

Supplementary Materials

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

Genes	Primers*	Sequences (5'-3')
KP34 RNAP	F	ACTGG GCTAG CATGATTAGCGCCCTAAGTAC GGTAGTAGTACC
	R	ACTGG CGGCGC GCTTAGCAGAAGAAGAACG 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 <u>AGGAGAACCTTAAGGT</u> <u>TTAACTTTAAGACCCTT</u> <u>AAGTG</u>	Figure 2C Annealed oligonucleotides as template
	R	CACTTAAGGGTCTTAA AGTTAAACCTTAAGGT TCTCCTATAGTGAGTCG TATT	
KP34-S1-37	F	TTAATGTTACAGGAGT <u>AGGAGAACCTTAAGGT</u> <u>TTAACTTTAAGACCCTT</u> <u>AAGTG</u>	Figure 2C Annealed oligonucleotides as template
	R	CACTTAAGGGTCTTAA AGTTAAACCTTAAGGT TCTCCTACTCCTGTAAC ATTAA	
KP34-S2-37	F	TTGATGTTACAGGAGT <u>AGGAGAACCTTAAGGT</u> <u>TTAACTTTAAGACCCTT</u> <u>AAGTG</u>	Figure 2C Annealed oligonucleotides as template
	R	CACTTAAGGGTCTTAA AGTTAAACCTTAAGGT TCTCCTACTCCTGTAAC ATCAA	
KP34-W-37	F	TACTTTGGACATCCGT <u>CAAGTGGAGAACCTTA</u> <u>AGGTTTAACTTTAAGA</u> <u>CCCTTAAGTG</u>	Figure 2C Annealed oligonucleotides

	R	CACTTAAGGGTCTTAA AGTTAAACCTTAAGGT TCTCCACTTGACGGATG TCCAAAGTA	as template
T7-50	F	TAATACGACTCACTAT <u>AGCAAAGCTTCGGCTG</u> <u>GTGCAGTGGCCTCATA</u> <u>AGAGGCGGCCCTAAC</u> <u>AGG</u>	Figure 3A Annealed oligonucleotides as template
	R	CCTGTTAGGGGCCGCC TCTTATGAGGCCACTGC ACCAGCCGAAGCTTTG CTATAGTGAGTCGTATT A	
KP34-50	F	TTAATGTTACAGGAGT <u>AGCAAAGCTTCGGCTG</u> <u>GTGCAGTGGCCTCATA</u> <u>AGAGGCGGCCCTAAC</u> <u>AGG</u>	Figure 3A Annealed oligonucleotides as template
	R	CCTGTTAGGGGCCGCC TCTTATGAGGCCACTGC ACCAGCCGAAGCTTTG CTACTCCTGTAACATTA A	
Syn5-50	F	TATTGGGCACCCGTA <u>AGCAAAGCTTCGGCTG</u> <u>GTGCAGTGGCCTCATA</u> <u>AGAGGCGGCCCTAAC</u> <u>AGG</u>	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 indicated 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).

		1		10		20		30		40		
KP34		MISAL.....	STVVVPEEA	LVKR	OLE	LE	ET	TYKIR	GIE	RARK	LITDA	LQ
Syn5				MSFD	LIAR	OL	QRE	TEAAEL	ARK	RLQD	ARREANE	
T7	MNTINI	AKNDFS	DIELAA	IPFNTL	ADHYGER	LARE	OL	AL	EH	ESYEM	GEAR	FRKM
SP6				MQD	LHAI	OL	QLE	EMFNG	GIR	RRFEAD	QQRQ	IA

		50		60		70		80		90		
KP34	N	GGIMN	LPM	TQRML	TSAYEV	AA	AA	IDE	MRNV	KAPGI	G	G...
Syn5	RSY	ASS	NIE	SRKAI	ATFLDP	IA	QR	TGE	RLFT	LRRGT	G	AVDAAE
T7	AGE	VAD	NA	AKP	LITLL	PK	MI	AR	IND	WFEEV	KAKR	GK...
SP6	A	GSESD	TA	WNRRLL	SELI	AP	MA	EG	TQA	YKEEY	EGKK	G

		100		110		120		130		140		150
KP34	L	CTMFEAF	S	VAPGES	ASRRQ	TAQ	AVMSAL	GR	NV	QS	ELL	SLQ
Syn5	M	KTALD	VLG	GK.....	DPEP	QIQ	QLT	TAIGR	NI	QL	ELR	LT
T7	I	KTTLAC	LT	S.....	ADNT	TVQ	AVASA	IGRA	IED	EAR	FGR	IRDLEAKH
SP6	M	KVVMD	MLN	T.....	DA	TLO	AIAMS	V	AE	R	I	EDQVR

