Supplementary Materials

Supplementary Table 1. Oligonucleotide primers used for cloning and mutagenesis in this work.

Genes	Primers*	Sequences (5'-3')
KP34 RNAP	F	ACTG**GCTAGC**ATGATTAGCGCCCTAAGTAC GGTAGTAGTACC
	R	ACTG**GCGGCCGC**TTAGCAGAAGAAGAACG GGGATTCTAGCACTTG
T7 RNAP	F	TCACCATCACCATCACCATATGAACACGATT AACATCGCTAAGA
	R	AGTCCAAGCTCAGCTAATTTTACGCGAACGC GAAGTCCGACTCT
pQE-82L	F	AATTAGCTGAGCTTGGACTCCTGTTGATAG
	R	ATGGTGATGGTGATGGTGAGATCCTCTCAT
KP34-Y601F RNAP	F	GTATGACCTTCTTCTACAGCGCCACGG
	R	CTGTAGAAGAAGGTCATACTGGGGCGC
KP34-F602Y RNAP	F	TGACCTACTACTACAGCGCCACGGTGC
	R	GCGCTGTAGTAGTAGGTCATACTGGGG
KP34-Y603F RNAP	F	CCTACTTCTTCAGCGCCACGGTGCGTA
	R	GTGGCGCTGAAGAAGTAGGTCATACTG

Introduced restriction enzyme sites are indicated in bold.
*F refers to forward primer and R refers to reverse primer.

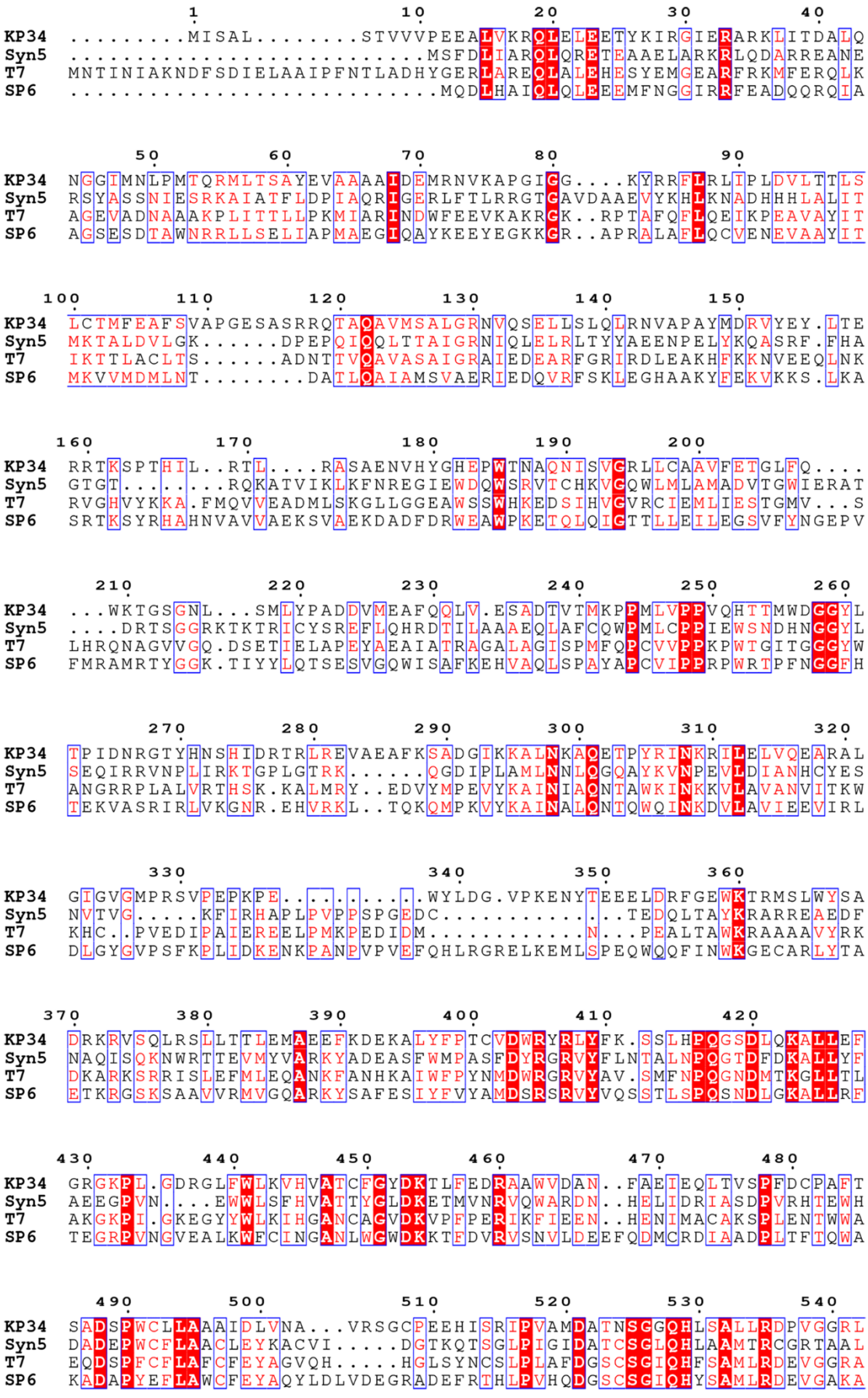
Supplementary Table 2. DNA templates used for *in vitro* transcription assays in this work.

