed to an X-ray film or visualized by phosphorImaging (Fujifilm, FLA 3100).

5. PCR amplification

PCR experiments were performed on an Eppendorf Mastercycler gradient. The reaction were done in an overall volume of 25 µL in Pwo-reaction buffer (1X) with the denoted additives (figure 2), using 200 µM modified nucleotides **1-4**, 25 pmol of each primer **P3'** and 5'-[^{32}P]-labelled primer **P5'**, 0.5 pmol template **fM79**, and 0.25 µL of Pwo DNA polymerase (5 U/µL). As a control PCRs were performed under the same conditions using 200 µM natural dNTPs instead of the modified nucleotides (figure S1). Amplification was performed through an initial denaturing at 99°C for 8 min, followed by 16 cycles of denaturing at 99°C for 2 min, primer annealing at 50°C for 2 min, and extension at 72°C for 2 min. A negative control without template has been carried out. 5'-[^{32}P]-labelled and denatured 100 bp DNA ladder (peqLab) served as a length standard. An aliquot of 10 µL of the PCR reactions was mixed with 30 µL formamide/water (4:1), containing 20 mM EDTA, and denatured at 99°C for 10 min. 5 µl of each reaction were analyzed by 10% denaturing PAGE (8M urea, 50-60°C), transferred to filter paper, dried under vacuum and visualized and quantified by phosphorImaging.

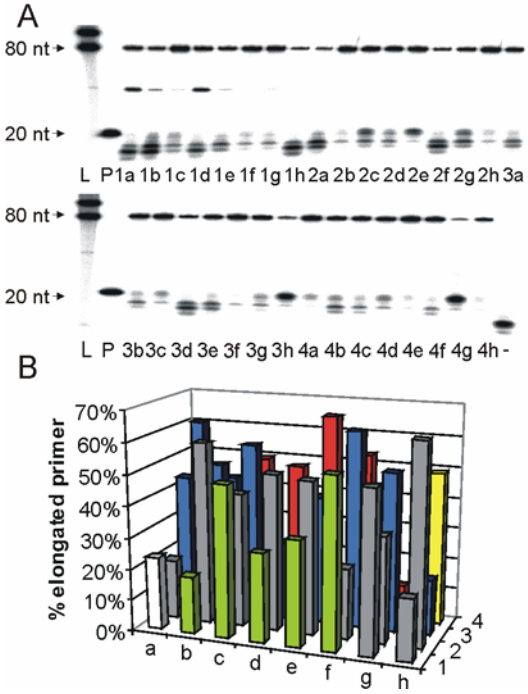

Figure S1. PCR experiments with ^{32}P-labeled primer **P5'**, dATP, dCTP, dGTP, TTP 200 µM each, template **fM79** and Pwo DNA Polymerase. Lane numbers refer to the different buffer conditions applied. PCR products were analyzed by 10% denaturing polyacrylamid electrophoresis. A**.** lanes L: length marker; P: primer **P5'**; 1a-4h: PCR reactions with respective buffer conditions (Figure 2); (-): control without template. B. Quantification of PCR products by phosphorImaging as percentage of total radioactivity.

6. Sequencing reaction

To sequence a double stranded fully functionalized DNA **fM59**, two PCR reactions were performed to biotinylate either the sense or antisense strand. Therefore, PCR reactions were carried out in an overall volume of 300 µL containing 1x Pwo-reaction buffer with 10% DMSO, 5% formamide, 0.75 M betaine and 50 mM TMAC, including 300 pmol of the 5'-biotinylated primer **BP5'** and **P3'** or **P5'** and biotinylated **BP3',** respectively**,** 6 pmol of template **M59** and 200 µM (each) of the modified nucleotides **1-4**. After addition of 15 U of Pwo DNA polymerase (5U/µL) and 1.5 U of thermostable inorganic pyrophosphatase of Tth (Genaxis, Germany) 20 cycles of PCR were performed (for PCR temperatures see above) in 50 µL reaction volumes.

For separation of the strands the PCR reactions were ethanol precipitated, dissolved in 50 µL wash buffer (150 mM NaCl, 0.1 mM EDTA, 50 mM HEPES, pH 7.0) and loaded on a pre-equilibrated streptavidin agarose column (50 µL column volume, Pierce, UltraLink™). After washing with 15 column volumes of wash buffer (150 mM NaCl, 0.1 mM EDTA, 50 mM HEPES, pH 7.0) at room temperature the unbiotinylated strands were received by basic elution with 3X 50 µL elution buffer (0.1 m NaOH, 150 mM NaCl). After neutralization with 5% acetic acid the single stranded fDNA was ethanol-precipitated and dissolved in 0.5X ThermoPol reaction buffer.

Sanger dideoxynucleotide sequencing was performed with a T7 DNA Polymerase Kit of USB. As a control M59 was PCR amplified with natural dNTPs (200 µM each) under the same conditions as described before (16 cycles of PCR) and sequenced following the protocol of the T7 DNA sequencing kit. For better resolution of short sequences 1 µL of a Mn^{2+}-solution (0.1 M $MnCl_2$ in 0.15 M sodium citrate) was included to the labelling reaction (figure S2B, lanes 1). As a second control the single stranded functionalized DNA fM59 was used as template in a PCR reaction with natural dNTPs (200 µM each) under the same conditions as described before (16 cycles of PCR) and the received native DNA was sequenced as described before. The result of the sequencing is shown in figure S2: B lanes 3.

The direct sequencing of the functionalized strands required some adjustment of the experimental conditions. 10 pmol of the modified template were incubated with 4 pmol of the corresponding primer (**P5'** or **P3')** in annealing buffer (provided by the supplier) containing 20 % DMSO for 8 min at 99˚C. The mixture was allowed to cool to room temperature over 1h. [α-32] dCTP (NEN) and 6.4 U of T7 DNA polymerase were used for labelling. For better resolution of short sequences 1 µL of a Mn^{2+}-solution (0.1 M $MnCl_2$ in 0.15 M sodium citrate) was added to the reaction. The labelling reaction was incubated for 15 min at room temperature and the termination reaction for 15 min at 37˚C.

Samples of 2 µL were analyzed by 8% denaturing PAGE (8M urea, 50-60°C), transferred to filter paper, dried under vacuum and visualized by phosphorImaging. (figure S2: B, lanes 2)

7. CD measurements

DNA for CD measurements was prepared as follows: double stranded functionalized **M59** was generated by large scale PCR in an overall reaction volume of 12 ml and natural **dsM59** by PCR in an overall volume of 8 ml. After PCR cycling the DNA was ethanol precipitated and purified by gelelectrophorese on 2.5 % low-melting point agarose gel (Sigma). Bands with the desired dsDNA were excised and eluted by gel extraction with phenol. The dsDNA was ethanol precipitated twice and desalted over a G25 column (Amersham Biosciences).

CD spectra were measured with a Yasco 810 spectrophotometer. CD spectra of oligonucleotide solutions (1.3 OD base concentration of natural dsDNA M59 and 1.8 OD base concentration of modified dsfDNA fM59 in 10 mM Phosphate buffer and 1 M NaCl, pH 7.0 at 25°C) were recorded using a 0.2 cm pathlength cell. A spectrum of the buffer was measured separately and substracted from the spectra resulting from the samples. An average of 7 spectra was recorded in each experiment.