Supplemental Material

Fig. S1. RT-qPCR test for transcription termination or arrest within homopolymeric tracts. A. Arrangement of RT-qPCR probes. Arrows indicate the direction and placement of PCR primers. Probes are labelled with their respective fluorophores 6-FAM (Blue) and HEX (Green). Black dots indicate quenchers (Q). B. A graph depicting the relative fold-expression of the upstream and downstream markers. A PCR product was used as a control. Calculations were done using the BioRad iQ5 software

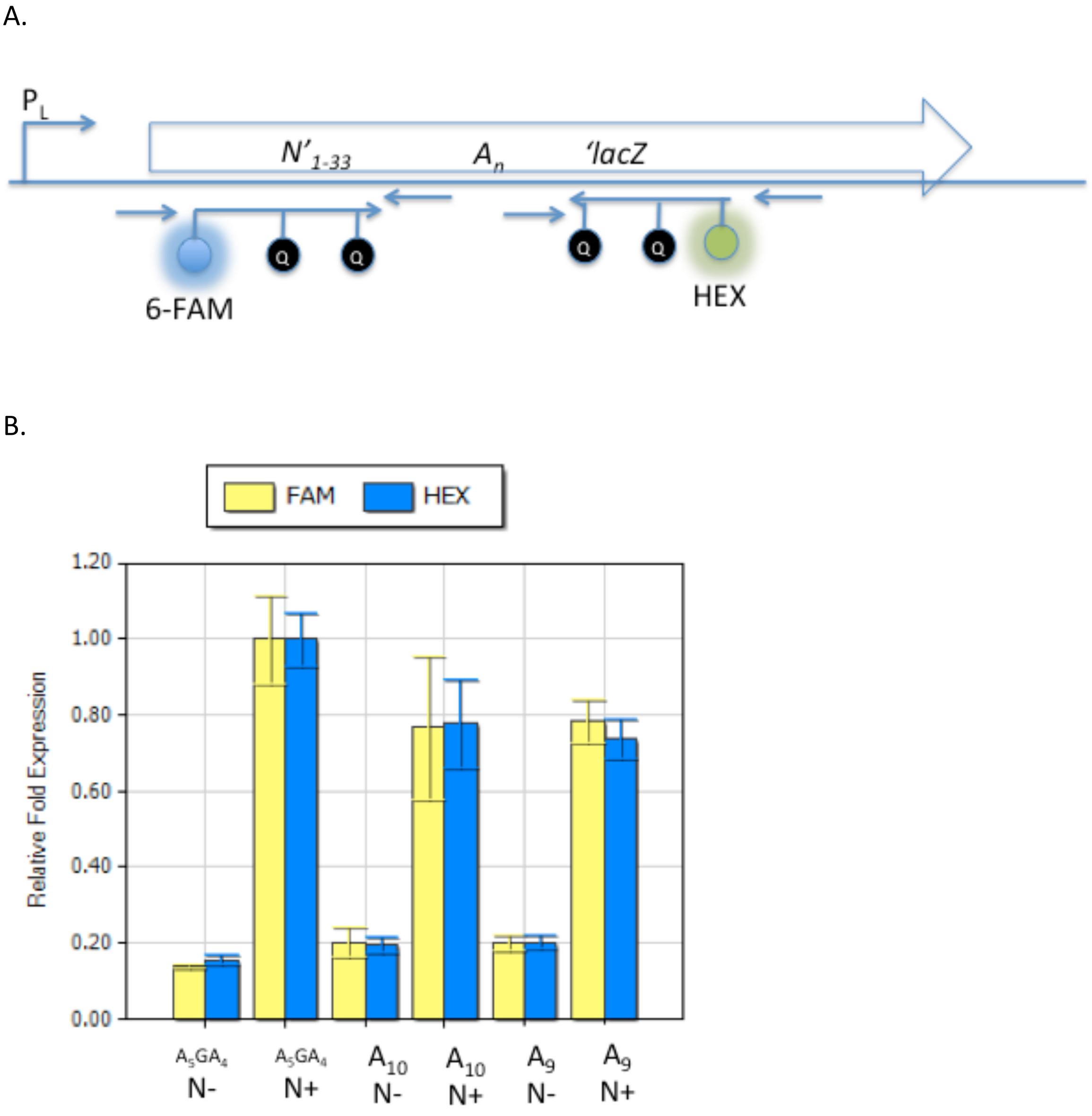


Fig. S2. Transcriptional slippage on U_{11} homopolymeric tracts in vitro. TECs were assembled as described in Figure 2 in the manuscript. Reactions were incubated with 1 mM UTP and sampled at the times shown in the figure. A MspI digest of pBR322 was run on either side of the samples for size comparison. Samples were run on a 6% denaturing gel and visualized by phosphor imaging.

