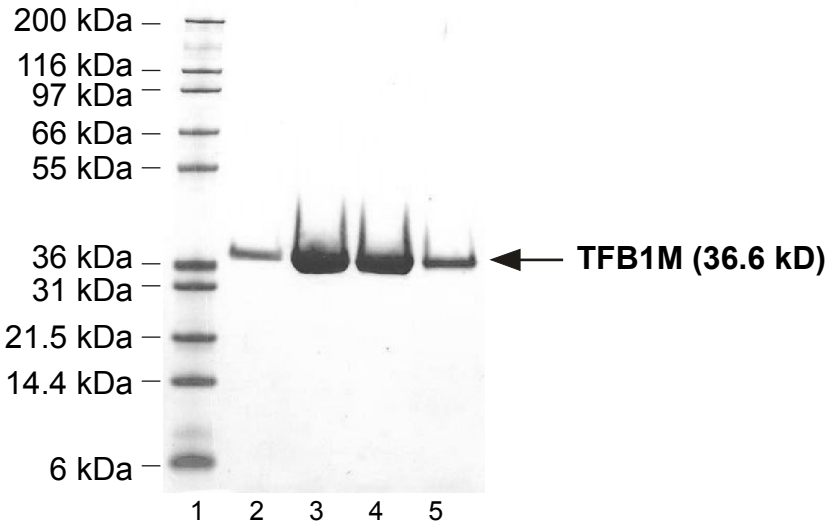
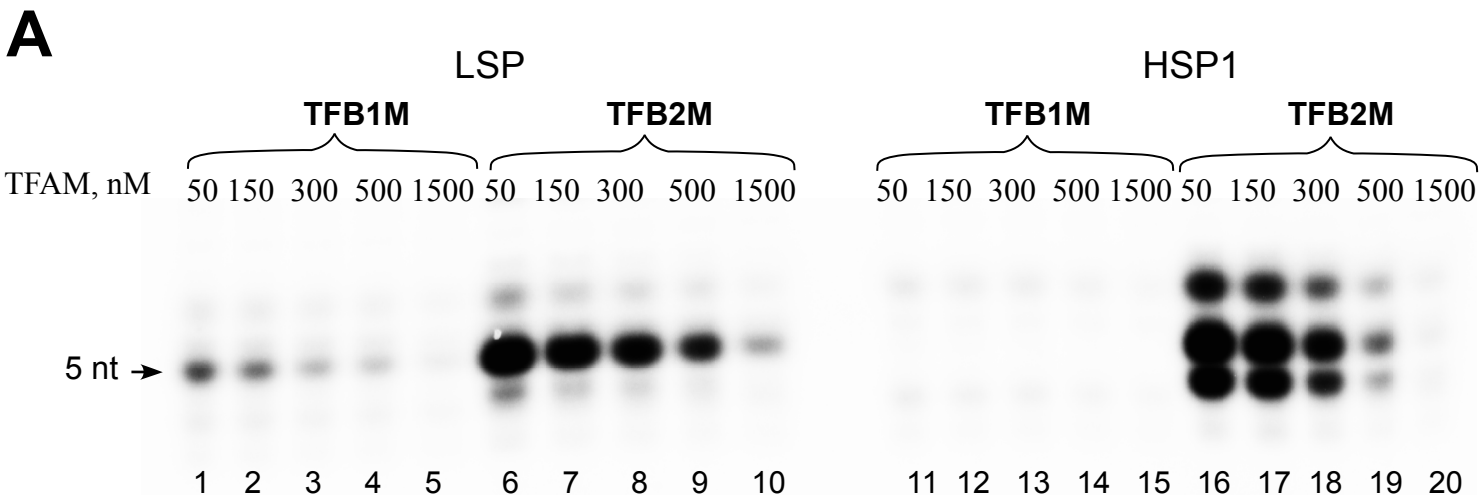


Figure S1. SDS- PAGE of TFB1M fractions after purification on heparin sepharose.

