

Kinetic Analysis of an Efficient DNA-dependent TNA Polymerase

Supplementary Material

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Steady State Reactions Experimental Methods.

α -L-Threofuranosyl nucleoside triphosphates were synthesized as described by Zou et. al¹. Stopped start single nucleotide incorporation kinetic experiments were conducted according to the method described by Goodman². Reactions were carried out in 20 μ l of 20 mM Tris-HCl, 10 mM KCl, 10 mM (NH₄)₂SO₄, 20 mM MgSO₄, 0.1% Triton X-100, 0.25 μ g/ μ l BSA, 100 μ M DTT with 0.05 units of Terminator DNA Polymerase (New England Biolabs) with or without the addition of freshly prepared MnCl₂. Reaction solutions were preheated at 55° C and initiated by the addition of 10 μ l of 2X NTP to an equal volume of solution containing both polymerase and P³² labeled primer template complex. Reaction times were designed to limit total primer extension to 20% (typically 1-3 min) at which time the reactions were quenched by the addition of stop buffer containing 8M urea, 100 mM EDTA, and 1X TBE buffer. Samples were analyzed using 20% denaturing PAGE followed by phosphorimaging and quantitation on a Bio-Rad Molecular Imager FX. The insertion kinetic parameters of V_{max} and K_m were determined using Hanes-Woolf plots to give an overall insertion efficiency for each NTP.

References

1. Zou, K.; Horhota, A.; Yu, B.; Szostak, J.W.; McLaughlin, L.W. *Org. Lett.* **2005**, In press.
2. (a) Boosalis, M. S.; Petruska, J.; Goodman, M. F. *J. Biol. Chem.* **1987**, 262, 14689-14696. (b) Goodman, M. F.; Creighton, S.; Bloom, L. B.; Petruska, J. *Crit. Rev. Biochem. Mol. Biol.* **1993**, 28, 83-126.

