Supplementary Data

Steric and Electrostatic Effects in DNA Synthesis by the SOS-Induced

DNA Polymerases II and IV of *Escherichia coli*

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Tables S1-S13. Representative incorporation data and Hanes-Woolf plots for Pols II and IV.



Figure S1. Gel showing incorporation of unnatural dNTPs opposite an "A" template with Pol II. Enzyme (200 nM for dHTP and dITP, 100 nM for other samples) was incubated with the indicated concentration of dNTP, and the reaction was quenched with stop buffer after the indicated time point.

<u>%</u>		<u>enzyme</u>		Normalized Incorporation	
incorporation	<u>time</u>	<u>multiplier</u>	<u>[dNTP]</u>	<u>(v)</u>	<u>[dNTP]/v</u>
0.101238	5	0.5	10	0.010124	987.774
0.182822	5	0.5	25	0.018282	1367.454
0.111259	2	0.5	50	0.027815	1797.613
0.184595	2	0.5	100	0.046149	2166.901
0.252109	2	0.5	150	0.063027	2379.924
0.278284	2	0.5	200	0.069571	2874.76
Vmax	0.111849	%/min			
Km	126.6022	uM			
Efficiency	883.4703	Vmax/Km			



Table S1. Incorporation of dHTP opposite "A" template with Pol II. Normalized incorporation is % incorporation times the enzyme multiplier (correction factor for different enzyme concentrations used in experiments) divided by time. The corresponding Hanes-Wolf plot from which values for Vmax and Km were derived is also shown.



Table S2. Incorporation of dFTP opposite "A" template with Pol II. Normalized incorporation is % incorporation times the enzyme multiplier (correction factor for different enzyme concentrations used in experiments) divided by time. The corresponding Hanes-Wolf plot from which values for Vmax and Km were derived is also shown.

		<u>enzyme</u>		Normalized	
				Incorporation	
incorporation	<u>time</u>	<u>multiplier</u>	<u>[dNTP]</u>	<u>(v)</u>	<u>[dNTP]/v</u>
0.069296	0.25	1	10	0.277182	36.07737
0.080131	0.25	1	25	0.320525	77.99708
0.082846	0.25	1	50	0.331383	150.8827
0.079033	0.25	1	100	0.316133	316.3226
0.102513	0.25	1	150	0.41005	365.8089
0.096134	0.25	1	200	0.384535	520.108
Vmax	0.402852	%/min			
Km	9.345365	uM			
Efficiency	43107.16	Vmax/Km			
	dLTP - A2	у =	2.4823x + 23.198		
600			R* = 0.9823		
500					
400	/	/			
400	· / ·		. Series1		
300			Linear (Series1)		
200					
100					
0	1 1				
0 50	100 150	200 25	50		

Table S3. Incorporation of dLTP opposite "A" template with Pol II. Normalized incorporation is % incorporation times the enzyme multiplier (correction factor for different enzyme concentrations used in experiments) divided by time. The corresponding Hanes-Wolf plot from which values for Vmax and Km were derived is also shown.



Table S4. Incorporation of dBTP opposite "A" template with Pol II. Normalized incorporation is % incorporation times the enzyme multiplier (correction factor for different enzyme concentrations used in experiments) divided by time. The corresponding Hanes-Wolf plot from which values for Vmax and Km were derived is also shown.

		<u>enzyme</u>		<u>Normalized</u>	
				Incorporation	
incorporation	<u>time</u>	<u>multiplier</u>	<u>[dNTP]</u>	<u>(v)</u>	<u>[dNTP]/v</u>
0.149672	0.5	0.5	10	0.149672	66.81283
0.176814	0.5	0.5	25	0.176814	141.3913
0.119531	0.25	0.5	50	0.239063	209.15
0.11731	0.25	0.5	100	0.23462	426.2204
0.114332	0.25	0.5	150	0.228664	655.9836
Vmax	0.218403	%/min			
Km	4.667045	uM			
Efficiency	46796.76	Vmax/Km			
		dITP - A2		y = 4.1573x + 21.369	
				R 0.390	
700			•		
600				-	
500				_	
400					
	/			 Series1 Linear (Series1) 	
300					
200	<u> </u>				
100					
0 20 4	0 60	80 100 1:	20 140	160	

Table S5. Incorporation of dITP opposite "A" template with Pol II. Normalized incorporation is % incorporation times the enzyme multiplier (correction factor for different enzyme concentrations used in experiments) divided by time. The corresponding Hanes-Wolf plot from which values for Vmax and Km were derived is also shown.