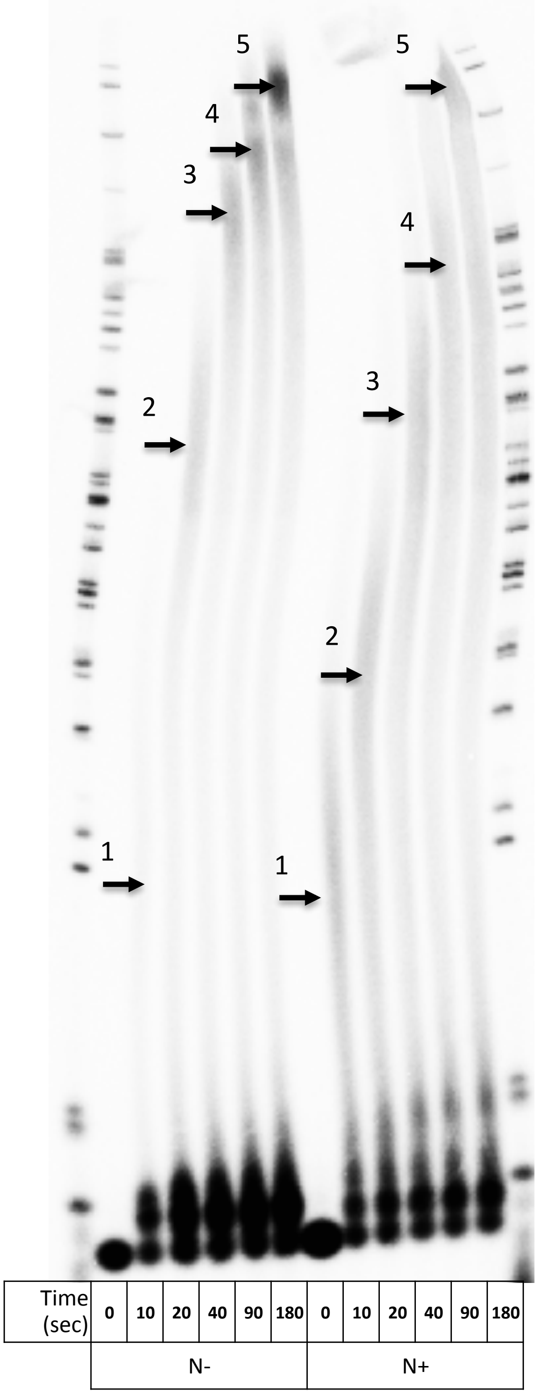
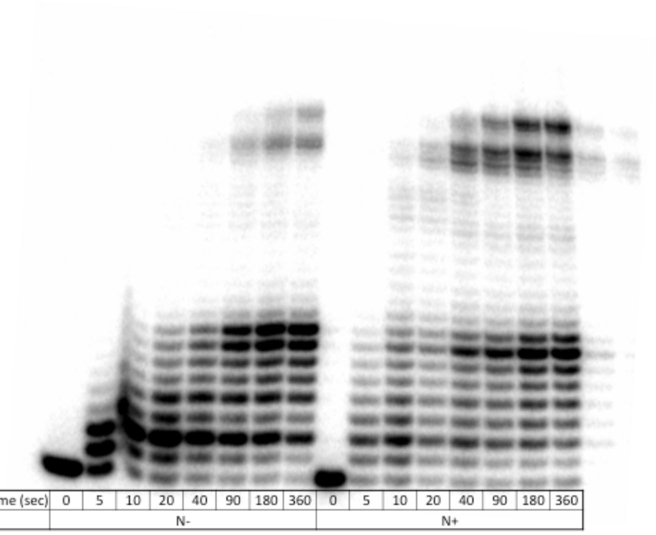


Fig. S3. Determination of the bulk elongation rate of TECs on Templates containing U10. TECs prepared as in Figure S2 were elongated using 5 uM each of all four nucleotides. Reactions were stopped at the times indicated. Samples containing N (N+) reached the end of the template in 2- to 4-fold less time than it took reactions containing none (N-).