		160		170		180		190		200	
KP34	R	RT	K	SPT	HIL	..RT	L	..R	ASA	ENVHYG	HEP
Syn5	G	TGT	...	...	RQ	KATVIK	L	KFN	REGIE	WDQ	WS
T7	R	VG	H	VYK	KA	FMQV	V	EADML	SKGL	LGGEA	WSS
SP6	S	RT	K	SYR	HA	HN	VA	V	AEKSV	A	EKDAD

		210		220		230		240		250		260
KP34	...	WKTGS	G	NL...	SM	L	YPAD	D	V	MEAFQ	QLV	ESAD
Syn5	...	DRTS	G	GRKTK	TR	I	CYSR	E	F	L	QHRD	T
T7	L	HRQ	NAGV	V	GQ	D	SET	I	E	LAP	EY	AE
SP6	F	M	RAMRTY	G	G	K	TIYY	L	Q	TSES	S	V

		270		280		290		300		310		320
KP34	T	PIDNR	GTY	HNS	H	I	DRTR	L	R	E	V	AEAFK
Syn5	S	E	QIRRV	N	P	L	I	R	K	T	G	P
T7	A	NGRR	P	L	A	L	V	R	T	H	S	K
SP6	T	E	K	V	A	S	R	I	R	L	V	K

		330		340		350		360
KP34	G	T	G	V	G	M	P	R
Syn5	N	V	T	V	G	...	...	...
T7	K	H	C	...	P	V	E	D
SP6	D	L	G	V	S	F	K	P

		370		380		390		400		410		420
KP34	D	R	K	R	V	S	Q	L	R	S	L	T
Syn5	N	A	Q	I	S	Q	K	N	W	R	T	T
T7	D	K	A	R	K	S	R	I	S	L	E	F
SP6	E	T	K	R	G	S	K	S	A	A	V	V

		430		440		450		460		470		480
KP34	G	R	G	K	P	L	G	D	R	G	L	F
Syn5	A	E	E	G	P	V	N	...	E	W	W	L
T7	A	K	G	K	P	I	G	K	E	G	Y	W
SP6	T	E	G	R	P	V	N	G	V	E	A	L

		490		500		510		520		530		540
KP34	S	A	D	S	P	W	C	L	L	A	A	I
Syn5	D	A	D	E	P	W	C	F	L	A	C	L
T7	E	Q	D	S	P	F	C	F	L	A	F	C
SP6	K	A	D	A	P	Y	E	F	L	A	W	C

Sequence alignment of KP34 RNAP, Syn5 RNAP, T7 RNAP and SP6 RNAP. The alignment shows conserved regions across the four sequences, with positions 550 to 820 highlighted. Conserved residues are indicated by red boxes, and gaps are indicated by dashes.

