DNA as a Recyclable Natural Polymer

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Nature has the ability of circularly re-using its components to produce the molecules and materials it needs. An example is the ability of most living organisms of digesting proteins they feed off into amino acids and then using such amino acids in the ribosomal synthesis of new proteins. Recently, it has been shown that such recycling of proteins can be reproduced outside living organisms. The key proteins’ feature that allows for this type of recycling is their being sequence-defined polymers. Arguably, nature’s most famous sequence-defined polymer is DNA. Here it is shown that it is possible, starting from sheared calf-DNA, to obtain all four nucleotides as monophosphate-nucleotides (dNMPs). These dNMPs are phosphorylated in a one-pot, multi-enzymes, phosphorylation reaction to generate triphosphate-nucleotides (dNTPs). Finally, dNTPs so achieved (with a global yield of ~60%) are used as reagents for PCR (polymerase chain reaction) to produce target DNA strands, and for the diagnosis of targeted DNA by quantitative PCR (qPCR). This approach is an efficient, convenient, and environmentally friendly way to produce dNTPs and DNA through recycling according to the paradigm of a circular economy.

1. Introduction

Nature can efficiently break down complex biomass into small molecules and circularly use them to fulfill its requirement of biosynthesis, a recycling masterpiece. For example, the bacteria in the stomach-intestinal system can efficiently digest food to obtain nutrition molecules for the synthesis of bioactive macromolecules.[1] Also, microorganisms used in fermentation processes can degrade organic nutrients to alcohol or other small molecules. Those functions have been widely utilized in alcoholic beverages or the dairy industry,[2,3] while most other approaches that Nature has to “recycle” proteins and nucleic acids have not yet been translated into laboratory settings. Recently we have shown that it is possible to reproduce outside living organisms the approach organisms use to “recycle” mixtures of n proteins into the (n+1) protein of interest, not necessarily related to the parent ones.[4] Briefly, protein mixtures were first enzymatically digested into their constitutive amino acids, and then a cell-free transcription-translation system was used to recycle the so obtained amino acids into fluorescent proteins (GFP, mScarlet-i), or bioactive proteins (catechol 2,3-dioxygenase), by means of the ribosomal expression.[5] Recycling unseparated mixtures of proteins into the protein of need, not necessarily related to the parent materials, can only work for sequence-defined polymers, where function derives from the sequence of monomers.

DNA is also a sequence-defined polymer where the function is derived from the exact order of the four nucleotides (bases) that determine its sequence. Hence, following the same principles illustrated for the proteins/amino acids system, one can conceive an approach where a random mixture of DNA can be “digested” into its nucleotides that can then be put back together into a new DNA sequence unrelated to the original one. In the case of DNA, the formation of the final DNA product can be made with a man-invented process (polymerase chain reaction, PCR).[5] When compared to protein recycling, DNA recycling requires an extra step.[6] DNA can be depolymerized through an enzymatic hydrolysis step to break the inter-nucleotide phosphodiester backbone, but the product of such a reaction is dNMPs. Unfortunately, dNMPs cannot be directly used as reagents to produce new DNA sequences, one needs to first convert them into triphosphate-nucleotides (dNTPs) by introducing additional phosphate groups. In this work, we used Nature’s available DNA (calf DNA) as a starting material to mimic Nature’s recycling of DNA. We first established a method to depolymerize DNA into dNMPs with an efficiency exceeding 80%. We then developed a convenient, efficient synthesis to phosphorylate the mixture of the four dNMPs into the respective dNTPs with yields of >95% (commercialized dNMPs) and ~80% (recycled dNMPs). Finally, we proved that the so produced dNTPs could be used for the biosynthesis of new DNA sequences by PCR amplification, and for nucleic acid testing by quantitative PCR (qPCR). This methodology could bring a totally new path to obtain monomeric nucleotide materials, which might replace their chemical synthesis.[7]
2. Result and Discussion