Time (sec)	0	5	10	20	40	90	180	360	0	5	10	20	40	90	180	360
	N-								N+							

Fig. S4. NusA does not affect slippage. Samples were taken at 0, 10, 20, 45, 90, and 180 seconds, with increasing time represented by the black triangle. NusA was added at 4-fold greater concentration than TECs. Peak bands in the first time-point are emphasized with arrows.

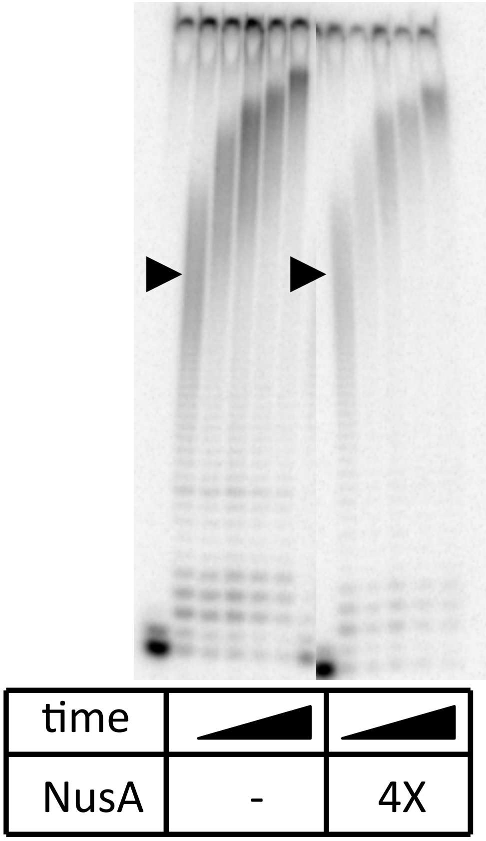


Table S1. Oligonucleotides for strain construction and RT-qPCR

Sequence	Description
5'- AAT GGA AAG CAG CAA ATC CCC TGT TGG TTG GGG TAA GCG CAA AAC CAG TTA AAA AGA AAA CGA CTG GGA A -3'	N-homology A_5GA_4 -1 frame
5'- ATG TGC TGC AAG GCG ATT AAG TTG GGT AAC GCC AGG GTT TTC CCA GTC GTT TTC TTT TTA ACT GGT TTT G -3'	LacZ-homology A_5GA_4 -1 frame
5'- AAT GGA AAG CAG CAA ATC CCC TGT TGG TTG GGG TAA GCG CAA AAC CAG TTA AAA AGA AAA GCC GAC TGG GAA-3'	N-homology A_5GA_4 +1 frame
5'- ATG TGC TGC AAG GCG ATT AAG TTG GGT AAC GCC AGG GTT TTC CCA GTC GGC TTT TCT TTT TAA CTG GTT TTG -3'	LacZ-homology A_5GA_4 +1 frame
5'- AAT GGA AAG CAG CAA ATC CCC TGT TGG TTG GGG TAA GCG CAA AAC CAG TTA AAA AGA AAA CCG ACT GGG AA -3'	N-homology A_5GA_4 in-frame
5'- ATG TGC TGC AAG GCG ATT AAG TTG GGT AAC GCC AGG GTT TTC CCA GTC GGT TTT CTT TTT AAC TGG TTT TG -3'	LacZ-homology A_5GA_4 in-frame
5'- AAT GGA AAG CAG CAA ATC CCC TGT TGG TTG GGG TAA GCG	N-homology