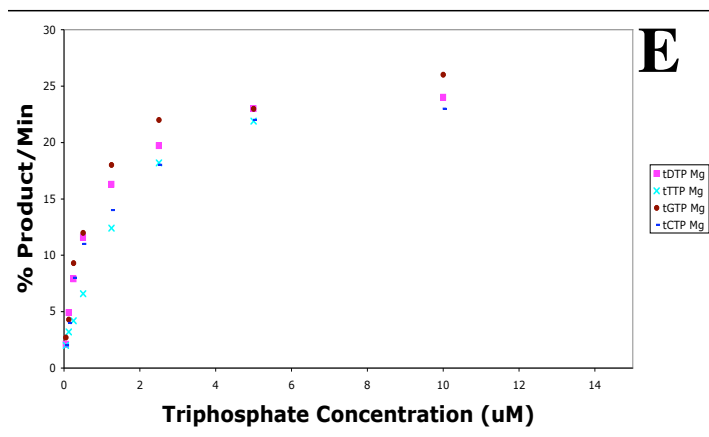
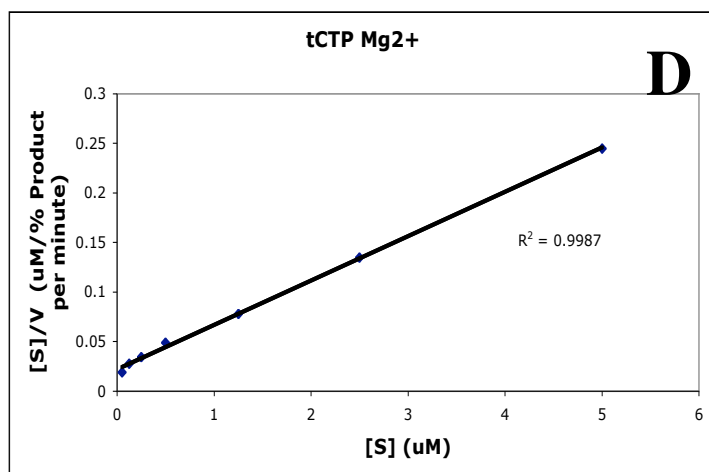
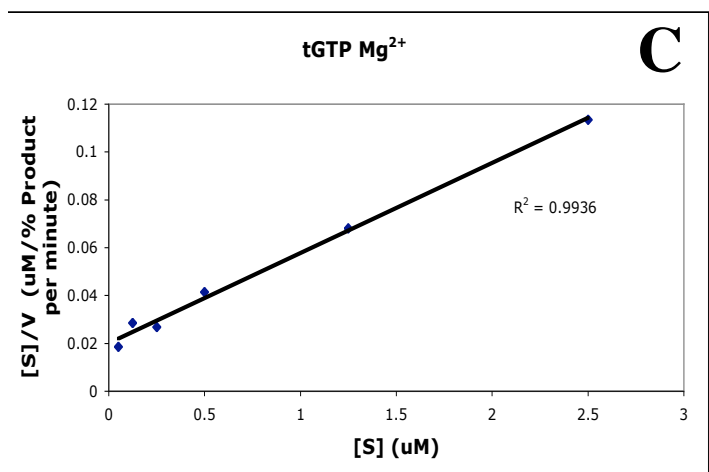
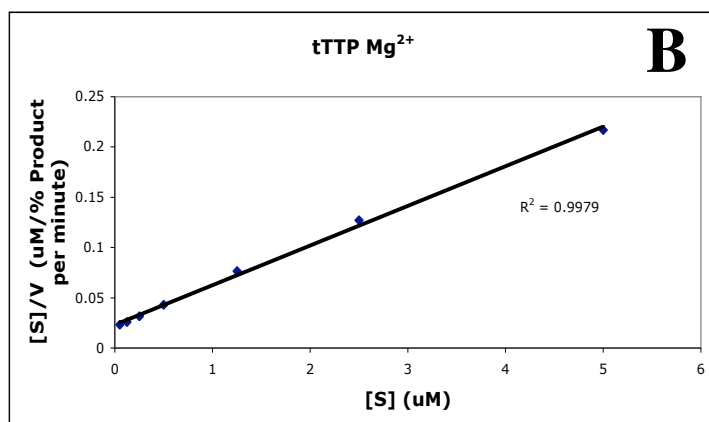
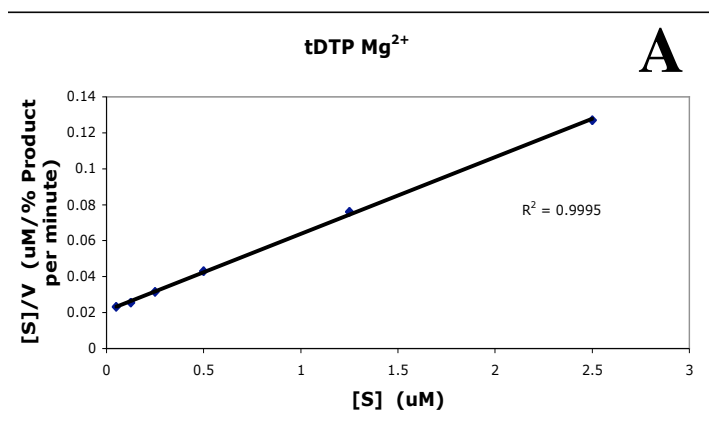


Fig. 1 (supplementary). A-D are Steady-State Hanes-Woolf plots for the incorporation of tNTPs by Terminator DNA Polymerase in the presence of Mg²⁺ cations from which V_{max} and K_m values were determined to yield an overall insertion efficiency. **A.** tDTP opposite T. **B.** tTTP opposite A. **C.** tGTP opposite C. **D.** tCTP opposite G. **E.** Michaelis-Menten nonlinear regression plot showing all four tNTPs insertion velocities relative to substrate concentration.