2.1. DNA De-Polymerization

Nature is a vast storage of polymeric DNA materials, e.g., the maximum DNA content can be 10% (dry mass basis) from bacterioplankton. Also, Nature can rapidly and constantly produce DNA, which can be considered as a sustainable resource to recycle monomeric nucleotide materials. In this work, commercially available calf thymus DNA (calf DNA) with known content of CG 41.9% and AT 58.1% (specifications from Sigma-Aldrich) was used as a natural DNA source for the enzymatic hydrolysis to obtain nucleotide monomers (Figure 1a). To efficiently hydrolyze calf DNA and release dNMPs, it is important to choose the cleavage site at the 3′-terminal of the phosphodiester bonds, so that the monophosphate group can be kept at the 5′-terminal of the released monomeric nucleotides. To obtain the desired hydrolysis product, we chose Exonuclease III (Exo III) and Exonuclease I (Exo I) with the required 3′-phosphophomonooesterase activity. Exo III is a double-strand DNA (dsDNA) specific exonuclease, which can catalyze the stepwise removal of dNMPs from the 3′-terminal of dsDNA with a blunt end or 5′-overhang. Exo I is a single-strand DNA (ssDNA) specific exonuclease, which can catalyze the stepwise removal of dNMPs from ssDNA in the 3′-to-5′ direction. The mixture of calf DNA, Exo III, and Exo I was incubated in 1× Exo III buffer at 37 °C overnight for hydrolysis (Figure 1a, step 1). Afterward, unhydrolyzed, and hydrolyzed calf DNA was loaded into a 2% agarose gel. In Figure 1b, we show a representative image of a gel containing the starting materials as well as the hydrolyzed one. The initial calf DNA shows a smeared band (lane 2, sequence length between 100 and 2000 and above 2000 base pair), the hydrolyzed one has an almost absent band (lane 3), indicating the relatively high hydrolysis efficiency.

To identify the DNA hydrolysis product, and to determine the hydrolysis efficiency, we used LC–MS for qualitative as well as quantitative analysis. The calibration curves from dNMPs standard solutions can be found in supporting information (Figure S1a, Supporting Information). The plot of XIC (extracted-ion chromatogram) of the hydrolysis product is shown in Figure 2a. It illustrates that the mixture of Exo III and Exo I could efficiently hydrolyze DNA to release dNMPs. The removal of dNMPs from the 3′-terminal of ssDNA with a blunt end or 5′-overhang. Exo I is a single-strand DNA (ssDNA) specific exonuclease, which can catalyze the stepwise removal of dNMPs from ssDNA in the 3′-to-5′ direction. The mixture of calf DNA, Exo III, and Exo I was incubated in 1× Exo III buffer at 37 °C overnight for hydrolysis (Figure 1a, step 1). Afterward, unhydrolyzed, and hydrolyzed calf DNA was loaded into a 2% agarose gel. In Figure 1b, we show a representative image of a gel containing the starting materials as well as the hydrolyzed one. The initial calf DNA shows a smeared band (lane 2, sequence length between 100 and 2000 and above 2000 base pair), the hydrolyzed one has an almost absent band (lane 3), indicating the relatively high hydrolysis efficiency.

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average hydrolysis efficiency of four dNMPs was 83.9 ± 0.6% (Figure S1b, Supporting Information). The possible reason for non-completely hydrolysis could be the hydrolysis efficacy of nuclease Exo III, which can hydrolyze dsDNA with a blunt end and 5’- overhang but cannot hydrolyze dsDNA with 3’-overhang. As the calf DNA was mechanically sheared, it was not possible to exclude the existence of dsDNA with a 3’-overhang in the starting material of the DNA hydrolysis reaction. Overall, the calf DNA hydrolysis was relatively efficient with the desired product and high yield. The established hydrolysis method is non-selective for sequence; hence we believe that it will be applicable to all kinds of sheared DNA.

