SUPPLEMENTAL DATA:

Association of ω with the C-terminal region of β' subunit is essential for assembly of RNA polymerase in *Mycobacterium tuberculosis*

Running title: The essential role of ω in *Mtb* RNAP assembly

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Supplementary Methods

Plasmid construction

Plasmids and oligonucleotides used in this study are listed in Tables S1 and S2, respectively. PCR fragments were inserted into linearized vectors with a ClonExpress II One Step Cloning Kit (Vazyme, China). Mutations in genes were constructed with a QuikChange II XL Site-Directed Mutagenesis Kit (Stratagene). Details are described as below.

The pMtRc expressing *Mtb* RNAP core (with His-tag at the C-terminal of β' subunit) was constructed based on the pMR4 plasmid (1), in which a T7 promoter was inserted upstream of the *rpoC* gene. Primer paris MtbrpoB-BsrGI-F/MtbrpoB-R and T7p-MtbrpoC-F/ MtbrpoC-NotI-R were used to amplify two fragments covering the C-terminal of *rpoB* gene and the N-terminal of *rpoC* gene of *Mtb* from the pMR4 plasmid. These two fragments were fused into one fragment by overlap PCR, in which a T7 promoter was introduced upstream of the *rpoC* gene. This fragment was then inserted into the pMR4 plasmid between the BsrGI and NotI sites. The pMtRcΔω plasmid expressing *Mtb* coreΔω was constructed based on pMtRc, in which the *rpoZ* gene was deleted using a Site-Directed Mutagenesis Kit. Mutation of β'CTD in *Mtb* core were introduced based on pMtRc as indicated in Table S1 using the Site-Directed Mutagenesis Kit.

Plasmids (pPaRc, pTtRc, pEcRc) expressing RNAP core enzymes (all with with His-tag at the C-terminal of β' subunit) from *Pae*, *Tth* and *Eco* were constructed in a similar way. Briefly, the *rpoA* and *rpoZ* genes from each bacterium were amplified and fused into one fragment named *rpoA-rpoZ* by overlap PCR, in which a ribosomal binding site (RBS) was introduced upstream of the *rpoZ* gene. The *rpoB* and *rpoC* genes from each strain were also amplified respectively. All these fragments were inserted into pET21a plasmid using the ClonExpress II One Step Cloning Kit to obtain pET21a-AZ, pET21a-B, and pET21a-C plasmids, respectively. Next, a fragment covering the T7 promoter and the *rpoC* gene from the pET21a-C plasmid was

amplified and inserted into the pET21a-AZ (downstream of the *rpoZ* gene) to obtain a plasmid named pET21a-AZC. Similarly, a fragment covering the T7 promoter and the *rpoB* gene from the pET21a-B plasmid was amplified and inserted into the pET21a-AZC (upstream of the *rpoC* gene) to obtain a plasmid named pET21a-AZBC, which was renamed as pEcRc, or pPaRc, or pTtRc depending on the source of the RNAP genes. Each of these plasmids carries a T7 promoter (T7p), followed with an RBS and the *rpoA* gene, an RBS and the *rpoZ* gene, the second T7p, an RBS and the *rpoB* gene, the third T7p, an RBS and the *rpoC* gene, and a T7 terminator. For plasmids expressing core enzymes lacking the ω subunit, the *rpoZ* gene was omitted as described above.

Plasmids expressing RNAP core from *Msm* and *Sco* were constructed based on a two-plasmid system. A p15A ori plasmid with Kan resistance gene was used to express α, ω (wherever it is present) and β subunits, and pET21a was used to express β' subunit. All these clones were constructed as described above using the ClonExpress II One Step Cloning Kit.

Plasmids expressing ω^{Flag} (without other tag) was constructed base on pET28a using the ClonExpress II One Step Cloning Kit. Plasmids expressing each of RNAP subunits (α^{His}, β, β', ω^{His}) used in RNAP *in vitro* reconstitution were constructed based on pET21a or pET28a respectively. Tag peptides (His-tag, His^{TEV}-tag, or His-$SUMO^{TEV}$-tag) were introduced into pET21a or pET28a using the QuikChange II XL Site-Directed Mutagenesis Kit or ClonExpress II One Step Cloning Kit, which were then used to express *Mtb* σ^A, σ^B and RbpA respectively as shown in Table S1. The pET21a plasmid was used to express Eco σ^{70}. All these clones were constructed as described above using the ClonExpress II One Step Cloning Kit.

Protein expression and purification

E. coli BL21(DE3) Δ*rpoZ* mutant was constructed by the CRISPR-Cas9 system as previously described (2). Briefly, the pTargetF plasmid (2) expressing the sgRNA targeting the *Eco rpoZ* gene sequence (GCGCTGCGCGAAATCGAAGA), and the donor DNA for homologous

recombination, were co-electroporated into electrocompetent *E. coli* cells harboring the pCas plasmid. Mutants were identified by colony PCR and confirmed by DNA sequencing.

Plasmids expressing the RNAP core, coreΔω, or other core mutants from *Mtb*, *Msm*, *Eco*, *S. coelicolor* (*Sco*), *P. aeruginosa* (*Pae*), or *T. thermophilus* (*Tth*), which all encode a C-terminal His-tag of β' subunit, were transformed into the *E. coli* BL21(DE3) Δ*rpoZ* strain. Protein expression was induced by the addition of 0.3 mM isopropyl β-D-thiogalactopyranoside (IPTG) at an OD_{600} of ~0.6, followed by another incubation at 20 ℃ for 15 h. Cells were harvested by centrifugation, re-suspended in buffer A (40 mM Tris-HCl pH 7.9, 500 mM NaCl, 5% glycerol) supplemented with 10 mM imidazole and 1 mM PMSF, and lysed by sonication. The lysate was centrifuged, and the supernatant was loaded onto a 5 mL Ni^{2+}-affinity column (GE Healthcare) equilibrated in buffer A containing 10 mM imidazole. The column was then washed with 6 column volume (CV) buffer A containing 30 mM imidazole and eluted with 4 CV buffer A containing 80 mM imidazole. Next, the elution was diluted and then loaded on a 5 mL heparin column (GE Healthcare) equilibrated with buffer B (20 mM Tris-HCl pH 7.9, 5% glycerol, 1 mM EDTA, 1 mM 2-mercaptoethanol) plus 300 mM NaCl. The column was washed with 4 CV buffer B containing 300 mM NaCl and eluted with 3 CV buffer B containing 600 mM NaCl. The sample was further purified by anion-exchange chromatography (Mono Q 5/50 GL, GE Healthcare) using 15 CV buffer B with linear gradient of 300-500 mM NaCl. Finally, fractions containing RNAP was purified by size exclusion chromatography (Superdex 200, GE Healthcare) equilibrated in 20 mM Tris-HCl pH 7.9, 300 mM NaCl, 5% glycerol, 1 mM dithiothreitol.

