Supporting Information

Polymerase-Directed Synthesis of 2′-Deoxy-2′-fluoro-β-D-arabinonucleic Acids

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Contents

Experimental Section

1. Nucleotides. 2'-Deoxyribonucleoside 5'-triphosphates (dNTPs) were purchased from Fermentas. 2'F-araN were synthesized by published procedures.[1] The introduction of the 5'-triphosphate moiety was conducted by Rasayan, Inc. (Encinitas, California, USA) and 2'F-rUTP and 2'F-rCTP were also purchased from Rasayan, Inc. The structures of the fluorinated nucleoside 5'-triphosphates are shown in Figure S1.

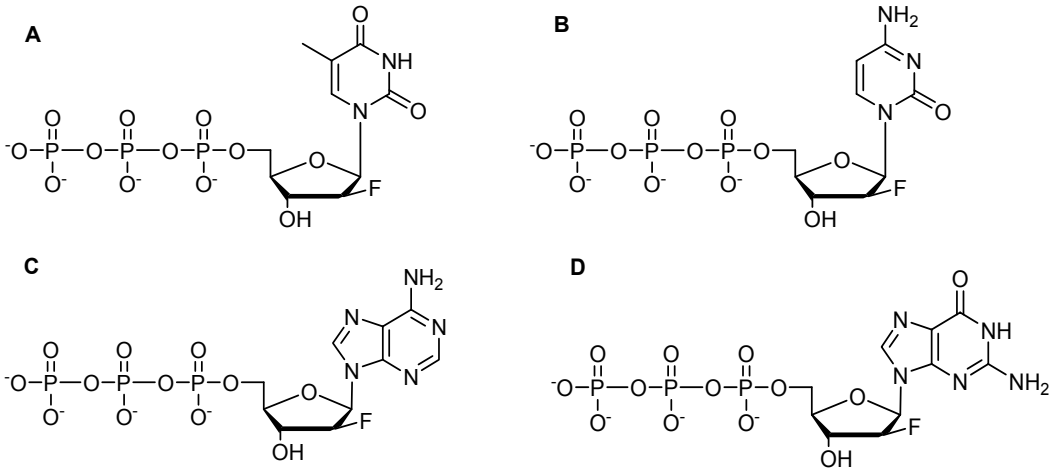

Figure S1. Structure of 2'F-araNTPs. **A**: 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)thymine 5'-triphosphate (2'F-araTTP); **B**: 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)cytosine 5'-triphosphate (2'F-araCTP); **C**: 9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)adenine 5'-triphosphate (2'F-araATP); **D**: 9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)guanine 5'-triphosphate (2'F-araGTP);

2. Template and Primer Design. DNA and FANA-DNA templates and primers used in this study are shown in Table S1**.** The primer (PF20) is a 17nt DNA sequence 5'-*TAATACGACTCACTATA*-3'. Each template strand comprises three sequence segments: a 17nt long primer binding sequence *5'-TATAGTGAGTCGTATTA-3'*, a 10nt long running start sequence 5'-CTCTTCTCCC-3', and a variable "test sequences" to test 2'F-araNTP incorporation (PF33), FANA template mediated DNA synthesis (PF31, PF34) and the fidelity of 2'F-araATP (PF41), 2'F-araGTP (PF43), 2'F-araTTP (PF21), 2'F-araCTP (PF23). Other control sequences (PF39, PF33) are also listed in Table S1. DNA sequences were obtained from commercial sources (Integrated DNA Technologies). Oligonucleotide FANA-DNA templates were synthesized according to the published procedures.[2]

3. General Procedure of Primer Extension Reaction. Primer extension assays were used to evaluate the polymerase activity for incorporation of 2'F-araNTPs, and for template mediated DNA biosynthesis. Unless otherwise noticed in this study, the conditions used in the primer extension assays, such as dNTP concentrations, reaction temperatures, concentration of Mg^{2+}, and reaction buffers (provided by the manufacturers, except for the HIV-RT buffer which was prepared in-house) are shown in Table S2. The DNA primer (PF20) was first labeled by a radioactive phosphorous probe (^{32}P) at the 5'-hydroxyl terminus according to published procedure.[3] Unlabeled DNA primer was also used to adjust the primer concentration. Then primer to template was mixed together with the final concentration at 100 nM for the primer and the template (1:1 molar ratio). The primer and template were heated at 95ºC for 5min and annealed at 4ºC at least two hours before use. In an microtube, the mixture of 5x or 10x buffer,