2.2. One-Pot dNMPs Phosphorylation

As previously mentioned, the DNA hydrolysis products dNMPs could not be directly applied for the biosynthesis of new DNA sequence, as this required dNTPs. To address this problem, we implemented a phosphorylation step to convert the dNMPs into dNTPs. Since the DNA hydrolysis product was a mixture of four dNMPs, it was important to establish a phosphorylation approach that could convert the four dNMPs into dNTPs in a one-pot reaction. We looked for multi-enzymes bio-catalysis as an efficient approach.[12,13] In the biosystem, the phosphorylation of nucleotides is catalyzed by intracellular phosphotransferase and kinase.[14,15] Therefore, cell lysate is an excellent catalyst and has been applied for this nucleotide phosphorylation reaction,[16,17] but the reported phosphorylation yields are relatively low (especially for dTTP). We thought that a possible reason for such low yields could be the imbalanced content of four nucleotides in the intracellular nucleotide pool. It is known that such a pool is rich in A and G bases as they are also needed for energy storage, signaling, and apoptosis.[18] Hence one can postulate that enzymes to phosphorylate the T and C bases are less present than the ones to perform the same task in the other bases.[19] To improve the phosphorylation yield of this one-pot reaction, we added T4-nucleotide monophosphate Kinase (T4), which can specifically catalyze the phosphorylation of dTMP, dCMP, and dGMP.[20] We performed the one-pot phosphorylation in an acetyl-phosphate/ATP dual-phosphate-donors system (Figure 1a, step 2, details see insert), with the mixture of E. coli S30 cell extract (S30) and T4 as a catalyst. S30 is a commercial cell lysate extract product,[21–23] that has been originally established for cell-free protein expression.[24] ATP was used as a phosphate donor and a cofactor, that was continuously consumed and re-generated, see insert in Figure 1a. Acetyl-phosphate (AceP) was applied as the phosphate donors to regenerate ATP. During this phosphorylation step, a phosphate group was introduced to dNMPs, with dNDPs...
nucleotide diphosphate) formed as intermediate products, and further a second phosphate group was introduced to dNDPs to generate the mixture of dNTPs.

To evaluate the phosphorylation efficiency of this one-pot reaction, we first performed the reaction with commercial dNMPs mixtures with equal content of four bases (0.4 × 10⁻³ m, 0.2 mL scale). The enzyme mixture was carefully adjusted to achieve the best phosphorylation efficiency of all four dNMPs. The phosphorylation product referred to as dNTPs_dNMPs was quantified by HPLC[25] (retention time of the dNTPs products in Figure 2b, line 2; full retention time of all components in Figure S2, line 4, Supporting Information). After the one-pot phosphorylation reaction, the dNMPs residues were not detectable anymore, and the amount of intermediate phosphorylation product dNDPs was quite low. A relatively high average phosphorylation yield was achieved (96.5 ± 1.6%, Figure 2c), which is higher than the reported nucleotide phosphorylation by chemistry methods.[7,26,27] In addition to the relatively good phosphorylation efficiency, this one-pot enzymatic reaction has many other advantages, such as mild reaction condition, green chemistry, all aqueous medium without any organic solvent, one-pot reaction condition suitable for multiple phosphate-receptors (four dNMPs and four dNDPs). In the phosphorylation mixture, final content of dNTPs was 76.6 ± 1.4%, the d(r)NMPs residue was 0%, d(r)NDPs residue was 3.9 ± 1.6% (with the concentration of each dNDPs in Figure S3c, Supporting Information), and ATP residue was 19.4 ± 0.1% (Figure 2d). A larger scale and higher concentration of this one-pot dNMPs phosphorylation reaction (10 × 10⁻³ m, 0.5 mL, total amount of all dNTPs products about 10 mg) were tested keeping the same ratio of all components. An average phosphorylation efficiency of 86.8 ± 6.3% was obtained, with the phosphorylation efficiency of all four bases exceeding 80% (Figure S3e, Supporting Information). There is a ~ 10% decrease in the phosphorylation efficiency for the 10 × 10⁻³ m scale reaction. A possible reason could be the increased concentration of enzymes, which brings additional components (for example, 50% glycerol from the enzyme storage condition) to the reaction and affect the catalysis performance.