TFB1M (amino acids 28-345) was purified as described in the Experimental Procedures using affinity chromatography on chitin beads followed by purification on heparin sepharose in 300-1500 mM NaCl gradient (lanes 2-5). Fraction in lanes 3 and 4 (98% pure TFB1M) were combined and used in subsequent transcription assays. Molecular weight markers (Mark 12, Invitrogen) are indicated in lane 1.



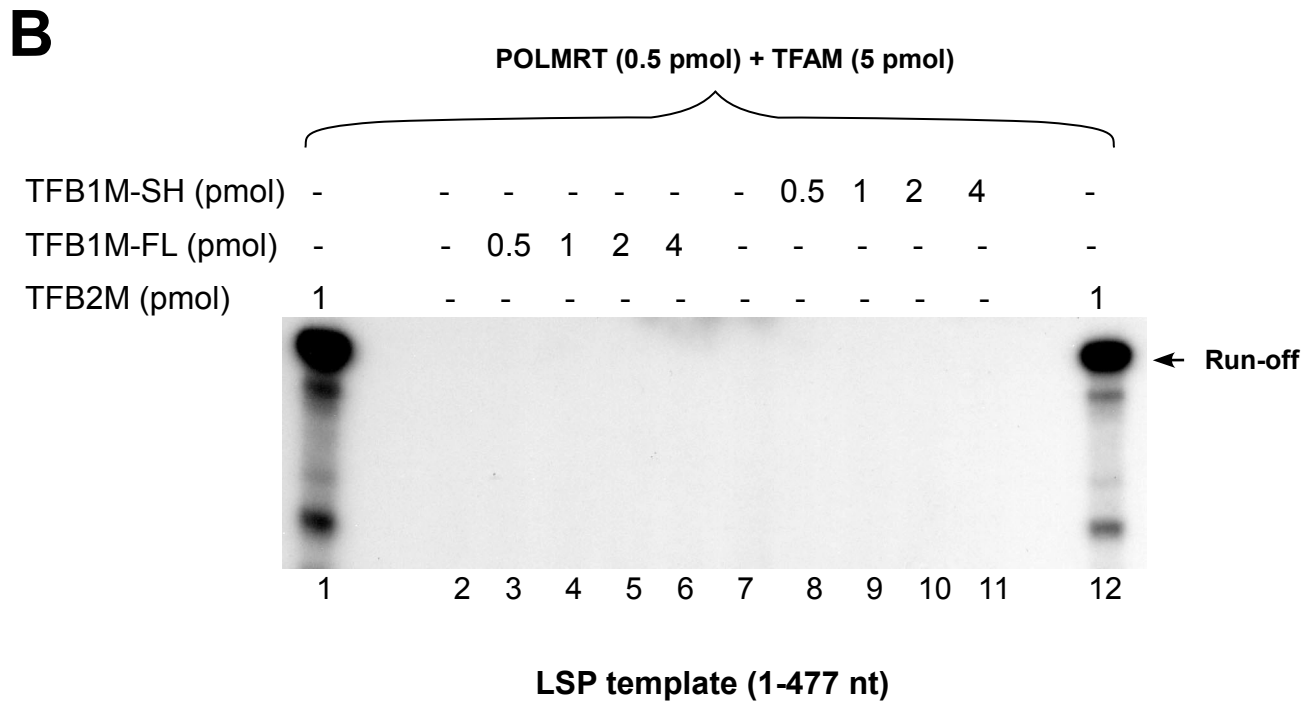


Figure S2. TFB1M does not activate in vitro transcription.

A. Abortive initiation assay. Reaction were performed using ApA primer (200 μM), ATP (10 μM), [α-P^{32}]ATP and GTP (0.3 mM) in the presence of POLMRT (150 nM), TFB2M (150nM) and TFAM, as indicated using LSP (lanes 1-10) and HSP1 (lanes 11-20) promoter.