To express ω^{Flag} together with *Mtb* RNAP coreΔω, the pET28a-*Mtb*rpoZ-Flag plasmid or its derivatives (pET28a-*Mtb*rpoZ-E^{loop}-Flag, pET28a-*Mtb*rpoZ-T^{loop}-Flag, pET28a-*Mtb*rpoZ-M1-Flag, pET28a-*Mtb*rpoZ-M2-Flag, pET28a-*Mtb*rpoZ-M3-Flag, pET28a-*Mtb*rpoZ-M4-Flag, pET28a-*Mtb*rpoZ-M5-Flag. Information for these plasmid is listed in Table S1) were co-transformed with the pMtRcΔω plasmid into the *E. coli* BL21(DE3) Δ*rpoZ* strain. Protein expression and purification were performed as described above.

Mtb σ^A, σ^B, and RbpA were expressed from pET-His^{TEV}-σ^A, pET-sumo^{TEV}-σ^B, and pET-His^{TEV}-RbpA in *E. coli* BL21(DE3) and purified by Ni^{2+}-affinity column (GE Healthcare), followed by TEV protease cleavage (3) and size exclusion chromatography (Superdex 200, GE Healthcare). *Eco* σ^{70} was expressed from pET21a-*Eco*σ^{70} and purified by Ni^{2+}-affinity column (GE Healthcare) and size exclusion chromatography (Superdex 200, GE Healthcare).

Supplementary figures and legends:

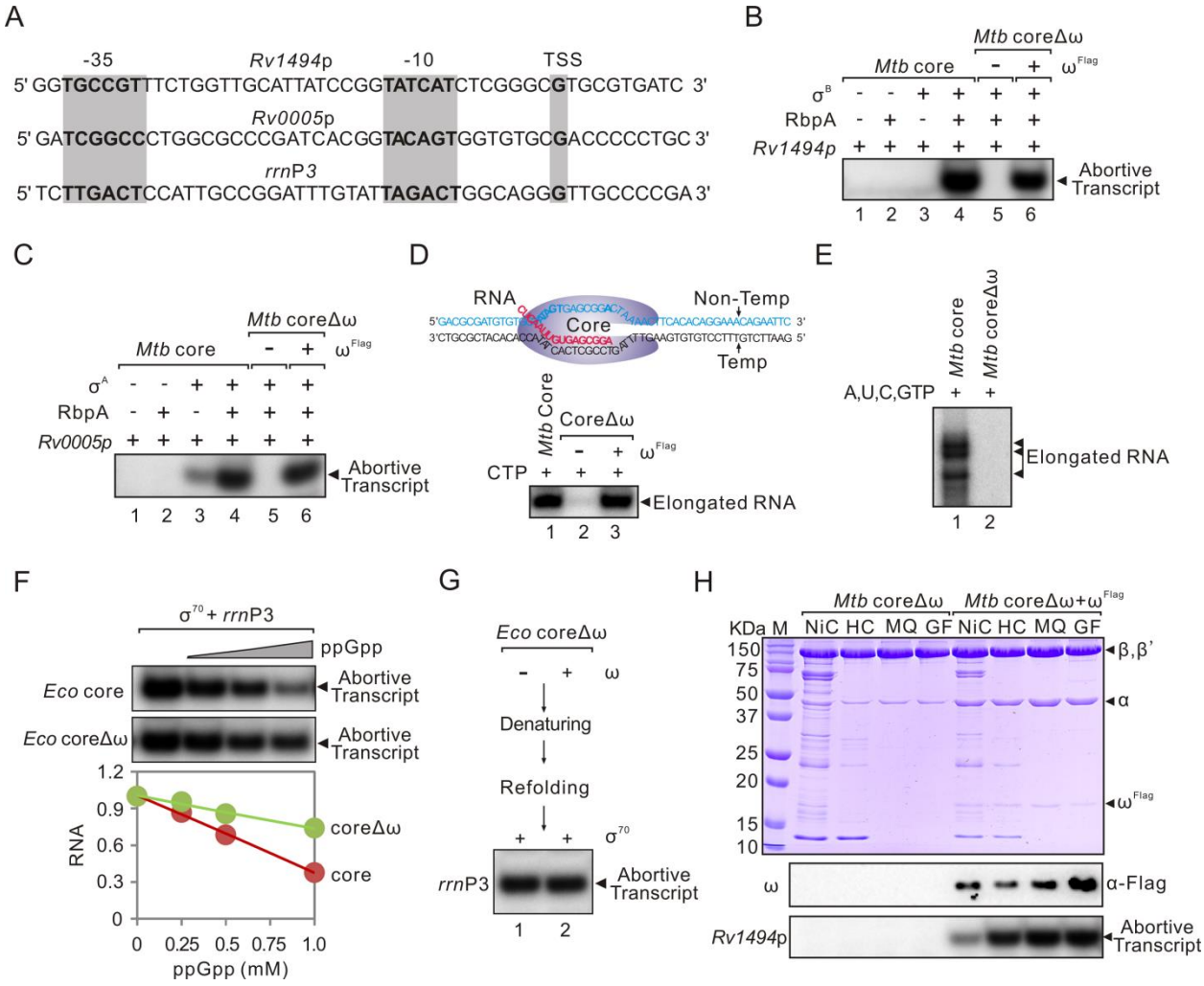

Figure S1. Transcriptional activity of *Mtb and Eco* core and coreΔω.