Table S1. Base Sequences of Oligonucleotide Templates and Primer

Code	Type	Sequence*	N-mer
PF20	Primer	5'-*TAATACGACTCACTATA*-3'	17
PF21	DNA temp.	5'-TTTGCC-A-CTCTTCTCCC*TATAGTGAGTCGTATTA*-3'	34
PF23	DNA temp.	5'-TTTACC-G-CTCTTCTCCC*TATAGTGAGTCGTATTA*-3'	34
PF31	FANA-DNA temp.	5'-**TTACCTTT**CTCTTCTCCC*TATAGTGAGTCGTATTA*-3'	35
PF32	DNA temp.	5'-CTCTATGTGCACGCACTCTTCTCCC*TATAGTGAGTCGTATTA*-3'	42
PF33	DNA temp.	5'-TCGGTGGATCATAGACAGTACTCTTCTCCC*TATAGTGAGTCGTATTA*-3'	47
PF34	FANA-DNA	5'-CTCT**A**T**G**T**G**C**A**C**G**C**A**CTCTTCTCCC*TATAGTGAGTCGTATTA*-3'	42
PF39	DNA control	5'-TAATACGACTCACTATAGGGAGAAGAG-3'	27
PF41	DNA temp.	5'-AGAGCC-T-GAGAAGAGAG*TATAGTGAGTCGTATTA*-3'	34
PF43	DNA temp.	5'-AAATGG-C-GAGAAGAGAG*TATAGTGAGTCGTATTA*-3'	34

*Note: **Bold and capital letter: FANA units;** Capital letters: DNA; *Italized sequence: primer binding sequence and primer (17nt);* Underlined sequence: a running start (10nt).

Table S2. Polymerase Information and Reaction Buffer Conditions

Polymerase*	Short name	Activity (units/μL)	Reaction buffer (x1)
Thermophilic DNA polymerase (reaction temperature at 55°C in this study)			
Deep Vent (3'→5' exo-) DNA polymerase	DV	2	20 mM Tris-HCl (pH 8.8 at 25°C), 10 mM $(NH_4)_2SO_4$, 10 mM KCl, 2 mM $MgSO_4$, 0.1 % Triton X-100; 0.2 mM dNTPs
9° N_m DNA polymerase	9N	2	Same as above
Therminator DNA polymerase	Th	2	Same as above
Bst DNA polymerase large fragment	*Bst*	8	Same as above
Taq DNA polymerase	*Taq*	5	Same as above
Phusion High-Fidelity DNA polymerase	Ph	2	5X Phusion™ HF Buffer; 0.2 mM dNTPs
Mesophilic DNA polymerase (reaction temperature at 37°C in this study)			
Klenow fragment (3'→ 5' exo-) DNA polymerase	KF	5	10 mM Tris-HCl (pH 7.5 at 25°C), 5 mM MgCl2, 7.5 mM DTT; 0.033 mM dNTPs
Reverse transcriptase (reaction temperature at 37°C in this study)			
HIV-1 RT (recombinant)	HIV	27.3	50 mM Tris-HCl (pH 7.8 at 25°C), 60 mM KCl, 2.5 mM $MgCl_2$; 0.2 mM dNTPs
Moloney Murine Leukemia Virus RT	MMLV	10	50 mM Tris-HCl (pH 8.3 at 25°C), 50 mM KCl, 4 mM $MgCl_2$, 10mM DTT; 0.5 mM dNTPs

* Enzyme sources: all from New England Biolabs (NEB) except for Ph from Finnzymes (distributed by NEB); HIV-1 RT from Worthington Biochemical Corp.

