

# Supporting Information

## Structural and Functional Characterization of New Ssopox Variant Points to the Dimer Interface as a Driver for the Increase in Promiscuous Paraoxonase Activity

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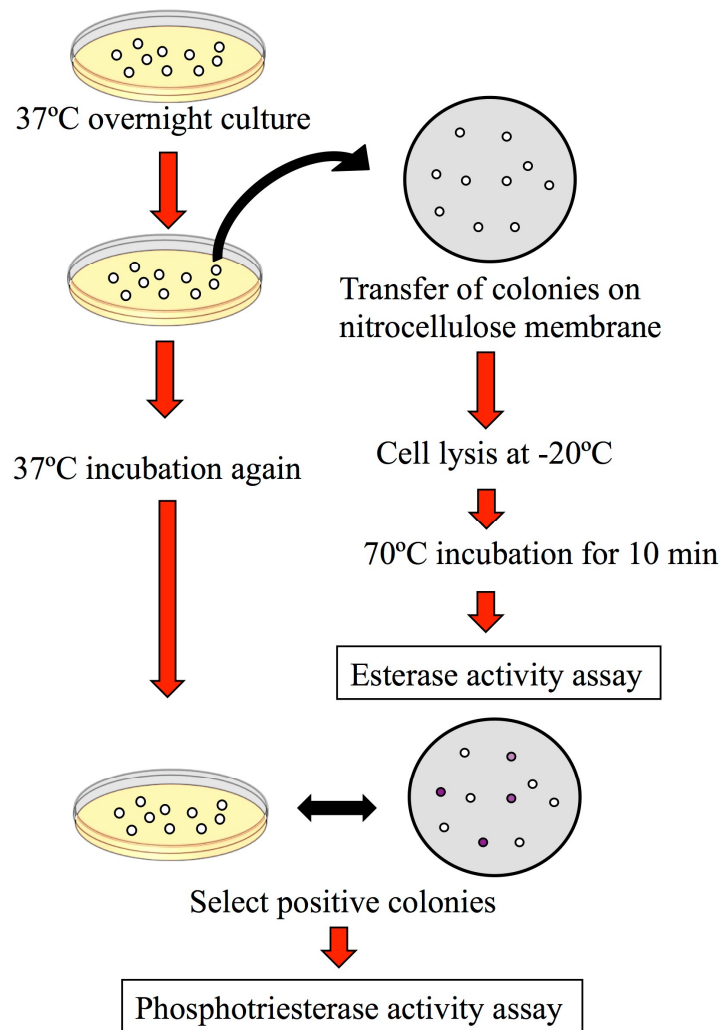
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SsoPox3Mut	ATGAGAA-TA--CCA--TTAGTTGGGAAAGATTCAATAGAATCTAAGGACATAGGATTTACGCTAATTCATGAACATTTAAGAGT-TTTTAGCGAA----
SisLac	ATGAGAA-TA--CCA--TTAGTTGGGAAAGAGCCAAATAGAAGCTGAGGATATGGGATTTACGCTGATTCATGAACATTTAAGAGT-TTTTAGTGAA----
SacPox	ATGACAAAAATTCTCT--CTTGAGGAAAAGGTGAAATATCACCTGGAGAAATGGGTTTACTTTAATACATGAGCATTTAAGGGT-CTTCAGTGAA----
VmoLac	ATGGTACGCA--TCAGCATCGTGGTGGAAACGAAATGTATCCGGGTAGTATGGGTTAACCCCTGTTTCATGAGCATTTACGCCCT-GATCACCAGAA----
GKL	ATGGCGGAGA--TGGTAGAAACGGTATGCGGGCGGTCGCGGTGGAAACAGCTTGGCAAACGCTCATCCACGAGCATTTCTCTTCGGTTATCCAGGGTT
Dr0930	ATGACGG-CA-----CA--GACGGTGACGGCGCAGTCGCGGCGCTCAACTCGGAGCGAGCTGCCCAACGAACACGTGATTTTCGGTACCCCGGCTA
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SsoPox3Mut	GCGGTCAGACAACAATGGCCCCATCTATATAACGAAGATGAGGAGTTCAG-AAACGCTGTAATGAGGTTAAAAGGGCAATGCAATTTGGAGTAAAGACT
SisLac	GCAGTCAGATATCAATGGCCTCATTGTACAATGAAGATGAGGAGTTAAG-AAACGCTGTAATGAGGTTAAAAGGGCTATGCAATTTGGAGTAAAGACT
SacPox	CCAGTTAGATATCAATGGCCACATCTTTATAATGAAGATGAGGAGTTAAA-AAATCGAGTAAATGAAGTAAAGACAATAATGTCATATGGTGTAAAGACC
VmoLac	ATCGGTGATCCCACTGTCA-TGGGTTAGGGAGAGACATTAGATTCAGTG
GKL	ATCATCGACTTGAACCTGTGCTGGGATTG-GCTGTGACGTTCGCTTTAACG
Dr0930	TTGTCGATCCGAGCCGA-ACGATTTGCGGGCGCAACCCGGCTTTTTCG
	CGCGGGCGAGTGCAG--CTCGGGCCATTGCACCACGCGCAGCGCTCGCAAGCTGCA-CCGAAACGGCGCGGCTGCTGGCGCGCGGTATTTCAGACG
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SsoPox3Mut	ATAGTAGATCCCAGTGTAA-TGGGATTGGGTAGGGACATCAGATTTATG
SisLac	ATAGTGGATCCCTACAGTAA-TGGGATTGGGAAGGGACATTAGATTTATG
SacPox	ATCGGTGATCCCACTGTCA-TGGGTTAGGGAGAGACATTAGATTCAGTG
VmoLac	ATCATCGACTTGAACCTGTGCTGGGATTG-GCTGTGACGTTCGCTTTAACG
GKL	TTGTCGATCCGAGCCGA-ACGATTTGCGGGCGCAACCCGGCTTTTTCG
Dr0930	GTGGTGAGCCACCCCA-ACGACTGCGGACGCAACCCGGCTTCCTCG
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SsoPox3Mut	TTAC-ATATATATCGAC---TTACCTTCTATTCTTAAATAGG-----TCAATTGATGAGATAGCTGACTTGTTTATTATCATGATATA
