

Supporting Information

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A Mpa Ligase alignment

Mpa Lig KMEQ---PEWEEPMLATLT-SKRFDHGWAFGHKLDGARCLAYRK
Mtu Lig KDQKVFEDNLAPMLATHGTVAGLKASQWAFEGKWDGYRLLVEAD
Msm Lig KNAR---FEDFAPMLATEGSAKYKAKQWAFEGKWDGYRIIDAD
Pae Lig KTPM---PEWIEELASL--VEQPPHGQWAYELKLDGYRLMSRIE

Mpa Lig DGHVRLMSRTRHEMNASYF EVADALMAQKANDFIIDG EIVALDEN
Mtu Lig HGAVRLRSRSGRDVTAEYPLRLA LAEDLADHHVVLDGEAVVLDSS
Msm Lig HQQLQIRSRTRGREVTGEYPLFKALAADLAEHHVVLDGEAVVLDSS
Pae Lig DGHVRLMLTNGHDWSERLHLKKA LAGLGLERSWLDGELVVLDDE

Mpa Lig GNSKFEELQPRMGLLEDPAEARRTGIPVHYVFDILFYDGYEITRL
Mtu Lig GVPFSFSQMNRRG-----RDTRVFEWAFD LLYLDGRA LLGT
Msm Lig GVPFSFGQMNRRA-----RSTRVFEWAFD ILWLDGRS LLRA
Pae Lig GRDFQALQNAFE-----EGHGENILYFLFDLPYHEGEDLRDV

Mpa Lig PLEERKHILEKTLDTYGD--DTRVFLIEDRLGGGLGLFDEACKKGEWEG
Mtu Lig RYQDRRKLLETLANAT---SLTVPELLPGDGAQAFACSRKHGWEG
Msm Lig KYSDRRKILEALADGG---GLIVPDQLPGDGP EAM EHVRRKR FEG
Pae Lig ALEERRARLEALLDGQDEDP LRF SATLAENPRD LLA SACLGLLEG

Mpa Lig LIAKRAYGPIYVH-GRSDEWLKFKCVQNEFVIGGYTEPHGKR I-E
Mtu Lig VIAKRRDRYQPGRRCA SWVKDKHWNTQEVVIGGWRAGEGGRSSG
Msm Lig VVAKKWDSTYQPGRRSSSWIKDKIWNTEV VIGGWRQEGEGGRSSG
Pae Lig VIGKRLG SVYRS-RRSNDWIKLKCQLRQEFVIVGYTEPKGSR-H

Mpa Lig FGAIVLGYQD--GKLMYAGKVTGDEEKT LRS LMDKFRPIERK
Mtu Lig VGSLLMGIPGF--GG LQFAGRVTGSLSERELANLKEMLAPLHTD
Msm Lig IGAIVLGLIPGF--EGLQFVGRVTGTEKE LSKLKDMLKPLHTD
Pae Lig IGALLGLLYSPDEERRIRYAGKVTGTEAASLKKVHERLAPLAIN

Mpa Lig ASPFAEE--VREKEVHWLEPKLVAEIGFEWTDYGKLRQPRYDG
Mtu Lig ESPFDVPLPARDKGIYV KPA LVAEVRYSEWTFEGR LRQSSWRG
Msm Lig ESPENAPLPKVDARGVT FVRPELVG EVRYSER TSDGR LRQPSWRG
Pae Lig SPLAKVPPARETG VQWVRPERLCEVSYAQMTRG GIIRQAVFHG

Mpa Lig LRYDKDPHSVIREVP
Mtu Lig LRPDKKPSSEVVR---
Msm Lig LRPDKTEDEVVW---
Pae Lig LREDKPAR E V TGERP