Further, phosphorylation of recycled dNMPs_calf DNA was performed by the same approach, and the product referred to as dNTPs_calf DNA was quantified by HPLC (chromatography of the dNTPs products in Figure 2b, line 3; full chromatography of all components in Figure S2, line 5, Supporting Information). The average phosphorylation efficiency of dNMPs recycled from calf DNA was calculated to be 79.0 ± 0.7% (Figure 2c). There was an 18.1% decrease in phosphorylation efficiency of dNTPs_calf DNA in comparison to the dNTPs_dNMPs standards. The possible reason could be the unequal ratio of four nucleobases from recycled dNMPs, which led to unequal catalysis efficiency in this one-pot, competing for phosphorylation reaction. Nevertheless, the phosphorylation efficiency is still relatively good. In the phosphorylation mixture, dNTPs_calf DNA content was 60.6 ± 0.3%, d(r)NMPs residue was 4.6 ± 0.4%, d(r)NDPs residue was 16.7 ± 0.1% (with the concentration of each dNDPs in Figure S3c, Supporting Information), and ATP residue was 17.9 ± 0.1% (Figure 2d). The concentration of all four dNTPs_calf DNA was in the range of 190–280 μM (on average 243.7 ± 0.9 × 10⁻⁶ m, Figure S3d, Supporting Information), which was suitable to be directly used for the synthesis of new DNA by PCR. Calculated from the total mass of starting material calf DNA, the average recycling efficiency of dNTPs_calf DNA was 61.5 ± 0.2% (all four dNTPs with recycling yield in the range of 58.1–67.2%, Figure 2e), which was relatively good after two steps of hydrolysis and phosphorylation reactions.

2.3. DNA Re-Polymerization

As a mimic of Nature DNA material circulation, we further re-polymerized the recycled dNTPs_calf DNA into a new DNA sequence by PCR (Figure 1a, step 3). In this work, we decided to use dNTPs_calf DNA directly for PCR after a simple filtration step without any further purification. We are aware that by doing so we did not remove ATP and dNDPs from dNTPs_calf DNA. Such impurities could potentially affect the efficiency of qPCR and also insert into the new sequence produced.[28] We judged that the level of such impurity was so low that an extra purification step was not justified. To test this hypothesis, we performed qPCR by adding 0.2 × 10⁻³ m ATP to a qPCR substrate that used commercial dNTPs and observed only a slight decrease in qPCR efficiency (Figure S5, Supporting Information). We re-polymerized the recycled dNTPs by PCR amplification of a linear DNA template encoding GFP (Figure 1c, duplicate, lanes 2 and 3). Commercially available dNTPs were used as a positive control for PCR (Figure 1c, duplicate, lanes 4 and 5). The so achieved DNA was purified, and fed into a commonly used cell-free transcription-translation (TX-TL) system[29] (PUREfex™, Kaneka Eurogentec SA, see Supporting Information) for verifying the “transcription”, and “translation” of the GFP sequence from a DNA template polymerized with recycled nucleotides. Upon feeding a DNA template, the protein of interest was expressed in the TX-TL system. As shown in Figure 1d, a good yield for GFP expression was achieved, proving that the re-polymerized DNA from the hydrolyzed DNA precursor encodes new genetic information for protein expression.

The GFP expression yield of recycled DNA was about 20% lower than the positive control. There are many potential reasons for this, among them we can mention the insertion of 5mC (5-methylcytosine) into the new GFP sequence given that 5mC is an epigenetic factor for the regulation of gene expression.[30] In our case, 5mC could originate from dNMPs_calf DNA. Although 5mC was not detected from dNMPs_calf DNA by LC–MS, its natural abundance (about 0.88% in mammals’ genomic DNA[31]) cannot be neglected, and the possibility of circulating the 5mC from calf DNA into the new GFP sequence cannot be excluded. We tested by Nanopore sequencing the linear DNA template we used to express GFP, and it showed a certain amount of 5mC. Further investigation must be performed to fully understand this issue.