(**A**) Sequences of the promoters used in *in vitro* transcription assays. (**B**) *In vitro* abortive transcription of *Mtb* core or coreΔω reconstituted with σ^B at *Rv1494*p. RbpA protein was added into reaction where indicated. (**C**) *In vitro* abortive transcription activity of *Mtb* core or coreΔω at *Rv0005*p template. RbpA and σ^A were added as indicated. (**D**) Transcriptional activities of *Mtb* core and coreΔω in a σ-independent transcription assay. A scheme for this assay is shown at the upper part. CTP was used as the substrate in these reactions. (**E**) Activities of *Mtb* core and coreΔω in σ-independent transcription reactions using a mixture of ATP, UTP, CTP and GTP as substrates. (**F**) *In vitro* abortive transcription of *Eco* core or coreΔω at *rrn*P3 promoter. The σ^{70}

was added to reconstitute RNAP for promoter-dependent transcription. ppGpp was added at concentrations of 0, 0.25, 0.5 and 1.0 mM. Quantifications of RNA products are shown at the bottom. (**G**) *In vitro* abortive transcription of *in vitro* reconstituted *Eco* core in the presence or absence of the ω subunit at *rrn*P3 promoter. (**H**) SDS-PAGE analysis of purified *Mtb* core and coreΔω from the Ni-column (NiC), Heparin-column (HC), Mono Q (MQ), and Superdex 200 (GF) steps are shown in the top section. A western-blot for the Flag-tagged ω subunit is shown in the middle section. *In vitro* abortive initiation transcription of these RNAPs at *Rv1494*p are shown in the bottom section. RbpA and σ^A were added in these reactions to reconstitute RNAP for promoter-dependent transcription.

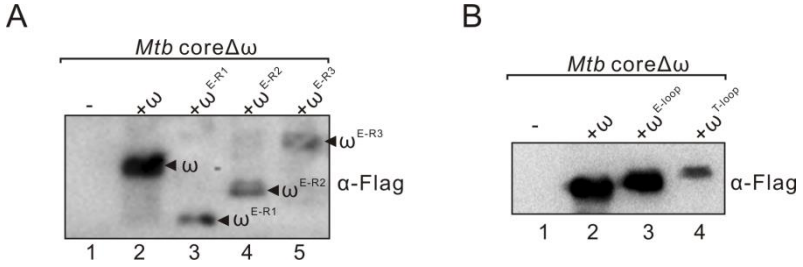

Figure S2. Soluble expression of mutated ω subunit in RNAP expression system.

The lysate supernatants of *Mtb* coreΔω co-expressed with mutated ω subunit (A: $\omega^{E\text{-}R1}$, $\omega^{E\text{-}R2}$ and $\omega^{E\text{-}R3}$; B: $\omega^{E\text{-}loop}$ and $\omega^{T\text{-}loop}$) were analyzed by western blotting via anti-Flag antibody to confirm the soluble expression of these ω derivatives.

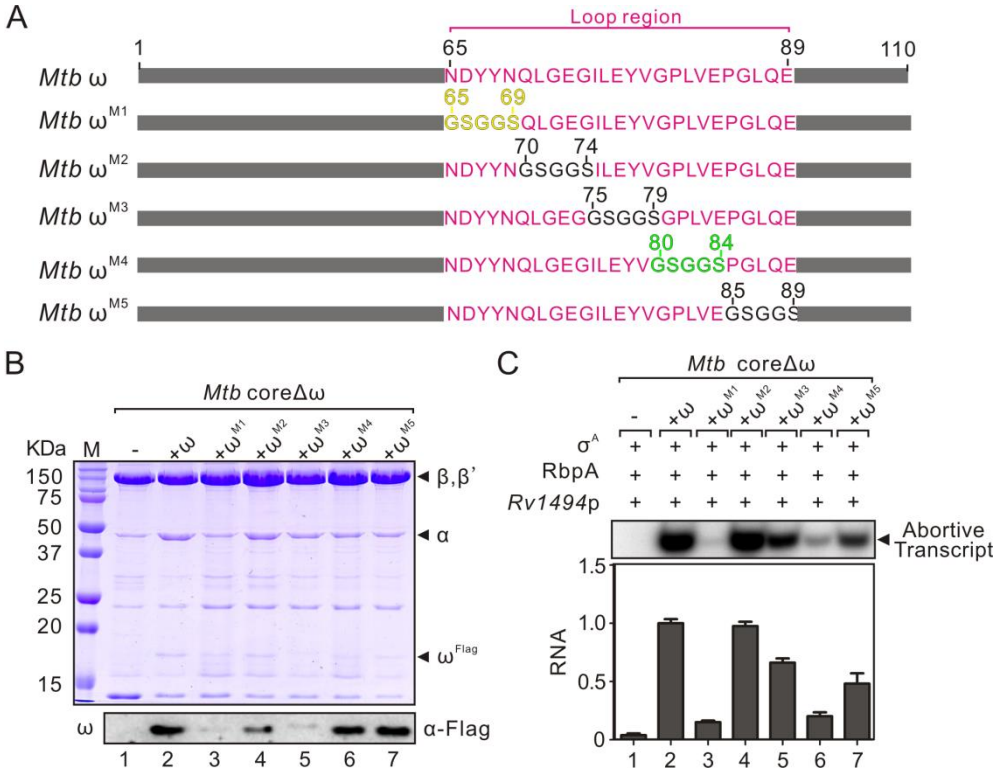

Figure S3. The loop region of ω is important for *Mtb* core.

(**A**) Mutations of *Mtb* ω loop regions by GSGGS linker replacement. (**B**) SDS-PAGE analysis of purified *Mtb* coreΔω co-expressed with the loop-mutated ω subunit is shown at the upper part. Western-blot for ω^{Flag} is shown in the bottom section. (**C**) *In vitro* abortive transcription of purified *Mtb* coreΔω derivatives at *Rv1494*p. RbpA and σ^{A} were added in each reaction in this promoter-dependent transcription assay. Quantifications of transcripts from three independent tests are shown in the bottom section.

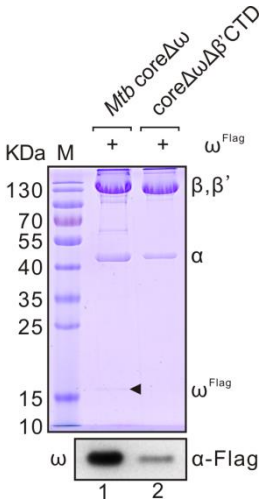

Figure S4. Purified *Mtb* coreΔω and coreΔωΔβ'CTD co-expressed with Flag-tagged ω subunit.

SDS-PAGE of purified *Mtb* coreΔω and coreΔωΔβ'CTD is shown in the upper panel and western blot analysis of ω^{Flag} subunit using a Flag-tag antibody is shown at the bottom panel.

