

Fig 3 – Supplement 3: substrate single strands do not spontaneously re-anneal at 30° C

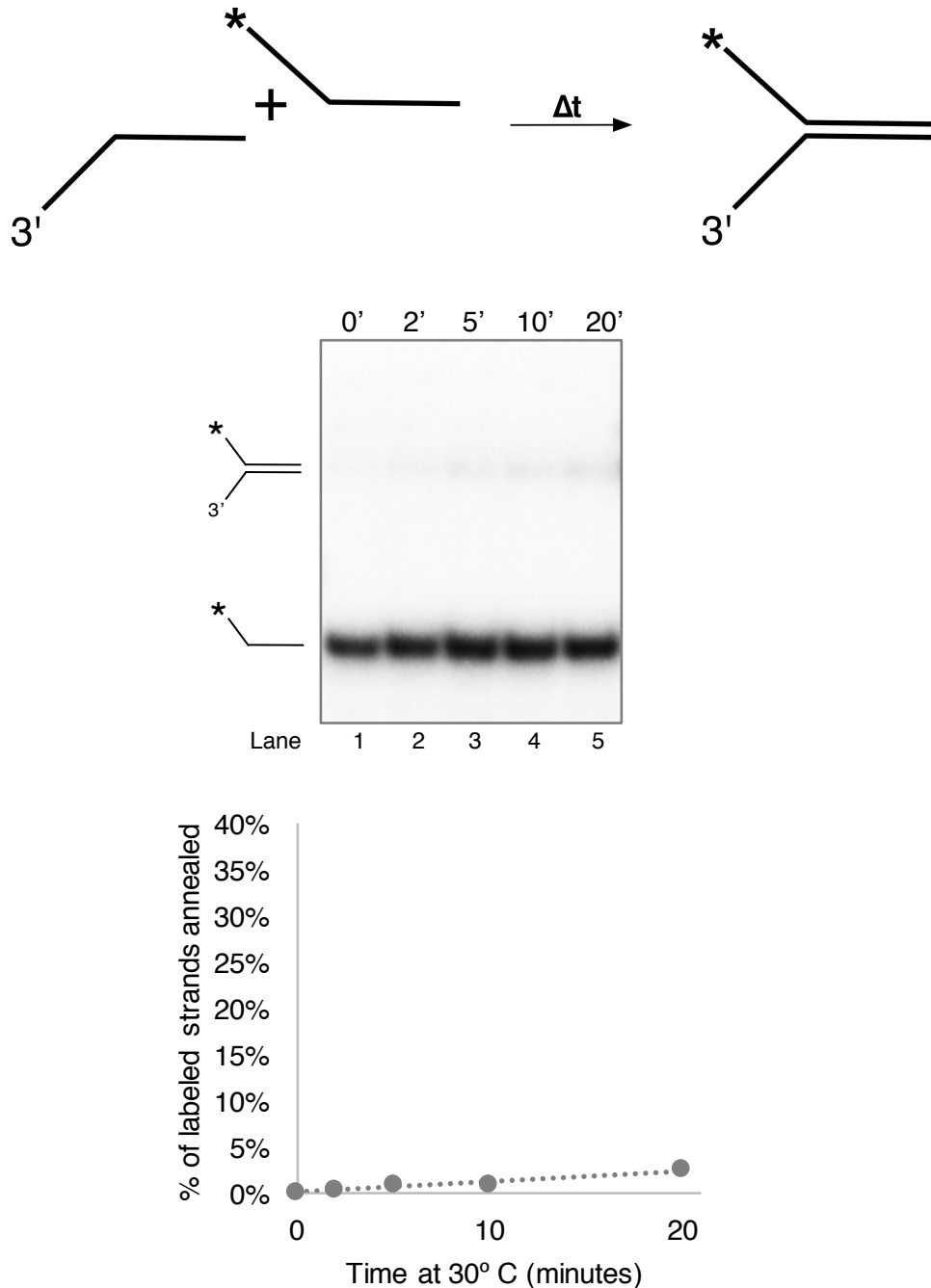


Figure 3 – Supplement 3: **Substrate single strands do not spontaneously reanneal at 30° C.**

To determine whether unwound substrate oligos spontaneously reanneal under our reaction conditions, we mixed 0.5 nM final concentration of radiolabeled 50duplex LAG oligo (Table I) with 0.5 nM unlabeled 50 duplex LEAD oligo under reaction conditions identical to those of Figure 3 in a total reaction volume of 55 μ l. Complete reactions were mixed on ice and started by incubating at 30° C. At the indicated time points, 10 μ l aliquots were removed and flash frozen in liquid nitrogen. Lack of re-annealing of the unwound strands allowed us to perform unwinding reactions in Figure 3 and subsequent Figures in the absence of a trap, eliminating potential side interactions between CMG and the unlabeled trap DNA.