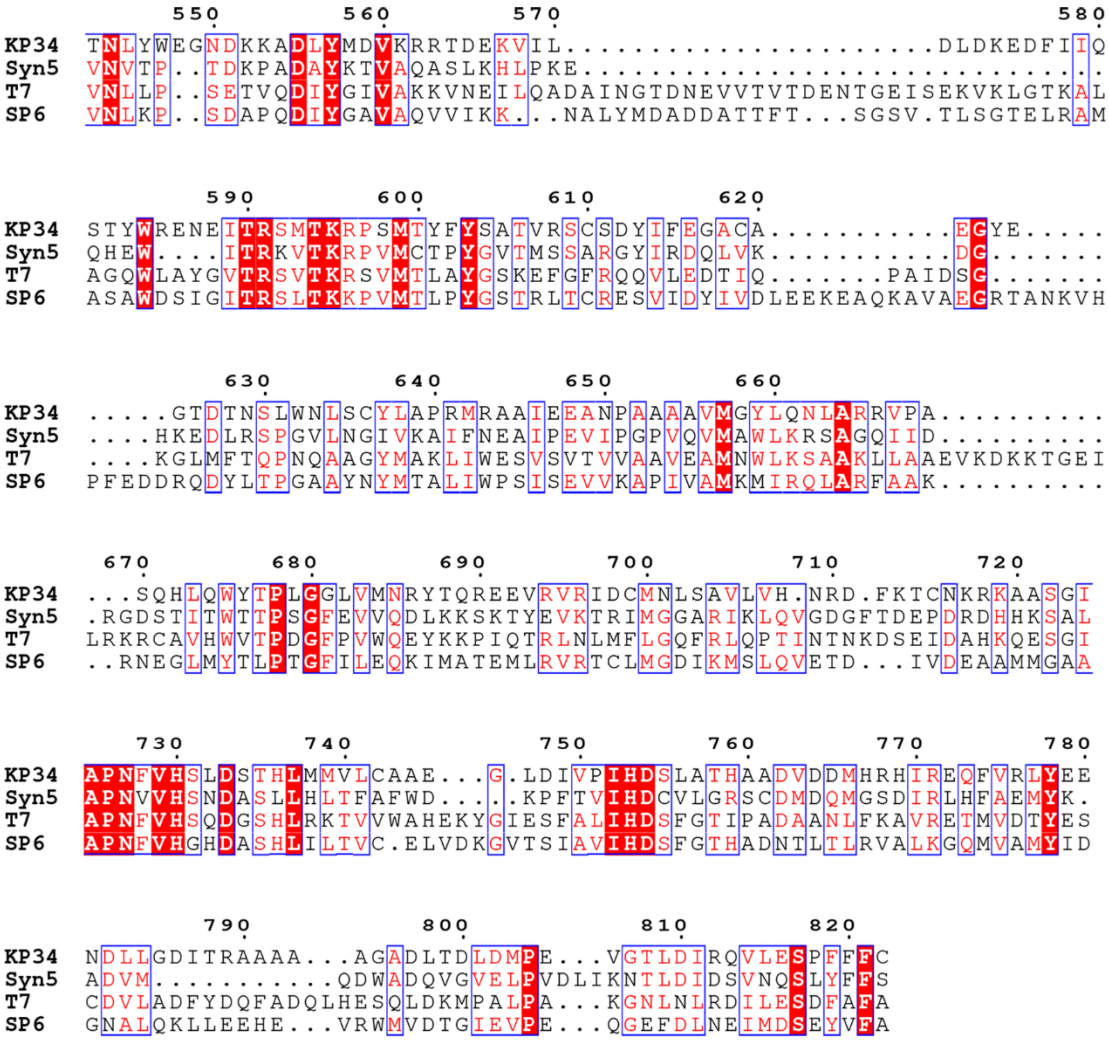
DNA templates	Oligos*	Sequences (5'-3')	Usage
Template 1	F	GGTTCCACGGTAGTGC AGTGGG	Figure 2A Primers to amplify PCR fragments
	R	CACTACATCATCGCCCT CATAG	
Template 2	F	ACTGGCTAGCATGATT AGCGCCCTAAGTACGG TAGTAGTACC	Figure 2A Primers to amplify PCR fragments
	R	GACGCCTGCGTCCTGG TCCTTC	
T7-37	F	**TAATACGACTCACTAT A**GGAGAACCTTAAGGT TTAACTTTAAGACCCTT AAGTG	Figure 2C Annealed oligonucleotides as template
	R	CACTTAAGGGTCTTAA AGTTAAACCTTAAGGT TCTCCTATAGTGAGTCG TATT	
KP34-S1-37	F	T**TAATGTTACAGGAGT A**GGAGAACCTTAAGGT TTAACTTTAAGACCCTT AAGTG	Figure 2C Annealed oligonucleotides as template
	R	CACTTAAGGGTCTTAA AGTTAAACCTTAAGGT TCTCCTACTCCTGTAAC ATTAA	
KP34-S2-37	F	T**TGATGTTACAGGAGT A**GGAGAACCTTAAGGT TTAACTTTAAGACCCTT AAGTG	Figure 2C Annealed oligonucleotides as template
	R	CACTTAAGGGTCTTAA AGTTAAACCTTAAGGT TCTCCTACTCCTGTAAC ATCAA	
KP34-W-37	F	T**ACTTTGGACATCCGT CAA**GTGGAGAACCTTA AGGTTTAACTTTAAGA CCCTTAAGTG	Figure 2C Annealed oligonucleotides