2.4. Recycling DNA for qPCR

Quantitative PCR (qPCR) is a powerful molecular diagnostic tool to quantify gene expression,[32] which is largely consumed for nucleic acid testing during the SARS-CoV-2 pandemic.[33]
Since Nature DNA can be efficiently recycled, next, we tested the possibility of using recycled dNTPs as a substrate for qPCR. The cycles of threshold (C_T) are defined as the qPCR cycle at which the fluorescent signal of the reporter dye crosses an arbitrarily placed threshold, indicating the exponential phase of qPCR amplification. [34] The C_T inversely depends on the DNA amplicon amount in the reaction mixture (the more DNA template, the lower the C_T value). With an equal amount of amplicons, the C_T value as a reference of the qPCR performance is influenced by the other components, i.e., the concentration of dNTPs involved in this polymerization process, [35–37] and the content of impurities in the PCR substrate. Therefore, in this work, the performance of qPCR was used to evaluate the quality of recycled dNTPs.

Since the DNA recycling process is applicable to all genomic DNA, E. coli DNA as an alternative DNA resource was applied for the recycling of monomeric dNTPs. The E. coli DNA was hydrolyzed, phosphorylated by the same protocol of calf DNA recycling (details see Supporting Information). The recycled product is referred to as dNTPs_E. coli DNA was also applied for nucleic acid testing by qPCR amplification. Self-made qPCR kits were prepared with a mixture of DreamTaq polymerase, Sybr dye, recycled dNTPs (dNTPs_calf DNA or dNTPs_E. coli DNA). The average concentration of dNTPs was adjusted to 0.2 × 10^{-13} M. A commercially available qPCR kit was applied as a positive control. The self-made qPCR kits were used for the amplification of a fragment with 133 base pairs from a luciferase DNA template. Primers were designed by IDT PrimerQuest™ Tool. As shown in Figure 3a, similar C_T values of self-made qPCR kits and positive control were obtained with low content of DNA template (0.001 ng). With a higher content of template DNA (1, 0.1, 0.01 ng), the detection performance of self-made qPCR kits was slightly lower than positive control with one or two more circles of C_T needed (Figure 3a, amplification plots see Figure S4, Supporting Information). The potential effect of ATP and dNDPs residues on the qPCR performance was minimal (Figure S5, Supporting Information). Since the commercially available qPCR kit (the positive control) is in a fully optimized condition, the analytical performance of the self-made qPCR kit from recycled dNTPs is still considered relatively good. For the no template control (NTC) sample, the C_T value of the commercially available qPCR kit was 35.3. The self-made qPCR assay was 30.8 and 30.4 (qPCR kit prepared from dNTPs_calf DNA and dNTPs_E. coli DNA), respectively. The detect limitation of self-made qPCR kits (template DNA content down to 0.001 ng) was not affected by NTC results. The qPCR amplification products were loaded to a Page-gel, showing the amplification products with desired length (Figure 3b). As a molecular diagnosing tool, the preparation of self-made qPCR from recycled dNTPs is relatively convenient and its analytical performance is very good.

3. Conclusion and Outlook

In this work, we have established an efficient approach to recycle DNA. We have shown that DNA can be de-polymerized to generate dNMPs, which in turn can be phosphorylated to generate dNTPs. The so obtained dNTPs can be re-polymerized by using PCR to achieve a new DNA sequence, hence information, that is completely different from the parent one. The obtained dNTPs can also be used as a substrate for qPCR with very good DNA detection performance. In addition, this work provides a new top-down method to obtain monomeric nucleotides from Nature available DNA. The conventional de novo chemical synthesis of nucleotides (dNMPs and dNTPs) still has several disadvantages; most methods require multi-steps of synthesis and purification, and quite a few use toxic chemicals and solvents. [7,26,27,38,39] This top-down, one-pot, two-steps of enzymatical hydrolysis and phosphorylation of Nature available DNA could become a totally new path to obtain monomer nucleotides materials with respect to costs, efficiency, reaction condition, and sustainability. Since all the used materials of DNA and enzyme mixtures are abundant in Nature, there is a possibility to scale up this process at an affordable cost. Also, during the current pandemic situation, large-scale PCR waste is accumulating from the massive covid test, which can be circularly recycled with the same approach. As DNA technology has become a very well-established technique for sequencing, bioengineering, and molecular diagnoses, [40,41] our DNA recycling methodology can be easily adapted to recycle modified nucleotides (e.g., fluorescence-labeled dNTPs for sequencing).

