General compatibility of nucleoside triphosphates with rolling circle amplification

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Introduction

• Besides PCR, rolling circle amplification (RCA) is the most used amplification method of DNA. In RCA, single-stranded DNA nanocircles serve as virtually infinite templates for short primers, generating long, ssDNA products containing multiple copies of the reverse complement of the template.[1,2] RCA has found a variety of applications including sensing, diagnostics, and nanotechnology.[2]

• Modified nucleoside triphosphates (dN*TPs) represent a versatile vector for the introduction of chemical diversity into nucleic acids.[3] Surprisingly, while dN*TPs have been engaged in a large palette of applications including the selection of aptamers[4] and electrochemical tagging of nucleic acids,[5] only few examples combining dN*TPs and RCA have been reported.[6]

• Herein, the compatibility of dN*TPs modified at any position of the scaffold with rolling circle amplification method was assessed. This method was used to generate long, nuclease-resistant and fully-modified cytosine-rich mimics of telomeric DNA.[7]

Design of the method

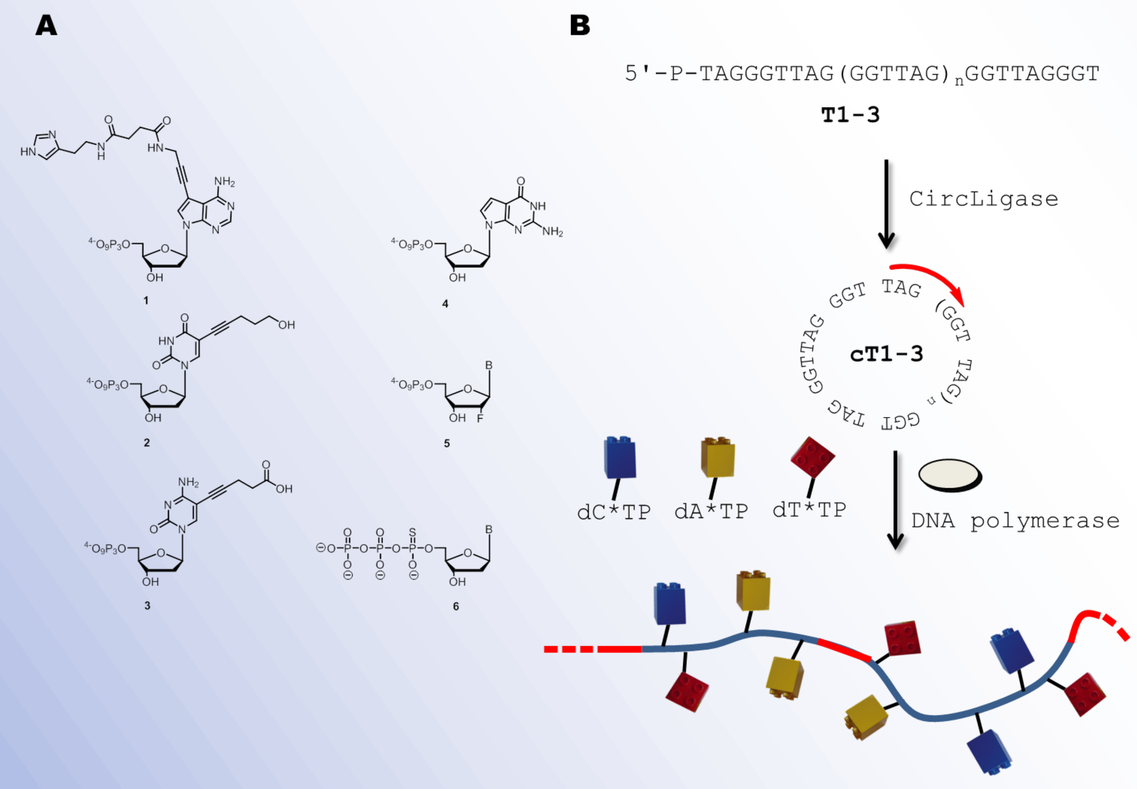

Figure 1. A) Chemical structures of the modified nucleoside triphosphates used in rolling circle amplification; B) Schematic representation of RCA with dN*TPs: the linear template precursors **T1-3** are circularized with CircLigase™. The nanocircles **cT1-3** served as templates for RCA, along with a suitable primer, dN*TPs, and a DNA polymerase.