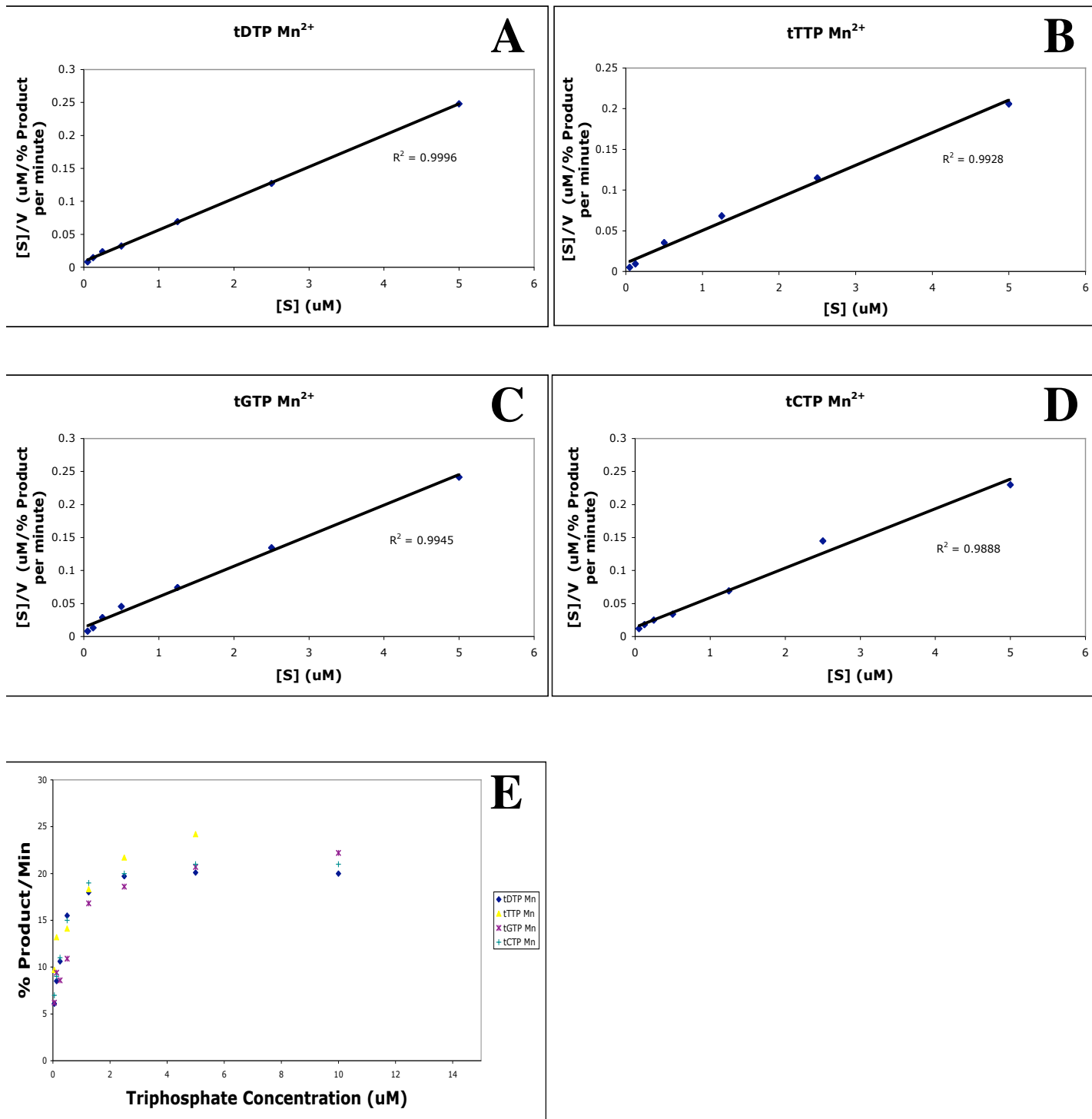


Fig. 2 (supplementary). A-D are Steady-State Hanes-Woolf plots for the incorporation of tNTPs by Therminator DNA Polymerase in the presence of Mn²⁺ cations from which V_{max} and K_m values were determined to yield an overall insertion efficiency. **A.** tDTP opposite T. **B.** tTTP opposite A. **C.** tGTP opposite C. **D.** tCTP opposite G. **E.** Michealis-Menten nonlinear regression plot showing all four tNTPs insertion velocities relative to substrate concentration.

