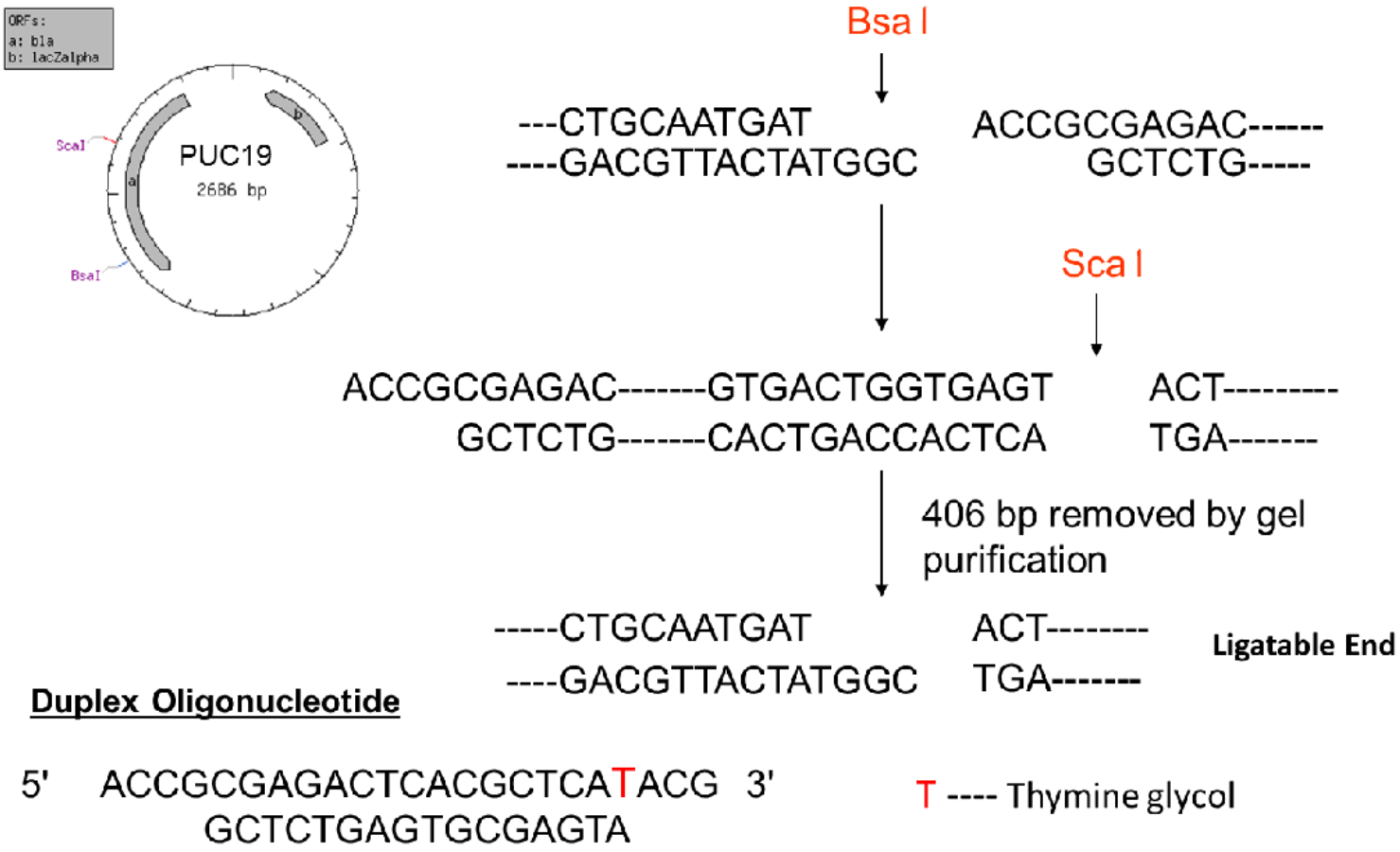
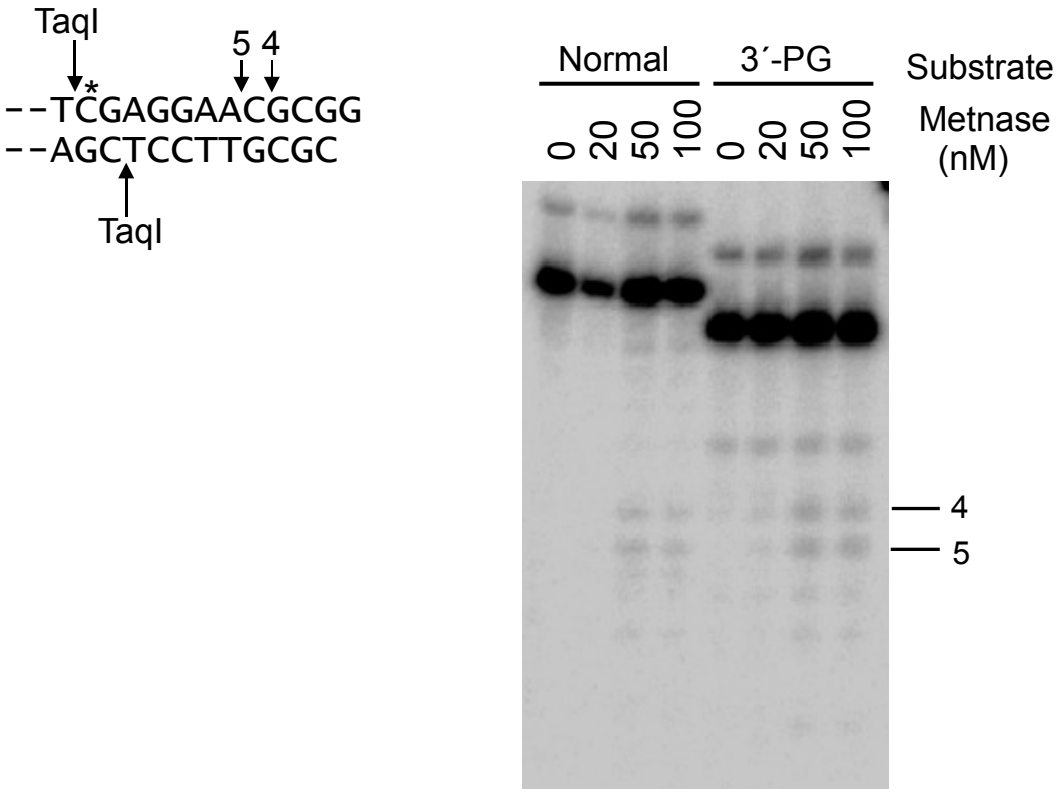
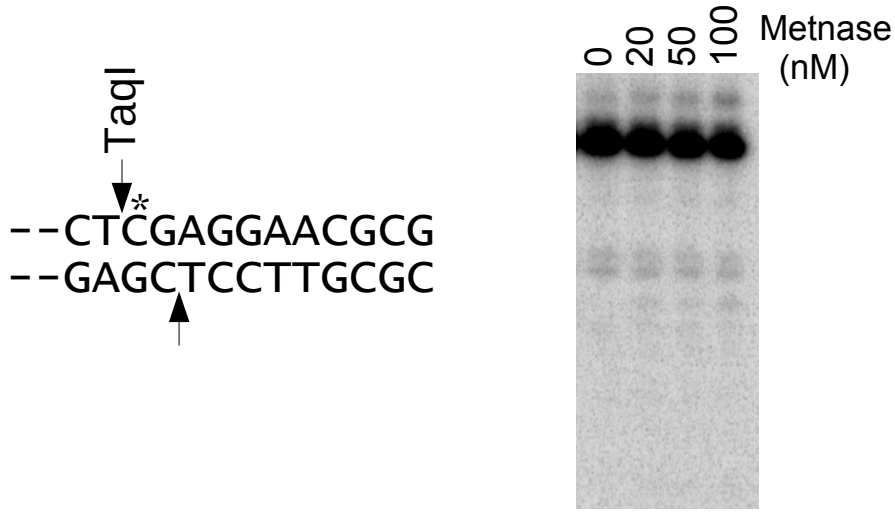


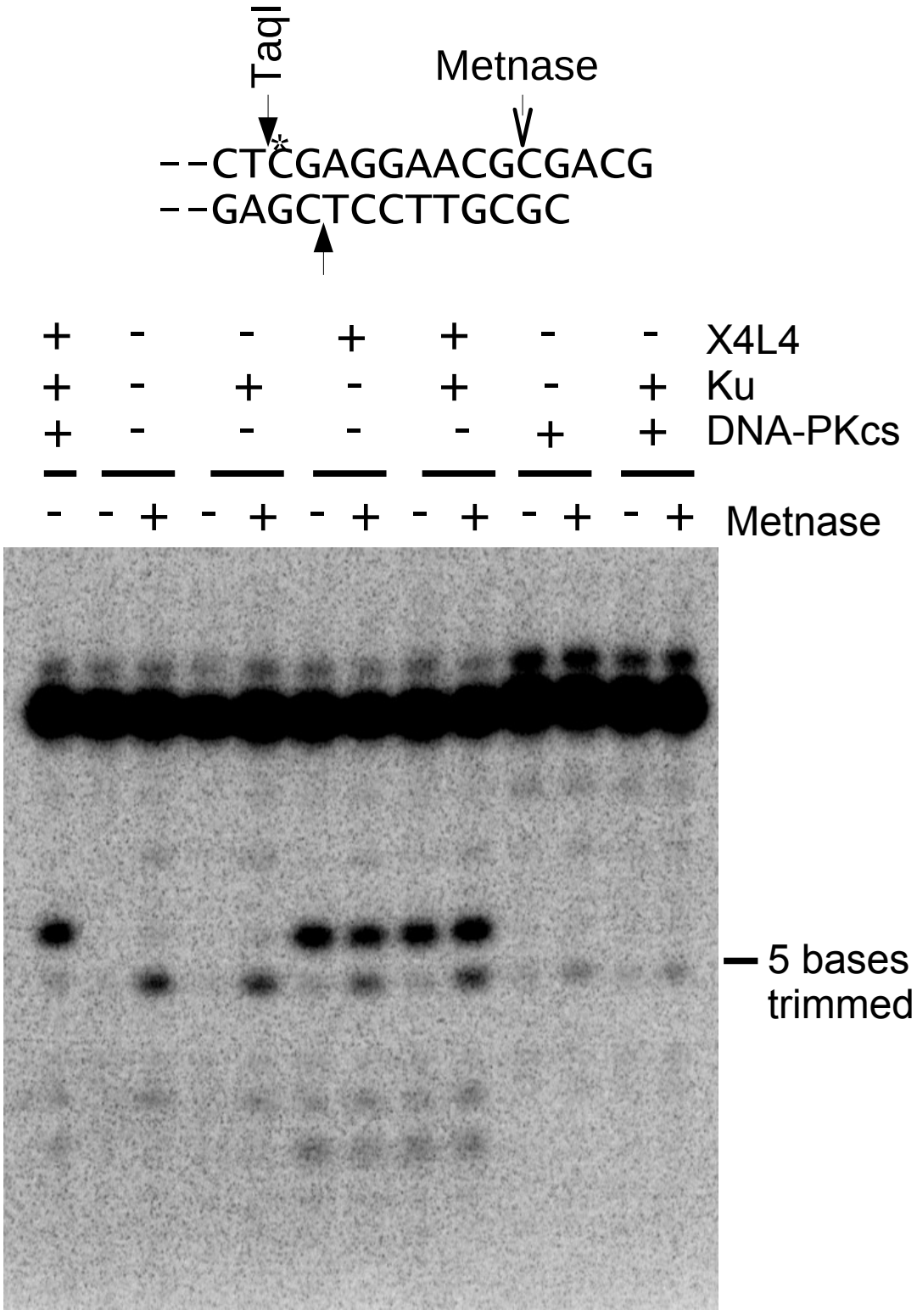
Supplemental Figure 1. Construction of thymine glycol-containing substrates. A 2.3-kbBsaI/ScaI restriction fragment was prepared from pUC19 and purified gel-purified. A 5'-end-labeled Tg-containing oligomer was annealed to a complementary strand, leaving an ACCG- 5' overhang that was ligated onto the cohesive end of the pUC19 fragment, and ligation products were gel-purified.