B Mpa Pol alignment

Mpa Pol HDIKVTNPDKVLFPEDGITKGE LVDYYRRISGVMVPLVGRGP
Mtu Lig QRVTLTNADKVLYPATGTTKSDIFDYYAGVAEVM LGH IAGRP
Msm Lig ERVRLTNPDKVLYPATGTTKAEVFDYYLSIAQVMVPH IAGRP
Pae Lig AGVRI SHPRRLIDP S IQA SKLELA EFHARYAD LLLRLDRERP

Mpa Pol MTMQRFEDGIGKEGFFQKEASDY-FFDWHR-ATLELGKGGI
Mtu Lig ATRKRWFNGVDQPAFFEKQLALS-APPWLSR-ATVAHRS GTT
Msm Lig VTRKRWFNGVAEEAFFEKQLASS-APSWLER-GSITHKSGTT
Pae Lig VSLV RGFEDGIGGELFRQKHAAARLKI PGIVQLDPA LDPGHPP-

Mpa Pol QHQVVCDDAATVYVLA SQAMITPHVFLSRIDK-----V--H
Mtu Lig TYP-IIDSATGLAWIAQQAAL EHVHPQWR FVAEPGSGELNPG
Msm Lig TYP-IINTREGLAWVAQQA SLEVHVHPQWR FED-----GDQG
Pae Lig --LLQIRSAEALV GAVQMGSI EFTWNASLAN-----L--E

Mpa Pol YPDRLIFDLDLFP-DNNFET-VRSAAKTIFEA LDAEGYPVYLM T
Mtu Lig PATRLVFDLDLPG-EGVMMAQLAEVARAVRD LLDIGLVTFPVT
Msm Lig PATRIVFDLDLPG-EGVTMTQLCEIAHEVRALMTDLDLETYP LT
Pae Lig RPDRLFVLDLDRDPA LPWKR-MLEATQLS LTLLEDELGLRA FLKT

Mpa Pol TGSRLHVVVPLDRSADFDTVRAFARGFGKLTKKYPDRFTI
Mtu Lig SGSKGLHLTYPLDEPVSSRGATV LAKRVAQRLEQAMPALVTS
Msm Lig SGSKGLHLTYVPLAEP ISSRGA SVLARRVAQQLEQAMPKLVTA
Pae Lig SGGKGMHLLVPLERRHGWDEVKAFQAISQHLARLMPERFSA

Mpa Pol ELSKEKRRGRLEFDYLRNSYGQTVGAPYGVRRASGAPVATPIT
Mtu Lig TMTKSLRAGKVFVDW SQNSGSKT IAPYSLRGRTHPTVAAPRT
Msm Lig AMTKSLRAGKVFVDW SQNNAAKTTIAPYSLRGRDHTVAAAPRT
Pae Lig VSGPRNRVGR I EVDYLRNSRGA STVAAVSVRRAREGLPVS VV

Mpa Pol WDELDDISGSQ EYNIRNIMGRMDKRG--DAWKYID
Mtu Lig WAELDDPALR-QLSYDEV LTRIARDG--DLLERLD
Msm Lig WDEIADPELR-HLRFDEVLDR LDEYQ--DLLAPLD
Pae Lig REELDSLQGANQWNLRSLPQR LDEELAGD DPWADYA

Fig. S1. (Continued)