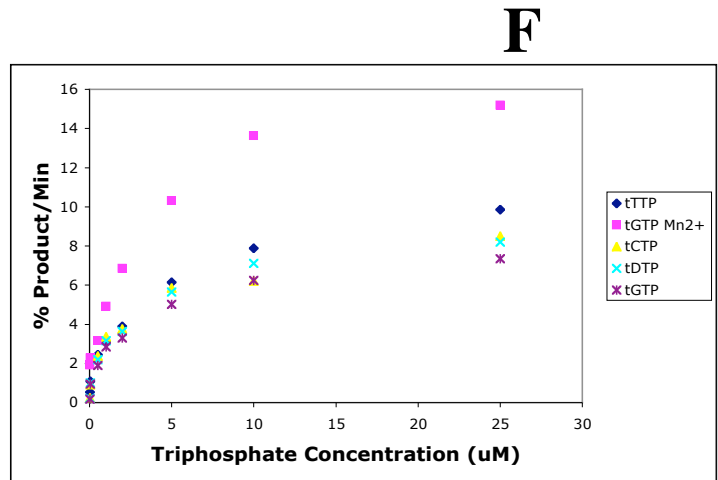
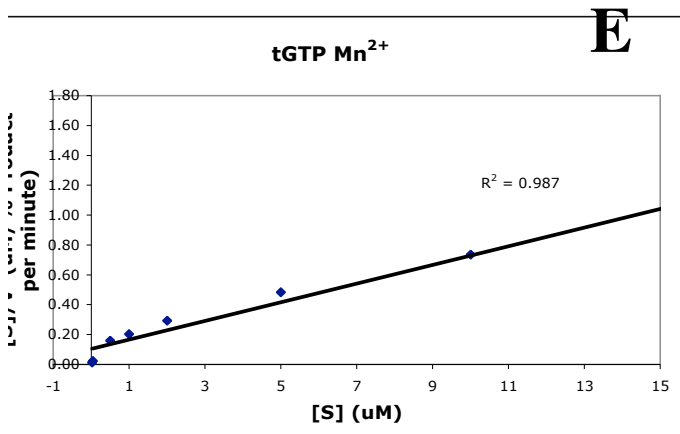
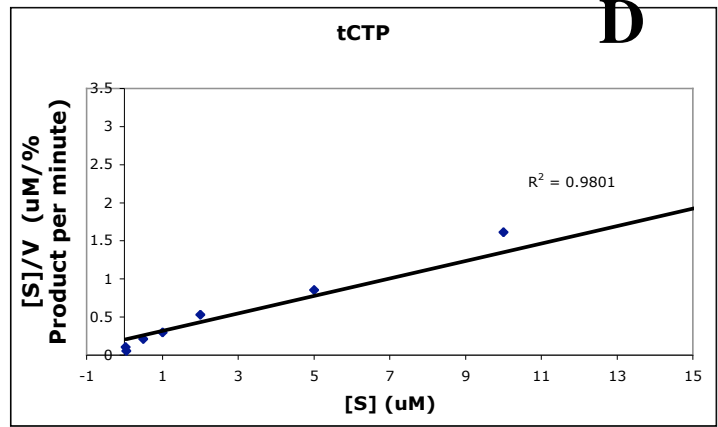