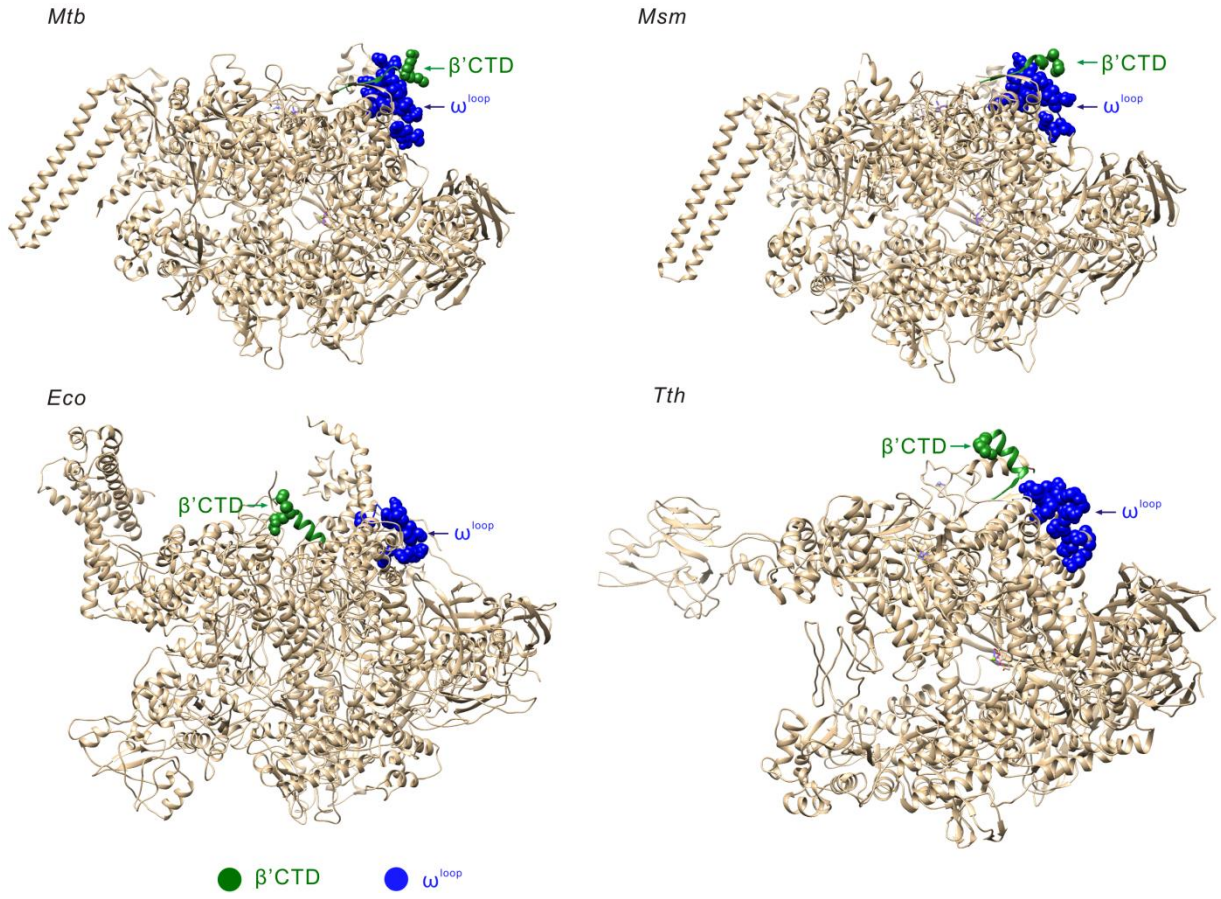

Figure S5. Locations of β'CTD and ω loop regions on bacterial RNAP structures.

Structures of RNAP from *Mtb* (PDB: 5UH8), *Msm* (PDB: 5TW1), *Eco* (PDB: 4YG2) and *Tth* (PDB: 1IW7) are shown. The ω loop in structure is indicated in blue and the β'CTD region is indicated in green.

Supplementary tables:

Table S1. Genomic DNA and plasmids used in this study

Name	Description*	Resource
Genomic DNA		
M. tuberculosis H37Rv		BEI Resources
M. smegmatis mc^2155		Dr. Jiaoyu Deng
S. coelicolor M145		Dr. Gang Liu
E. coli K12 MG1655		Dr. Qingsheng Qi
P. aeruginosa PAO1		Dr. Dongru Qiu
T. thermophiles HB8		Dr. Yu Zhang
Plasmids		
pET28a	Protein expression plasmid, T7 promoter, Kan^R	Lab collection
pET21a	Protein expression plasmid, T7 promoter, Amp^R	Lab collection
pMtRc	Plasmid expressing all subunits of *Mtb* RNA polymerase core enzyme, with His-tag at the C-terminal of β' subunit, Amp^R	This study
pMtRcΔω	Plasmid expressing the α, β, and β' subunits of *Mtb* RNA polymerase, with His-tag at the C-terminal of β' subunit, Amp^R	This study
pMtRcΔβ'CTD	Based on pMtRc, with a deletion of *Mtb*β'CTD (1271-1316 aa), Amp^R	This study
pMtRcΔωΔβ'CTD	Based on pMtRcΔω, with a deletion of *Mtb*β'CTD (1271-1316 aa), Amp^R	This study
pET28a-*Mtb*rpoA	pET28a expressing the α subunits of *Mtb* RNA polymerase with N-terminal His-tag, Kan^R	This study
pET21a-*Mtb*rpoB	Plasmid expressing the β subunits of *Mtb* RNA polymerase, without tag, Amp^R	This study
pET21a-*Mtb*rpoC	pET21a expressing the β' subunits of *Mtb* RNA polymerase, without tag, Amp^R	This study
pET21a-*Mtb*rpoZ	pET21a expressing the ω subunits of *Mtb* RNA polymerase with C-terminal His-tag, Amp^R	This study
pET28a-His^{TEV}-σ_A	pET28a expressing *Mtb* σ^A (with deletion of 1-165aa), with N-terminal His^{TEV}-tag, Kan^R	This study
pET28a-$sumo^{TEV}$-σ^B	pET28a expressing *Mtb* σ^B protein with N-terminal $sumo^{TEV}$-tag, Kan^R	This study
pET28a-His^{TEV}-RbpA	pET28a expressing *Mtb* RbpA with N-terminal His^{TEV}-tag, Kan^R	This study
pET28a-*Mtb*rpoZ-Flag	pET28a expressing *Mtb* ω subunit with C-terminal Flag-tag (without His-tag), Kan^R	This study
pET28a-*Mtb*rpoZ-EcR1-Flag	pET28a expressing *Mtb* ω^{EcR1} (deletion of *Mtb* ω 1-35 aa) subunit with C-terminal Flag-tag (without His-tag), Kan^R	This study
pET28a-*Mtb*rpoZ-EcR2-Flag	pET28a expressing *Mtb* ω^{EcR2} (*Mtb* ω 36-103 aa was replaced by *Ec* ω 2-58 aa) subunit with C-terminal Flag-tag (without His-tag), Kan^R	This study
pET28a-*Mtb*rpoZ-	pET28a expressing *Mtb* ω^{EcR3} (*Mtb* ω 104-110 aa was	This study