CAA AAC CAG TTA AAA ATA AAA CGA CTG GGA A -3'	A_5UA_4 -1 frame
5'- ATG TGC TGC AAG GCG ATT AAG TTG GGT AAC GCC AGG GTT TTC CCA GTC GTT TTA TTT TTA ACT GGT TTT G -3'	LacZ-homology A_5UA_4 -1 frame
5'- AAT GGA AAG CAG CAA ATC CCC TGT TGG TTG GGG TAA GCG CAA AAC CAG TTA AAA AUA AAA GCC GAC TGG GAA-3'	N-homology A_5UA_4 +1 frame
5'- ATG TGC TGC AAG GCG ATT AAG TTG GGT AAC GCC AGG GTT TTC CCA GTC GGC TTT TAT TTT TAA CTG GTT TTG -3'	LacZ-homology A_5UA_4 +1 frame
5'- AAT GGA AAG CAG CAA ATC CCC TGT TGG TTG GGG TAA GCG CAA AAC CAG TTA AAA AUA AAA CCG ACT GGG AA -3'	N-homology A_5UA_4 in-frame
5'- ATG TGC TGC AAG GCG ATT AAG TTG GGT AAC GCC AGG GTT TTC CCA GTC GGT TTT ATT TTT AAC TGG TTT TG -3'	LacZ-homology A_5UA_4 in-frame
5'- AAT GGA AAG CAG CAA ATC CCC TGT TGG TTG GGG TAA GCG CAA AAC CAG TTA AAA AAA GCA CTG GGA A -3'	N-homology A_7 -1 frame
5'- ATG TGC TGC AAG GCG ATT AAG TTG GGT AAC GCC AGG GTT TTC CCA GTG CTT TTT TTA ACT GGT TTT G -3'	LacZ-homology A_7 -1 frame
5'- AAT GGA AAG CAG CAA ATC CCC TGT TGG TTG GGG TAA GCG CAA AAC CAG TTA AAA AAA GCG GAC TGG GAA -3'	N-homology A_7 +1 frame
5'- ATG TGC TGC AAG GCG ATT AAG TTG GGT AAC GCC AGG GTT TTC CCA GTC CGC TTT TTT TAA CTG GTT TTG -3'	LacZ-homology A_7 +1 frame
5'- AAT GGA AAG CAG CAA ATC CCC TGT TGG TTG GGG TAA GCG CAA AAC CAG TTA AAA AAAGCG ACT GGG AA -3'	N-homology A_7 in-frame
5'- ATG TGC TGC AAG GCG ATT AAG TTG GGT AAC GCC AGG GTT TTC CCA GTC TTT TTT AAC TGG TTT TG -3'	LacZ-homology A_7 in-frame
5'- AAT GGA AAG CAG CAA ATC CCC TGT TGG TTG GGG TAA GCG CAA AAC CAG TTA AAA AAA AGA CTG GGA A -3'	N-homology A_8 -1 frame
5'- ATG TGC TGC AAG GCG ATT AAG TTG GGT AAC GCC AGG GTT TTC CCA GTC TTT TTT TTA ACT GGT TTT G -3'	LacZ-homology A_8 -1 frame
5'- AAT GGA AAG CAG CAA ATC CCC TGT TGG TTG GGG TAA GCG CAA AAC CAG TTA AAA AAA AGG GAC TGG GAA -3'	N-homology A_8 +1 frame
5'- ATG TGC TGC AAG GCG ATT AAG TTG GGT AAC GCC AGG GTT TTC CCA GTC CCT TTT TTT TAA CTG GTT TTG -3'	LacZ-homology A_8 +1 frame
5'- AAT GGA AAG CAG CAA ATC CCC TGT TGG TTG GGG TAA GCG CAA AAC CAG TTA AAA AAA AGG ACT GGG AA -3'	N-homology A_8 in-frame
5'- ATG TGC TGC AAG GCG ATT AAG TTG GGT AAC GCC AGG GTT TTC CCA GTC CTT TTT TTT AAC TGG TTT TG -3'	LacZ-homology A_8 in-frame
5'- AAT GGA AAG CAG CAA ATC CCC TGT TGG TTG GGG TAA GCG CAA AAC CAG TTA AAA AAA AAG TGA CTG GGA A -3'	N-homology A_9 -1 frame
5'- ATG TGC TGC AAG GCG ATT AAG TTG GGT AAC GCC AGG GTT TTC CCA GTC ACT TTT TTT TTA ACT GGT TTT G -3'	LacZ-homology A_9 -1 frame
5'- AAT GGA AAG CAG CAA ATC CCC TGT TGG TTG GGG TAA GCG CAA AAC CAG TTA AAA AAA AAG GAC TGG GAA -3'	N-homology A_9 +1 frame
5'- ATG TGC TGC AAG GCG ATT AAG TTG GGT AAC GCC AGG GTT TTC CCA GTC CTT TTT TTT TAA CTG GTT TTG -3'	LacZ-homology A_9 +1 frame