550 560 570 580

KP34 TNL Y WEG ND KKA DLY MD V KRR TDE KV IL DLDKEDFI IQ

Syn5 VNV TP . . . TD KPA DAY KT VA QASLK HLP KE

T7 VNL LP . . . SE TVQ DIY GI V AKKVNEI LQ ADA ING TD NE VVT VTD ENT GEI SEKV KLG TK AL

SP6 VNL K P . . . SD APQ DIY GA VA QVVIK K . . . NAL YMD ADD AT T FT . . . SGSV . T LSG TEL RA M

590 600 610 620

KP34 STY W RENE I TR SMT KRP SMT YF Y SA TVR SC SDY I F E G A C A EG YE

Syn5 QHEW I TR KVT KRP VMC TP YG VT MS SARGY I R D Q LV K DG

T7 AGQ W LAY G V TR SVT KRS VMT LA YG SKEF GFR QQ VL ED TI Q PAIDS G

SP6 ASA W DSIG I TR SLT KKP VMT LP YG S TRL TC RES V I DY IV D LEE KEA QKAVA EG RTANKVH

630 640 650 660

KP34 GT D TN SLWN L SC YL AP RM RAA TE E AN P A A A A VM G Y L Q N L AR R VP A

Syn5 HKE D LR SP GV L NGI V KA I F NEA I P EV I PG PV Q VM A WL KRS AG Q I I D

T7 KGL M FT Q P NQ A AG YMAK LI WES VSV TVV A AVE AMN WLK SAA K L LA AE VKD KKT GEI

SP6 P F E D DR Q D YL TP GA AYN YMTA LI WPS IS EVV K AP I V AM KM I R Q L AR F AA K

670 680 690 700 710 720

KP34 . . . SQH L Q WY TP L GGL VMN RRY TQ REEV RVR IDC MNLS AV L VH . NRD . FKTC NKR KAA SGI

Syn5 . RGD ST I T W T TP S GFE VVQ D LK KSK TY EVK TR I MG GA RI K LQV GDG FT DEP DRD HHK SAL

T7 LRK RCA V H W V TP D G F P V WQ EY K KPI QT RL N LMF LG QF RL QPT I INT NKD SEI DAH KQESGI

SP6 . . RNEG L M Y T L P T G F I L E Q K I M A T E M L RVR T C L M G D I K M S LQV ETD . . . IV D E A A M M G A A

730 740 750 760 770 780

KP34 APN EVHS L D S THL M MV L CAAE . . . G . LD I VP I HD S LA TH AA DV D DM HRRH IRE QF V R L YEE

Syn5 APN VVHS NDAS LL H L T FAFWD KPFT VI HD CVL GR SC DM D QMGSD IRL HF A E M Y K .

T7 APN EVHS QD G SHL RK TV VWA HEKY G IES F ALI HD S FGT I P A D A N L FKA VRE TM VDT YES

SP6 APN EVH GH DA SHL I LTV C . ELVDK GVT SI AVI HD S FGT H ADNTL TL RVA LKG QM VAM YID

790 800 810 820

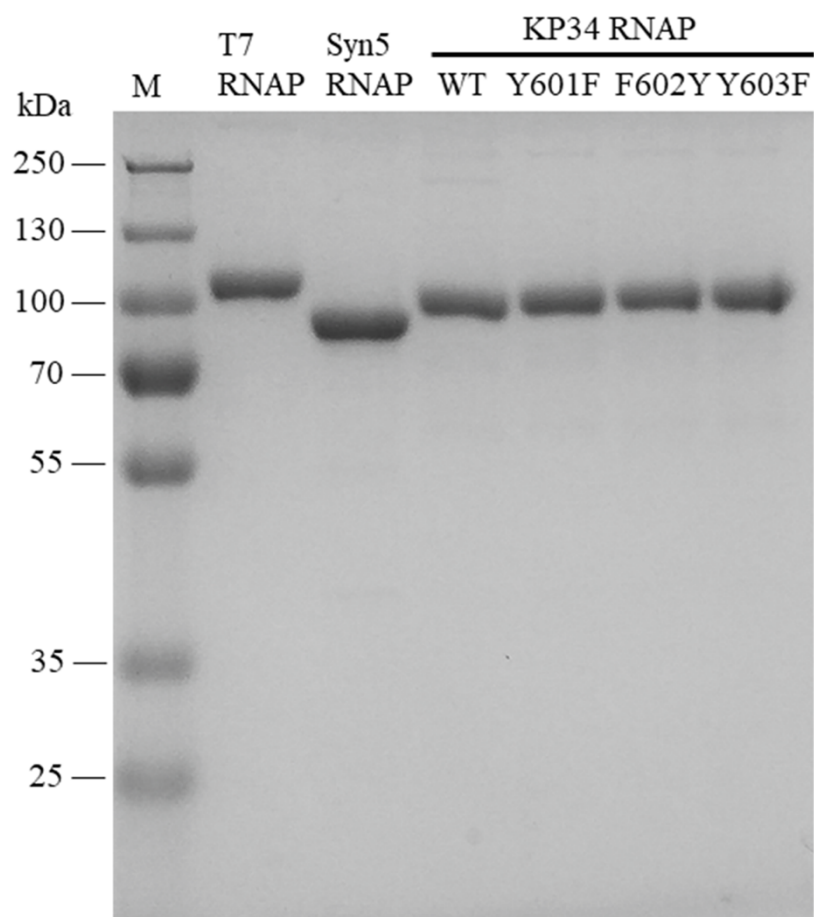
KP34 N D L L G D I T R A A A A . . . AG A D L T D L D M P E . . . V G T L D I R Q V L E S P F F F C