	R	CACTTAAGGGTCTTAA AGTTAAACCTTAAGGT TCTCCACTTGACGGATG TCCAAAGTA	as template
T7-50	F	**TAATACGACTCACTAT A**GCAAAGCTTCGGCTG GTGCAGTGGCCTCATA AGAGGCGGCCCCTAAC AGG	Figure 3A Annealed oligonucleotides as template
	R	CCTGTTAGGGGCCGCC TCTTATGAGGCCACTGC ACCAGCCGAAGCTTTG CTATAGTGAGTCGTATT A	
KP34-50	F	T**TAATGTTACAGGAGT A**GCAAAGCTTCGGCTG GTGCAGTGGCCTCATA AGAGGCGGCCCCTAAC AGG	Figure 3A Annealed oligonucleotides as template
	R	CCTGTTAGGGGCCGCC TCTTATGAGGCCACTGC ACCAGCCGAAGCTTTG CTACTCCTGTAACATTA A	
Syn5-50	F	T**ATTGGGCACCCGTA A**GCAAAGCTTCGGCTG GTGCAGTGGCCTCATA AGAGGCGGCCCCTAAC AGG	Figure 3A Annealed oligonucleotides as template
	R	CCTGTTAGGGGCCGCC TCTTATGAGGCCACTGC ACCAGCCGAAGCTTTG CTTACGGGTGCCCAAT A	
EGFP sgRNA	F	CATATGCGGTGTGAAA TACCGCACAGATGC	Figure 4A Primers to amplify PCR fragments
	R	AAAAAAAGCACCGACT CGGTGCCACTTTTTCAA G	