B. Run-off transcription assay. Increasing amounts of TFB1M-FL (aa 1 - 345) or TFB1M-SH (aa 28 - 345) failed to support mitochondrial transcription in the presence of POLRMT and TFAM. TFB2M was used as a positive control. The individual transcription reaction mixtures contained POLRMT (400 fmol), TFB2M (400 fmol), TFAM (2.5 pmol) and the indicated mtDNA template (85 fmol) as described previously (Gaspari et al, EMBO J, 2004).

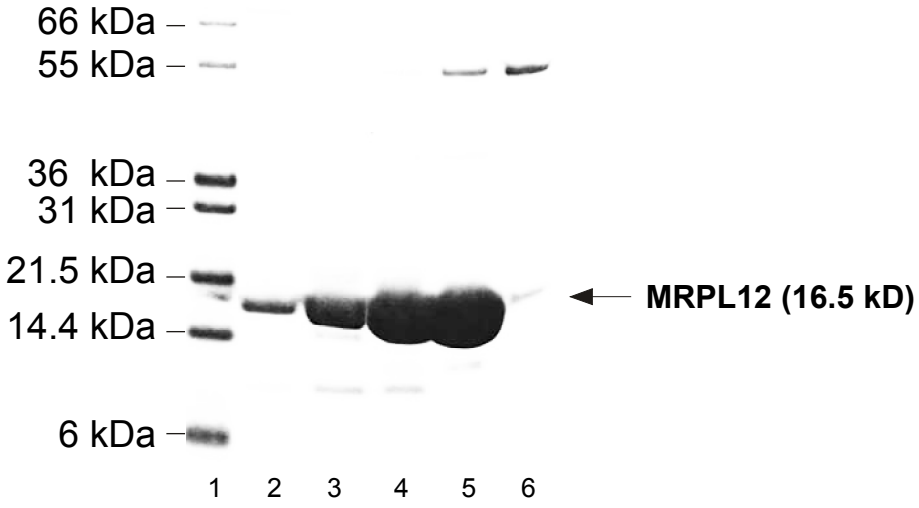


Figure S3. SDS- PAGE of MRPL12 fractions after purification on MonoQ.

MRPL12 was purified as described in the Experimental Procedures using affinity chromatography on chitin beads followed by anion exchange chromatography on MonoQ in 50-300 mM gradient of NaCl (lanes 2-6). Fraction in lanes 4 and 5 (95-98% pure MRPL12) were combined and used in subsequent transcription assays. Molecular weight markers Mark12 (Invitrogen) are shown in lane 1.