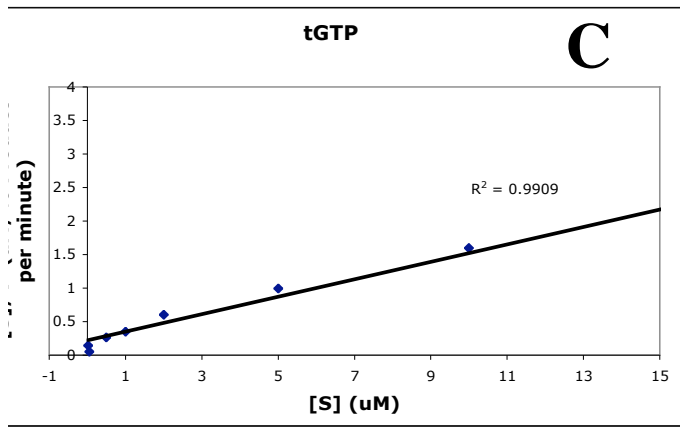
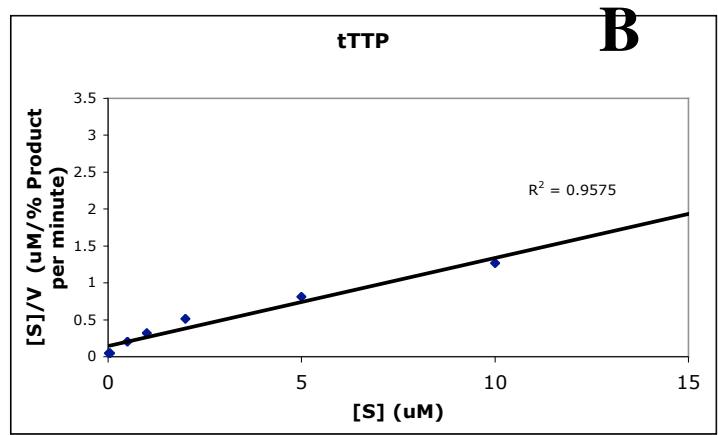