Figure S2. Gel showing incorporation of natural dATP or dTTP opposite unnatural templates, as indicated, by Pol IV. Enzyme (1000 nM) was incubated with the indicated concentration of dNTP, and the reaction was quenched with stop buffer after the indicated time point.



Table S6. Incorporation of dATP opposite "H" template with Pol IV. Normalized incorporation is % incorporation times the enzyme multiplier (correction factor for different enzyme concentrations used in experiments) divided by time. The corresponding Hanes-Wolf plot from which values for Vmax and Km were derived is also shown.

		<u>enzyme</u>		<u>Normalized</u> Incorporation	
incorporation	<u>time</u>	<u>multiplier</u>	[dNTP]	<u>(v)</u>	[dNTP]/v
0.052046	20	0.25	50	0.000651	76854.78
0.097885	20	0.25	150	0.001224	122592.9
0.140853	20	0.25	200	0.001761	113593.3
0.083364	12	0.25	250	0.001737	143946.7
0.085068	12	0.25	300	0.001772	169276.4
0.117203	12	0.25	400	0.002442	163817.7
0.139043	12	0.25	500	0.002897	172608.4
Vmax	0.004758	%/min			
Km	390.0186	uM			
Efficiency	12.19839	Vmax/Km			



Table S7. Incorporation of dTTP opposite "H" template with Pol IV. Normalized incorporation is % incorporation times the enzyme multiplier (correction factor for different enzyme concentrations used in experiments) divided by time. The corresponding Hanes-Wolf plot from which values for Vmax and Km were derived is also shown.



Table S8. Incorporation of dATP opposite "F" template with Pol IV. Normalized incorporation is % incorporation times the enzyme multiplier (correction factor for different enzyme concentrations used in experiments) divided by time. The corresponding Hanes-Wolf plot from which values for Vmax and Km were derived is also shown.



Table S9. Incorporation of dTTP opposite "F" template with Pol IV. Normalized incorporation is % incorporation times the enzyme multiplier (correction factor for different enzyme concentrations used in experiments) divided by time. The corresponding Hanes-Wolf plot from which values for Vmax and Km were derived is also shown.

			<u>enzyme</u>		Normalized		
					Incorporation in the second se	on	
incorp	<u>ooration</u>	<u>time</u>	<u>multiplier</u>	<u>[dNTP]</u>	<u>(v)</u>		[dNTP]/v
0	.082928	4	0.25	50	0.005	183	9646.878
0	.114042	4	0.25	100	0.007	128	14029.94
0	.128377	4	0.25	150	0.008)24	18694.94
0	.146734	4	0.25	200	0.009	171	21808.18
0	.179946	4	0.25	250	0.0112	247	22228.95
	0.1781	4	0.25	300	0.011	131	26951.16
Vmax		0.01532	%/min				
Km		114.442	uM				
Efficie	ncy	133.8652	Vmax/Km				
			L - dATP	у =	65.275x + 7470.2]	
20000					R ² = 0.9622		
1 3000 T					~		
25000							
20000				· ·			
≧							
[dAT 12000 1		_					
10000	•						
5000							
0	50	100	150 200	250	300 350		

[dATP]

Table S10. Incorporation of dATP opposite "L" template with Pol IV. Normalized incorporation is % incorporation times the enzyme multiplier (correction factor for different enzyme concentrations used in experiments) divided by time. The corresponding Hanes-Wolf plot from which values for Vmax and Km were derived is also shown.

