Appendix

Appendix Supplementary Methods

Time course analysis of telomerase primer-extension assay. Five microliters of RRL reconstituted TF telomerase enzyme was assayed in a master mix containing 50 μL reaction containing 1X telomerase reaction buffer, 40 μM the pre-annealed DNA/RNA duplex, 2 or 200 μM dGTP and 0.165 μM α-^{32}P-dATP. The master mix was aliquoted to 10 μL reactions, incubated at 30°C, and reactions terminated at 0, 20, 40, 60, or 80 min by phenol/chloroform extraction, followed by ethanol precipitation. The DNA products were resolved on a 15% (wt/vol) polyacrylamide/8 M urea denaturing gel, dried, exposed to a phosphorstorage screen, imaged on a Bio-Rad FX-Pro phosphorimager and quantitated using Quantity One (Bio-Rad) software. The intensities of specific products were normalized to the recovery control for total product intensity or the total product intensity for nucleotide incorporation efficiency.

Quality assessment of nucleotide reagents. Commercially purchased nucleotides (Sigma) were analyzed by matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) using a Voyager DE STR (Applied Biosystems) operated in negative ion mode. For this analysis, 2 μL of the sample-analyte solution (200 nmoles of dGTP, dGDP or dGMP in 100 μM Ammonium Acetate) was combined with 10 μL of freshly prepared 0.5 M ethanol solution of 2,4,6-trihydroxyacetophenone (matrix) and 1 μL was applied to the mass spectrometer auto-sampler plate and air dried prior to analysis.