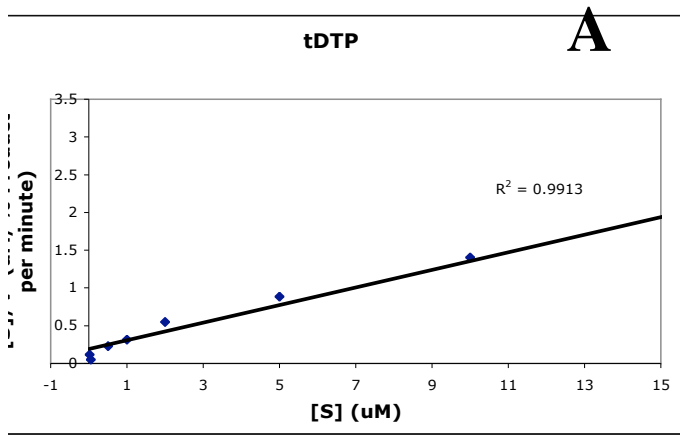
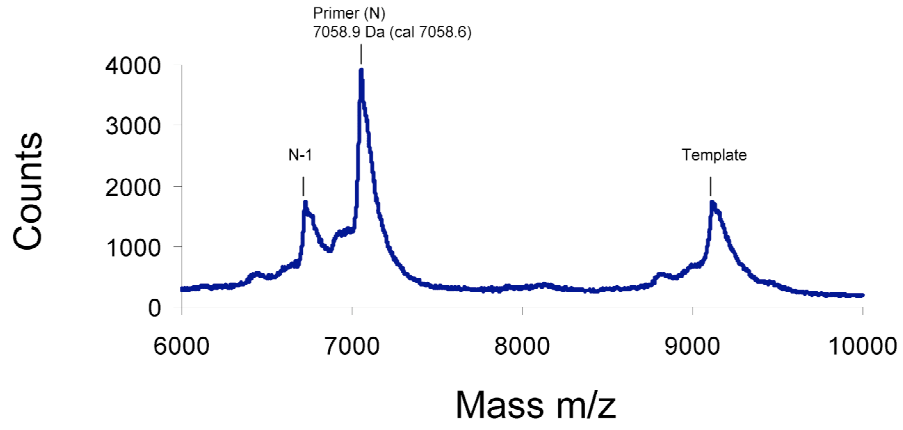
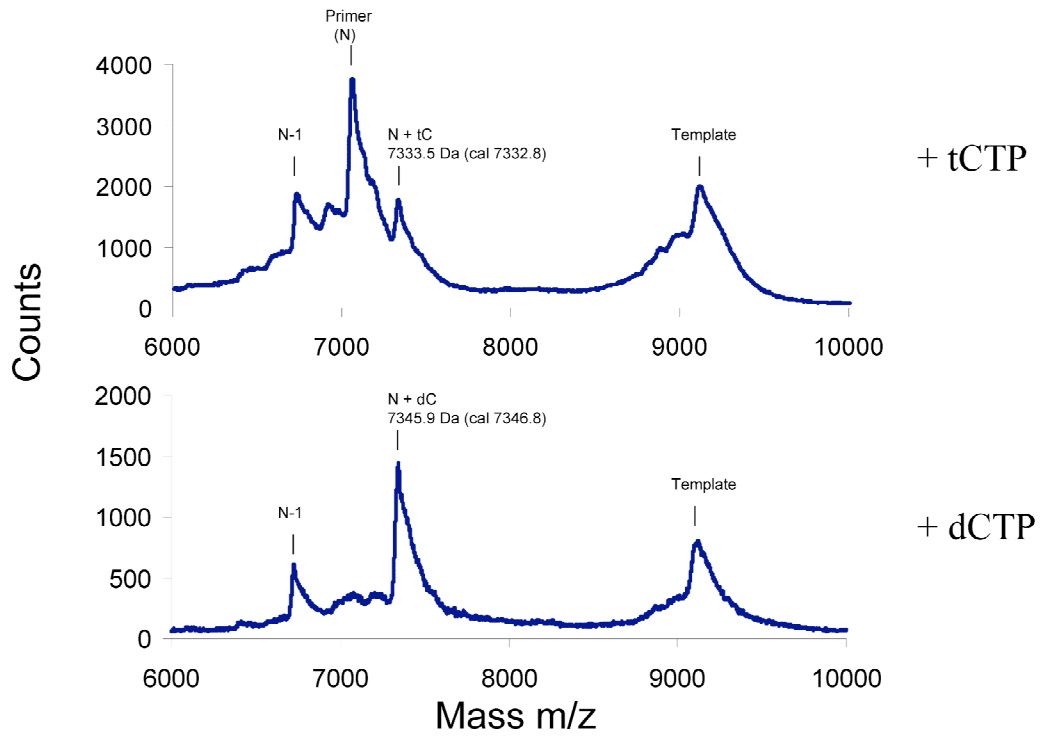


