Enzymatic Incorporation of Biotin-16-AA-dNTPs

TriLink BioTechnologies, Inc.
Research and Development

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Overview

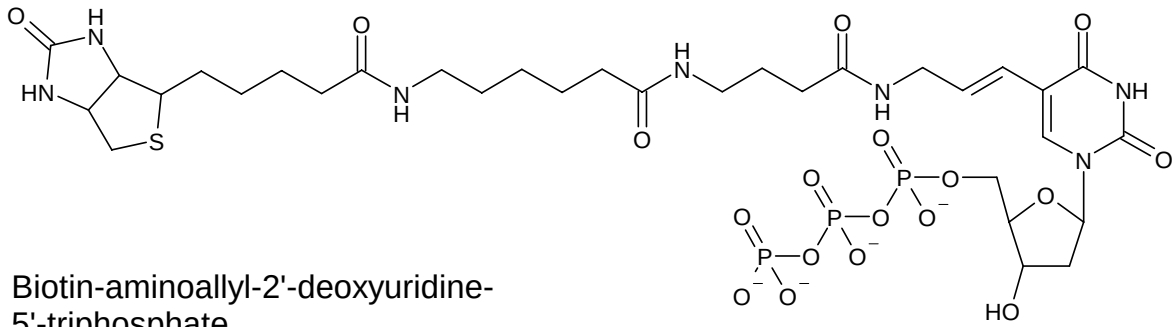
Biotin is a naturally occurring cofactor that binds very tightly to the tetrameric protein, avidin. This strong association between biotin and avidin has been used in a number of biotechnology applications, which include detection and isolation of a molecule of interest. One approach for introducing biotin modifications into DNA is by the enzymatic incorporation of nucleoside 5′-triphosphates that have been chemically modified with biotin.

Outline

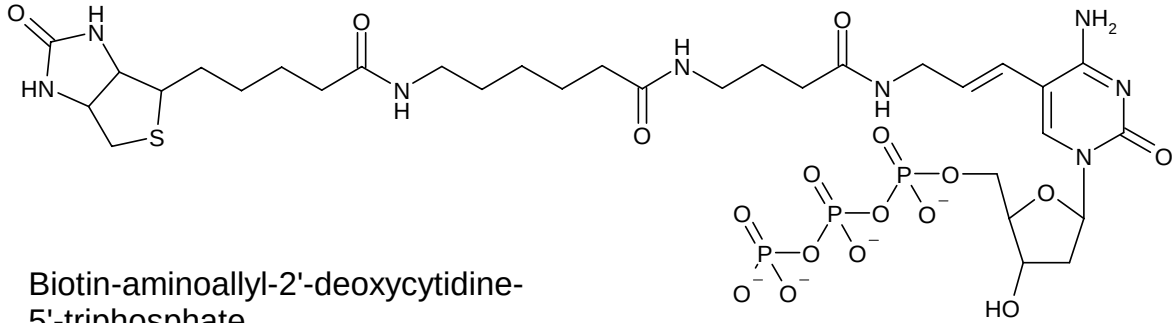
- Goal: Evaluate the enzymatic incorporation of Biotin-16-AA-dNTPs.

- Approach:
  - Evaluate Biotin-16-AA-dNTP incorporation in primer extension experiments using either MMLV reverse transcriptase or Klenow(exo-) DNA polymerase.
  - Evaluate the incorporation of Biotin-16-AA-dNTPs in PCR.