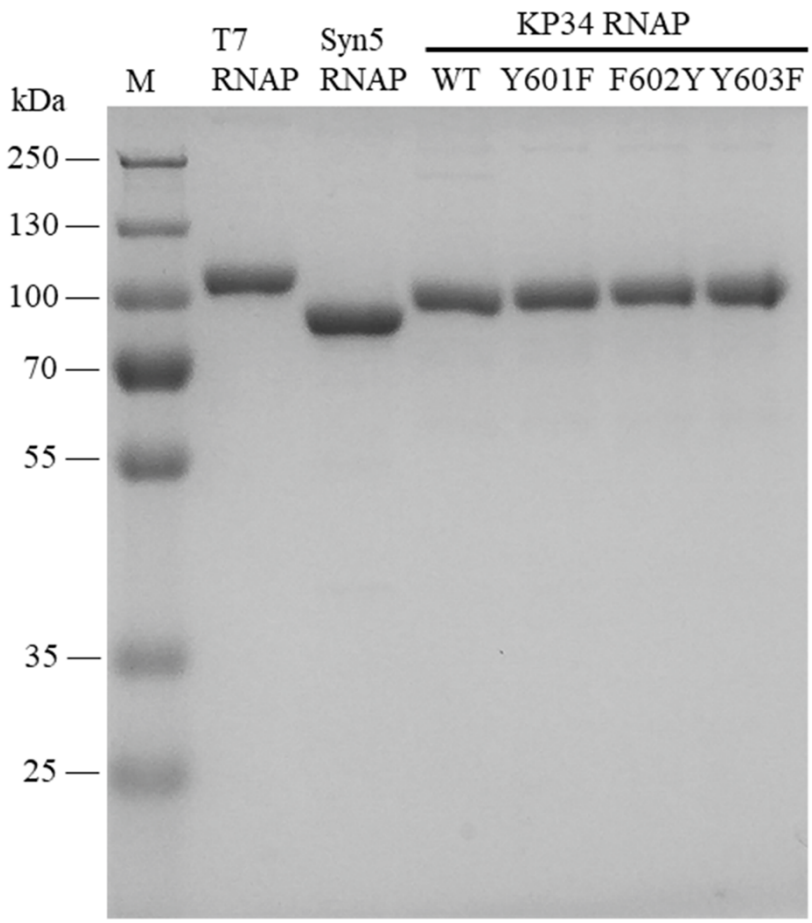
The promoter sequences are indicted in bold and the nucleotides corresponding to the run-off RNA sequences are underlined.

*F indicates forward primers (for PCR fragments) or sense strands (for annealed oligos) while R indicates reverse primers (for PCR fragments) or antisense strands (for annealed oligos).

