Supplementary Information for

Template and primer requirements for DNA Pol θ-mediated end joining

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Materials and Methods

DNA oligonucleotides

Non-self-complementary oligos were designed to test DNA synthesis activity of Polθ and the base-pair requirement between template and primer (Table S1). Initially, mixed purine and pyrimidine sequences, or purines or pyrimidines only were used. To make unique template and primer pairs and avoid unwanted intra- and inter-strand base pairing, we eventually designed primers, which contained mostly dCs except for one or two dTs for distinct register, and the templates, which contained dGs and dAs to pair with the primers and an overhang of six dAs for directing dTTP incorporation. DNA oligonucleotides were purchased from Integrated DNA Technologies (IDT) or the Facility for Biotechnology Resources (FBR) and PAGE purified. Because there were no more than 6 bp, template and primer at a 1:1 molar ratio were simply mixed in TE buffer (pH 8.0) before mixing with Polθ.

Expression and purification of human Pol θ

Cloning and expression of human Pol θ were reported previously (23). Briefly, human POLQ cDNA was extracted from HEK293 cells by RT-PCR. Two versions of the polymerase domain, consisting of residues 1822-2590 (POLQ86) and residues 1792-2590 (POLQ90) (19-21), were amplified and cloned into the mammalian expression vector pLEXm (26). To improve protein solubility and ease purification, a His_8 tag and two tandem copies of the maltose-binding protein (2MBP) followed by a PreScission protease cleavage site were fused to the N-terminus of POLQ86. Because both truncated Pol θ exhibit similar polymerase activities (Fig. S5 in reference #23), we chose to use the

shorter version (Pol θ86) in this study. The 8His-2MBP–tagged Pol θ86 variant (86 kDa), which is simply referred to as Polθ in this paper, was overexpressed in HEK293GNTI cells according to the protocol described previously (23). His_8-2MBP–tagged Polθ was first purified with amylose resin (NEB). After cleaving off the 8His-2MBP tag by PreScission protease, Polθ was further purified over a HiTrap Heparin HP column and followed by Superdex 200 (GE Healthcare). Purified proteins were concentrated to ~4 mg/ml and stored at –20 °C in 20 mM Tris, pH 8.0, 0.15 M NaCl, 0.5 mM TCEP and 30% glycerol. All purification steps were performed at 4°C. Polθ expressed in mammalian culture appears to be much more active than E. coli expressed protein (20,23,27).

DNA synthesis assay

Polθ activities were measured as previously described (23). Briefly, the DNA primer was ^{32}P-labeled at the 5′ end. Reactions were carried out in 10 µL of the standard reaction buffer (20 mM Tris, pH 8.0, 100 mM NaCl, 0.5mM TCEP and 5 mM $MgCl_2$) with 100 nM DNA substrates, 10 nM or 100 nM Polθ, and 400 µM of mixed four dNTPs or 100 µM of a single dNTP at either 25°C or 37°C. After pre-incubation of the protein-DNA mixture for 5 min, reactions were initiated by addition of desired nucleotides and 5 mM $MgCl_2$. After incubation for a desired time (0~20 min), each reaction was terminated by addition of equal volume (10 µL) of 90% formamide quench buffer. Samples were denatured at 95°C for 5 min, and the reaction products were resolved on 20% or 25% polyacrylamide-urea gels in 1× TBE buffer, and visualized on a Typhon Trio (GE Healthcare). All products were quantified using ImageQuant TL (GE Healthcare).