Chemical Structures of the Biotin-16-AA-dNTPs

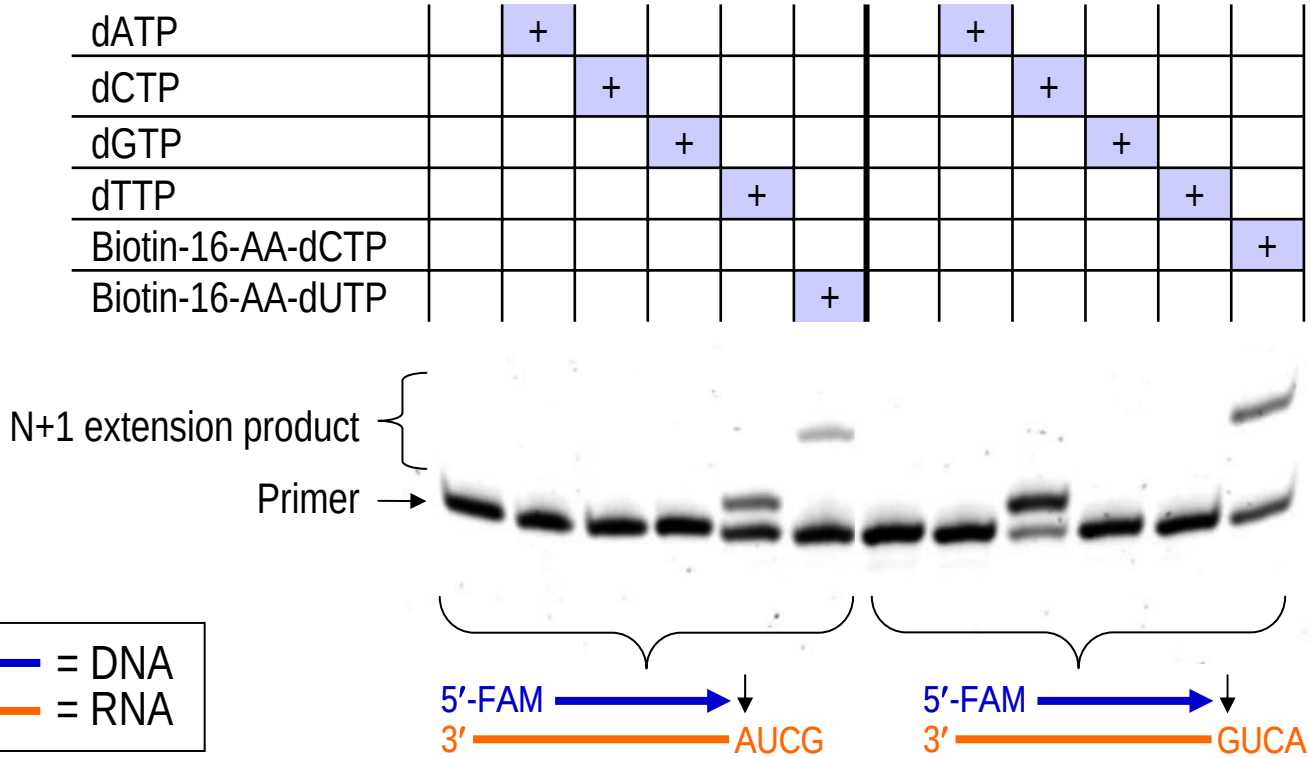


Biotin-aminoallyl-2'-deoxyuridine-5'-triphosphate



Biotin-aminoallyl-2'-deoxycytidine-5'-triphosphate

Single Nucleotide Incorporation of Biotin-16-AA-dNTPs by MMLV Reverse Transcriptase



All biotinylated dNTPs are substrates for MMLV reverse transcriptase and produce an extension product with lower mobility.

Experimental Conditions: 1x First strand synthesis buffer (50 mM Tris-HCl (pH 8.3 @ 25°C), 50 mM KCl, 3.0 mM $MgCl_2$, 5 mM DTT), 5'-FAM-labeled primer (6.25 μM), RNA template (10 μM), Ambion MMLV Reverse transcriptase (0.2 U/μL), 0.1 mM dNTP. Thermal cycling parameters: 42°C @ 20 min; 98°C @ 2 min.

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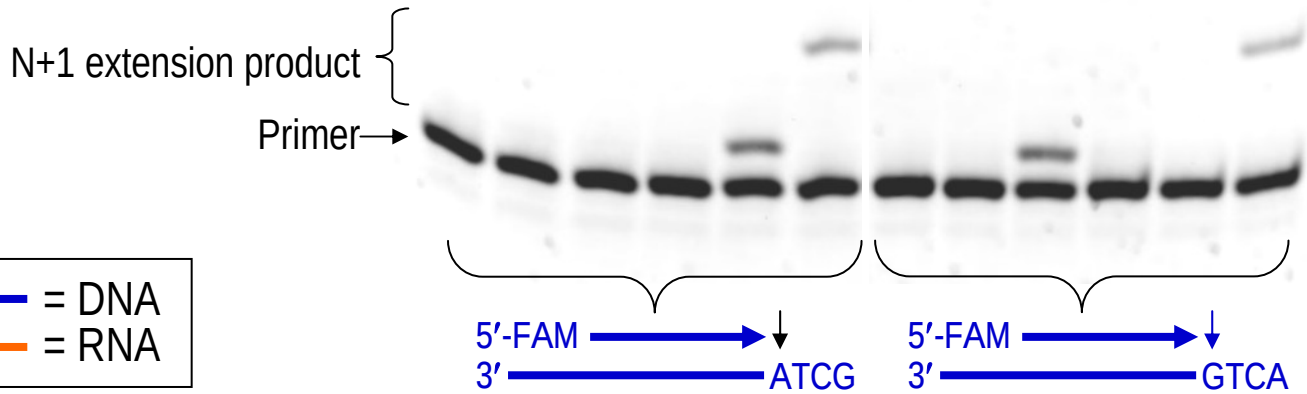
Single Nucleotide Incorporation of Biotin-16-AA-dNTPs by Klenow(exo-) DNA Polymerase