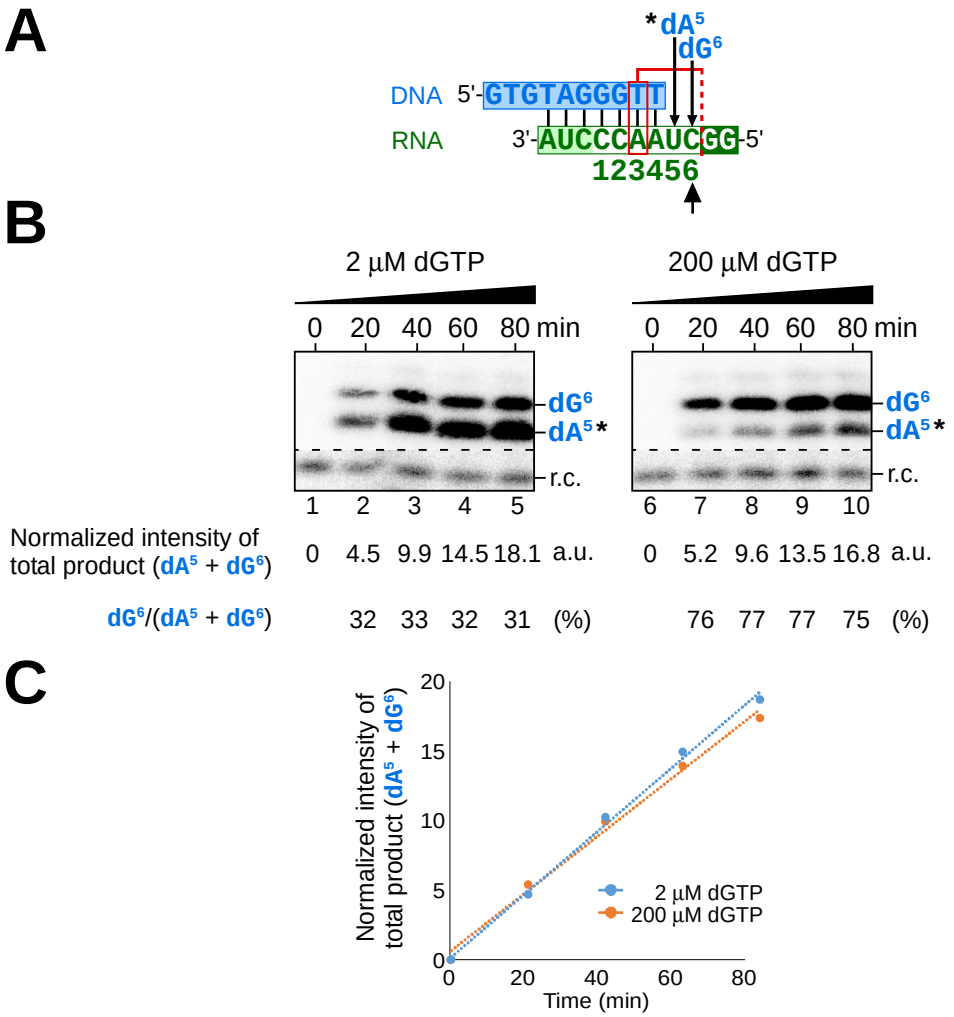

Figure S1. Time course analysis of telomerase primer-extension assay at 2 and 200 µM.
A Sequence of the DNA/RNA hybrid substrate, position of the pause signal and pause site (red). *In vitro* reconstituted template-free (TF) telomerase were analyzed with specific DNA/RNA hybrid substrate to determine linear product formation for the K_M measurements of nucleotide incorporation over time (0, 20, 40, 60, and 80 min). Numbers below the DNA/RNA duplex indicate positions corresponding to the telomerase template and the order of nucleotides incorporated. The nucleotide incorporation K_M values measured are indicated (black arrow). The DNA products were labeled by incorporating a ^{32}P-dATP (0.166 µM, asterisk) prior to the K_M measurement.
B Representative gel for linear product formation for the K_M measurements of nucleotide incorporation over time. A ^{32}P end-labeled DNA recovery control (r.c.) was added before product purification and precipitation. The intensity of total product formation (dA^5 and dG^6) at various times were normalized to the loading control were measured with arbitrary units (a.u.) and were linear. The ratio intensity of dG^6 over total (dA^5 and dG^6) was unchanged with time.
C Plots derived from the normalized intensity of total product formation (dA^5 and dG^6) at various times revealed a linear relationship between product formation and time.

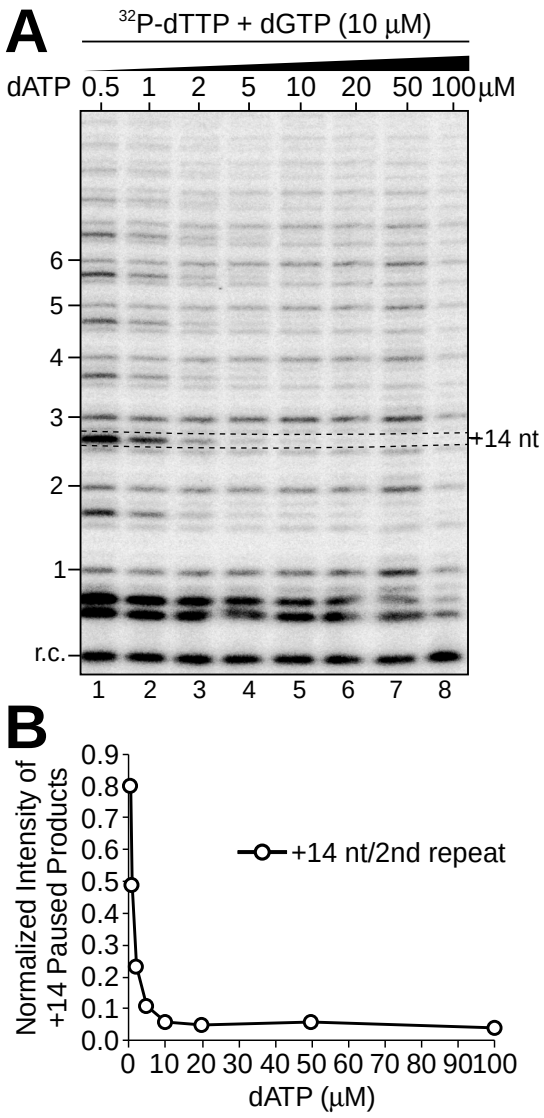

Figure S2. Minimal nucleotide concentration for processive nucleotide addition.
A Direct primer extension assays were performed with native telomerase reconstituted in vivo and immuno-purified. The DNA primer substrate was extended in the presence of 0.166 µM 32P-dTTP (asterisk), 10 µM dTTP, 10 µM dGTP and a range of dATP concentrations (0.5, 1, 2, 5, 10, 20, 50 and 100 µM).
B Quantitation of intermediate product accumulation from dATP insufficiency. Plot of band intensity for the stalling +14 nt DNA product (14 nucleotides added, two repeats and 4 nucleotides) over the major 2nd repeat product (two repeats added) and normalized for total telomerase activity.