triphosphates (dNTPs or 2'F-araNTPs), water, the primer and template mixture was prepared according to pre-calculated reaction volume and concentrations (see buffer conditions in Table S2). Mineral oil (20 μL) was used to prevent evaporation. The reaction mixture was incubated at either 37ºC or 55ºC (see temperature conditions in Table S2) and one of the various enzymes tested was added to initiate the primer extension reactions. Time points were obtained by taking 4 μL or 8 μL aliquot from the reaction mixture and quenching the aliquot by the same volume of a stopping dye solution (98% deionized formamide, 10 mM EDTA, 1 mg/ml bromophenol blue and 1 mg/ml xylene cyanol). The product pattern from each time point was analyzed by 12% denaturing polyacryamide gel electrophoresis (PAGE) and subsequent autoradiography analysis.

REFERENCES

(1) Elzagheid, M. I., et al., Synthesis of protected 2'-Deoxy-2'-fluoro-β-D-arabinonucleosides. In *In Current Protocols in Nucleic Acid Chemistry,* John Wiley & Sons: **2002**, pp1.7.1-19.

(2) Viazovkina, E. V., et al., Solid-phase synthesis of 2'-deoxy-2'-fluoro-β-D-oligoarabinonucleotides (2'F-ANA) and their phosphorothioate derivatives. In *Current Protocols in Nucleic Acid Chemistry*, John Wiley & Sons, Inc. **2002**, pp 4.15.1-22.

(3) Galarneau, A., et al., Assay for evaluating ribonuclease H-mediated degradation of RNA-antisense oligonucleotide duplexes. In *Methods Mol. Biol.*, Totowa, NJ, United States, **2005**, 288, pp 65-80.