![Figure 3.](image-url)
We believe the established approach has paved the road for the circulation of DNA materials, which can further boost materials recycling for the development of a circular economy, as well as the establishment of related enzymatic reaction systems. In this work, only the recycling of monomeric nucleotides is discussed. It is also very meaningful to think of the circular recycling of oligonucleotides, which are used as PCR primers and experiencing a bottleneck problem regarding the testing kit-shortage situation during the current Covid pandemic.

**Supporting Information**

Supporting Information is available from the Wiley Online Library or from the author.

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**Conflict of Interest**

The authors declare no conflict of interest.

**Data Availability Statement**

The data that support the findings of this study are openly available in Zenodo at https://doi.org/10.5281/zenodo.5898370, reference number 5898370.

**Keywords**

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[1] H. N. Munro, J. B. Allison, *Mammalian Protein Metabolism*, Academic Press, New York And London 1964.
[2] H. Shigechi, J. Koh, Y. Fujita, T. Matsumoto, Y. Sito, M. Ueda, E. Satoh, H. Fukuda, A. Kondo, *Appl. Environ. Microbiol.* 2004, 70, 5037.
[3] M. E. Sharpe, *Int. J. Dairy Technol.* 1979, 32, 9.
[4] S. Giaveri, A. M. Schmitt, L. Roset Julià, V. Scamarcio, A. Murello, S. Cheng, L. Menin, D. Ortiz, L. Patiny, S. Bolisetty, R. Mezzenga, S. J. Maerk, F. Stellacci, *Adv. Mater.* 2021, 33, 2104581.
[5] J. M. S. Bartlett, D. Stirling, *PCR Protoc.* 2003, 226, 545.
[6] J. Martinussen, M. Willemoës, M. Kistrup, *Compr. Biotechnol.* 2011, 6, 91.
[7] B. Roy, A. Depaix, C. Périgaud, S. Peyrottes, *Chem. Res.* 2016, 116, 7854.
[8] D. K. Button, B. R. Robertson, *Appl. Environ. Microbiol.* 2001, 67, 1636.
[9] Q. Xu, A. Cao, L. Zhang, C. Zhang, *Anal. Chem.* 2012, 84, 10845.
[10] C. D. Mol, C.-F. Kuo, M. M. Thayer, R. P. Cunningham, J. A. Tainer, *Nature* 1995, 374, 381.
[11] I. R. Lehman, A. L. Nussbaum, *J. Biol. Chem.* 1964, 239, 2628.
[12] C. D. Richter, D. Niftis, P. W. R. Broadhurst, K. J. Weissman, *Nat. Chem. Biol.* 2008, 4, 75.
[13] N. C. Dubey, B. P. Tripathi, *ACS Appl. Bio Mater.* 2021, 4, 1077.
[14] A. N. Lane, T. W.-M. Fan, *Nucleic Acids Res.* 2015, 43, 2466.
[15] V. Pareek, A. M. Pedley, S. J. Benkovic, *Crit. Rev. Biochem. Mol. Biol.* 2021, 56, 1.
[16] A. Alissandratos, K. Caron, T. D. Loan, J. E. Hennessy, C. J. Easton, *ACS Chem. Biol.* 2016, 11, 3289.
[17] T. D. Loan, C. J. Easton, A. Alissandratos, *Sci. Rep.* 2019, 9, 15621.