EcR3-Flag	replaced by *Ec* ω 59-91 aa) subunit with C-terminal Flag-tag (without His-tag), Kan^R	
pET28a-*Mtb*rpoZ-E^{loop}-Flag	pET28a expressing ω subunit carrying the loop region from *E. coli* ω(*Mtb* ω 65-89 aa was replaced by *Ec* ω 31-44 aa), with C-terminal Flag-tag (without His-tag), Kan^R	This study
pET28a-*Mtb*rpoZ-T^{loop}-Flag	pET28a expressing ω subunit carrying the loop region from *T. thermophiles* ω(*Mtb* ω 65-89 aa was replaced by *Tth* ω 31-58 aa), with C-terminal Flag-tag (without His-tag), Kan^R	This study
pET28a-*Mtb*rpoZ-M1-Flag	pET28a expressing *Mtb* ω^{M1} (*Mtb* ω 65-69 aa was replaced by GSGGS linker) subunit with C-terminal Flag tag and without His tag, Kan^R	This study
pET28a-*Mtb*rpoZ-M2-Flag	pET28a expressing *Mtb* ω^{M2} (*Mtb* ω 70-74 aa was replaced by GSGGS linker) subunit with C-terminal Flag-tag (without His-tag), Kan^R	This study
pET28a-*Mtb*rpoZ-M3-Flag	pET28a expressing *Mtb* ω^{M3} (*Mtb* ω 75-79 aa was replaced by GSGGS linker) subunit with C-terminal Flag-tag (without His-tag), Kan^R	This study
pET28a-*Mtb*rpoZ-M4-Flag	pET28a expressing *Mtb* ω^{M4} (*Mtb* ω 80-84 aa was replaced by GSGGS linker) subunit with with C-terminal Flag-tag (without His-tag), Kan^R	This study
pET28a-*Mtb*rpoZ-M5-Flag	pET28a expressing *Mtb* ω^{M5} (*Mtb* ω 85-89 aa was replaced by GSGGS linker) subunit with C-terminal Flag-tag (without His-tag), Kan^R	This study
pEcRc	Plasmid expressing all subunits of *E.coli* RNA polymerase core enzyme, with His-tag at the C-terminal of β' subunit, Amp^R	This study
pEcRcΔω	Plasmid expressing the α, β, and β' subunits of *E.coli* RNA polymerase, with His-tag at the C-terminal of β' subunit, Amp^R	This study
pET21a-*Ec*rpoA	pET21a expressing the α subunits of *E.coli* RNA polymerase with C-terminal His-tag, Amp^R	This study
pET21a-*Ec*rpoB	pET21a expressing the β subunits of *E.coli* RNA polymerase, without His-tag, Amp^R	This study
pET21a-*Ec*rpoC	pET21a expressing the β' subunits of *E.coli* RNA polymerase, without His-tag, Amp^R	This study
pET21a-*Ec*rpoZ	pET21a expressing the ω subunits of *E.coli* RNA polymerase with C-terminal His-tag, Amp^R	This study
pET21a-*Ec*σ^{70}	pET21a expressing the σ^{70} subunit of *E.coli*, with C-terminal His-tag, Amp^R	This study
pET21a-*Ms*rpoA B	pET21a expressing the α and β subunits of *Ms* RNA polymerase, without His-tag, Amp^R	This study
pET21a-*Ms*rpoA BZ	pET21a expressing the α , β, and ω subunits of *Ms* RNA polymerase, without His-tag, Amp^R	This study
pET21a-*Ms*rpoA BZ-Flag	pET21a expressing the α , β, and ω (with C-terminal Flag-tag) subunits of *Ms* RNA polymerase, Amp^R	This study
pET28a-*Ms*rpoC	pET28a expressing the β' subunits of *Ms* RNA polymerase, with C-terminal His-tag kan^R	This study
pPaRc	Plasmid expressing all subunits of *P. aeruginosa* RNA	This study

	polymerase core enzyme, with His-tag at the C-terminal of β' subunit, Amp^R	
pPaRcΔω	Plasmid expressing the α, β, and β' subunits of *P. aeruginosa* RNA polymerase, with His-tag at the C-terminal of β' subunit, Amp^R	This study
pTthRc	Plasmid expressing all subunits of *T. thermophilus* RNA polymerase core enzyme, with His-tag at the C-terminal of β' subunit, Amp^R	This study
pTthRcΔω	Plasmid expressing the α, β, and β' subunits of *T. thermophilus* RNA polymerase, with His-tag at C-terminal of β' subunit, Amp^R	This study
pET28a-*Tth*rpoZ-Flag	pET28a expressing *Tth* ω subunit with C-terminal Flag-tag, without His-tag, Kan^R	This study
pET28a-*Ec*rpoZ-Flag	pET28a expressing *Eco* ω subunit with C-terminal Flag-tag, without His-tag, Kan^R	This study
pET21a-*Sco*AZB	pET21a expressing the α, ω and β subunits of *S. coelicolor* RNA polymerase, without His-tag, Amp^R	This study
pET21a-*Sco*AB	pET21a expressing the α and β subunits of *S. coelicolor* RNA polymerase, without His-tag, Amp^R	This study
pKT-T7-ScoC	Plasmid expressing the β' subunit of *S. coelicolor* RNA polymerase, with C-terminal His-tag, Kan^R	This study
pRK793	Plasmid expressing TEV protease enzyme, Amp^R	(3)
pCas	Plasmid expressing the Cas9 and λ Red recombination system in *E. coli*, Kan^R	(2)
pTargetF	Plasmid transcribing sgRNA in *E. oli*, Str^R	(2)
pTarget-EcrpoZ	Plasmid transcribing sgRNA targeting to *Ec rpoZ*, Str^R	This study

167 *Amp^R, Kan^R, and Str^R represent resistance to ampicillin, kanamycin and streptomycin,

168 respectively.

