

Table S1. Highly conserved residues					
Shared region	Structural feature	Residue ^a	BLOSUM62 information score ^b	Sequence identity (%)	Role
β subunit					
α4	β1	G131	0.9829	96.69	
a8	FL2	R428	0.9992	100	
		G446	0.9946	99.62	
		G450	0.9988	99.92	
a10		R557	0.9961	99.85	<ul style="list-style-type: none"> • Interacts with substrate γ-phosphate • Participates in E-site nucleotide binding
		Q567	0.9953	99.85	<ul style="list-style-type: none"> • Interacts with RNA O3' at the -3 position
a11		G669	0.9959	99.69	
		G681	0.9932	99.00	
		D686	1	100	<ul style="list-style-type: none"> • Interacts with β'D739/F740/D741 (in β'-NADFDGD motif) • Interacts with βR879 • Interacts with substrate γ-phosphate • Chelates MgII
a12	flap	G741	0.9948	99.54	

Shared region	Structural feature	Residue ^a	BLOSUM62 information score ^b	Sequence identity (%)	Role
a14		G836	0.9995	99.92	
		D837	0.9985	99.92	• Interacts with β H999
		K838	1	100	• Interacts with RNA phosphate backbone at -1 position
		K846	0.9990	99.92	• Interacts with RNA phosphate backbone at -1 position • Participates in E-site nucleotide binding
		G847	0.9978	99.85	
		P859	0.9920	99.77	
		G864	0.9985	99.92	
		R879	0.99920	100	• Interacts with β D686 • Interacts with substrate γ -phosphate • Participates in E-site nucleotide binding
		E887	0.9852	99.15	• Participates in buried salt-bridge with β R842/ β H843

Shared region	Structural feature	Residue ^a	BLOSUM62 information score ^b	Sequence identity (%)	Role	
a15		G990	0.9916	99.31		
		L997	1	100		
		H999	0.9919	99.69	• Interacts with RNA O2' at -3 position	
		K1004	0.9963	99.85	• Interacts with RNA O2' at -2 position	
		R1008	0.9985	99.92		
	Sw3		G1011	0.9950	99.38	• lines RNA exit channel
			Q1019	0.9932	99.92	
			G1023	0.9987	99.92	• lines path of template DNA
			G1028	1	100	• lines path of template DNA
			G1029	0.99920	99.92	• lines path of template DNA
		G1033	1	100	• Interacts with β 'Sw2 region • lines path of template DNA	
		E1034	1	100	• Participates in salt bridge with β 'R615 (in β 'Sw2 region)	
		M1035	1	100	• Interacts with template DNA at -1 position	
		E1036	1	100		
		G1044	0.9973	99.77	• Interacts with β 'G1475	
		L1053	0.9851	97.85		
β '						
a3		C57	0.9924	99.92		
a8		R525	0.9956	99.92		

Shared region	Structural feature	Residue ^a	BLOSUM62 information score ^b	Sequence identity (%)	Role	
a11	Sw2	K610	0.9992	100	• Interacts with template DNA phosphate backbone at -1/+1 positions	
		R615	0.9992	100	• Interacts with template DNA phosphate backbone at +2 position	
		R622	0.9973	99.83	• Interacts with template DNA phosphate backbone at -3 position	
			R628	1	100	• Interacts with template DNA phosphate backbone at -3 position
			V630	0.9965	99.83	
			P645	0.9984	99.92	
			L652	0.9892	97.31	

Shared region	Structural feature	Residue ^a	BLOSUM62 information score ^b	Sequence identity (%)	Role
a12	NADFDGD motif	R704	1	100	<ul style="list-style-type: none"> • Interacts with β'N737/A738/D743 of β'-NADFDGD motif • Interacts with RNA O2' at -1 position • Interacts with substrate
		P706	1	100	
		L708	1	100	• Interacts with β 'T1234 in the TL
		H709	0.9934	99.83	
		N737	1	100	• Interacts with substrate O2'/O3'
		A738	0.9881	98.82	
		D739	1	100	<ul style="list-style-type: none"> • Interacts with βD686 • Chelates MgI/MgII
		F740	0.9937	99.75	<ul style="list-style-type: none"> • Interacts with βD686 • Participates in hydrophobic core behind active site
		D741	1	100	<ul style="list-style-type: none"> • Interacts with βD686 • Chelates MgI • Interacts with RNA at -1 position
		G742	1	100	
		D743	1	100	<ul style="list-style-type: none"> • Chelates MgI • Interacts with RNA at -1 position
		E758	1	100	
		D784	0.9931	99.58	

Shared region	Structural feature	Residue ^a	BLOSUM62 information score ^b	Sequence identity (%)	Role
a15		G1027	0.9907	99.07	
		G1043	0.9946	99.49	
		G1064	0.9970	99.92	
	Bridge helix	R1078	0.9952	99.74	• Interacts with β R428
		G1080	0.9811	96.46	• Interacts with β 'F1241 of the TL
		D1083	0.9813	98.65	
		T1088	0.9986	99.92	• Interacts with template DNA at the +1 position • Interacts with β 'T1234 of the TL
		G1092	1	100	• Gly required at this position to make room for the template DNA
		R1096	0.9992	100	• Interacts with the template DNA phosphate backbone at the +2 position
a16	TL helix1	G1222	1	100	
		T1234	1	100	• Interacts with β 'L708 • Interacts with β 'T1088 of the bridge helix
		Q1235	1	100	
	TL	F1241	0.9977	99.83	
		H1242	1	100	• Interacts with substrate phosphates
a20		L1447	0.9847	98.40	
		G1475	0.9931	99.41	• Interacts with β G1044

^a According to *Thermus* RNAP sequence numbering.

^b As calculated using the program PFAAT {Caffrey, 2007 #1608}

Table S2. Switch regions¹.

Switch	Subunit		Residues		Mean Blosum62 information score	Absolutely conserved residues
	eRNAP II	bRNAP	<i>Sce</i> RNAP II	<i>Thermus</i> RNAP		
1	Rpb1	β'	1384-1406	1384 – (not shared)	-	-
2	Rpb1	β'	328-246	606 - 624	0.85	K610 R615 R622
3	Rpb2	β	1107-1129	1010-1031	0.81	G1011 Q1019 G1023 G1028 G1029
4	Rpb2	β	1152-1159	1054-1061	0.68	-
5	Rpb1	β'	1431-1433	1469-1471	0.76	-

¹ As defined by Gnatt et al. ¹¹