5'- AAT GGA AAG CAG CAA ATC CCC TGT TGG TTG GGG TAA GCG CAA AAC CAG TTA AAA AAA AAG CCG ACT GGG AA -3'	N-homology A_9 in-frame
5'- ATG TGC TGC AAG GCG ATT AAG TTG GGT AAC GCC AGG GTT TTC CCA GTC GGC TTT TTT TTT AAC TGG TTT TG -3'	LacZ-homology A_9 in-frame
5'- AAT GGA AAG CAG CAA ATC CCC TGT TGG TTG GGG TAA GCG CAA AAC CAG TTA AAA AAA AAA CGA CTG GGA A -3'	N-homology A_{10} -1 frame
5'- ATG TGC TGC AAG GCG ATT AAG TTG GGT AAC GCC AGG GTT TTC CCA GTC GTT TTT TTT TTA ACT GGT TTT G -3'	LacZ-homology A_{10} -1 frame
5'- AAT GGA AAG CAG CAA ATC CCC TGT TGG TTG GGG TAA GCG CAA AAC CAG TTA AAA AAA AAA GCC GAC TGG GAA-3'	N-homology A_{10} +1 frame
5'- ATG TGC TGC AAG GCG ATT AAG TTG GGT AAC GCC AGG GTT TTC CCA GTC GGC TTT TTT TTT TAA CTG GTT TTG -3'	LacZ-homology A_{10} +1 frame
5'- AAT GGA AAG CAG CAA ATC CCC TGT TGG TTG GGG TAA GCG CAA AAC CAG TTA AAA AAA AAA CCG ACT GGG AA -3'	N-homology A_{10} in-frame
5'- ATG TGC TGC AAG GCG ATT AAG TTG GGT AAC GCC AGG GTT TTC CCA GTC GGT TTT TTT TTT AAC TGG TTT TG -3'	LacZ-homology A_{10} in-frame
5'- AAT GGA AAG CAG CAA ATC CCC TGT TGG TTG GGG TAA GCG CAA AAC CAG TTA AAA AAA AAA AGA CTG GGA A -3'	N-homology A_{11} -1 frame
5'- ATG TGC TGC AAG GCG ATT AAG TTG GGT AAC GCC AGG GTT TTC CCA GTC TTT TTT TTT TTA ACT GGT TTT G -3'	LacZ-homology A_{11} -1 frame
5'- AAT GGA AAG CAG CAA ATC CCC TGT TGG TTG GGG TAA GCG CAA AAC CAG TTA AAA AAA AAA ACC GAC TGG GAA -3'	N-homology A_{11} +1 frame
5'- ATG TGC TGC AAG GCG ATT AAG TTG GGT AAC GCC AGG GTT TTC CCA GTC GGT TTT TTT TTT TAA CTG GTT TTG -3'	LacZ-homology A_{11} +1 frame
5'- AAT GGA AAG CAG CAA ATC CCC TGT TGG TTG GGG TAA GCG CAA AAC CAG TTA AAA AAA AAA ACG ACT GGG AA -3'	N-homology A_{11} in-frame
5'- ATG TGC TGC AAG GCG ATT AAG TTG GGT AAC GCC AGG GTT TTC CCA GTC GTT TTT TTT TTT AAC TGG TTT TG -3'	LacZ-homology A_{11} in-frame
5'- AAT GGA AAG CAG CAA ATC CCC TGT TGG TTG GGG TAA GCG CAA AAC CAG TAT TTT TTT TTT CGA CTG GGA A -3'	N-homology U_{10} -1 frame
5'- ATG TGC TGC AAG GCG ATT AAG TTG GGT AAC GCC AGG GTT TTC CCA GTC GAA AAA AAA AAT ACT GGT TTT G -3'	LacZ-homology U_{10} -1 frame
5'- AAT GGA AAG CAG CAA ATC CCC TGT TGG TTG GGG TAA GCG CAA AAC CAG TTC CCC CCC CCC GGA CTG GGA A -3'	N-homology C_{10} -1 frame
5'- ATG TGC TGC AAG GCG ATT AAG TTG GGT AAC GCC AGG GTT TTC CCA GTC CGG GGG GGG GGA ACT GGT TTT G -3'	LacZ-homology C_{10} -1 frame
5'- AAT GGA AAG CAG CAA ATC CCC TGT TGG TTG GGG TAA GCG CAA AAC CAG TTG GGG GGG GGG CGA CTG GGA A -3'	N-homology G_{10} -1 frame
5'- ATG TGC TGC AAG GCG ATT AAG TTG GGT AAC GCC AGG GTT TTC CCA GTC GCC CCC CCC CCA ACT GGT TTT G -3'	LacZ-homology G_{10} -1 frame
5'- AAT GGA AAG CAG CAA ATC CCC TGT TGG TTG GGG TAA GCG CAA AAC CAG TAT TTT TTT TTT CCG ACT GGG AA -3'	N-homology U_{10} in-frame
5'- ATG TGC TGC AAG GCG ATT AAG TTG GGT AAC GCC AGG GTT TTC	LacZ-homology