Syn5 A D V M QD W A D Q V G V E L P V D L I K N T L D I D S V N Q S L Y F F S

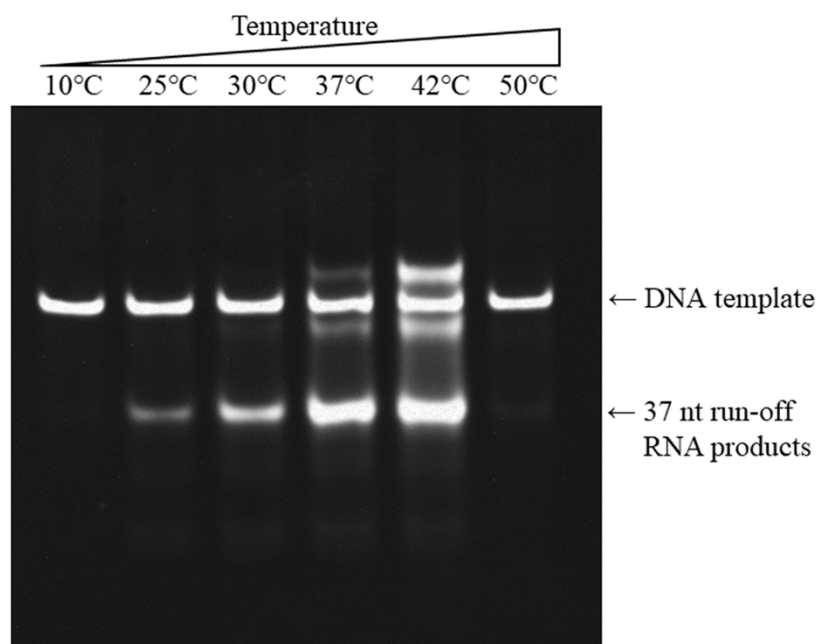
T7 C D V L A D F Y D Q F A D Q L H E S Q L D K M P A L P A . . . K G N L N I R D I L E S D F A F A

SP6 G N A L Q K L L E E H E . . . V R W M V D T G I E V P E . . . Q G E F D L N E I M D S E Y V F A

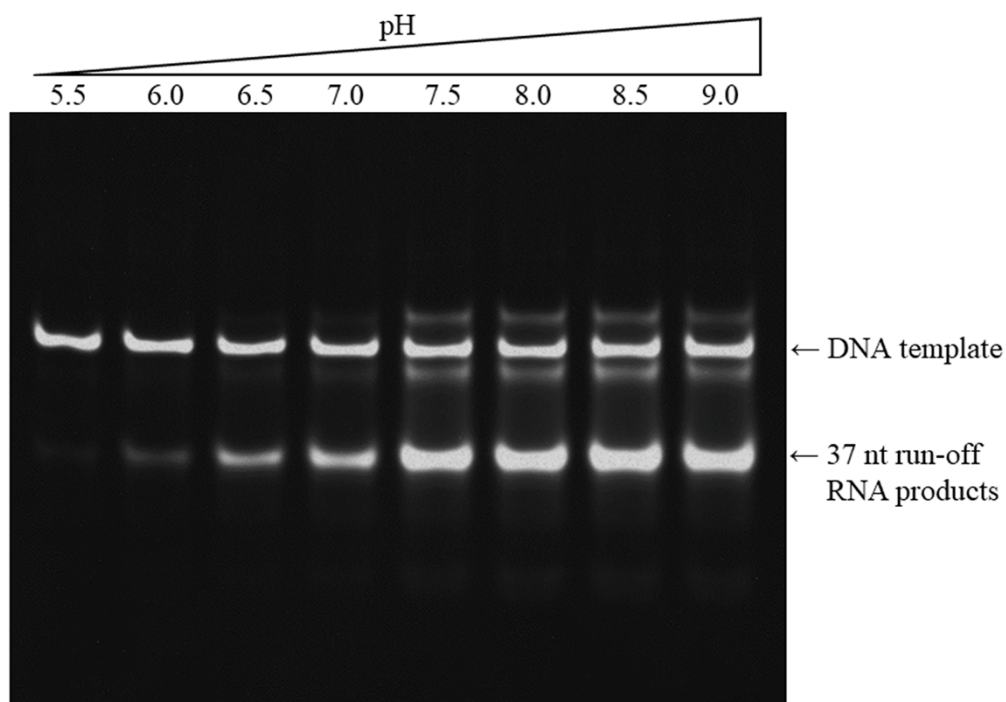
Supplementary Figure 1. Sequence alignment of the KP34 RNAP, Syn5 RNAP, T7 RNAP and SP6 RNAP was performed using Clustal Omega. The alignment was processed for publication using the ESPRIPT server v 3.0. The amino acid sequence of KP34 RNAP shares a similarity of 24.39%, 27.28% and 25.54% with Syn5,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