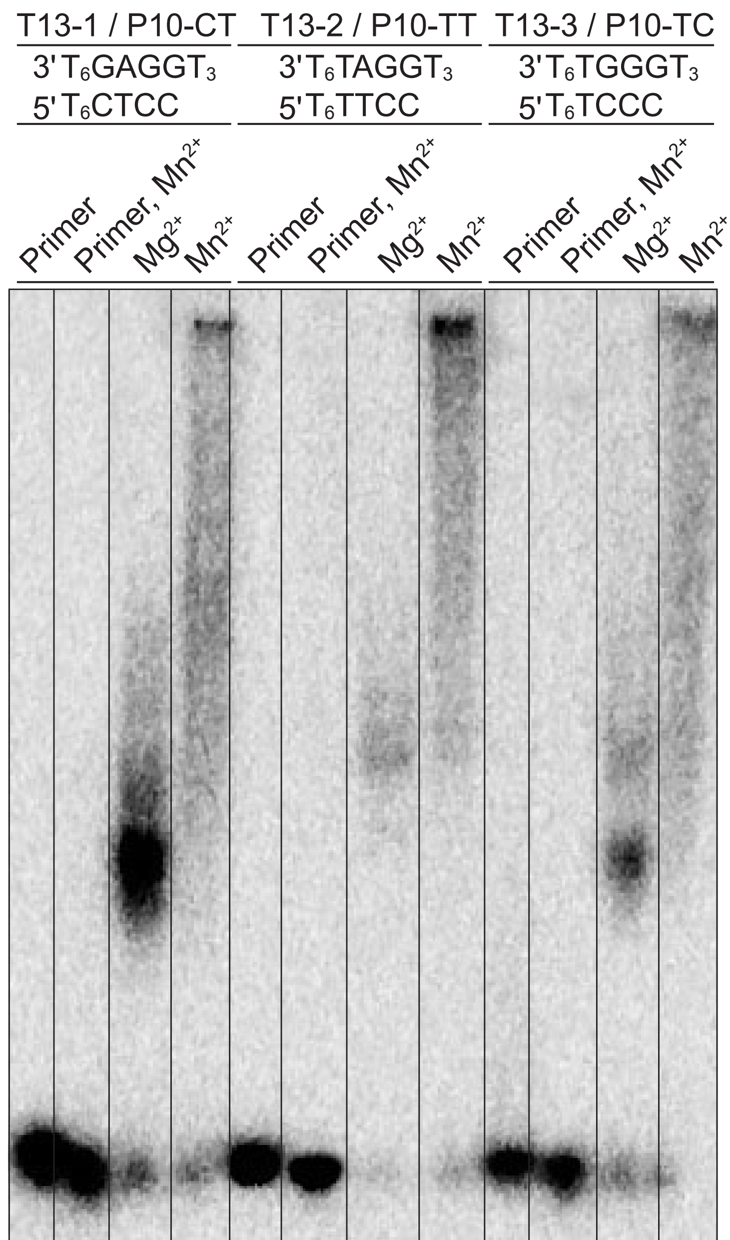

Figure S1. With PCR-like reaction, Polθ made more and longer products with Mn^{2+} than with Mg^{2+} at 37°C. For each substrate, first lane is primer alone without dNTPs, second lane is primer alone as substrate in the presence of Mn^{2+}, third lane is template / primer with Mg^{2+}, fourth lane is DNA substrate with Mn^{2+}. The reaction conditions is 37°C, 30 min, 50 nM Polθ, 100 nM DNA substrate, 0.1 mM of all four dNTPs in the standard reaction buffer.