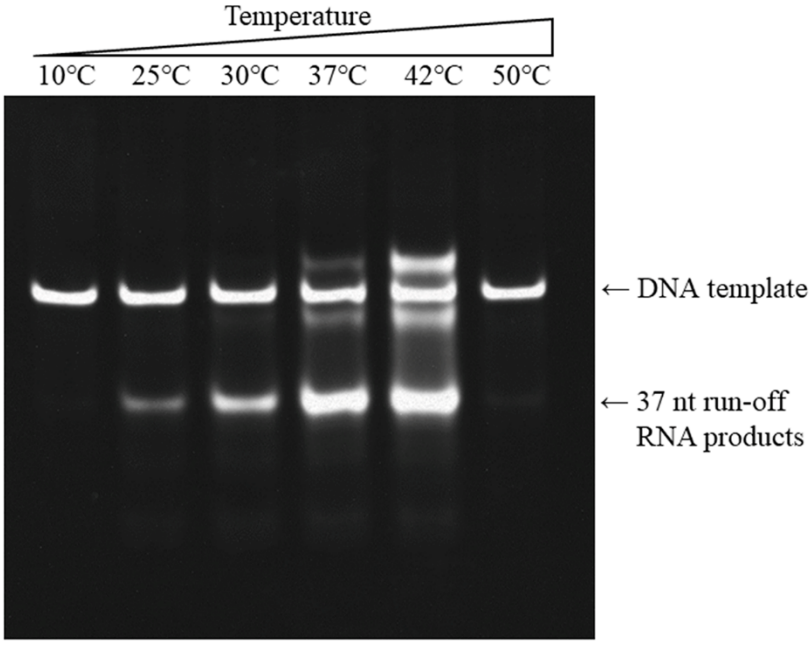

KP34
Syn5
T7
SP6



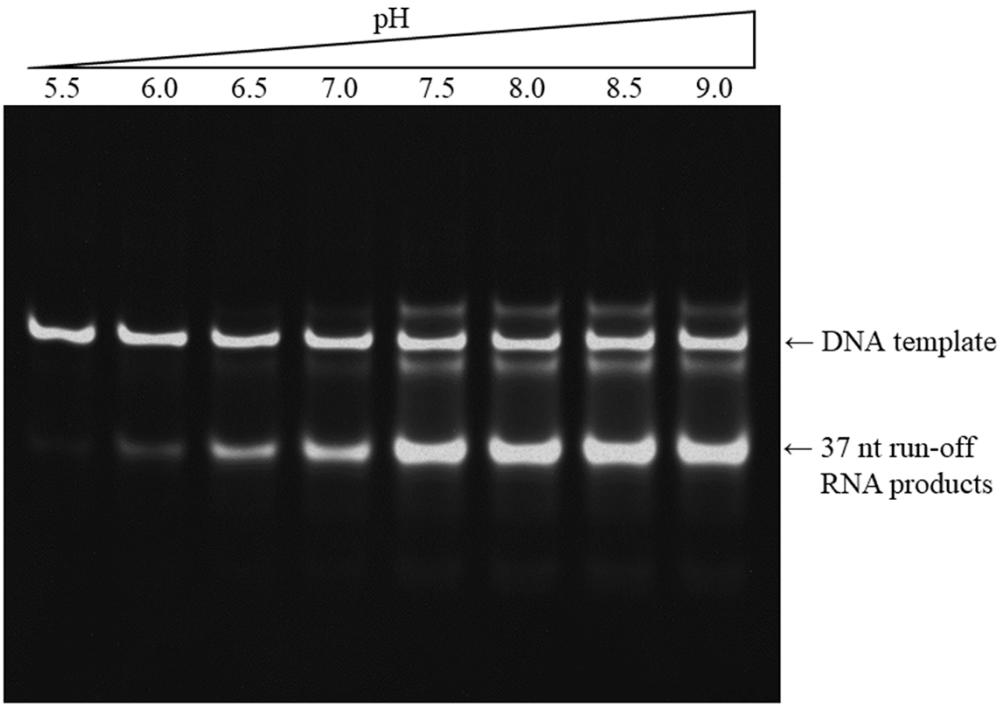
Supplementary Figure 1. Sequence alignment of the KP34 RNAP, Syn5 RNAP, T7 RNAP and SP6 RNAP was performed using Clustal Omega. The alignment was processed for publication using the ESPRIPT server v 3.0. The amino acid sequence of KP34 RNAP shares a similarity of 24.39%, 27.28% and 25.54% with Syn5, T7 and SP6 RNAP, respectively.



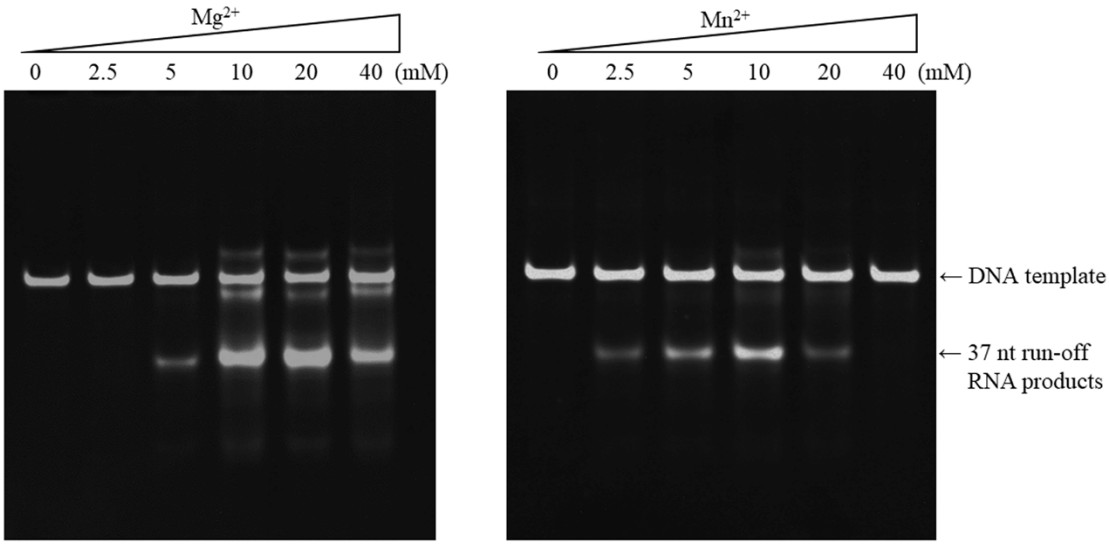
Supplementary Figure 2. SDS-PAGE analysis of all RNA polymerases in this work. All enzymes are N-terminal His-tagged and purified through Ni-NTA-agarose column and gel filtration column. Proteins were stained with Coomassie Blue. M: molecular mass marker.