[18] D. Chandra, S. B. Bratton, M. D. Person, Y. Tian, A. G. Martin, M. Ayres, H. O. Fearnhead, V. Gandhi, D. G. Tang, *Cell* 2006, 125, 1333.
[19] A. Hofer, M. Crona, D. T. Logan, B.-M. Sjöberg, *Crit. Rev. Biochem. Mol. Biol.* 2012, 47, 50.
[20] G. S. Brush, S. K. Bhatnagar, M. J. Besman, *J. Bacteriol.* 1990, 172, 2935.
[21] D. Y. Liu, J. F. Zawada, J. R. Swartz, *Biotechnol. Prog.* 2005, 21, 460.
[22] S. K. Dondapati, M. Stech, A. Zemella, S. Kubick, *BioDrugs* 2020, 34, 327.
[23] A. Lesley, M. J. Tymms, *Vitro Transc. Transl. Protoc.* 1995, 265.
[24] B. J. L. Dopp, D. D. Tamiev, N. F. Reuel, *Biotechnol. Adv.* 2019, 37, 246.
[25] M. H. Buckstein, J. He, H. Rubin, *J. Bacteriol.* 2008, 190, 718.
[26] A. R. Kore, M. Shannugam, A. Senthivelan, B. Srinivasan, *Curr. Protoc. Nucleic Acid Chem.* 2012, Chapter 13, Unit13.10.
[27] K. Burgess, D. Cook, *Chem. Rev.* 2000, 100, 2047.
[28] S. A. Nick McElhinney, B. E. Watts, D. Kumar, D. L. Watt, E.-B. Lundström, P. M. J. Burgers, E. Johansson, A. Chabes, T. A. Kunkel, *Proc. Natl. Acad. Sci.* 2010, 107, 4949.
[29] Y. Shimizu, T. Kanamori, T. Ueda, *Methods* 2005, 36, 299.
[30] L. D. Moore, T. Le, G. Fan, *Neuropsychopharmacology* 2013, 38, 23.
[31] K. Jabbari, S. Cacció, J. P. Pas de Barros, J. Desgrés, G. Bernardi, *Gene* 1997, 205, 109.
[32] C. A. Heid, J. Stevens, K. J. Livak, P. M. Williams, *Genome Res.* 1996, 6, 986.
[33] B. Udugama, P. Kadhiresan, H. N. Kozlowski, A. Malekjahani, M. Osborne, V. Y. C. Li, H. Chen, S. Mubareka, J. B. Cubbay, W. C. W. Chan, *ACS Nano* 2020, 14, 3822.
[34] T. D. Schmittgen, K. J. Livak, *Nat. Protoc.* 2008, 3, 1101.
[35] J. E. Szabó, É. V. Surányi, B. S. Mébold, T. Trombitás, M. Cserepes, J. Tóth, *Nucleic Acids Res.* 2020, 48, e45.
[36] B. Kim, L. A. Nguyen, W. Daddacha, J. A. Hollenbaugh, *J. Biol. Chem.* 2012, 287, 21570.
[37] B. Breiner, K. Johnson, M. Stolarek, A.-L. Silva, A. Negrea, N. M. Bell, T. H. Isaac, M. Dethlefsen, J. Chana, L. A. Ibbotson, S. W. Ragsdale), Academic Press, 2011, pp. 219–231.
[38] L. Appy, A. Depaix, X. Bantreil, F. Lamaty, S. Peyrottes, B. Roy, *Phosphorus Sulfur Silicon Relat. Elem.* 2020, 195, 930.
[39] Q. Sun, J. P. Edadhil, R. Wu, E. D. Smidansky, C. E. Cameron, B. R. Peterson, *Org. Lett.* 2008, 10, 1703.
[40] D. Yang, M. R. Hartman, T. L. Derrien, S. Hamada, D. An, K. G. Yancey, R. Cheng, M. Ma, D. Luo, *Acc. Chem. Res.* 2014, 47, 1902.
[41] M. R. Jones, N. C. Seeman, C. A. Mirkin, *Science* 2015, 347, 1260901.
[42] J. G. Ferry, in *Methods Enzymol.* (Eds.: A.C. Rosenzweig, S.W. Ragsdale), Academic Press, 2011, pp. 219–231.