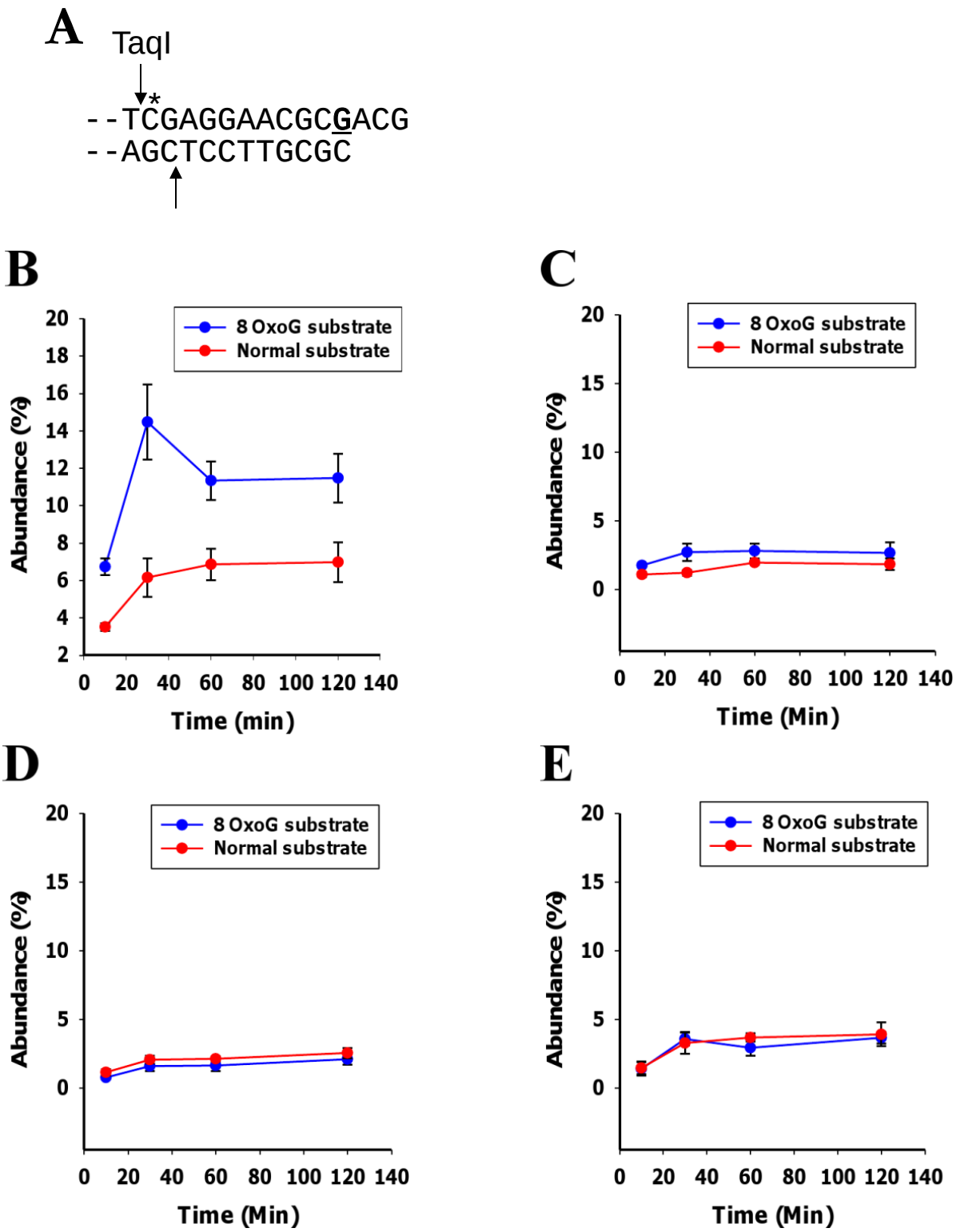
Supplemental Figure 2. Weak cleavage of a PG-terminated or hydroxyl-terminated 3' overhang by Metnase. The substrate shown was treated with Metnase for 2 hr, cut with TaqI, and analyzed on a sequencing gel. Cleavage at 4 and 5 bases from the terminus was <1% at 100 nM Metnase for both substrates.