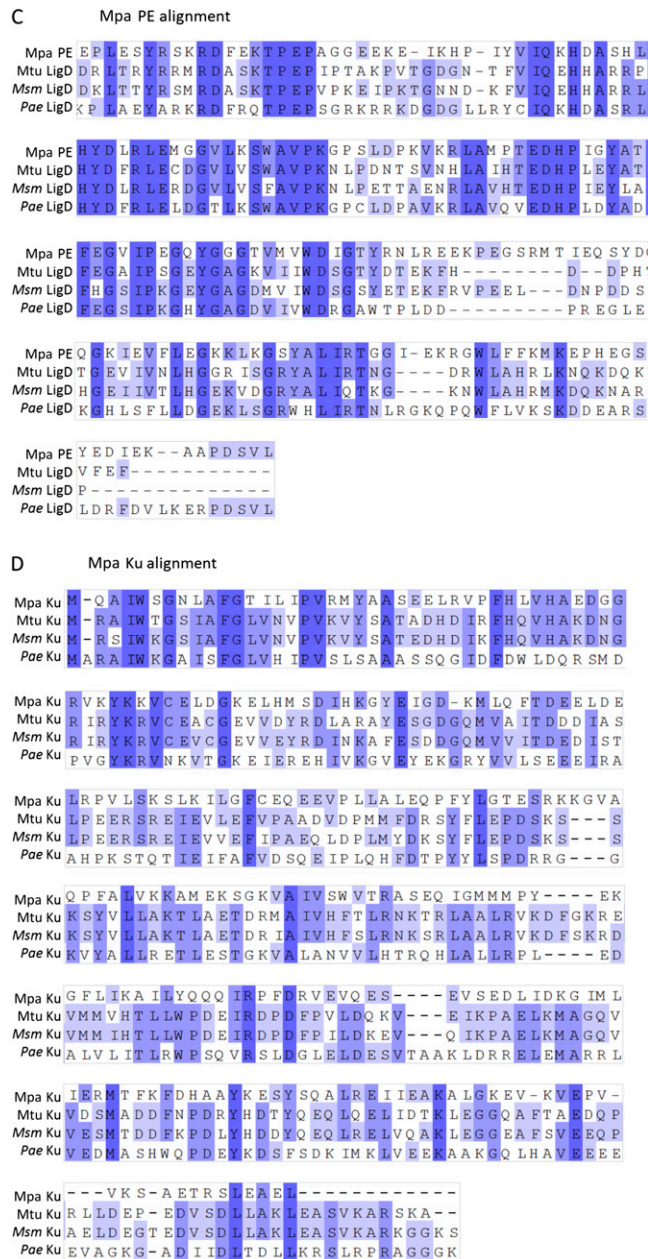


Fig. S1. Protein sequence alignments of *Methanocella paludicola* (Mpa) ligase (Lig), polymerase (Pol), phosphoesterase (PE), and Ku with *Mycobacterium tuberculosis* (Mtu), *Mycobacterium smegmatis* (Msm), *Pseudomonas aeruginosa* (Pae) ligase D (LigD), and Ku. (A) Mpa Lig protein sequence alignment with LigD ligase domains (LigDom) of Mtu, Msm, and Pae. (B) Mpa Pol protein sequence alignment with LigD Pol domains (PolDom) of Mtu, Msm, and Pae. (C) Mpa PE protein sequence alignment with LigD PE domains (PEDom) of Mtu, Msm, and Pae. (D) Mpa Ku protein sequence alignment with Ku of Mtu, Msm, and Pae. All alignments were made with Toffee and edited in JalView.

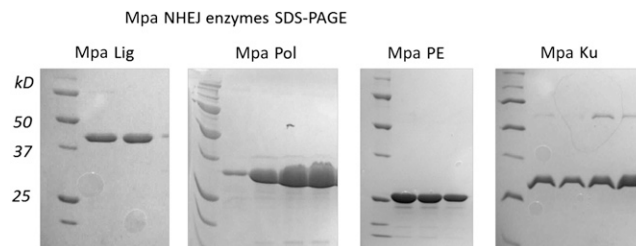


Fig. S2. SDS/PAGE Mpa Lig, Pol, PE, and Ku. SDS/PAGE of gel filtration chromatography fractions of respective purifications of Mpa Lig, Pol, PE and Ku. Migration sizes of the proteins were Mpa Lig ~40; Mpa Pol ~34 kD; Mpa PE ~24 kD; and Mpa Ku ~30 kD (monomer) and ~60 kD (dimer). The gels demonstrate the high purity of the proteins.