Polymerase screen

Various DNA polymerases (A- and B-family) were screened to assess whether they could extend the primer in the presence of one or multiple dN*TPs under RCA conditions. Only the 9°N_m DNA polymerase is capable of extending the primer in the presence of *all* modifications with high yields.

The 9°N_m DNA polymerase was also shown to be compatible with a broad variety of templates (Figure 2) and could be used to generate fully modified, long, cytosine-rich mimics of telomeric DNA.

Primer extension reactions with linear templates and a Sanger-sequencing experiment clearly demonstrated that the products emanate from a rolling circle mechanism.

Generation of fully modified RCA products

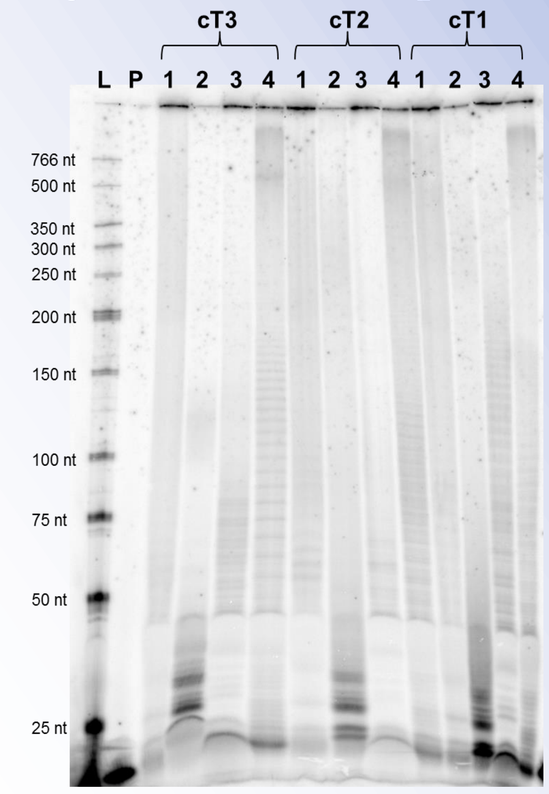

Figure 2. Analysis (PAGE 5%) of RCA products obtained with templates **cT1-3** and the 9°N_m DNA polymerase. Lane 1: α-thio dNTPs **6**; lane 2: 2'-fluoro-rNTPs **5**; lane 3: base-modified dN*TPs **1**–**3**; lane 4: natural dNTPs.

Serum resistance

The modified RCA products all display a higher stability in FBS than the corresponding unmodified products:

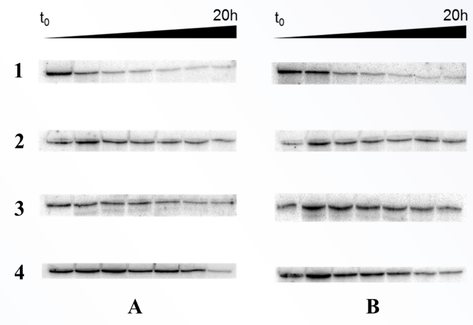

Figure 3. Gel image (PAGE 5%) showing the stability of RCA products in 10% FBS (37°C). Line 1 natural dNTPs; line 2: dN*TPs **1**–**3**; line 3: α-thio-dNTPs **6**; line 4: 2'-fluoro-rNTPs **5.** A:template **cT1**; B: template **cT3**.

Conclusions

- dN*TPs are compatible with RCA, regardless of the location and nature of the modification and the topology of the template.
- The combination of RCA and dN*TPs yields long, single-stranded and modified products that have an increased stability in serum.
- This method could be used to develop new biosensors, biocatalysts based on DNAzymes, and nanomaterials.

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