169

170 **Table S2. Oligonucleotides used in this study.**

Name	Sequence (5'→3')	Application
MtbrpoB-BsrGI-F	TACCCGGTCACGGTTGGCTACATG	pMtRc
MtbrpoB-R	GATCGAGAGAGAATTACGCAAGATCCTCGACA	
T7p-MtbrpoC-F	GTAATTCTCTCTCGATCCCGCGAAATTAATACGA	
MtbrpoC-NotI-R	GTCTCGGCCATCCACGCATCGC	
MtRc-drpoZ-F	GAACAGCTTTAGCACGCCGATCTGCTCGAGCACACCGAGGGCGAGT	pMtRcΔω
MtRc-drpoZ-R	GATCGGCGTGCTAAAGCTGTTCGGTTTCGGCGTAGTCCTGCTCG	
Δβ'CTD-F	TCAACCGCTACCGCAACATCCACCACCACCACCACCACTGAGATGGCCGC	pMtRcΔβ'CTD pMtRcΔωΔβ'CTD
Δβ'CTD-R	TCAGTGGTGGTGGTGGTGGTGGATGTTGCGGTAGCGGTTGATACCGGTACC	
pET28a-R2	CATCACCAGGCCGCTGCTGTG	pET28a-sumo^{TEV}
pET28a-F2	GAGAACCTGTACTTCCAATCC	
SUMO-F	CAGCGGCCTGGTGATGTCGGACTCAGAAGTC	
SUMO-R	GGAAGTACAGGTTCTCTCCACCAATCTGTTCTCTGTG	
pET28a-TEV-F	GCGCGGCAGCGAGAACCTGTACTTCCAATCCCCATATGGCTAGCATGACTGGTGGA	pET28a-His^{TEV}
pET28a-TEV-R	CTAGCCATATGGGATTGGAAGTACAGGTTCTCGCTGCCGCGCGGCACCAGGCC	
pET-F	TAACAAAGCCCGAAAGGAAGCTGA	pET backbone
TEV-R	CATGGATTGGAAGTACAGGTTCTC	
pET-R	CATGGTATATCTCCTTCTTAAAGTTAAAC	
pET21a-F	TAAGCGGCCGCACTCGAGCACCACCACC	
pET21a-R	CATATGTATATCTCCTTCTTAAAGTTAAAC	
MtbrpoA-F	ACCTGTACTTCCAATCCATGCTGATCTCACAGCGCCCCACCCTG	pET28a- *Mtb*rpoA
MtbrpoA- R	CTTCCTTTCGGGCTTTGTTAAAGCTGTTCGGTTTCGGCGTAGTCCTG	
MtbrpoB-21a-F	TAAGAAGGAGATATACATATGGTGTTGGCAGATTCCCGCCAGAG	pET21a-*Mtb*rpoB
MtbrpoB-21a-R	CTTCCTTTCGGGCTTTGTTACGCAAGATCCTCGACACTTGCGG	
MtbrpoC-21a-F	TAAGAAGGAGATATACATATGCTCGACGTCAACTTCTTCGATG	pET21a-*Mtb*rpoC
MtbrpoC-21a-R	CTTCCTTTCGGGCTTTGTTAGCGGTAGTCGCTGTAGCCGTAGTC	
*Mtb*rpoZ-F	AAGAAGGAGATATACATATGAGTATCTCGCAGTCCG	pET21a-*Mtb*rpoZ
*Mtb*rpoZHis-R	CTTCCTTTCGGGCTTTGTTAGTGGTGGTGGTGGTGGTGCTCGCCCTCGGTGTGCTCGA	
TEV-SigA-Δ165-F	ACCTGTACTTCCAATCCATGGAAGACCACGAAGACCTCGAAGC	pET28a-His^{TEV}-SigAΔ165

Tev-SigA-Δ165-R	TCCTTTCGGGCTTTGTTAGTCCAGGTAGTCGCGCAGGAC	pET28a-sumo^{TEV}-SigB pET28a-His^{TEV}-RbpA
TEV-SigB-F	ACCTGTACTTCCAATCCATGGCCGATGCACCCACAAG	
Tev-SigB-R	TCCTTTCGGGCTTTGTTAGCTGGCGTACGACCGCAG	
TEV-RbpA-F	ACCTGTACTTCCAATCCATGGCTGATCGTGTCCTGAGG	
TEV-RbpA-R	TCCTTTCGGGCTTTGTTAGCCGCGCCGACGTGACCGAATGAG	
*Mtb*rpoZ-F	AAGAAGGAGATATACATATGAGTATCTCGCAGTCCG	pET28a-*Mtb*rpoZ
*Mtb*rpoZ-R	CTTCCTTTCGGGCTTTGTTACTCGCCCTCGGTGTGCTCGA	
*Mtb*rpoZflag-F	CGAGGGCGAGGACTACAAAGACGATGACGACAAGTAACAAAGCCCGAAAGGAAGCTGAG	pET28a-*Mtb*rpoZ-Flag
*Mtb*rpoZflag-R	GGCTTTGTTACTTGTCGTCATCGTCTTTGTAGTCCTCGCCCTCGGTGTGCTCGAGCAGA	
MtbrpoZ-TEV-Y67-F	CAACGACTACGAGAACCTGTACTTCCAATCCTACAACCAGCTTGGCGAGGGCATCCTCG	pET28a-*Mtb*rpoZ^{TEV}-Flag
MtbrpoZ-TEV-Y67-R	GCTGGTTGTAGGATTGGAAGTACAGGTTCTCGTAGTCGTTGATCTGCCGGGCACGCTTT	
MtbrpoZ-d35-F	AAGAAGGAGATATACCATGCTGAATCCGCCCATCGACGAGTTGCTG	pET28a-*Mtb*rpoZ-EcR1-Flag
V-EcR2-F	CTCGAGCACACCGAGGGCGAG	pET28a-*Mtb*rpoZ-EcR2-Flag
V-EcR2-R	GATGCCCAGCGGGGTGTCGTAG	
EcR2-F	ACGACACCCCGCTGGGCATCGCACGCGTAACTGTTCAGGACGCTG	
EcR2-R	TCGCCCTCGGTGTGCTCGAGCAGACCTTCTTCGATTTCGCGCAGC	
MtbrpoZ-103-R	CTGGTTGTTGATCAGATCGGCGTGGATCTCGCGC	pET28a-*Mtb*rpoZ-EcR3-Flag
EcrpoZ-C-F	GATCTGATCAACAACCAGATCCTCGACG	
EcrpoZ-28a-R	CTTCCTTTCGGGCTTTGTTAACGACGACCTTCAGCAATAG	
V-Eloop-F	AAGAAGGAGATATACATATGAGTATCTCGCAGTCCGACG	pET28a-*Mtb*rpoZ-E^{loop}-Flag
V-Eloop-R	CGGTACCAGCGGATCCTTTCCGCCTACCTGGATCTGCCGGGCACGCTTTGCCGCATAG	
Eloop-F	GAAAGGATCCGCTGGTACCGGAAGAAAACGATAAGCCGTTGTCCATCGCGTTGCGCGAG	
Eloop-R	CTTCCTTTCGGGCTTTGTTACTTGTCGTCATCGTCTTTGTAG	
V-Tloop-F	AAGCCGTTGTCCATCGCGTTGCGCGAGATC	pET28a-*Mtb*rpoZ-T^{loop}-Flag
V-Tloop-R	GATCTGCCGGGCACGCTTTGCCGCATAG	
Tloop-F	AAAGCGTGCCCGGCAGATCCTCCGCCACGGCTTCAAGAAC	
Tloop-R	AACGCGATGGACAACGGCTTGGGATCGTCAAAAAGCCCCTC	