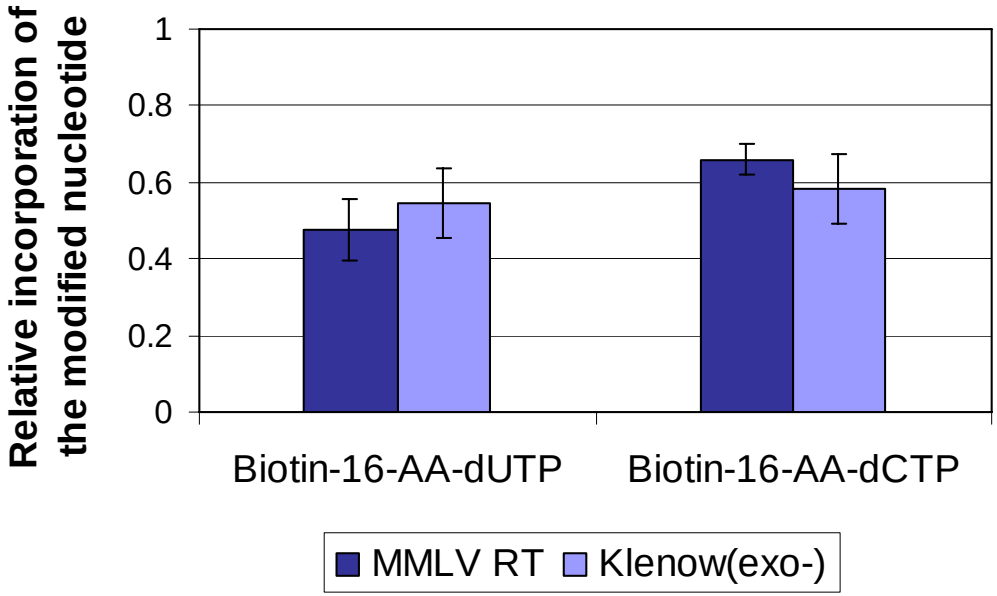
dATP		+						+				
dCTP			+						+			
dGTP				+						+		
dTTP					+						+	
Biotin-16-AA-dCTP												+
Biotin-16-AA-dUTP						+						



All biotinylated dNTPs are substrates for Klenow(exo-) DNA polymerase and produce an extension product with lower mobility.

Experimental Conditions: 1X NEBuffer 2 (10 mM Tris-HCl (pH 7.9 @ 25°C), 50 mM NaCl, 10 mM $MgCl_2$, 1 mM DTT), 5'-FAM-labeled primer (10 μM), DNA template (17 μM), New England Biolabs Klenow(exo-) DNA polymerase (0.025 U/μL), 20 μM dNTP. Thermal cycling parameters: 37°C @ 20 min; 72°C @ 20 min.

Summary of biotin-16-AA-dNTP Single Nucleotide Incorporation Studies



$$\text{Relative modified nucleotide incorporation} = \frac{\%\ \text{n+1 extension product (modified dNTP)}}{\%\ \text{n+1 extension product (natural dNTP)}}$$

Relative to their natural counterpart, biotinylated dCTP and dUTP analogs are incorporated with ~50% lower efficiency at the conditions examined.

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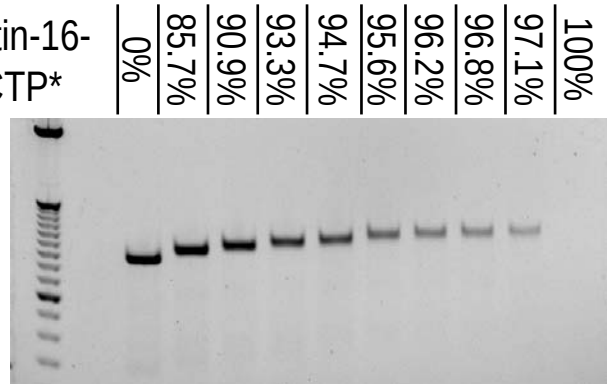
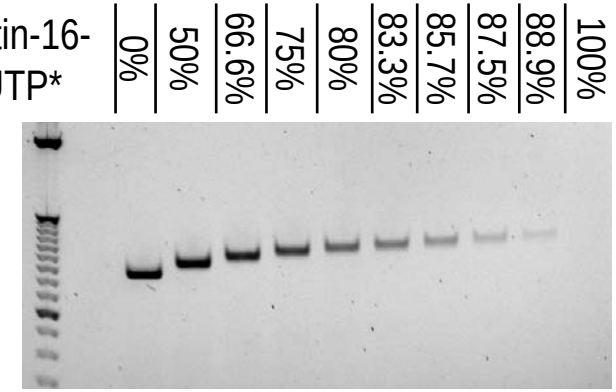
Incorporation of Biotinylated dNTPs by *Taq* DNA Polymerase in PCR

% Biotin-16-AA-dCTP*



% Biotin-16-AA-dUTP*



*Total concentration of biotinylated + natural dNTP was maintained at 0.2 mM. The percentage of biotinylated dNTP was titrated between 0 and 100%.