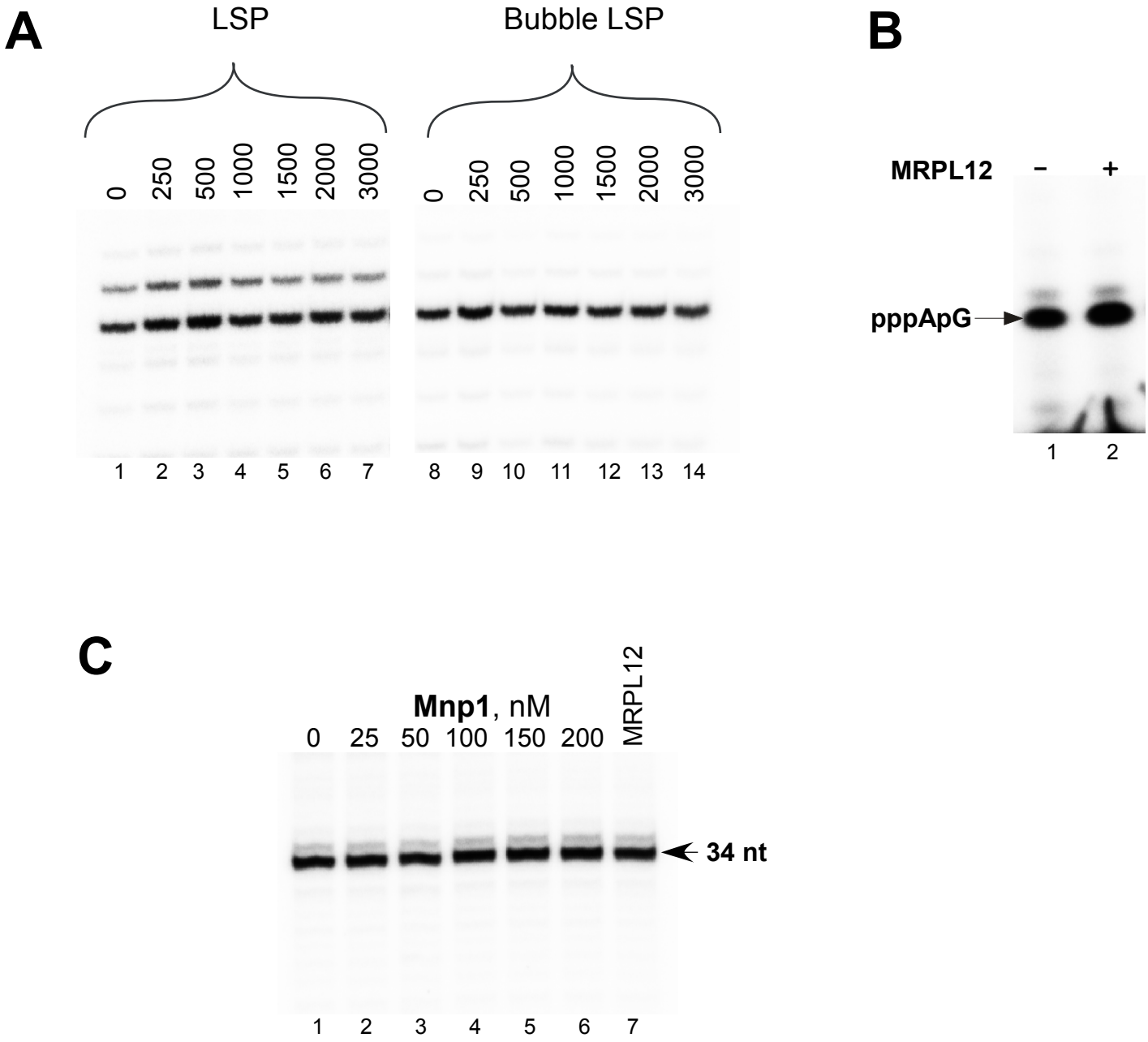


Figure S4. Human mitochondrial ribosomal protein MRPL12 and its yeast analog Mnp1 do not stimulate *in vitro* transcription.

A. MRPL12 does not stimulate factor-dependent and factor-independent transcription. Transcription reactions were performed using ApA primer (200 μM), ATP (10 μM), [α-P^{32}]ATP, UTP (0.3 mM) and GTP (0.3 mM) in the presence of POLMRT (150 nM), TFB2 (150 nM) and TFA (50 nM) (linear LSP template, lanes 1-7) or just POLMRT (bubble LSP template, lanes 8-14) and MRPL12 concentrations indicated.

B. MRPL12 does not affect formation of the first phosphodiester bond. Abortive initiation assay was done using templates having mutant LSP-AGU promoter (Sologub et al , Cell, 2009) in the presence of GTP (0.3 mM), ATP (10 μM) and [α-P^{32}]ATP.

C. Mnp1 does not stimulate yeast mitochondrial transcription. Transcription reactions containing RPO41 and Mtf1 were performed as described in Savkina et al (JBC, 2010) and supplemented with Mnp1 as indicated (lanes 1-6). MRPL12 (100 nM) was added to the reaction mixture containing RPO41 and Mtf1 (lane 7).

