

SUPPLEMENTARY TABLES FOR NAR-00703-2002.R1

Template (+2)	Substrate Polymerase Primer	% primer extension by dNTP					
		KF- P1	KF- SP1	KF+ SP1	T4+ SP1	T7- P1	T7+ SP1
LTA (T)	dTTP	29	23	13	-	-	-
LTC (A)	dGTP	39	32	-	-	-	-
LTG (A)	dCTP	77	60	8	-	-	-
LTT (A)	dATP	91	85	32	-	-	-
	Incorrect avg	59	50	13	0	0	0

Table S1. Incorrect double addition of dNTP substrates by exonuclease-deficient (-) and proofreading (+) variants of Klenow fragment (KF), T4 DNAP (T4) and T7 DNAP (T7). The identity of the template nucleotide for misincorporation is shown in brackets. “-“ entries indicate values below the limit of detection (< 5%).

	Substrate	% primer extension by acyNTP	
	Polymerase	Vent exo minus	Vent exo plus
	Primer	P1	SP1
	Buffer metal	Mn <sup>2+</sup>	Mn <sup>2+</sup>
LTA	dATP	74	13
	dCTP	57	22
	dGTP	21	-
	<b>dTTP</b>	<b>100</b>	<b>79</b>
LTC	dATP	84	24
	dCTP	15	-
	<b>dGTP</b>	<b>100</b>	<b>80</b>
	dTTP	100	28
LTG	dATP	55	8
	<b>dCTP</b>	<b>100</b>	<b>72</b>
	dGTP	75	14
	dTTP	93	22
LTT	<b>dATP</b>	<b>100</b>	<b>69</b>
	dCTP	74	21
	dGTP	65	8
	dTTP	65	9
	Incorrect avg	64	14
	Correct avg	100	75
	I/C <sub>avg</sub> %	64	19

Table S2. Effect of Mn<sup>2+</sup> on deoxynucleotide incorporation by Vent DNA polymerase. Extension reactions were conducted in the presence of 2 mM MnSO<sub>4</sub> in place of the 2 mM MgSO<sub>4</sub> in the standard reaction buffer. Correct nucleotide incorporation appears in bold. “-” entries indicate values below the limit of detection (< 5%). Reaction conditions consisted of 10 μM primer, 4 μM template, 200 μM substrate, 2 U polymerase, 35 cycles of 30 s at 85 °C, 1 min at 53 °C, 1 min at 63 °C.

Template	Substrate	% primer extension		
	Polymerase	exo <sup>-</sup> Klenow		
	Primer	SP1		
	Exonuclease III	0 units	1 unit	5 units
LTT <sup>a</sup>	dATP	87	94	82
<b>LTA<sup>a</sup></b>	<b>dATP</b>	<b>46</b>	<b>20</b>	<b>9</b>
LTC <sup>a</sup>	dGTP	92	94	90
<b>LTT<sup>a</sup></b>	<b>dGTP</b>	<b>63</b>	<b>45</b>	<b>7</b>
LTG <sup>b</sup>	ddCTP	100	100	100
<b>LTG<sup>b</sup></b>	<b>ddGTP</b>	<b>42</b>	<b>40</b>	-
LTT <sup>b</sup>	ddATP	81	83	80
<b>LTT<sup>b</sup></b>	<b>ddCTP</b>	<b>66</b>	<b>57</b>	-

Table S3. Effect of exonuclease III addition on misincorporation by exonuclease-deficient KF. Incorrect nucleotide incorporation for each template is shown in bold. “-” entries indicate values below the limit of detection (< 5%). In all cases the buffer used was the standard buffer supplied for DNA Pol I.

- a. 10 μM primer, 25 μM template, 200 μM substrate, 1 U polymerase, 60 min at 37 °C.  
b. 10 μM primer, 25 μM template, 1 mM substrate, 2 U polymerase, 4 h at 37 °C.