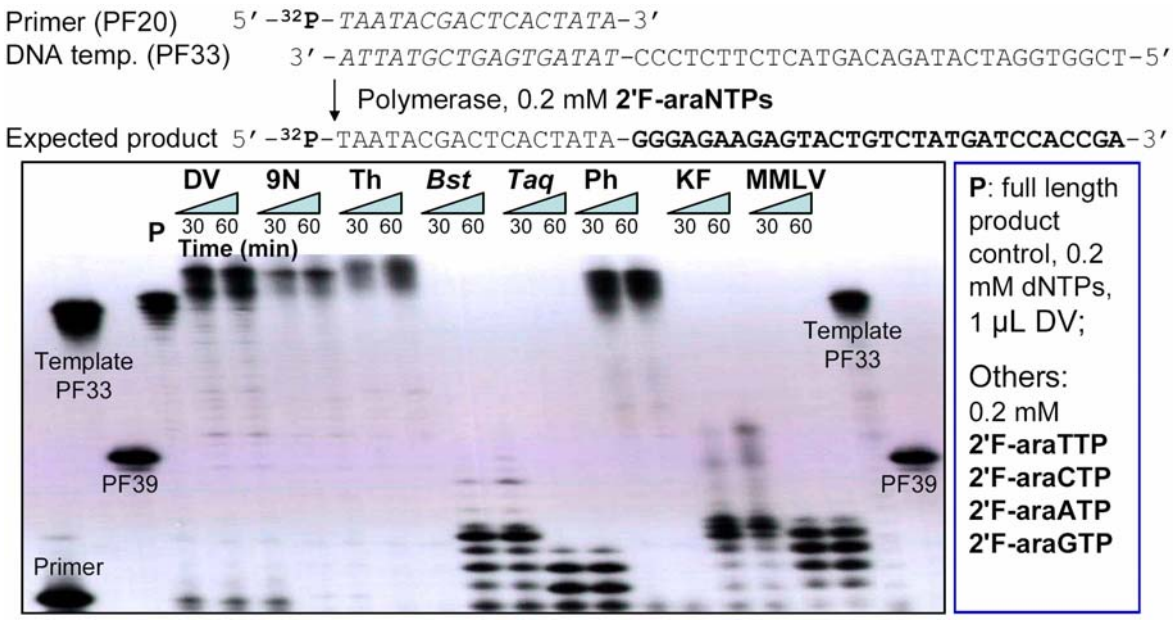

Figure S2. Incorporation of 2'F-araN units (7x 2'F-araG, 11x 2'F-araA, 6x 2'F-araT, 6x 2'F-araC) catalyzed by DNA polymerases on a DNA template PF33. Primer extension reaction conditions were performed with 100 nM primer/template (1:1 molar ratio), 0.2 mM 2'F-araNTPs (shown above) and 1 μL DV, 9N, Th, *Bst* and *Taq*, KF, 0.5 μL Ph and MMLV, respectively in 20 μL reaction volume, at 55^{o}C (DV, 9N, Th, *Bst*, *Taq*, Ph) or 37^{o}C (KF and MMLV). Reaction progress over time was analyzed by 12% denaturing PAGE. Lane **P** is the product control obtained in a similar primer extension assay with 1 μL DV, 0.2 mM dNTPs at 55^{o}C for 30min, on the same template PF33; PF39 is a 27-nt DNA control (see Table S1).