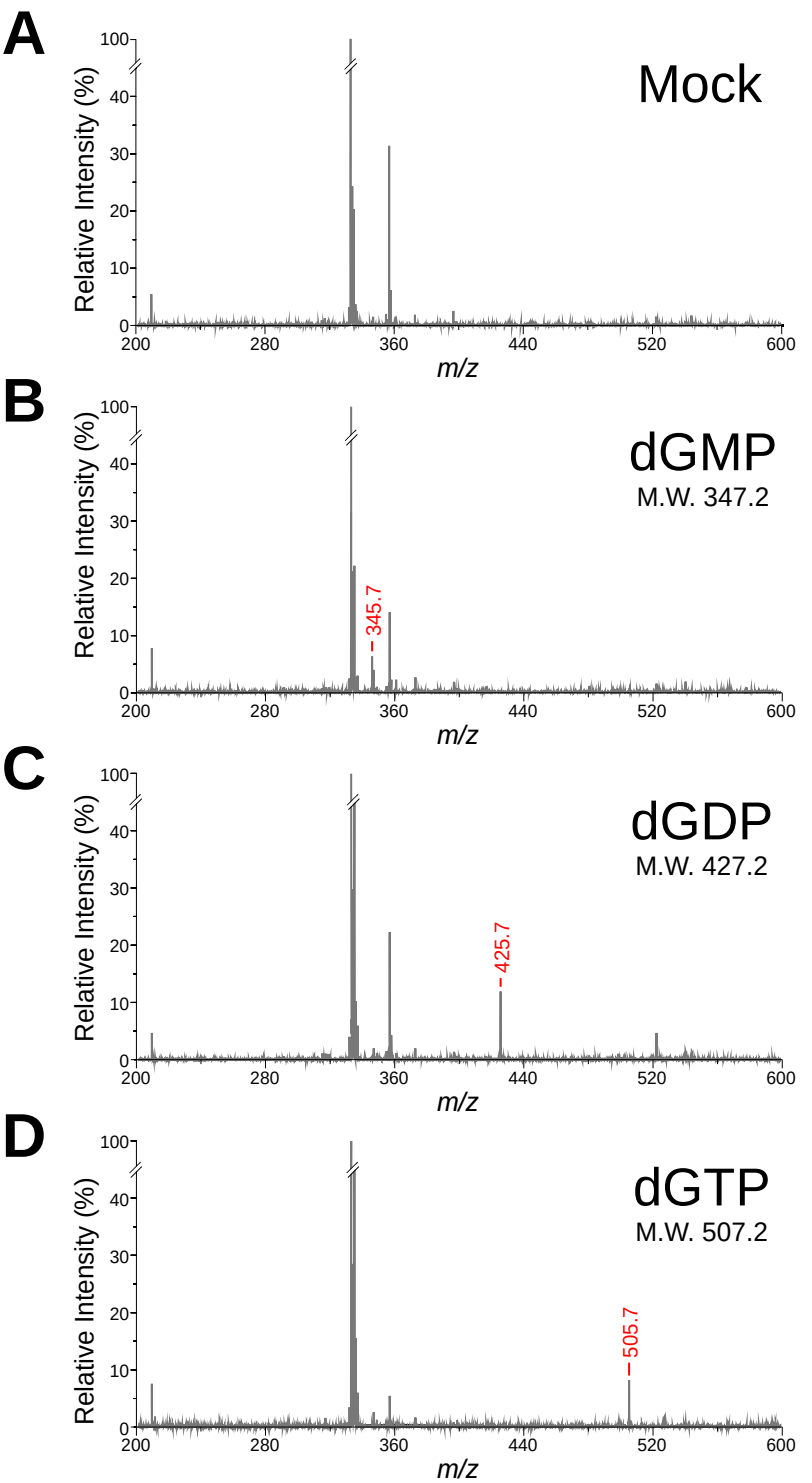

Figure S3. Purity assessment of nucleotides.
A-D MALDI-TOF mass spectra of ammonium acetate analyte solution in 0.5 M ethanol solution of 2,4,6-trihydroxyacetophenone (matrix) as mock (A) with either 200 nmoles of dGMP (B), dGDP (C), or dGTP (D). The mass of the single addition peak (red) compared to the mock spectrum is denoted and corresponds to the expected masses of the nucleotides with the loss of a single proton.