AZ-RC-F	GTTGGCTGCTGCCACCGCTG	pPaRc and pTthRc backbone
AZ-RC-R	TCAGCTTCCTTTCGGGCTTT	
B-RC-F	AAGCCCGAAAGGAAGCTGAGATCTTCCCCATCGGTGATG	
B-RC-R	TTTCATAGCTGTCTCCTTCTCAGCTTCCTTTCGGGCTTT	
PArpoA-F	TAAGAAGGAGATATACATATGCAGAGTTCGGTAAATGAGTT	pPaRc
PArpoA-R	CTATATCTCCTTCTTGGATCTCAGGCAGTGGCCTTGTCGTC	
PArpoZ-F	GATCCAAGAAGGAGATATAGATATGGCCCGCGTCACCGTTGAAG	
PArpoZ-R	CTTCCTTTCGGGCTTTGTTACAGGGCCTCGGTGTTGGC	
PArpoB-F	TAAGAAGGAGATATACATATGGCTTACTCATACACTGAGA	
PArpoB-R	CTTCCTTTCGGGCTTTGTTATTCGGTTTCCAGTTCGATGTC	
PArpoC-F	TGCTAACTCCGACGGGAGCAAATCCATGAAAGACTTGCTTAATCTGTTGA	
PArpoC-R	CTTCCTTTCGGGCTTTGTTAGTGGTGGTGGTGGTGGTGGTTACCGCTCGAGTTCAGCG	
PArpoC-RC-F	AGAAGGAGACAGCTATGAAAGAC	
pPaRC-dZ-F	CACTGCCTGACGACGACGAGGCCAACACCGAGGCCCTGTAAC	pPaRcΔω
pPaRC-dZ-R	CTCGTCGTCGTCAGGCAGTGGCCTTGTCGTCTTTCTTAAG	
TtrpoA-F	TAAGAAGGAGATATACATATGTTGGATTCCAAGCTCAAGGCC	pTthRc
TtrpoA-R	ATCTCCTTCTTAAGTCTGCTTACTCCTTCAGGGTGAAGCCCTTC	
TtrpoZ-F	CAGACTTAAGAAGGAGATATAGATATGGCGGAACCGGGCATTGACAAGC	
TtrpoZ-R	CTTCCTTTCGGGCTTTGTTACTCCTCCCGCTCCACCGGGTAG	
TtrpoB-F	TAAGAAGGAGATATACATATGGAGATCAAGCGGTTCGGTCGC	
TtrpoB-R	CTTCCTTTCGGGCTTTGTTACCGCTTGGAGGCCAACCCCTCA	
TtrpoC-F	AAAGCCCGAAAGGAAGCTGAAGAAGGAGACAGCTATGAAAAAAGAGGTTCGTAAGGTTC	
TtrpoC-R	CAGCGGTGGCAGCAGCCAACTTAGTGGTGGTGGTGGTGAGCCTGCTTGCCGGGCTG	
TtrpoC-RC-F	AGAAGGAGACAGCTATGAAAAAAGAG	
TtrpoA-R2	CTTCCTTTCGGGCTTTGTTACTCCTTCAGGGTGAAGCCCTTC	pTthRcΔω
EcrpoA-F	GAAGGAGATATACATATGCAGGGTTCTGTGACAGAGTTTC	pEcRc pEcRcΔω
EcrpoA-R	TTCCTCAGTCGCTGACAAGTCCACGGATCGGGGATCCGGTTACTCGTCAGCGATGCTTG	

EcrpoZ-F	GTCAGCGACTGAGGAAGACTTAAGAAGGAGATTAATGTATGGCACGCGTAACTGTTCAG	
EcrpoZ-R	GTGCTCGAGTGCGGCCGCTTAACGACGACCTTCAGCAATAG	
EcrpoBC-F	GTCAGCGACTGAGGAAACAGACCATGGATCCCCGATCCGTCGACTTGTCAGCGAGCTG	
EcrpoBC-His-R	CTTAAGTCTGCAGTCATTTAATGGTGATGGTGATGGTGCACCTCGAGCTCGTTATCAGA	
EcAZ-F	ATGACTGCAGACTTAAGAAGGAGATTAATG	
MsrpoA-F	TAAGAAGGAGATATACATATGCTGATCTCTCAGCGTCCCACC	pET21a-*Ms*rpoAZB
MsrpoA-R	ATCTCCTTCTTAAGTCTGCTTAAAGCTGCTCGGTCTCGGCGTAG	
MsrpoZ-F	CAGACTTAAGAAGGAGATATAGATATGAGCACCCCGCACGCCGATGCGCAG	
MsrpoZ-R	CTTCCTTTCGGGCTTTGTTATTCGCCTTCGGTGTGCTCGAG	
MsrpoB-F	TAAGAAGGAGATATACATATGCTGGAAGGATGCATCTTGGCAG	
MsrpoB-R	CTTCCTTTCGGGCTTTGTTACGCGAGATCCTCGACGGACGCGGAT	
MsrpoA-R2	CTTCCTTTCGGGCTTTGTTAAAGCTGCTCGGTCTCGGCGTAG	pET21a-*Ms*rpoAB
*Ms*rpoZ-Flag-F	CGAAGGCGAAGACTACAAAGACGATGACGACAAGTAACAAAGCCCGAAAGGAAGCTGAG	pET21a-*Ms*rpoABZ-Flag
*Ms*rpoZ-Flag-R	GGCTTTGTTACTTGTCGTCATCGTCTTTGTAGTCTTCGCCTTCGGTGTGCTCGAGCAGG	
MsrpoC-F	CTTTAAGAAGGAGATATACCATGCTAGACGTCAACTTCTTCGATGAAC	pET28a-*Ms*rpoC
MsrpoC-R	CTTCCTTTCGGGCTTTGTTAGTGGTGGTGGTGGTGGTGGCGGTAATCCGAGTAGCCGTA	
ScorpoA-F	TAAGAAGGAGATATACATATGCTGATCGCTCAGCGTCCCTCG	pET21a-*Sco*AZB
ScorpoA-R	ATCTCCTTCTTAAGTCTGCTTAGTACTGCTCGGTCTCCACGAAACC	
ScorpoZ-F	CAGACTTAAGAAGGAGATATAGATATGTCCTCTTCCATCTCCGCGCCCGAG	
ScorpoZ-R	CTTCCTTTCGGGCTTTGTTACTGCGCCGGGCCCTCGATGGCCTCGGA	
ScorpoB-F	TAAGAAGGAGATATACATATGGCCGCCTCGCGCAATGCCTCGACC	
ScorpoB-R	CTTCCTTTCGGGCTTTGTTAGACCTCTTCGACGCTGCTCGGCTCG	
Sco-B-RC-R	CAGCGGTGGCAGCAGCCAAC	
ScorpoA-R1	CTTCCTTTCGGGCTTTGTTAGTACTGCTCGGTCTCCACGAAACC	pET21a-*Sco*AB
ScorpoC-F	TAAGAAGGAGATATACATATGCTCGACGTCAACTTCTTCGACGAGCT	pKT-T7-*Sco*C