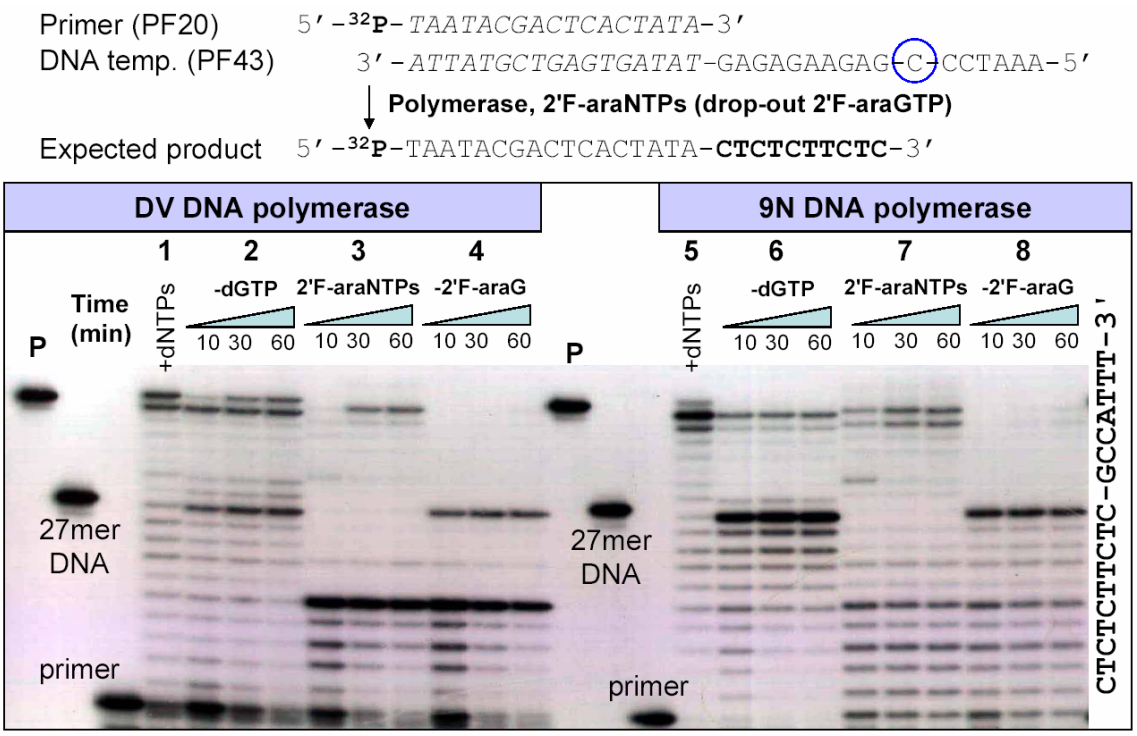

Figure S3. Fidelity study on 2'F-araGTP incorporation by DV and 9N DNA polymerases on DNA template PF43. Reaction conditions: primer/template (1:1 molar ratio): 100 nM; 0.4 μL (0.8 unit) DV, 9N, 25 μM triphosphate concentration at 55^{o}C in 30 μL reaction volume. Reaction time for dNTPs (group 1, 5) was 30 min and others were shown above. Group 1 & 5: dNTPs; 2 & 6: drop out dGTP (i.e. dTTP, dCTP and dATP), 3 & 7: 2'F-araNTPs, 4 & 8: drop out 2'F-araGTP; lane P is the template PF41 as the full-length product control; PF39 is a 27-nt DNA control (see Table S1). All samples were analyzed by 12% denaturing PAGE.