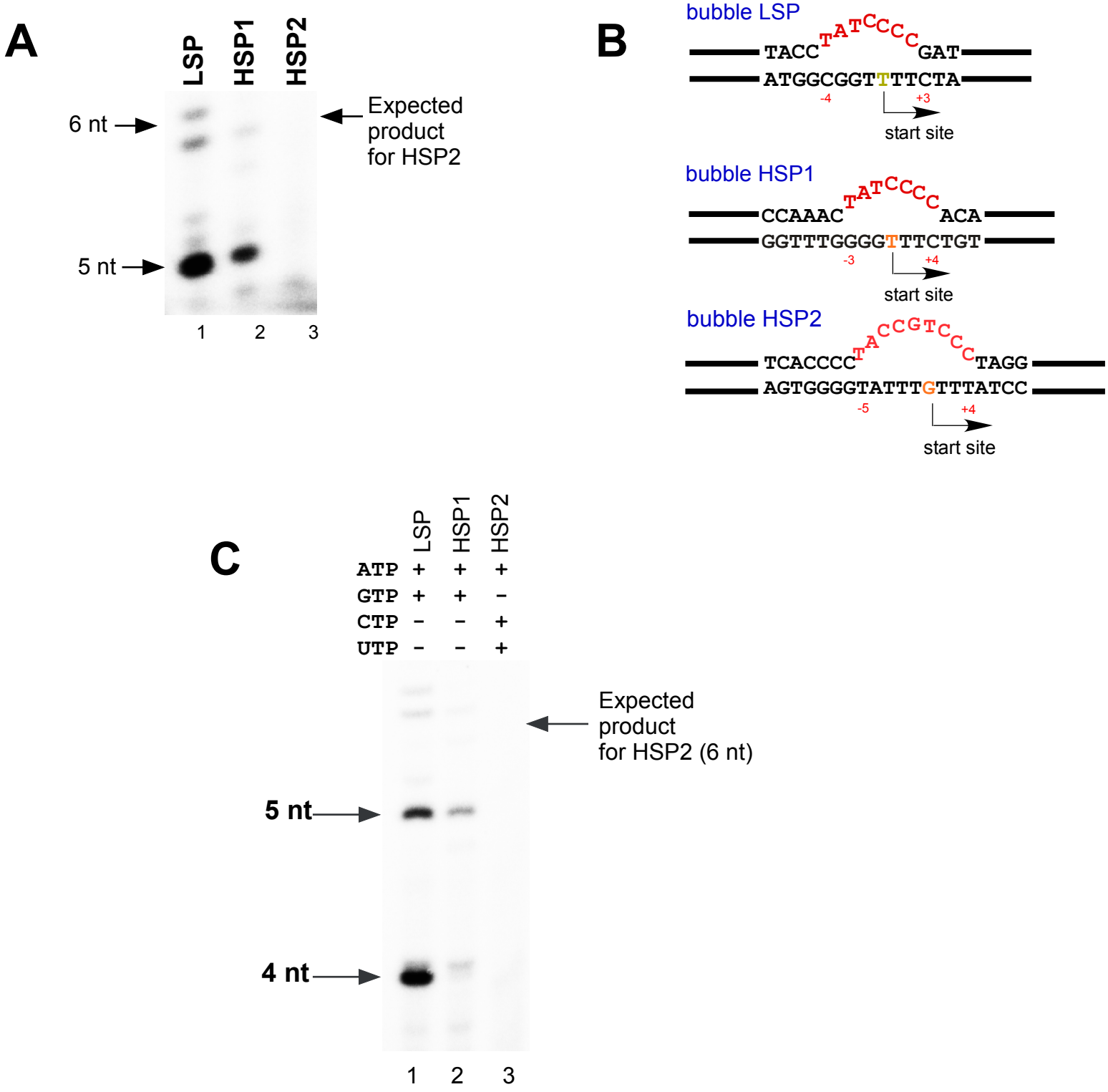


Figure S5. HSP2 is not active in in vitro transcription assays.
A. Abortive initiation assay. Reaction were performed as described in Figure S2, A using linear LSP, HSP1 and HSP2 templates described in the Experimental Procedures. NTPs mixtures used contained ATP and GTP (LSP and HSP1, lanes 1,2) and CTP, ATP and UTP (HSP2, lane 3).
B. Bubble templates containing LSP, HSP1 and HSP2.
C. Abortive initiation assay using factor-independent transcription. Reactions were performed using templates shown in panel B (all at 50 nM) and contained 150 nM POLMRT and nucleotide mixtures as in the experiment shown in panel A.