Fig 3 – Supplement 2: Time course of CMG unwinding on forked duplex

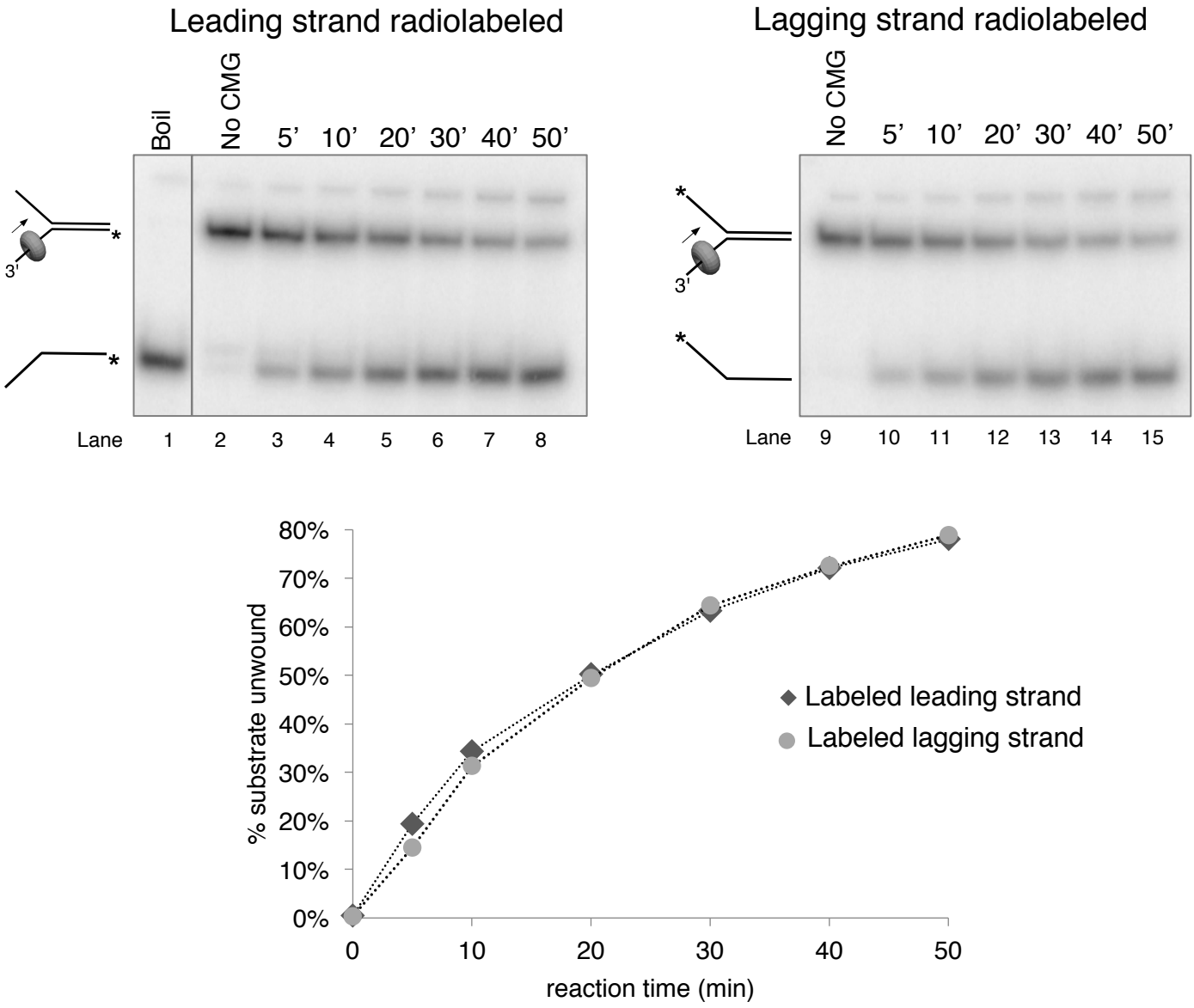

Figure 3 – Supplement 2: **Time course of CMG unwinding on forked duplex.**
Time course of unwinding of the forked duplex substrate like those shown in Figure 3 – Supplement 1 but with no biotin modifications. Lane 1 shows the position at which the unwound radiolabeled strand migrates (by boiling of the duplex substrate), lane 2 shows the migration of the substrate in the absence of CMG, and lanes 3-8 show substrate unwinding by CMG over a 50’ time course. The % of the radiolabeled strand unwound is shown in the graph at bottom. Lanes 9-15 are a repeat of this experiment with the radiolabel on the opposite strand, showing that unwinding is not affected by the position of the radiolabel. Reactions were mixed on ice and contained 0.5 nM radiolabeled DNA substrate and 20nM CMG in a total reaction volume of 90 µl. Reactions were started by incubating at 30° C and 12 µl aliquots were removed, stopped with EDTA/SDS, and flash frozen in liquid nitrogen at the time points indicated in the Figures to ensure no reannealing takes place. Reaction products were separated on 10% Native PAGE minigels and subsequently exposed to a storage phosphor screen that was scanned on a Typhoon 9400 laser imager (GE Healthcare). Scanned gels were analyzed using ImageQuant TL v2005 software.