RIBONUCLEOTIDE DISCRIMINATION AND REVERSE TRANSCRIPTION BY THE HUMAN MITOCHONDRIAL DNA POLYMERASE

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Supplemental Material

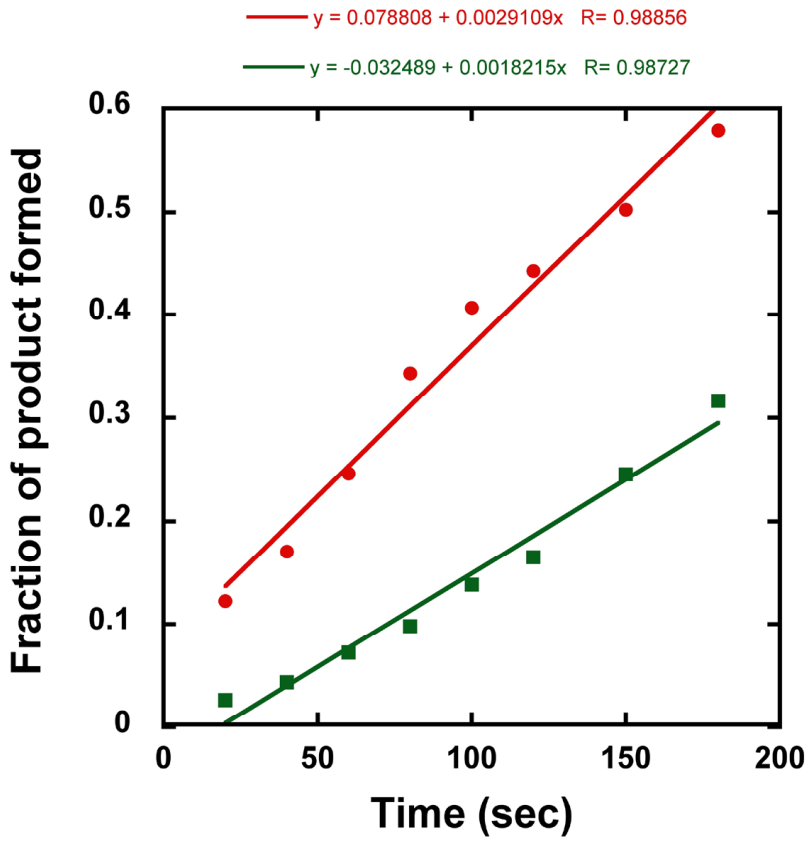

Figure S1. Incorporation of dTMP *vs.* rUMP opposite to dA in template by DNA polymerase γ. Single nucleotide incorporation assays were performed using primer-template substrate containing dAMP as the first template. Reactions (100 μl) contained 25 mM HEPES-KOH (pH 7.6), 2 mM 2-mercaptoethanol, 0.1 mM EDTA, 5 mM $MgCl_2$, 50 nM radiolabeled substrate, 10 nM exonuclease-deficient WT pol γ and 20 nM WT p55. The reaction was started by addition of 200 nM dTTP or 1 mM rUTP at 37 °C and 10 μl of the reaction was taken out and terminated with 10 μl of 95% deionized formamide and 10 mM EDTA after 20, 40, 60, 80, 100, 120, 150 and 180 sec. Samples (3 μl) were boiled for 5 min at 95 °C and resolved by electrophoresis on 12% polyacrylamide gels containing 6 M urea. Gels were dried, exposed to a phosporImager screen, and radioactive bands were detected with a Typhoon 9400 PhosphorImager (Molecular Dynamics) and quantified with NIH Image software. The fraction of product formed was plotted against the time and the y-intercept was determined by linear curve fitting. Red circles, incorporation of dTMP opposite to dA in template; Green squares, incorporation of rUMP opposite to dA in template. The equation of the line is shown at the top of the graph.