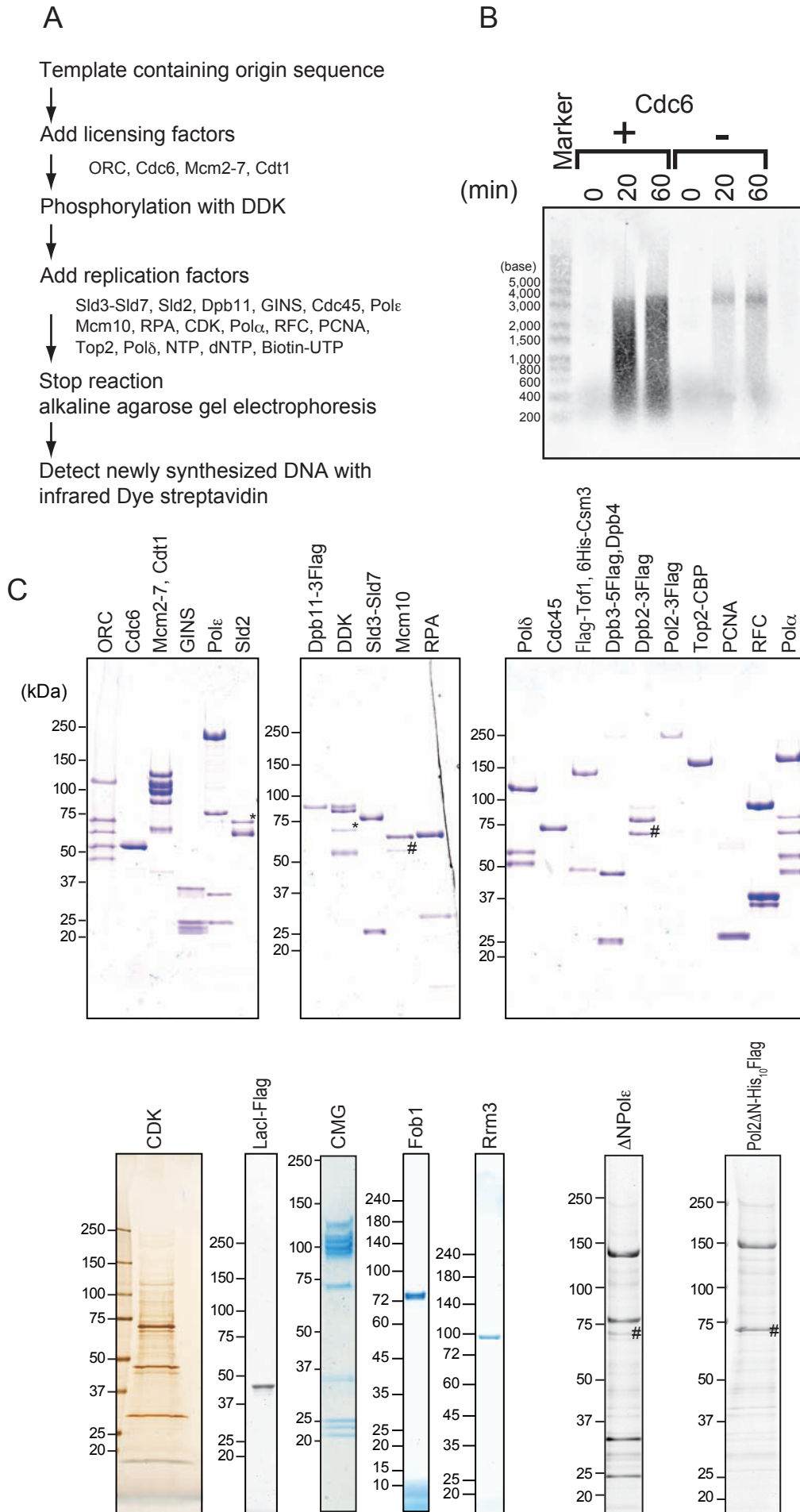


Hizume Supplementary Fig S1



Supplementary Figure S1. In vitro replication assay using purified proteins. (A) Experimental outline of the in vitro replication assay. Plasmid DNA containing the origin sequence of budding yeast was purified from *E. coli* and used in this assay as a template. First, in the presence of ATP, the template was mixed with ORC, Cdc6, Mcm2–7, and Cdt1 to complete replication licensing. After incubation with DDK, the replication factors that are required for the initiation and elongation of DNA replication were added. The reaction was stopped by the addition of alkaline buffer and applied to alkaline agarose gel electrophoresis. (B) In vitro replication assay with or without Cdc6. After the addition of the replication factors, the reaction was incubated for 0, 20, and 60 min. (C) Proteins used in the in vitro replication assay and CMG helicase assay. Proteins were detected using silver staining (CDK) or CBB (other proteins). The bands detected at 70 kDa in the lanes of Sld2 and DDK (indicated with *) were contaminated with Ssa1, which is a member of HSP70 family and is involved in protein folding, and the bands indicated with “#” in the lanes of Mcm10, Dpb2-3Flag, Δ NPoI ϵ and Pol2 ϵ Δ N-His₁₀Flag are unidentified contaminating proteins. Δ NPoI ϵ (PoI ϵ -pol2 Δ N) lacks the N-terminal amino acids 176-1135 of Pol2.