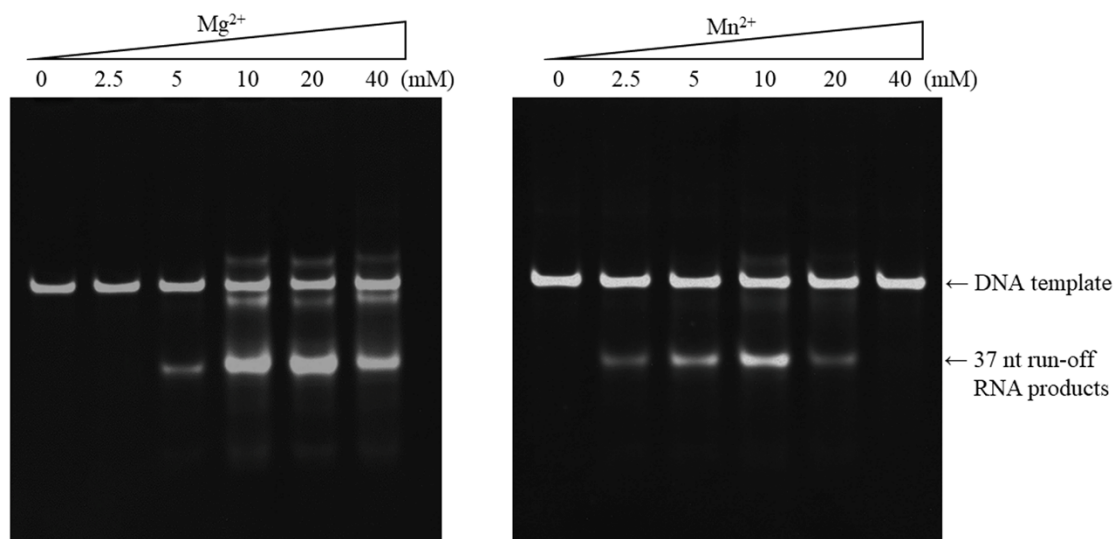
Supplementary Figure 2. SDS-PAGE analysis of all RNA polymerases in this work. All enzymes are N-terminal His-tagged and purified through Ni-NTA-agarose column and gel filtration column. Proteins were stained with Coomassie Blue. M: molecular mass marker.



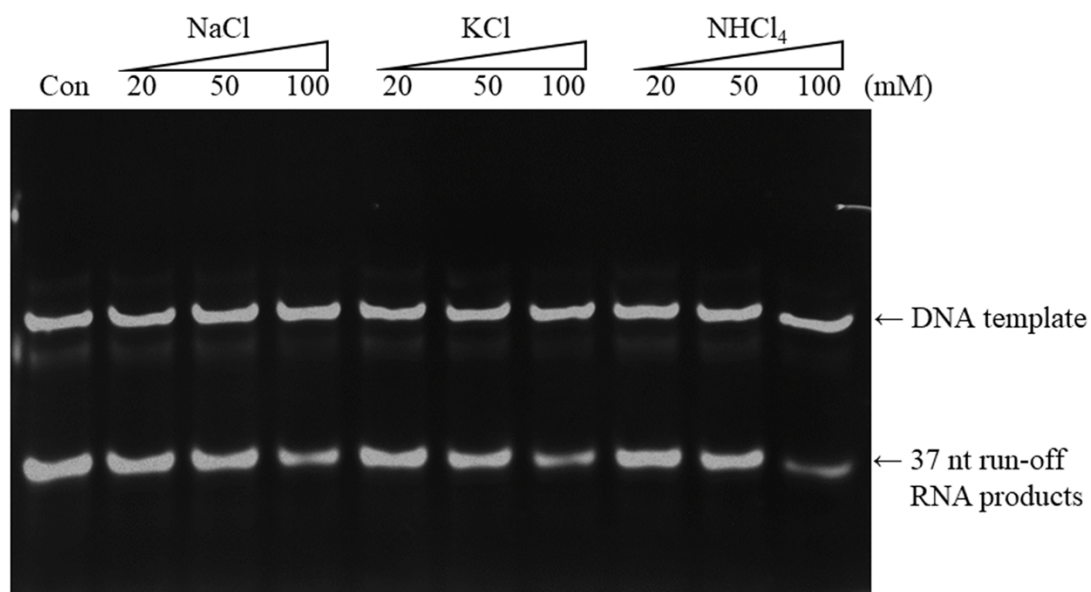
Supplementary Figure 3. Effect of reaction temperature on KP34 RNAP activity. The transcription assays of the KP34 RNAP (1 μ M) were performed at various temperatures in the presence of 40 mM Tris-HCl (pH 8.0), 20 mM MgCl₂, 2 mM spermidine, 20 mM DTT, 4 mM of each of the 4 NTPs and 2 μ M DNA template, 40 U/ μ l RNaseOUT™ Recombinant Ribonuclease Inhibitor, 0.04 U/ μ l *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.