Appendix Figure S4

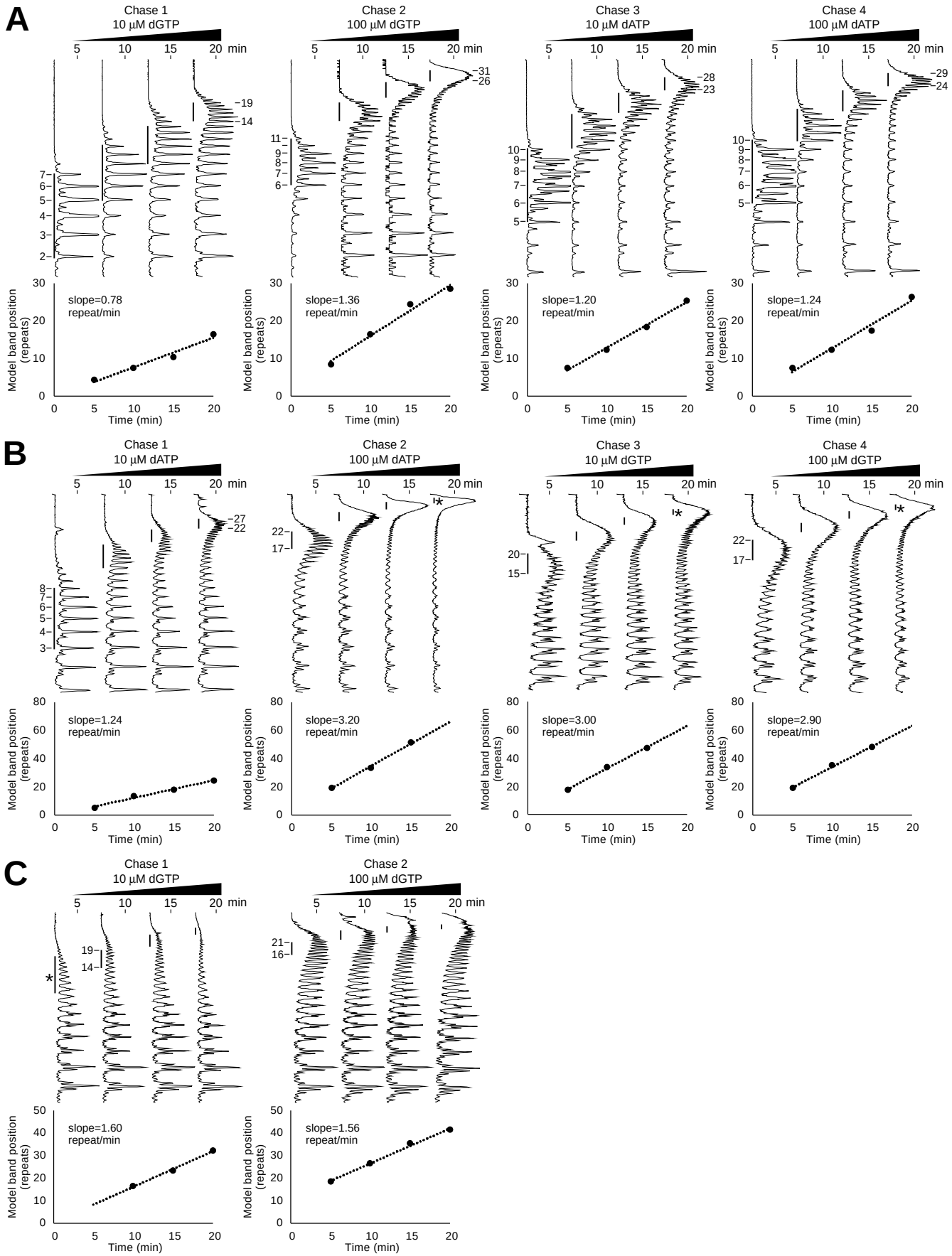

Figure S4. Quantification of telomerase repeat addition rate from pulse chase time-course assay. A-C Representative intensity trace plots from the gel from Fig 3E (A), Fig 5E (B), and Fig 8E (C). Repeat addition rate determined from the slope of the the average position of the modal band (denoted on intensity trace) over the reaction time. Dashed line is the regression line from the four time points measured. Asterisk (*) denoted when modal band could not be determined from the intensity trace.