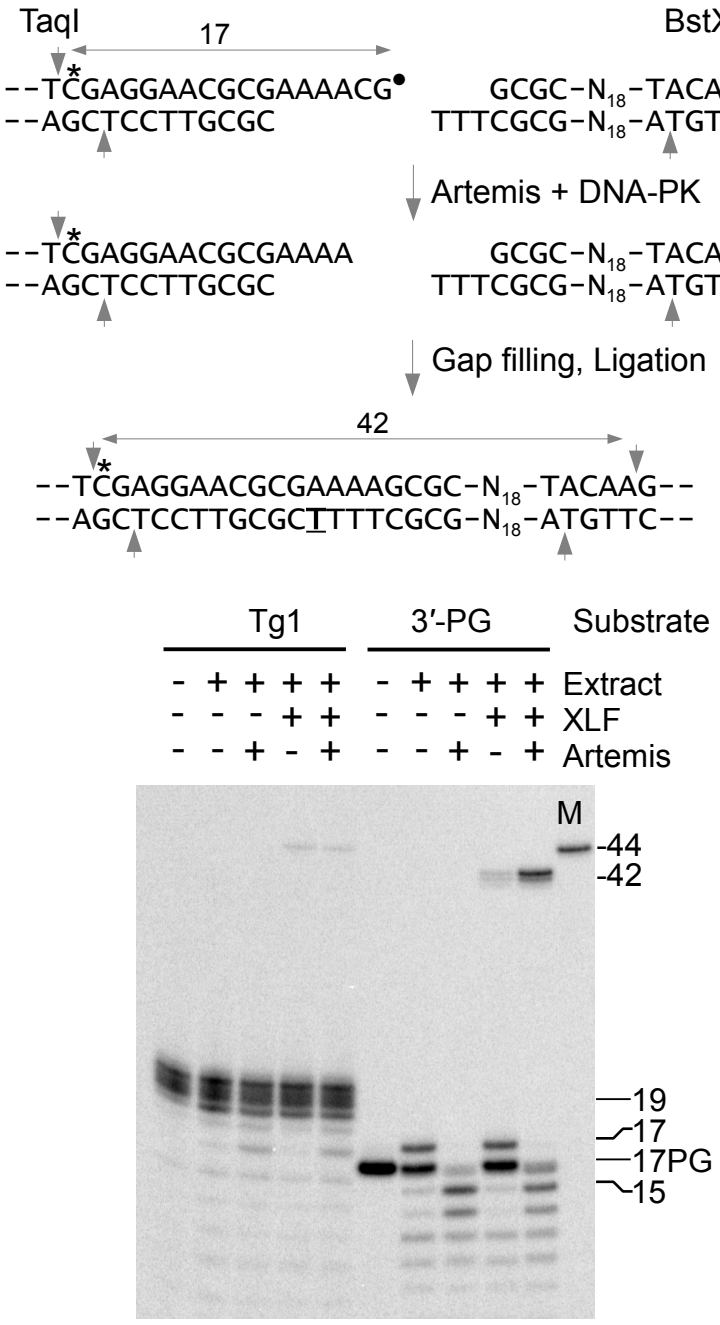
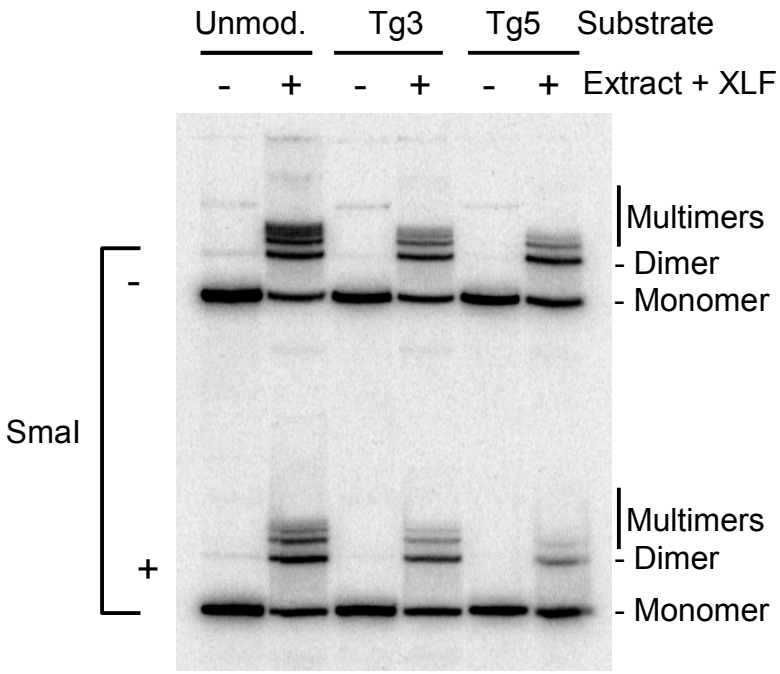
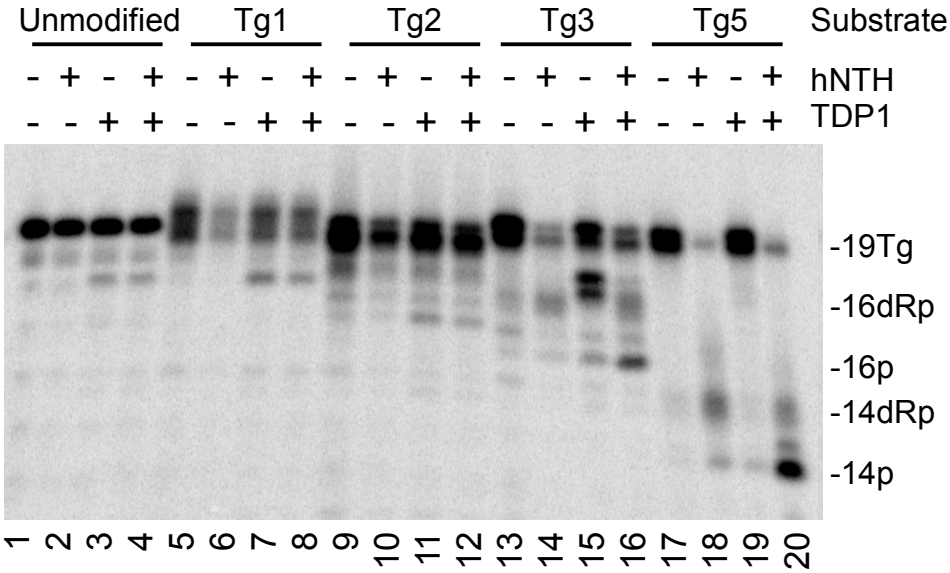