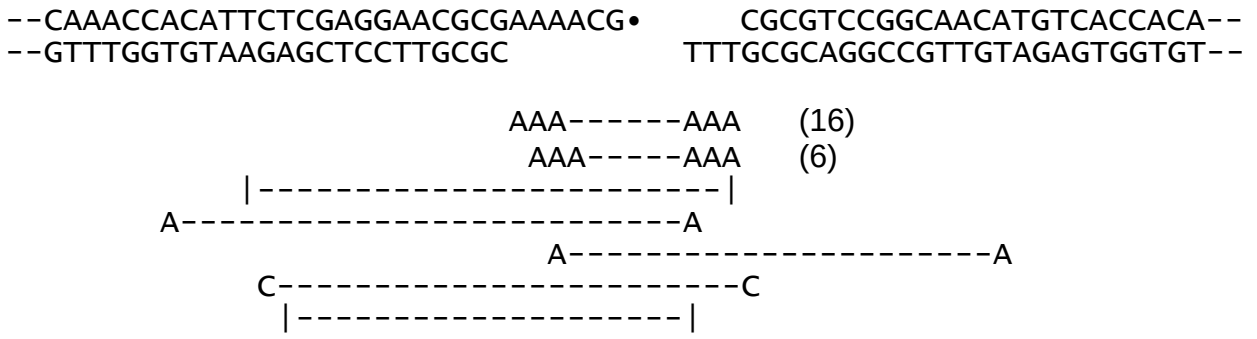
Supplemental Figure 3. No cleavage of a 3'-PG-terminated blunt end by Metnase. The substrate shown, bearing a 3'-PG terminus, was treated with Metnase for 2 hr, cut with TaqI, and analyzed on a sequencing gel.



Supplemental Figure 4. Effect of NHEJ proteins on Metnase-mediated cleavage of a PG-terminated 3-base 3' overhang. The substrate shown was treated with 40 nM Metnase for 2 hr in the presence of 40 nM X4L4, 10 nM Ku and/or 20 nM DNA-PKcs, as indicated, cut with TaqI, and analyzed on a sequencing gel. There was apparently some cleavage by a contaminating nuclease in the X4L4.



Supplemental Figure 5. Effect of 8-oxoG at the ss/ds junction on Artemis-mediated trimming of 3-base 3' overhang. The substrate shown (A), with or without substitution of 8-oxoG at the underlined **G**, was treated with Artemis (90 nM) in the presence of Ku (25 nM) and DNA-PKcs (65 nM) for the indicated times, then cut with TaqI and analyzed on a sequencing gel. The abundance of products representing trimming of 2 (B), 3 (C), 4(D), and 5 (E) bases was determined. Error bars represent the mean ±SEM for three independent experiments.



Supplemental Figure 6. Sequencing of the products of end joining in the presence of Artemis. The plasmid substrate shown, bearing a 3′-PG terminus (●) in one strand, was incubated in HeLa nuclear extracts supplemented with both X4L4 and Artemis. DNA from these incubations was deproteinized and transfected into competent *E. coli* (Bioline alfa gold select, genotype F^- *deo*R *end*A1 *rec*A1 *rel*A1 *gyr*A96 *hsd*R17(r_k^-, m_k^+) *sup*E44 *thi*-1 *pho*A Δ(*lac*ZYA-*arg*F)U169 Φ80*lac*ZΔM15 λ^-). Plasmids were isolated from individual ampicillin-resistant colonies, and their repair joints were sequenced using the primer TATGCTTGCTGTGCTTACTG, which lies 80 bp from the DSB site. Dashes and vertical lines show the extent of deleted sequences. Letters on both ends of a deletion show (in terms of the top strand) bases that could have come from either end of the break, with one copy retained in the repair joint. Numbers in parentheses indicate the number of clones with a particular repair joint sequence. For example, 16 clones had a -CGCGAAACGCG- repair joint.