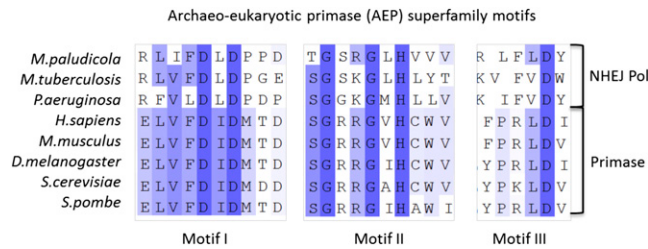


Fig. 53. Archaeo-eukaryotic primase (AEP) superfamily motif sequence alignment. Three key conserved motifs (I, II, and III) confer membership to the AEP superfamily. Here Mpa Pol is aligned with Mtu and Pae nonhomologous end-joining (NHEJ) Pols, and *Homo sapiens*, *Mus musculus*, *Drosophila melanogaster*, *Saccharomyces cerevisiae*, and *Schizosaccharomyces pombe* primase small subunits.

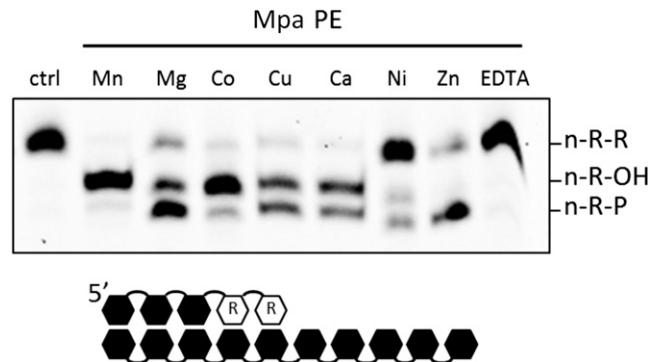


Fig. 54. Mpa PE ion preference. PE reaction mixtures contained 50 mM Tris (pH 7.5), 5 mM MnCl₂, MgCl₂, CoCl₂, CuCl₂, CaCl₂, NiCl₂, or Zn(O₂CCH₃)₂ where indicated, 30 nM 5'-fluorescein-labeled 16mer DNA/RNA (D14R2), and 300 nM Mpa PE protein. Reactions were incubated at 37 °C for 1.5 h and electrophoresed on a 15% denaturing polyacrylamide gel. n-R-R, unmodified substrate; n-R-P, removal of the terminal ribonucleotide monophosphate to yield a terminal phosphate group; n-R-OH, product after phosphate removal leaving a terminal OH group.

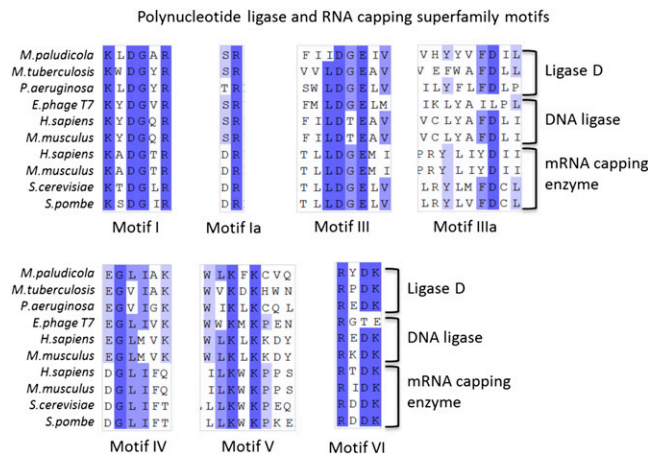


Fig. 55. Polynucleotide ligase and mRNA-capping superfamily motifs. Six key conserved motifs (I, Ia, III, IIIa, IV, and V) confer membership in the polynucleotide ligase and mRNA-capping superfamily. Motif VI also is included with highly conserved residues. Here Mpa Lig is aligned with Mtu and Pae LigD; Enterobacteria phage T7, *H. sapiens*, and *M. musculus* DNA ligase; and *H. sapiens*, *M. musculus*, *D. melanogaster*, *S. cerevisiae*, and *S. pombe* mRNA-capping enzymes.