CCA GTC GGC GGA AAA AAA AAA TAC TGG TTT TG -3'	U_{10} in-frame
5'- AAT GGA AAG CAG CAA ATC CCC TGT TGG TTG GGG TAA GCG CAA AAC CAG TTC CCC CCC CCC GCG ACT GGG AA -3'	N-homology C_{10} in-frame
5'- ATG TGC TGC AAG GCG ATT AAG TTG GGT AAC GCC AGG GTT TTC CCA GTC GCG GGG GGG GGG AAC TGG TTT TG -3'	LacZ-homology C_{10} in-frame
5'- AAT GGA AAG CAG CAA ATC CCC TGT TGG TTG GGG TAA GCG CAA AAC CAG TTG GGG GGG GGG CCG ACT GGG AA -3'	N-homology G_{10} in-frame
5'- ATG TGC TGC AAG GCG ATT AAG TTG GGT AAC GCC AGG GTT TTC CCA GTC GGC CCC CCC CCC AAC TGG TTT TG -3'	LacZ-homology G_{10} in-frame
5'- AAT GGA AAG CAG CAA ATC CCC TGT TGG TTG GGG TAA GCG CAA AAC CAG TAT TTT TTT TTT GCC GAC TGG GAA -3'	N-homology U_{10} +1 frame
5'- ATG TGC TGC AAG GCG ATT AAG TTG GGT AAC GCC AGG GTT TTC CCA GTC GGC AAA AAA AAA A TA CTG GTT TTG -3'	LacZ-homology U_{10} +1 frame
5'- AAT GGA AAG CAG CAA ATC CCC TGT TGG TTG GGG TAA GCG CAA AAC CAG TT C CCC CCC CCC GCC GAC TGG GAA -3'	N-homology C_{10} +1 frame
5'- ATG TGC TGC AAG GCG ATT AAG TTG GGT AAC GCC AGG GTT TTC CCA GTC GGC GGG GGG GGG GAA CTG GTT TTG -3'	LacZ-homology C_{10} +1 frame
5'- AAT GGA AAG CAG CAA ATC CCC TGT TGG TTG GGG TAA GCG CAA AAC CAG TTA GGG GGG GGG GCC GAC TGG GAA -3'	N-homology G_{10} +1 frame
5'- ATG TGC TGC AAG GCG ATT AAG TTG GGT AAC GCC AGG GTT TTC CCA GTC GGC CCC CCC CCC TAA CTG GTT TTG -3'	LacZ-homology G_{10} +1 frame
5'- CAT TCG CCA TTC AGG CTG CG-3'	lacZ reverse for amplifying N::lacZ
5'- TCA CCT ACC AAA CAA TGC CCC-3'	upstream pL for amplifying N::lacZ
5'- AGA ATC CAG ATG GAT GCA CA-3'	N-leader forward
5'- ATT TGC TGC TTT CCA TTG AGC-3'	N-leader reverse
5'- /56-FAM/CG AAC GTC G/ZEN/C GCA GAG AAA CAG /3IABkFQ/-3'	N-leader probe
5'- AAA CCC TGG CGT TAC CC-3'	LacZ forward
5'- CGG GCC TCT TCG CTA TTA C-3'	LacZ reverse
5'- /5HEX/CT TAA TCG C/ZEN/C TTG CAG CAC ATC CC/3IABkFQ/-3'	LacZ probe
5'- ATC CCC TGT TGG TTG GGG TAA GCG CAA AAC CAG TCA AAA AAA AGA CTG GGA AAA CCC TGG CGT TAC CCA ACT TAA TCG C-3'	5'-C -1 frame
5'- ATC CCC TGT TGG TTG GGG TAA GCG CAA AAC CAG TTA AAA AAA AGA CTG GGA AAA CCC TGG CGT TAC CCA ACT TAA TCG C-3'	5'-U -1frame
5'- ATC CCC TGT TGG TTG GGG TAA GCG CAA AAC CAG TGA AAA AAA AGA CTG GGA AAA CCC TGG CGT TAC CCA ACT TAA TCG C-3'	5'-G -1 frame
5'- ATC CCC TGT TGG TTG GGG TAA GCG CAA AAC CAG TCA AAA AAA AGG GAC TGG GAA AAC CCT GGC GTT ACC CAA CTT AAT CG-3'	5'-C +1 frame
5'- ATC CCC TGT TGG TTG GGG TAA GCG CAA AAC CAG TTA AAA AAA AGG GAC TGG GAA AAC CCT GGC GTT ACC CAA CTT AAT CG-3'	5'-U +1 frame
5'- ATC CCC TGT TGG TTG GGG TAA GCG CAA AAC CAG TGA AAA AAA AGG GAC TGG GAA AAC CCT GGC GTT ACC CAA CTT AAT CG-3'	5'-G +1 frame