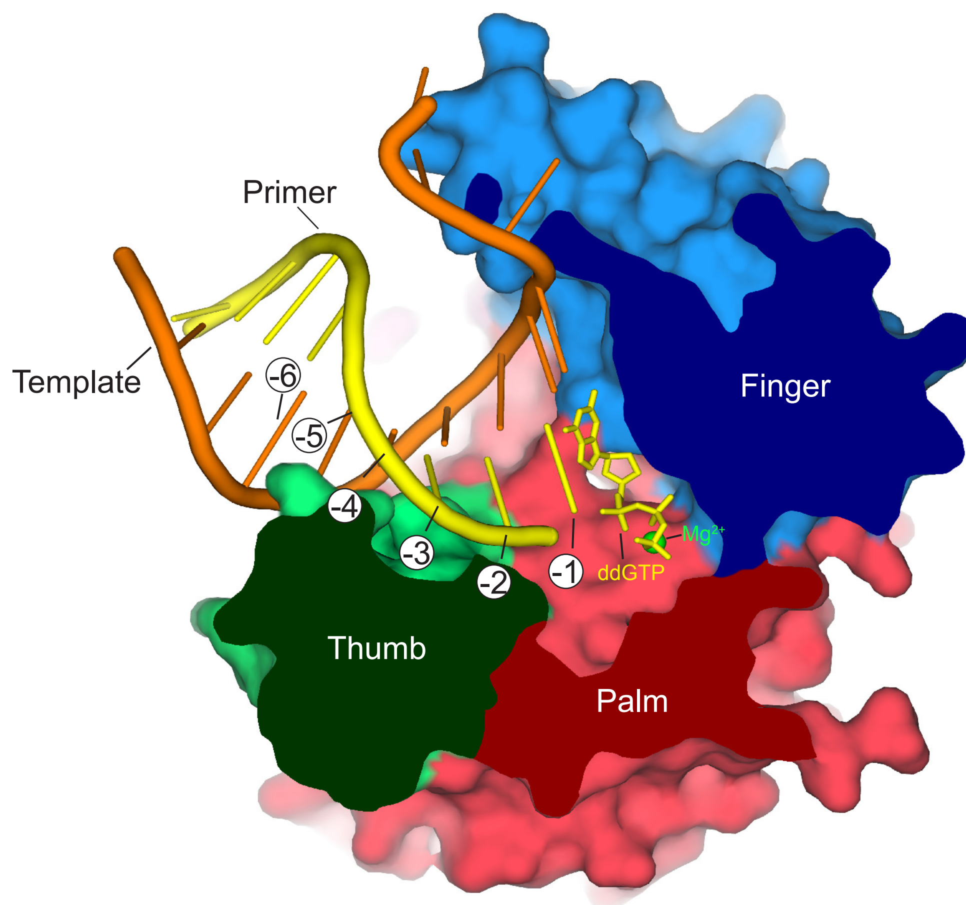

Figure S2. Crystal structure of Polθ-DNA-ddGTP complex (PDB: 4X0Q) (19). Polθ interacts with 6 bp upstream from the nascent base pair.