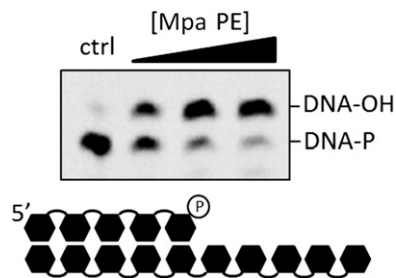


Fig. 58. Mpa PE phosphomonoesterase activity does not require 2'-OH. Phosphatase reaction mixtures contained 50 mM Tris (pH 7.5), 5 mM $MnCl_2$, 30 nM 5'-fluorescein-labeled 16mer DNA with 3' phosphate, and 250, 500, and 1,000 nM Mpa PE protein. Reactions were incubated at 37 °C for 1.5 h and electrophoresed on a 15% denaturing polyacrylamide gel.

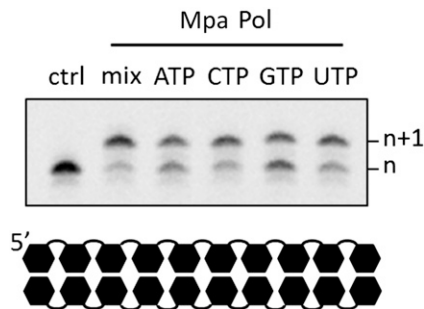


Fig. 59. Mpa Pol incorporation of nontemplated NTP. Pol nontemplated extension reaction mixtures contained 50 mM Tris (pH7.5), 5 mM $MnCl_2$, 62.5 μM ATP, CTP, GTP, UTP, or 250 μM mixed nucleoside triphosphates (NTPs) and, where indicated, 30 nM 5'-fluorescein-labeled 36mer DNA and 300 nM Mpa Pol protein. Reactions were incubated at 37 °C for 1 h and electrophoresed on a 15% denaturing polyacrylamide gel.

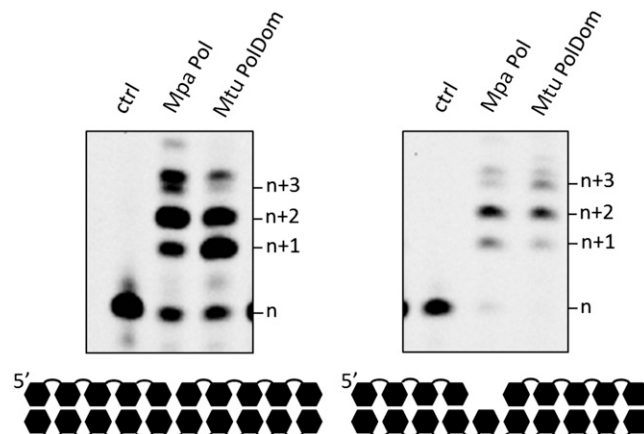


Fig. 510. Mtu Pol domain (PolDom) strand displacement. Polymerase strand displacement reaction mixtures contained 50 mM Tris (pH 7.5), 5 mM $MnCl_2$, 250 μM NTPs, 30 nM 5'-fluorescein-labeled 16mer DNA, and 300 nM Mpa Pol or Mtu PolDom protein where indicated. Reactions were incubated at 37 °C for 1 h and electrophoresed on a 15% denaturing polyacrylamide gel.