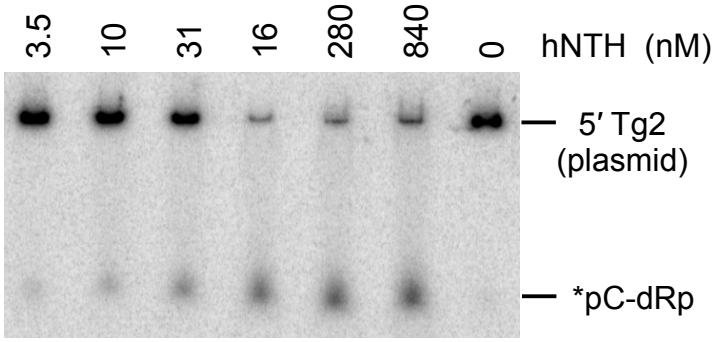
Supplemental Figure 1. Artemis catalyzes efficient trimming and promotes end joining of a PG-terminated 3'-overhang substrate but not a Tg-terminated substrate. Either the blunted-ended Tg-terminated Tg1 substrate or the PG-terminated substrate shown were incubated for 6 hr in Bustel whole-cell extracts supplemented with 100 nM XLF and/or 100 nM Artemis as indicated. The Tg1 substrate was cut with NdeI and PstI as in Fig. 2, while the PG substrate was cut with BstXI and TaqI, followed by denaturing gel electrophoresis. Artemis trimmed nearly all of the 3'-PG overhang resulting in a 2-base-shorter overhang (15-mer band) that was efficiently joined to a partially complementary overhang to yield the predicted 42-base fragment, whereas there was minimal trimming of the Tg1 substrate and no enhancement of end joining.



Supplemental Figure 2. Tg at the fifth position disrupts end joining in extracts. The indicated substrates were incubated for 6 hr in XLF-supplemented extracts (or heat-inactivated extracts), then treated (or not) with SmaI to eliminate tail-to-tail ligations, and finally subjected to electrophoresis on a 0.8% agarose gel, which was dried and analyzed by phosphorimaging. For SmaI-treated samples, end joining of Tg5 (23.8%) was substantially less than Tg3 (40.8%) or the unmodified substrate (49.3%). End joining as quantified by this assay is much greater than seen with the denaturing gel assay, first, because it is the sum of head-to-head and head-to-tail ligations; second, because each end joining event results in two labeled molecules being scored as a dimer or multimer; and third, because it includes minor products such as ligations accompanied by small deletions, that may not be seen on denaturing gels. Nevertheless, Tg5 clearly shows a lower level of end joining than the other substrates.



Supplemental Figure 3. Susceptibility of substrates harboring 3′-proximal Tg to cleavage by purified hNTH. Each substrate was treated with 840 nM hNTH for 1 hr and then with 10 μM TDP1 for 1 hr prior to NdeI cleavage and gel electrophoresis. There was no detectable hNTH-mediated cleavage in Tg1, Tg2 or the unmodified substrate. Because the 3′-dRp products resulting from Tg1 and Tg2 cleavage might not resolve from the initial substrate, samples were treated with TDP1 to convert 3′-dRp termini to 3′-phosphates. For all substrates, treatment with TDP1 alone produced variable amounts of 1-base-shorter fragments due to its weak 3′-nucleotidase activity, but hNTH did not increase the level of these products.



Supplemental Figure 4. Susceptibility of a substrate containing 5′-penultimate Tg to cleavage by purified hNTH. The 5′ Tg2 substrate was treated with the indicated concentrations of hNTH for 1 hr and analyzed on a 36% nondenaturing gel to assess release of the 5′-terminal nucleotide-ribose phosphate from the plasmid substrate, which remains in the well.