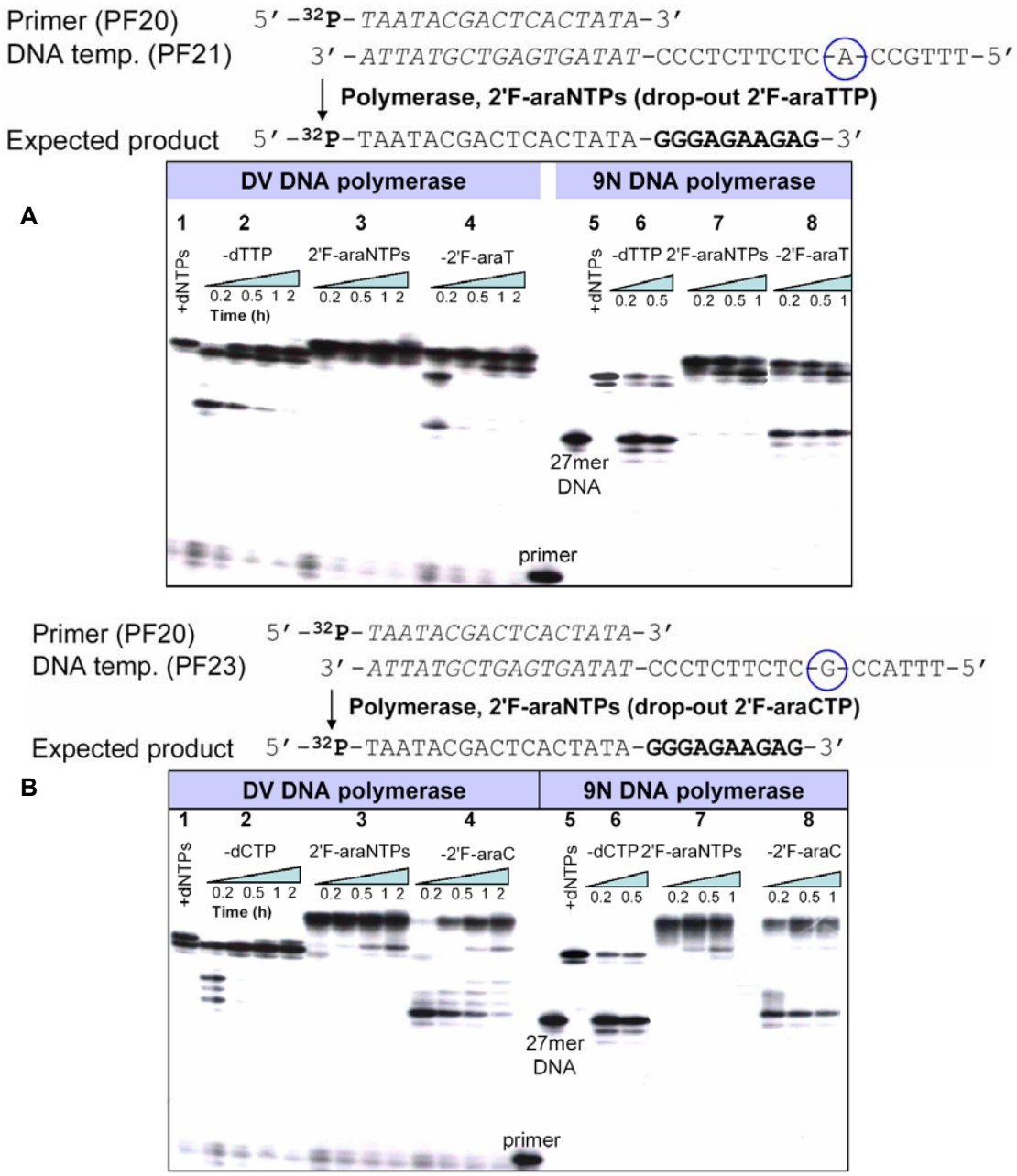

Figure S4 A & B. Fidelity study on (**A**) 2'F-araTTP and (**B**) 2'F-araCTP incorporation by DV and 9N DNA polymerases on a DNA template (**A**) PF21 and (**B**) PF23, respectively. Reaction conditions: 100 nM primer/template (1:1 molar ratio); 0.4 μL (0.8 unit) DV or 9N, 25 μM triphosphate concentration at 55 ^{o}C in 30 μL reaction volume. Reaction time for dNTPs (group 1, 5 in both **A** and **B**) was 30 min and others were shown above. In **A**: group 1 & 5: dNTPs; 2 & 6: drop out dTTP; 3 & 7: 2'F-araNTPs; 4 & 8: drop out 2'F-araTTPs; in **B**: group 1 & 5: dNTPs; 2 & 6: drop out dCTP; 3 & 7: 2'F-araNTPs; 4 & 8: drop out 2'F-araCTPs; PF39 is a 27-nt DNA control (see Table S1). All samples were analyzed by 12% denaturing PAGE.