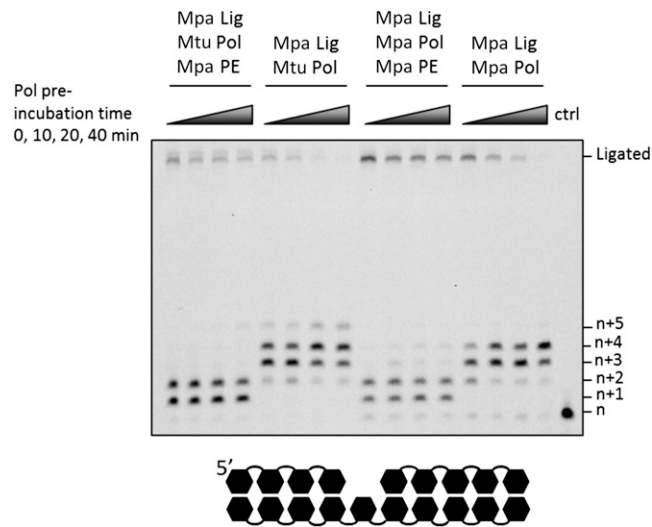


Fig. S11. Mpa PE can rescue NHEJ intermediates formed by Mtu PolDom and Mpa Pol. Gap-filling, PE, and ligation reaction mixtures contained 50 mM Tris (pH 7.5), 5 mM MnCl₂, 250 μM NTPs, 30 nM 5'-fluorescein-labeled 16mer DNA, and 300 nM Mpa Pol or Mtu PolDom, 300 nM Mpa Lig, and PE protein where indicated. Reactions were incubated at 37 °C for 1 h and electrophoresed on a 15% denaturing polyacrylamide gel.

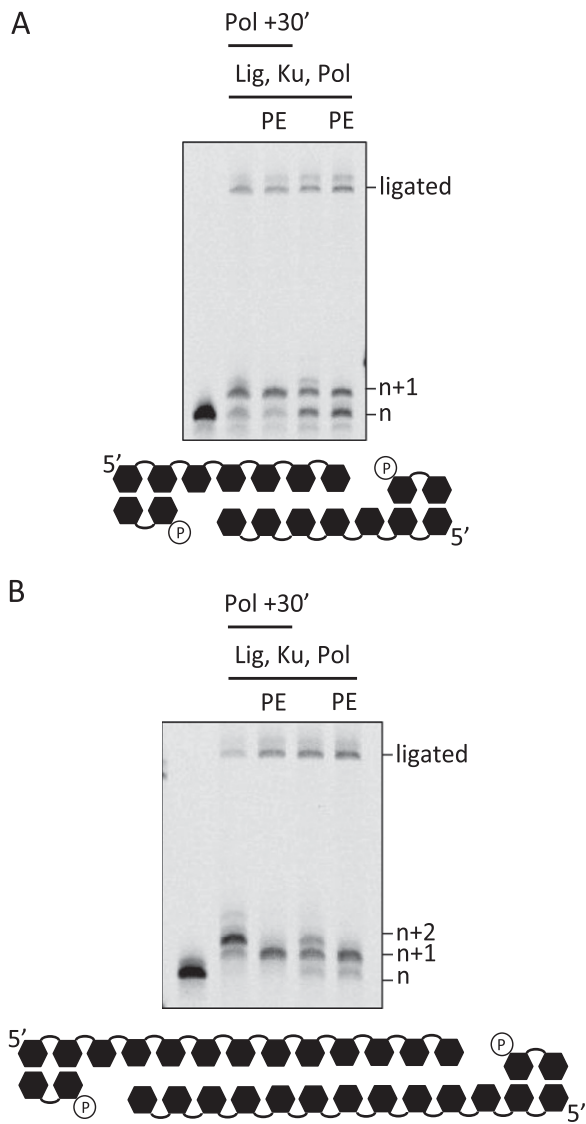


Fig. S12. Mpa PE resection of strand-displacement intermediates during 3' overhang NHEJ repair. Gap-filling, PE, and ligation reaction mixtures contained 50 mM Tris (pH 7.5), 5 mM MnCl₂, 250 μM NTPs, 30 nM 5'-fluorescein-labeled 42mer DNA (A) and 48mer DNA (B), and 300 nM Mpa Pol, Lig, and PE and 400 nM Ku protein where indicated above the lanes. Mpa Pol was preincubated with the substrate for 30 min where indicated above the lanes before the addition of Mpa Lig, PE and Ku. Electrophoresed on a 15% denaturing polyacrylamide gel.