Fig. 3 (supplementary). A-E are Steady-State Hanes-Woolf plots for the incorporation of tNTPs by Deep Vent DNA Polymerase from which V_{max} and K_m values were determined to yield an overall insertion efficiency. **A.** tDTP opposite T. **B.** tTTP opposite A. **C.** tGTP opposite C. **D.** tCTP opposite G. **E.** tGTP opposite C with Mn^{2+} . **F.** Michealis-Menten nonlinear regression plot showing all four tNTPs insertion velocities relative to substrate concentration.

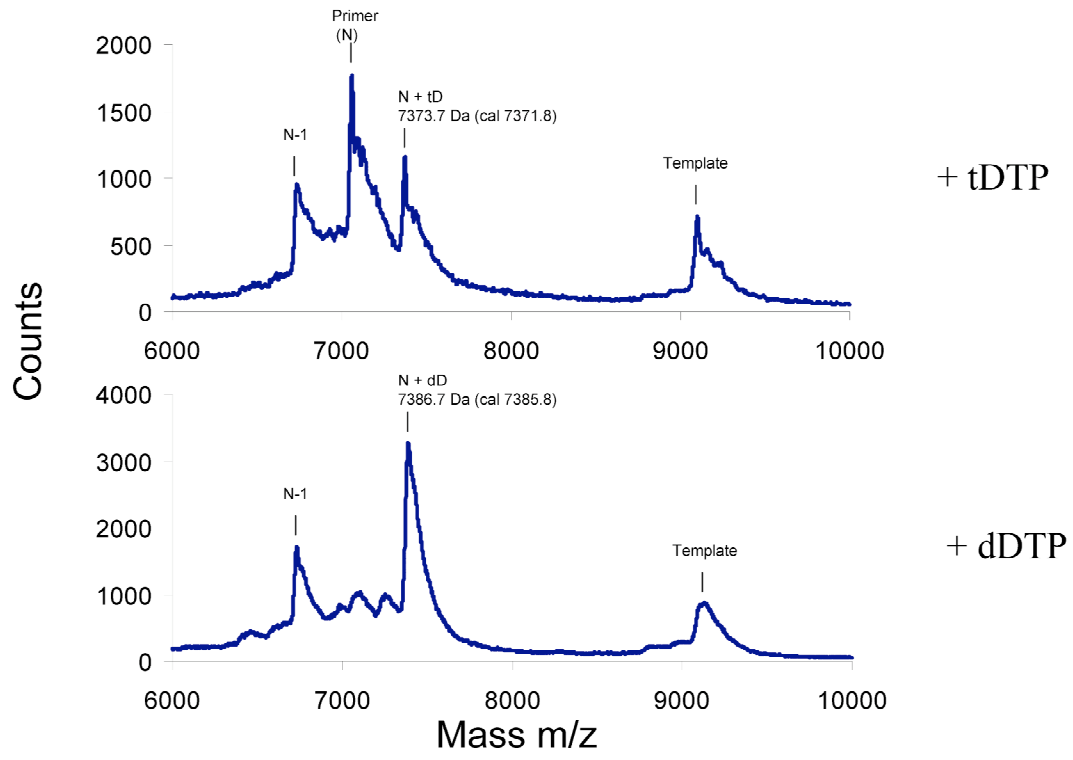
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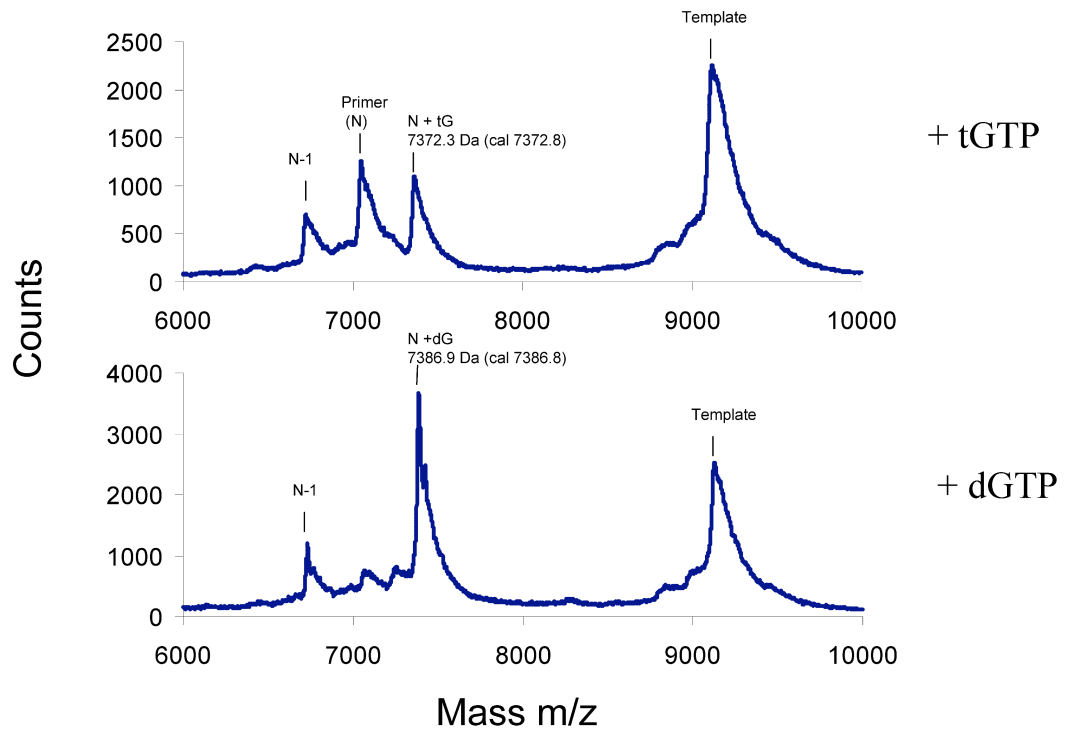
C



D



G



T