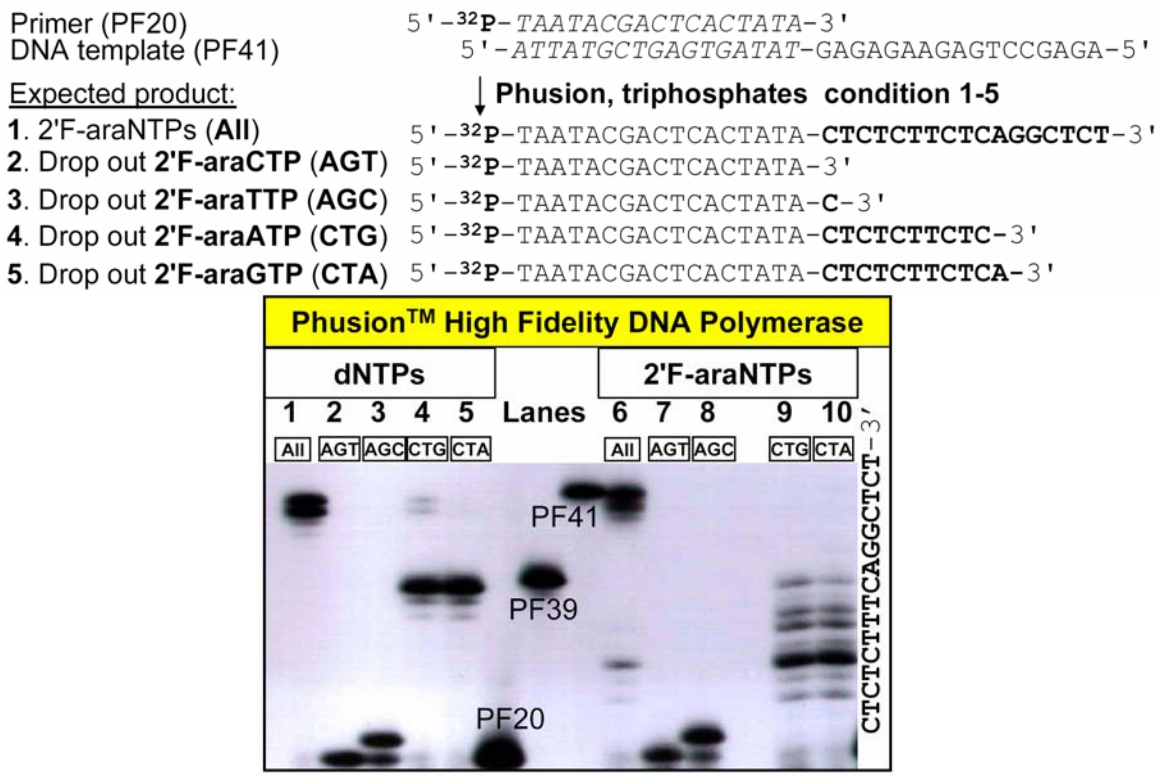

Figure S5. Fidelity study on 2'F-araNTP incorporation by Phusion High Fidelity polymerases on purine rich DNA template PF41. The base sequence of the primer, DNA template and resulting terminated products in different dropout conditions (condition 1-5) is shown. Primer extension conditions: 100 nM primer/template (1:1 molar ratio); for dNTPs (lanes 1-5): 0.4 μL (0.8 unit) Ph, 25 μM dNTP concentration; for 2'F-araNTPs (lanes 6-10): 0.8 μL (1.6 unit) Ph, 0.2 mM 2'F-araNTP at 55 ^{o}C in 30 μL reaction volume for 30 min. Lane 1: dNTPs; 2: drop out dCTP (i.e. dATP, dGTP and dTTP, shorten as AGT, same coding for lanes 3-5), lane 6: 2'F-araNTPs; 7: drop out 2'F-araCTP (i.e. 2'F-araATP, 2'F-araGTP, 2'F-araTTP, shorten as AGT; same coding for lanes 8-10). The template PF41 and the primer PF20 are shown; PF39 is a 27mer DNA control (see Table S1). All samples were analyzed by 12% denaturing PAGE.