5'- CAT GAA GGT GAC GCT CTT AAA AAT TAA GCC CAT TTC TTG GGC AGC ATT CAA AGC AGA AGG CTT TGG GGT G-3'	nutL7

Supplemental Methods

RT-qPCR

Slippage reporter strains were grown and induced as indicated above (see β-galactosidase assay). After cells were plunged on ice to stop further growth, cultures were treated with RNAprotect (Qiagen, Valencia, CA), and total RNA was isolated using the Qiagen RNeasy Mini Kit, according to the manufacturers recommendations (Qiagen, Valencia, CA). RNA samples were stored at -20 °C until use in RT-qPCR experiments. TaqManTM probes were designed using PrimerQuest® and purchased from IDT (Coralville, IA, USA), see supplemental information for primer and probe sequences. RT-qPCR was accomplished using the TaqMan® RNA-to-CT™ 1-Step Kit according to the manufacturers recommendations. Samples were processed using the iCycler iQ™ real-time PCR detection system, and quantified using the iQ5 software (Version 2.1.97.1001, Bio-Rad, Hercules, CA).

In vitro processive slippage assay on U_{11} tracts

TECs were prepared and radiolabelled as described in the body of the main text. For U_{11}-tract experiments, the template strand and non-template strands contained the sequences 5’-TTG GGT TCT CTA TTC GCC TCG AAA AAA AAA AAC CCT CTC GAT GGC TGT AAG TAT CCT ATA CC-3’, and 5’-GGT ATA GGA TAC TTA CAG CCA TCG AGA GGG TTT TTT TTT TTC GAG GCG AAT AGA GAA CCC AA-3’, respectively. One millimolar UTP was added and complexes were allowed to transcribe the U-tract for 10, 20, 40, and 90 sec, after which they were stopped by the addition of 2X loading buffer containing 7M Urea. Samples were analyzed by denaturing PAGE, visualized by phophorimaging (Molecular Dynamics).

In vitro processive slippage assay with NusA

TECs were prepared and radiolabelled as described in the body of the main text. The template strand and non-template strands contained the sequences 5’-TTG GGT TCT CTA TTC GCC TCG TTT TTT TTT TTG CCT CTC GAT GGC TGT AAG TAT CCT ATA CC-3’, and 5’-GGT ATA GGA TAC TTA CAG CCA TCG AGA GGC AAA AAA AAA AAC GAG GCG AAT AGA GAA CCC AA-3’, respectively. NusA was added at 4-fold concentration with respect to TECs, where present. ATP was added at a concentration of 10 μM and complexes were allowed to transcribe the A-tract for 10, 20, 30, 40, and 60 sec, after which they were stopped by the addition of 2X loading buffer containing 7M Urea. Samples were analyzed by denaturing PAGE, visualized by phophorimaging (Molecular Dynamics).