		<u>enzyme</u>		Normalized		
				Incorporation		
incorporation	<u>time</u>	<u>multiplier</u>	<u>[dNTP]</u>	<u>(v)</u>	<u>[dNTP]/v</u>	
0.051241	20	0.25	100	0.000641	156125	
0.069287	16	0.25	200	0.001083	184738.2	
0.053887	10	0.25	250	0.001347	185573.5	
0.062694	10	0.25	300	0.001567	191405.9	
0.079114	10	0.25	400	0.001978	202239.8	
0.092157	10	0.25	500	0.002304	217021.9	
Vmax	0.007226	%/min				
Km	1077.865	uM				
Efficiency	6.704435	Vmax/Km				
		L-dTTP		y=138.38x+14915	5	
250000				R ⁻ =0.942		
			_			
200000		• • •				
≥ 150000	•					
I T P						
- 100000						
50000						
0	100 2	00 300	400	500 6	1	
[4TD]						



		<u>enzyme</u>		Norma	alized	
				Incorp	oration	
incorporation	<u>time</u>	<u>multiplier</u>	[dNTP]	<u>(v)</u>		<u>[dNTP]/v</u>
0.059387	10	0.25	100		0.001485	67354.27
0.060803	10	0.25	150		0.00152	98679.49
0.080196	10	0.25	200		0.002005	99755.1
0.062637	6	0.25	250		0.00261	95789.32
0.067336	6	0.25	300		0.002806	106927.2
0.091478	6	0.25	400		0.003812	104942.9
0.118448	6	0.25	500		0.004935	101309.9
Vmax	0 017047	%/min				
Km	1371 838	uM				
Efficiency	12.42668	Vmax/Km				
	B	- dat P		9 66% 90472	1	
	5		y – 5 F	R ² = 0.3842		
120000						
100000	+ +	+	• •			
80000						
60000	•					
번 40000						
20000						
0	100 200	300 4	100 500	600		
		[dATP]				

Table S12. Incorporation of dATP opposite "B" template with Pol IV. Normalized incorporation is % incorporation times the enzyme multiplier (correction factor for different enzyme concentrations used in experiments) divided by time. The corresponding Hanes-Wolf plot from which values for Vmax and Km were derived is also shown.



Table S13. Incorporation of dTTP opposite "B" template with Pol IV. Normalized incorporation is % incorporation times the enzyme multiplier (correction factor for different enzyme concentrations used in experiments) divided by time. The corresponding Hanes-Wolf plot from which values for Vmax and Km were derived is also shown.



Table S13. Incorporation of dATP opposite "I" template with Pol IV. Normalized incorporation is % incorporation times the enzyme multiplier (correction factor for different enzyme concentrations used in experiments) divided by time. The corresponding Hanes-Wolf plot from which values for Vmax and Km were derived is also shown.

		<u>enzyme</u>		<u>Norn</u> Inco	<u>nalized</u> rporation	
incorporation	<u>time</u>	<u>multiplier</u>	[dNTP]	<u>(v)</u>		[dNTP]/v
0.052658	20	0.25	200		0.000658	303848.1
0.059938	20	0.25	250		0.000749	333680.3
0.076865	20	0.25	300		0.000961	312235.6
0.093173	20	0.25	400		0.001165	343447.3
0.112019	20	0.25	500		0.0014	357083.2
Vmax	0.006316	%/min				
Km	1754.497	uM				
Efficiency	3.599621	Vmax/Km				
	I - dī	TTP	y = 158.34x + R ² = 0.7	277807 545		
360000						
350000			/			
340000						
â 330000	•	/				
320000						
310000						
300000	•					
100 150	200 250 300	J 350 400	450 500	550		

Table S13. Incorporation of dTTP opposite "I" template with Pol IV. Normalized incorporation is % incorporation times the enzyme multiplier (correction factor for different enzyme concentrations used in experiments) divided by time. The corresponding Hanes-Wolf plot from which values for Vmax and Km were derived is also shown.