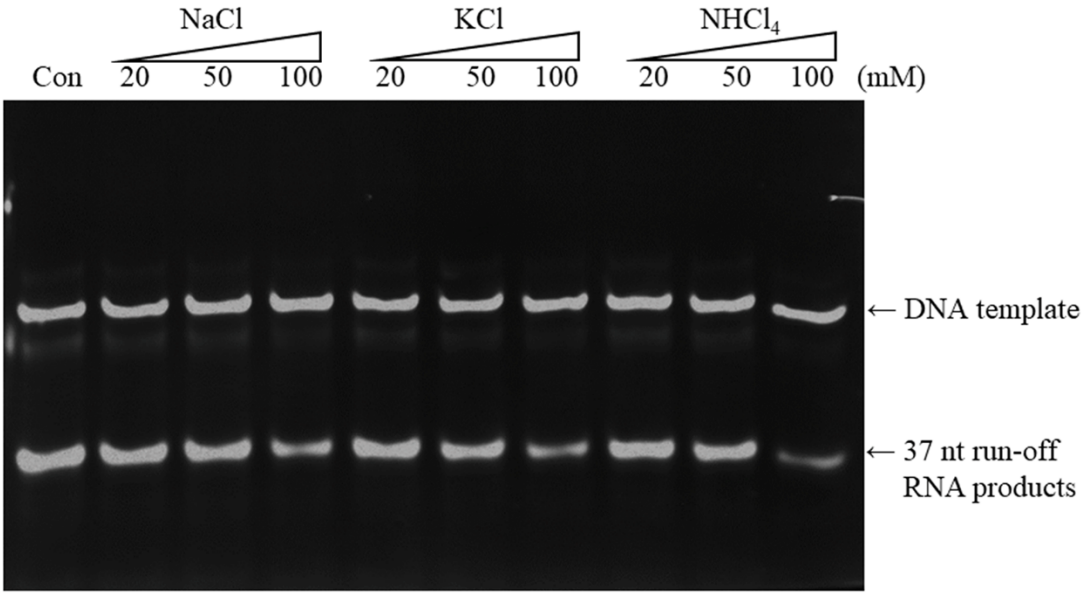
Supplementary Figure 3. Effect of reaction temperature on KP34 RNAP activity. The transcription assays of the KP34 RNAP (1μM) were performed at various temperatures in the presence of 40 mM Tris-HCl (pH 8.0), 20 mM $MgCl_2$, 2 mM spermidine, 20 mM DTT, 4 mM of each of the 4 NTPs and 2 μM DNA template, 40 U/μl RNaseOUT™ Recombinant Ribonuclease Inhibitor, 0.04 U/μl *E. coli* inorganic pyrophosphatase, for 1 h. DNA template and RNA products were separated on a 12% TBE native gel. Various temperatures for the reactions were shown at the top of the gel.



