Supporting Information

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SI Materials and Methods

Construction, Expression, and Purification of Chimerical TPs N-ϕ and N-Nf. To construct chimeras Nϕ-CNf and NNf-Cϕ, ϕ29 and Nf DNA gene sequence from nucleotide 1 to 173 (to render fragments I and II, respectively) were amplified by PCR using primers 1 (5′-CGCGGAATTCTGAAAGGAGATAACG) and 2 (5′-CTGTGTCATACGTCTATCGTAGTACTGAGGGTCTGTTCTCATCTCCAT) (for ϕ29 TP gene), and 3 (5′-CGCGGAATTCGTTAAAGGGGGTGA) and 4 (5′-CTGTATCATTTTCTTTTCATAATAATCTGGGGATGCTTTTTCTGCCAT) (for Nf TP gene), and the plasmid pT7–3 containing either the ϕ29 or Nf TP gene as template (1). Primers 1 and 3 incorporate an EcoRI restriction site 5′ of the TP ORF. In parallel, primers 5 (5′-TATTATGAAAAGAAAATGATACAG) and 6 (5′-CGCGGGATCCGGAGCCTAGAACC) were used to amplify the ϕ29 TP sequence from nucleotide 174 to 266 (fragment III), while primers 7 (5′-TACTACGATAGACGTATGACACAG) and 8 (5′-CGCGGGATCCGTGCTTTAAAACCC) amplified the Nf TP sequence from nucleotide 174 to 265 (fragment IV). Primers 6 and 8 insert a BamHI restriction site at the 3′ of the corresponding PCR fragment. By means of the sequence overlapping between the 3′ end of fragment I and the 5′ end of fragment IV, as well as between the 3′ end of fragment II and the 5′ end of fragment III, a second cycle of PCR was performed by mixing equimolar amounts of either fragments I and IV or fragments II and III as template. In the first case, the use of primers 1 and 8 allowed to obtain a 0.8 kb DNA containing the N- and C-terminal parts of ϕ29 and Nf TP genes, respectively (chimera Nϕ-CNf), whereas, in the second case, primers 3 and 6 amplified a DNA containing the N- and C-terminal parts of Nf and ϕ29 TP genes, respectively (chimera NNf-Cϕ). Both DNAs were EcoRI-BamHI digested and cloned into an EcoRI-BamHI digested pT7–3 plasmid. Chimerical TPs were expressed in the *E. coli* strain BL21(DE3) and further purified essentially as described for the wild-type ϕ29 TP (2).

1. Longás E, de Vega M, Lázaro JM, Salas M (2006) Functional characterization of highly processive protein-primed DNA polymerases from phages Nf and GA-1, endowed with a potent strand displacement capacity. *Nucleic Acids Res* 34:6051–6063.
2. Zaballos A, Salas M (1989) Functional domains in the bacteriophage ϕ29 terminal protein for interaction with the ϕ29 DNA polymerase and with DNA. *Nucleic Acids Res* 17:10353–10366.

Table S1. Compilation of the single-stranded oligonucleotides with the sequence of the template strand of Nf or ϕ29 DNA right origin used as template of the initiation and initiation + elongation reactions described in the main text

Name	Sequence of the template oligonucleotide
ori(12)Nf	3′-TTTCATTCCAAG
ori(12)Nf T1A	3′-**A**TTCATTCCAAG
ori(12)Nf T2A	3′-T**A**TCATTCCAAG
ori(12)Nf T3A	3′-TT**A**CATTCCAAG
ori(12)Nf T3G	3′-TT**G**CATTCCAAG
ori(12)Nf T1A/T2A	3′-**AA**TCATTCCAAG
ori(12)Nf T2A/T3A	3′-T**AA**CATTCCAAG
ori(29)Nf	3′-TTTCATTCCAAGTTTCGTTTTAGCTGGGT
ori(29)Nf T1A	3′-**A**TTCATTCCAAGTTTCGTTTTAGCTGGGT
ori(29)Nf T2A	3′-T**A**TCATTCCAAGTTTCGTTTTAGCTGGGT
ori(29)Nf T3A	3′-TT**A**CATTCCAAGTTTCGTTTTAGCTGGGT
ori(29)Nf T2G	3′-T**G**TCATTCCAAGTTTCGTTTTAGCTGGGT
ori(29)Nf T3G	3′-TT**G**CATTCCAAGTTTCGTTTTAGCTGGGT
ori(29)ϕ	3′-TTTCATCCCATGTCGCTGTTGTATGTGGT
ori(29)ϕ T1A	3′-**A**TTCATCCCATGTCGCTGTTGTATGTGGT
ori(29)ϕ T2A	3′-T**A**TCATCCCATGTCGCTGTTGTATGTGGT
ori(29)ϕ T3A	3′-TT**A**CATCCCATGTCGCTGTTGTATGTGGT
ori(29)ϕ T2G	3′-T**G**TCATCCCATGTCGCTGTTGTATGTGGT
ori(29)ϕ T3G	3′-TT**G**CATCCCATGTCGCTGTTGTATGTGGT

Oligonucleotides are named as ori(*n*)*x*, where *n* stands for the length in nucleotides and *x* refers to the specific phage. Point substitutions in the ori(*n*) sequence are indicated by three-symbol code: the first letter stands for the nucleotide to be changed, the number indicates its position from the 3′-end and the third letter refers to the nucleotide introduced. The mutations introduced are indicated with bold letters. The sequences of the oligonucleotides are written in the 3′ to 5′ direction for a straightforward comprehension. Oligonucleotides were obtained from Invitrogen. All of them were purified electrophoretically on 8 M urea/20% polyacrylamide gels.