All biotinylated dNTPs are substrates for Taq DNA polymerase in PCR.

As the percentage of biotinylated nucleotide increases, a corresponding decrease in the mobility of the amplicon is observed.

100% biotinylated dNTP substitution causes complete reaction inhibition.

Experimental Conditions: Control Lambda primers (0.2 mM), New England Biolabs *Taq* DNA Polymerase (1 U/50 mL rxn), 0.2 mM dNTPs (including Biotin and natural), 1x NEB buffer (10 mM Tris-HCl (pH 8.3 @ 25ºC), 50 mM KCl, 1.5 mM $MgCl_2$, Lambda genomic DNA (1 ng/50 μL rxn).
Primer sequences: 5'-CCTGCTTCTGCCGCTTCACGA and 5'-TCCGGATAAAAACGTCGATGACATTTGC.
Thermal cycling parameters: 95ºC @ 2 min; [95ºC @ 15 sec, 55ºC @ 15 sec, 72ºC @ 45 sec]25x; 72ºC @ 5 min.

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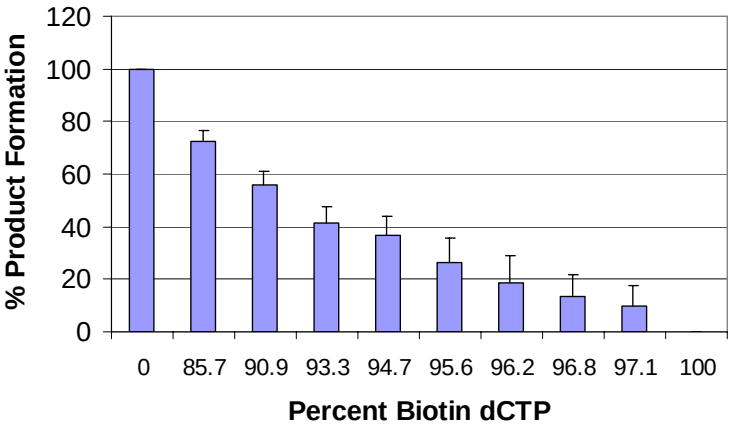
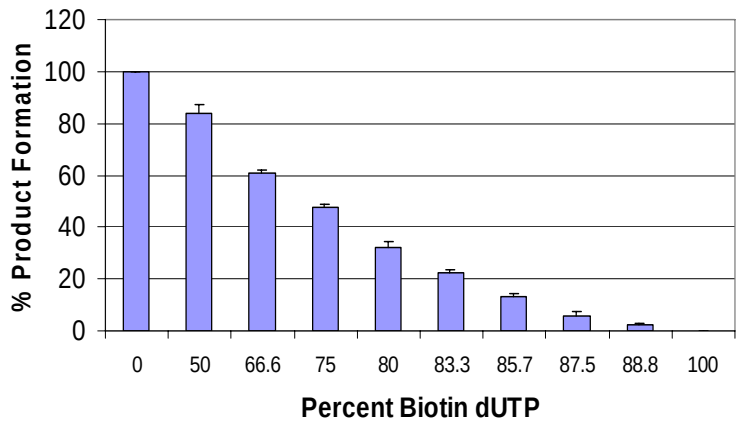
Incorporation of Biotinylated dNTPs by *Taq* DNA Polymerase in PCR

Biotin-16-AA-dCTP



Biotin-16-AA-dUTP



The percent product formation is the product yield, normalized to the yield with 100% unmodified dNTPs.

Extent of biotinylated dNTP substitution that yields ~50% PCR product formation:
Biotin dCTP = ~92%
Biotin dUTP = ~75%

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Summary

- *Biotin-16-AA-dCTP and biotin-16-AA-dUTP can be readily incorporated by reverse transcriptases and DNA polymerases in primer extension schemes.*
- *Biotinylated nucleotides are readily incorporated during PCR amplification schemes.*
- *Biotin-16-AA-dCTP can be substituted to a greater extent for its natural dNTP in PCR without compromising amplicon yield.*


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