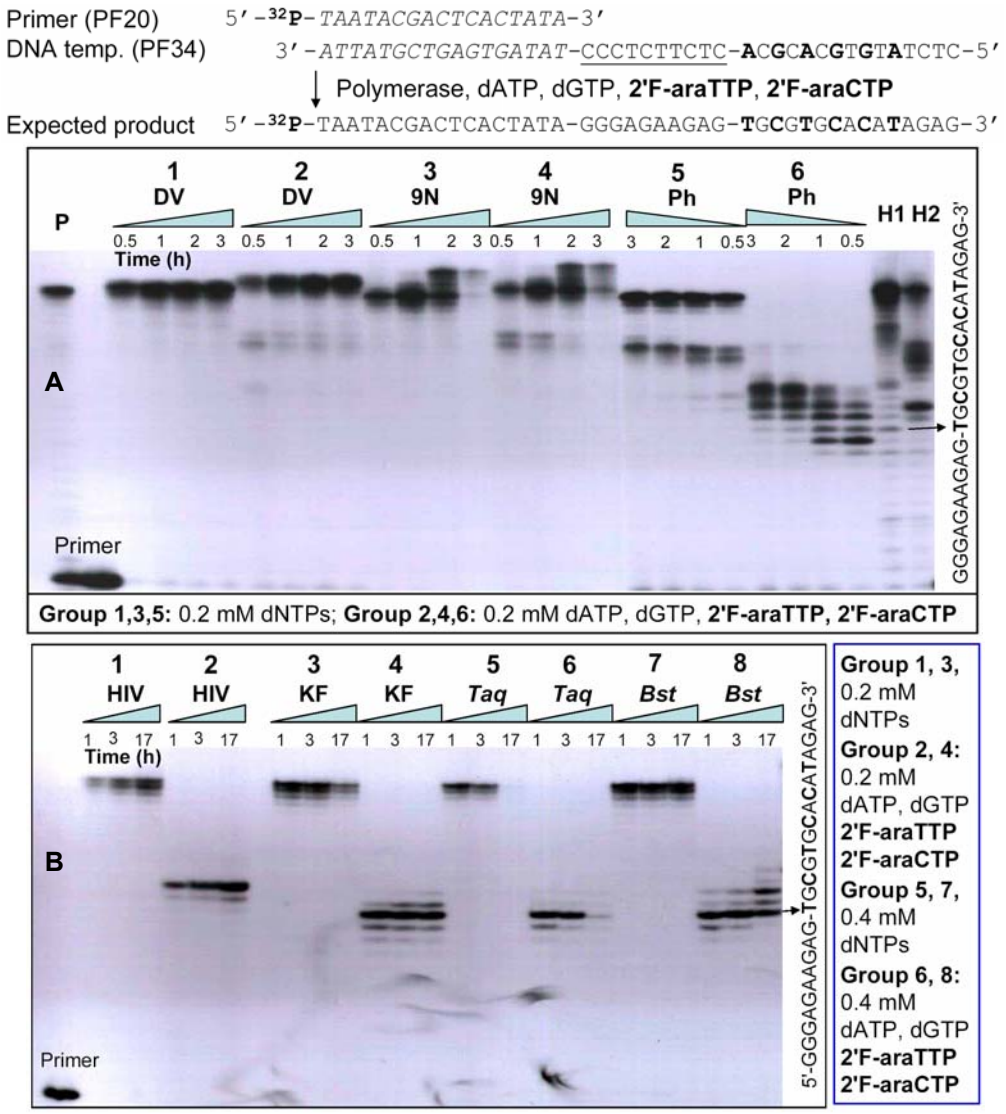

Figure S6. Incorporation of 2'F-araNTPs (3x 2'F-araT and 3x 2'F-araC) units catalyzed by DNA polymerases on a chimeric DNA-FANA template PF34. The identity of DNA polymerases, and the base sequence of the primer, chimeric DNA-FANA template (PF34), and resulting chimeric DNA-FANA product are shown. **A:** 20 μL reaction volume for all, 2 μL enzyme used; **B:** 20 μL reaction volume for all, 1.5 μL HIV-RT, 2 μL KF, *Taq*, *Bst*, respectively; H1 were formed through a chain termination assay with 0.1 mM dATP, dGTP, dCTP, dTTP + ddTTP (1:1 molar ratio), 0.4 μL HIV-RT in 20 μL reaction volume on a DNA template PF32 with the same sequence as PF34 (See Table S1); products shown on lane H2 were formed through a chain termination assay with 0.1 mM dATP, dGTP, dTTP, dCTP + ddCTP (1:1 molar ratio), 0.4 μL HIV-RT in 20 μL reaction volume on a DNA template PF32 with the same sequence as PF34 (See Table S1).

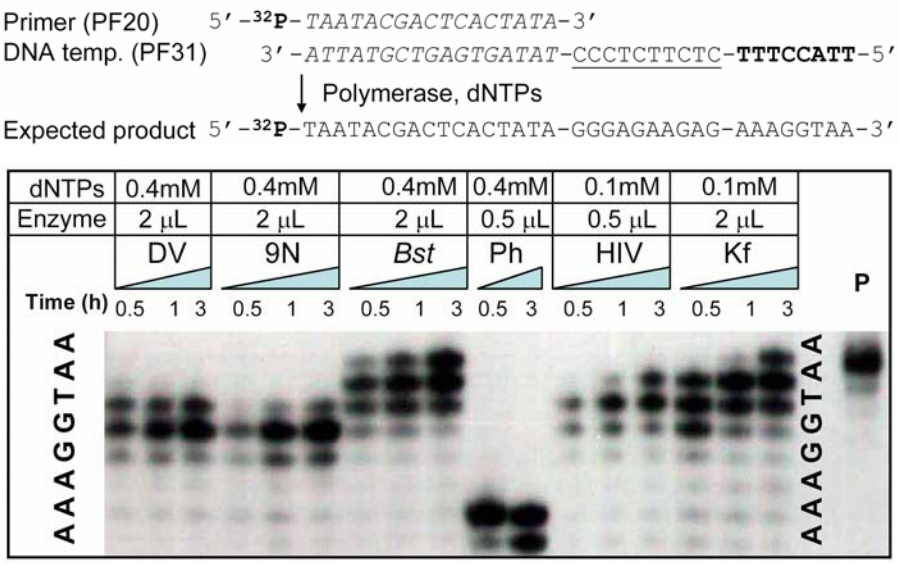

Figure S7. Assay to assess the activity of FANA as a template (PF31) for polymerase-directed DNA synthesis. The identity of the DNA polymerases, the base sequence of the primer, chimeric DNA-FANA template (PF31), and resulting DNA oligonucleotide product are shown. Reaction volume was 30 μL, and dNTP and enzyme amounts/concentrations are also shown.