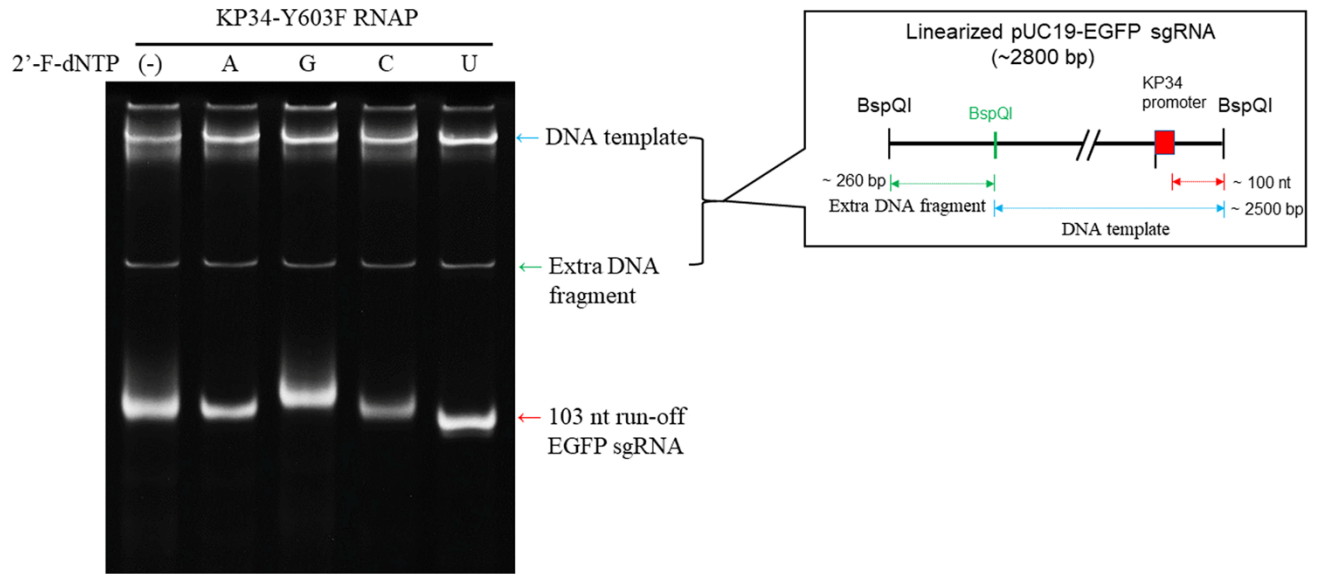
Supplementary Figure 4. Effect of pH on KP34 RNAP activity. The transcription assays of the KP34 RNAP (1 μ M) were performed using buffers with various pH at 37°C for 1 h in the presence of 20 mM MgCl₂, 2 mM spermidine, 20 mM DTT, 4 mM of each of the 4 NTPs and 2 μ M DNA template, 40 U/ μ l RNaseOUT™ Recombinant Ribonuclease Inhibitor, 0.04 U/ μ l *E. coli* inorganic pyrophosphatase. Three different buffers (all at 40 mM concentrations) were used to prepare different pHs: NaH₂PO₄-Na₂HPO₄ (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 (1 μ M) were performed with Mg^{2+}/Mn^{2+} at 37°C 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 μ M DNA template, 40 U/ μ l RNaseOUT™ Recombinant Ribonuclease Inhibitor, 0.04 U/ μ l *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 NHCl₄ on KP34 RNAP activity. NaCl, KCl or NHCl₄ 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 MgCl₂, 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 NHCl₄ 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.