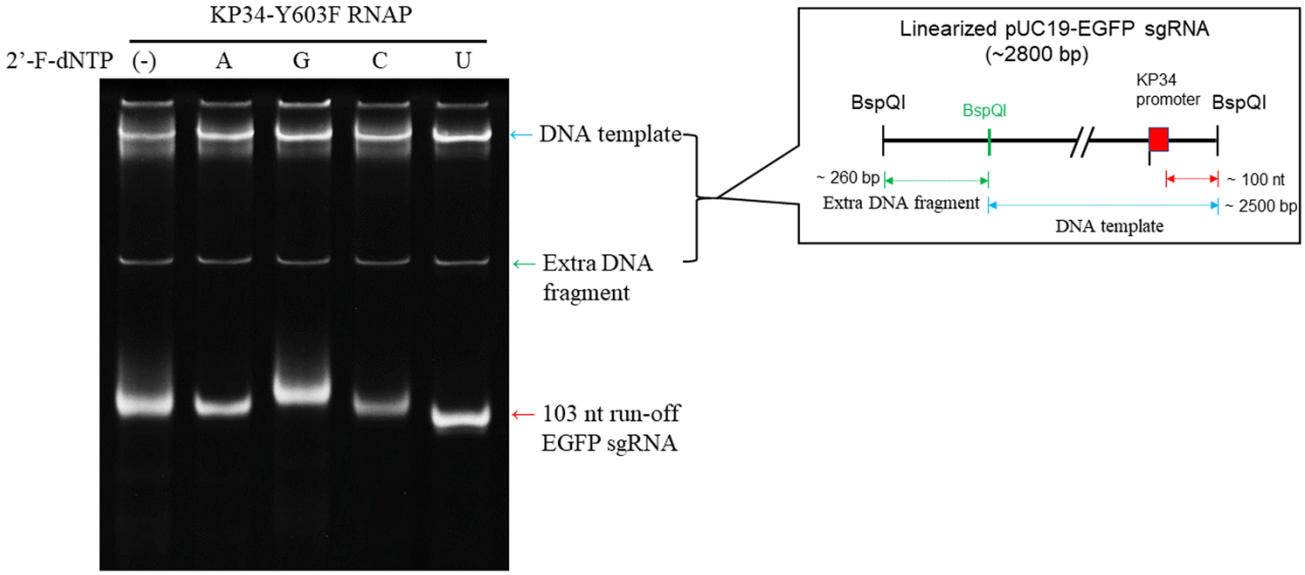
Supplementary Figure 4. Effect of pH on KP34 RNAP activity. The transcription assays of the KP34 RNAP (1μM) were performed using buffers with various pH at 37℃ for 1 h in the presence of 20 mM $MgCl_2$, 2 mM spermidine, 20 mM DTT, 4 mM of each of the 4 NTPs and 2 μM DNA template, 40 U/μl RNaseOUT™ Recombinant Ribonuclease Inhibitor, 0.04 U/μl *E. coli* inorganic pyrophosphatase. Three different buffers (all at 40 mM concentrations) were used to prepare different pHs: NaH_2PO_4-Na_2HPO_4 (pH 5.5, pH 6.0, pH 6.5, pH 7.0), Tris-HCl (pH 7.5 and pH 8.0), and Glycine-NaOH (pH 8.5 and pH 9.0). DNA template and RNA products were separated on a 12% TBE native gel. Various pH conditions for the reactions were shown at the top of the gel.



Supplementary Figure 5. Effect of $MgCl_2$ and $MnCl_2$ on KP34 RNAP activity. The transcription assays of the KP34 RNAP (1μM) were performed with Mg^{2+}/Mn^{2+} at 37℃ for 1 h in the presence of 40 mM Tris-HCl (pH 8.0), 2 mM spermidine, 20 mM DTT, 4 mM of each of the 4 NTPs and 2 μM DNA template, 40 U/μl RNaseOUT™ Recombinant Ribonuclease Inhibitor, 0.04 U/μl *E. coli* inorganic pyrophosphatase. DNA template and RNA products were separated on a 12% TBE native gel. Concentrations of Mg^{2+} and Mn^{2+} in the reactions were shown at the top of the gel.



Supplementary Figure 6. Effect of NaCl, KCl and $NHCl_4$ on KP34 RNAP activity. NaCl, KCl or $NHCl_4$ at various concentrations were added to the transcription assays contained 40 mM Tris-HCl (pH 8.0), 2 mM spermidine, 20 mM DTT, 20 mM $MgCl_2$, 4 mM of each of the 4 NTPs, 40 U/µl RNaseOUT™ Recombinant Ribonuclease Inhibitor, 0.04 U/µl *E. coli* inorganic pyrophosphatase, 2 µM DNA template and 1 µM KP34 RNAP. Reaction mixtures were incubated at 37°C for 1 h. DNA template and RNA products were separated on a 12% TBE native gel. Concentrations of NaCl, KCl and $NHCl_4$ in the reactions were shown at the top of the gel. Con: the reaction without salt.



Supplementary Figure 7. Incorporation of 2'-F-dNMPs into the 103 nt sgRNA transcript using KP34-Y603F RNAP. The position of the migration of the DNA template, an extra DNA fragment during template preparation, and the RNA products were marked on the right of the gel. In the reaction, none (-) or one of the four NTPs was replaced by its 2'-F-dNTP analog as indicated at the top of the gel. In the right panel box: schematic showing the preparation of transcription template by digestion of the plasmid using BspQI restriction enzyme.