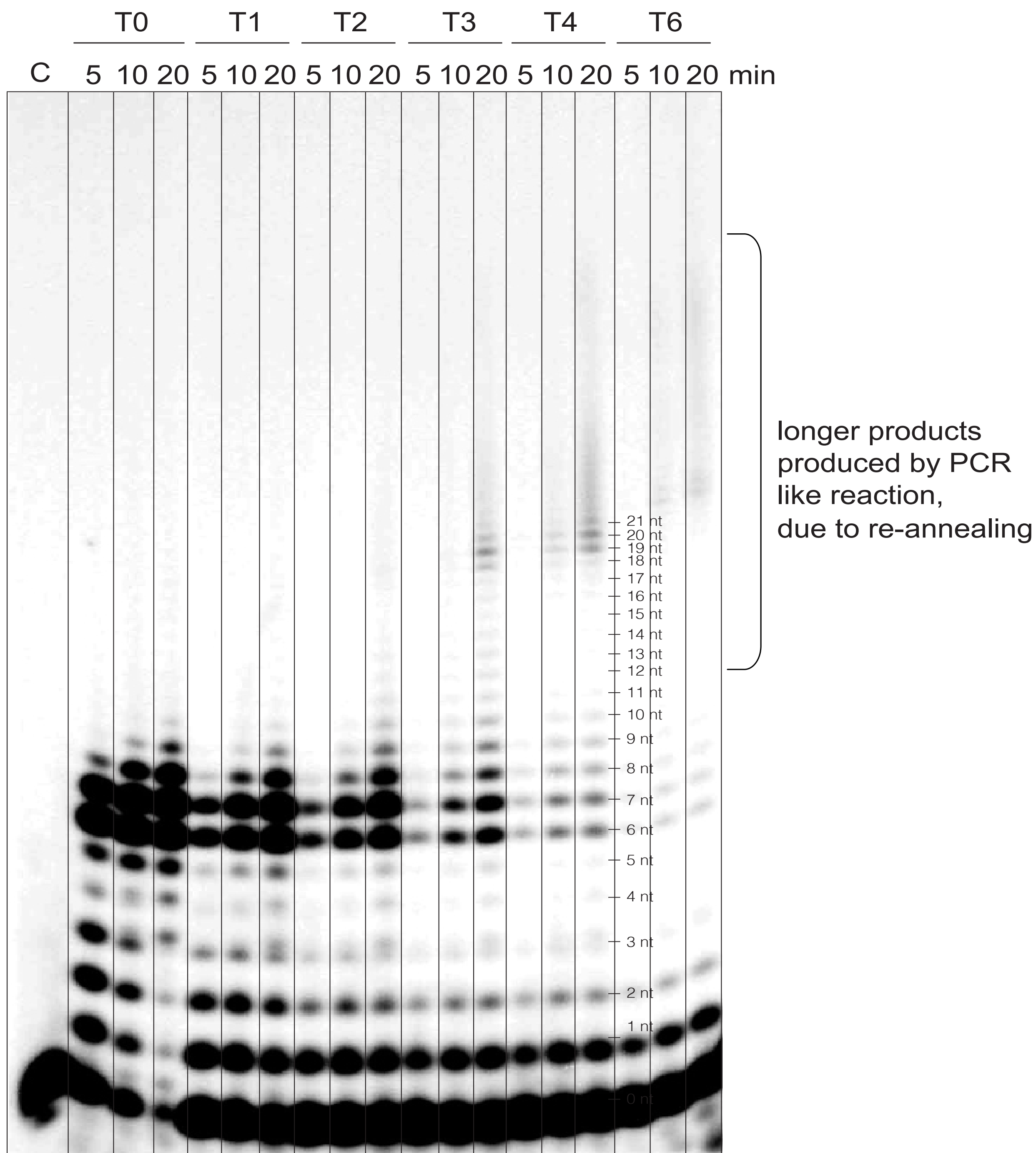

Figure S3. Higher contrast of Figure 4B reveals longer products due to re-annealing induced PCR-like reactions.

Supplemental Table 1. Oligos used in this work

Oligo name	Oligo sequence	DNA substrate	Figure
P0	5′ TCC		3A-C
P1	5′ CTCC		3A-C
P2	5′ CCTCC		3A-C
P3	5′ CCCTCC		3A-C
P4	5′ CCCCTCC		3A-C
P9-T	5′ TTTTTTTCC		1C-D
P9-C	5′ CCCCCCTCC	MM-4, T-6s, T-23s, 6s-17d, 6s-p17d	1C, 1E, 3A-C, 4A-C, 5A-C
P10-CT	5′ TTTTTTCTCC		S1
P10-TT	5′ TTTTTTTTCC		S1
P10-TC	5′ TTTTTTTCCC		S1
P-MM-1	5′ CCCCCTTCCC	MM-1	4A-C
P-MM-2	5′ CCCCCTTTC	MM-2	4A-C
P-MM-3	5′ CCCCCTCCC	MM-3	4A-C
P-17d	5′ TTGCATCGAATGACAGG	6s-17d	5A-C
P-p17d	5′p-TTGCATCGAATGACAGG	6s-p17d	5A-C
T1	3′ GAAAAAA		1C-D, 2A-C
T2	3′ GGAAAAAA		1C-D, 2A-C
T3	3′ AGGAAAAAA		1C-E, 2A-C, 3A-E, 3D-E
T4	3′ GAGGAAAAAA	T-6s	2A-D 5A-C
T5	3′ GGAGGAAAAAA		2A-C
T6	3′ GGGAGGAAAAAA		2A-C
T7	3′ AAGGAAAAAA		3D-E, 4A-C
T8	3′ AAAGGAAAAAA		3D-E
T9	3′ AAAAGGAAAAAA		3D-E
T10	3′ AAAAAGGAAAAAA		3D-E
T11	3′ AAAAAAAGGAAAAAA		3D-E
T13-1	3′ TTTTTTGAGGTTT		1B, S1
T13-2	3′ TTTTTTTAGGTTT		1B, S1
T13-3	3′ TTTTTTTGGGTTT		1B, S1
T-23S	3′GAGGAAAAAAAACGTAGCTTACTGTCC	T-23s, 6s-17d, 6s-p17d	5A-C