ScorpoC-R	CTTCCTTTCGGGCTTTGTTAGTGGTGGTGGT GGTGGTGCTGGTTGTACGGACCGTAGTC	
*Ec*rpoA-F	AAGAAGGAGATATACATATGCAGGGTTCTGT GACAGAGTTTC	pET21a-*Ec*rpoA
*Ec*rpoA-R	CTTCCTTTCGGGCTTTGTTAGTGGTGGTGGT GGTGGTGCTCGTCAGCGATGCTTGCCGG	
EcrpoB-21a-F	TAAGAAGGAGATATACATATGGTTTACTCC TATACCGAGAAAAAAC	pET21a-*Ec*rpoB
EcrpoB-21a-R	CTTCCTTTCGGGCTTTGTTACTCGTCTTCCA GTTCGATGTTG	
EcrpoC-21a-F	TAAGAAGGAGATATACATATGAAAGATTT ATTAAAGTTTCTGAAAG	pET21a-*Ec*rpoC
EcrpoC-21a-R	CTTCCTTTCGGGCTTTGTTACTCGAGCTCGT TATCAGAACCGCCCAG	
*Ec*rpoZ-F	AAGAAGGAGATATACATATGGCACGCGTAA CTGTTCAGGAC	pET21a-*Ec*rpoZ pET28a-*Ec*rpoZ-Flag
*Ec*rpoZ-R	CTTCCTTTCGGGCTTTGTTAGTGGTGGTGGT GGTGGTGACGACGACCTTCAGCAATAGC	
*Ec*rpoZflag-R	CTTTCGGGCTTTGTTACTTGTCGTCATCGTC TTTGTAGTCACGACGACCTTCAGCAATA	
*Tth*rpoZ-F	AAGAAGGAGATATACATATGGCGGAACCGG GCATTGACAAGC	pET28a-*Tth*rpoZ-Flag
*Tth*rpoZ-R	CTTTCGGGCTTTGTTACTTGTCGTCATCGTC TTTGTAGTCCTCCTCCCGCTCCACCGGG	
RpoZM1-F	CCGGCAGATCGGTAGCGGTGGTAGCCAGCT TGGCGAGGGCATCCTCGAATATGTCGGTC	pET28a-*Mtb*rpoZ-M1-Flag
RpoZM1-R	CGCCAAGCTGGCTACCACCGCTACCGATCT GCCGGGCACGCTTTGCCGCATAGATCACG	
RpoZM2-F	CTACTACAACGGTAGCGGTGGTAGCATCCT CGAATATGTCGGTCCGCTGGTTGAGCCGG	pET28a-*Mtb*rpoZ-M2-Flag
RpoZM2-R	ATTCGAGGATGCTACCACCGCTACCGTTGTA GTAGTCGTTGATCTGCCGGGCACGCTTT	
RpoZM3-F	TGGCGAGGGCGGTAGCGGTGGTAGCGGTCC GCTGGTTGAGCCGGGGTTGCAAGAGAAGC	pET28a-*Mtb*rpoZ-M3-Flag
RpoZM3-R	CCAGCGGACCGCTACCACCGCTACCGCCCT CGCCAAGCTGGTTGTAGTAGTCGTTGATC	
RpoZM4-F	CGAATATGTCGGTAGCGGTGGTAGCCCGGG GTTGCAAGAGAAGCCGTTGTCCATCGCGT	pET28a-*Mtb*rpoZ-M4-Flag
RpoZM4-R	GCAACCCCGGGCTACCACCGCTACCGACAT ATTCGAGGATGCCCTCGCCAAGCTGGTTG	
RpoZM5-F	GCTGGTTGAGGGTAGCGGTGGTAGCAAGCC GTTGTCCATCGCGTTGCGCGAGATCCACG	pET28a-*Mtb*rpoZ-M5-Flag
RpoZM5-R	ACAACGGCTTGCTACCACCGCTACCCTCAA CCAGCGGACCGACATATTCGAGGATGCCC	
pTargetF-EcrpoZ-F	GTATAATACTAGTGCGCTGCGCGAAATCGAA GAGTTTTAGAGCTAGAAATAGCAAGTT	pTarget-*Ec*rpoZ
pTargetF-EcrpoZ-R	GCTCTAAAACTCTTCGATTTCGCGCAGCGC ACTAGTATTATACCTAGGACTGAGCTAG	
EcrpoZ-Cm-F	CATGCCCAGTCATTTCTTCACCTGTGGAGCT TTTTAAGTAGATTGCAGCATTACACGTC	For constructing BL21(DE3) Δ*rpoZ*

EcrpoZ-Cm-R	TTCAGGCTTTCAAACAGATACAAGGGCGACCCGCTTTGTGATGTAACGCACTGAGAAGC	mutant
Rv1494p-F	CGCGGCGATTGACCTGCTGCACA	Amplify promoters for *in vitro* transcription
Rv1494p-R	GGCGCTGGCGAGTTTGCTGGTCGA	
Rv0005p-F	CTAGTCACGTCACGGCCGGGTAAAAACGAGGCCAGA	
Rv0005p-R	CCGAATACTCTCCTCAGGGGTTGCGTGCATGC	
rrnP3-F	GCCGGCTATGGATGACCGAACCTG	
rrnp3-R	CCCGGCGTTTCCGCCCGCTTCGG	

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