SisLac	TTAC-ATATACGTTGAC---TTACCTTCTATTCTTAAATAGG-----TCAATTGATGAGATAGCTGACCTATTTATTACAGATATA
SacPox	GTAT-ACCTACACTGAT---TTACCTTCTTCTTCAATGGAAGA-----TCATTGGAAGAGATTGCAAGAAATTATTAATACATGATATA
VmoLac	TTAC-ACGTATACCGAA---ATTCCGTTTACTTCAAGAATCGT-----GGGATTGACTCGTTAGTCGATGCCTTTGTACACGATATT
GKL	TTATTATGAA--GGGGAAGGGGCGCGCGCTACTTCCAATTCCGCGCGGTTCTC-GGAACA-GCAGAAGATGACATTTACGACATGTTTATGGCCGAGCTG
Dr0930	TTATTACGAG-GGCGAGGGGCCACGACCTACTTCAAGTTCCGC--GCTTCTCTGGGTGACGCCGAAAGCGAAATCTACGAGATGATGCGGACCGAGGTG
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SsoPox3Mut	AAAGAGGGAATACAAAGTACTCTCAATAAAGCTGGCTTCGTAAGATAGCT-GCAGATGAACCTGGGATCACAAGGATGTGGAGAAGGTAATAAGGGCT
SisLac	AAAGAGGGAATACAGGCTACTTCCAATAAAGGCTGGCTTCGTAAGATAGCT-GCAGACGAGCCAGGCATTACTAAAGATGTGGAGAAGGTAATAAGAGCA
SacPox	AAAAAGGGGAATACAAAGGACAATAATAGAGCAGCATTTCAATTAAAGGTTGCA-GCAGATGAGCCAGGTATAACGAGGATGTAGAGAGGGCAATAAGGGCA
VmoLac	ACCATTTGGCATTAAGGCACCAATACTCGCGCAGCATTCGTGAAAGCCGTA-ATCGATTCACGCGGCTGACGAAAGACGCTGGAAATGGCAATTCGTGCA
GKL	ACCGAGGGCATTGCCGATACCCGGAATCAAGGCGGGTGTCTCAAGCTGCC-TCGAGCAAAGGGCGCATCCGAGGTACAAAAGATGTTCTTCGCGGCC
Dr0930	ACCGAGGGCATCGCGGCACCGGCATCCGCGCGGGGTCTCAAGCTGGCGAGCAGCCGCA-CGCGCATACCCCTACGAGCAATGTTTTCCGGGGC
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SsoPox3Mut	GCTGCCATAGCAACAAAGAGACTAAAGTACCAATAATTACCACTCTAACGCTCACAATAACACCGGATTAGAACAGCAAGAATAATTGACTGAAGAAG
SisLac	GCAGCTATAACCCATAAGGAGGCTAAAGTACCTATAAATTACGCACTTCTAATGCTCACAATAACACTGGATTAGAAGAACAGAGAAATTAATGGAAGAAG
SacPox	GCTGCTATAGCTCAGAAGGAGACTAACGTACCCATAAATACACATCTCAATAGTCTATAACGGGACAGGCTTGAAGCAACAAAGGATCTTAATGAGGAGG
VmoLac	GCCCGCAAAGCGCACATCAAGACGGATGTGCCCATCATTAACCACTCTTTCGTTGGCAATAAGAGCTCTCTGGATCTGATTTCGCATTTTCAAAGAAGAAG
GKL	GCCGCCCGCGCGCAAAAGAGAGCGGGCGCGGTCTATCATCACCATACGCAAGAAG-AACG--ATGGGGCGGAAACAGCCGCCTATTGTCTGAGCAGC
Dr0930	GCGGCGCGGTGACGCGCAAAACCGCGTGCAGTATCACTACCCACACTAGGAAGGCCAGC--A-GGGACCGCAGCAAGCCGAGCTGCTGACCTCGCTCG
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SsoPox3Mut	GTGTTGATCCAGGGAATAATTAATAGGTCATTTAGGTGATACAGATAAATATAGATTAC-ATAAGAAGATAGCAGATAAAGGATCCTTTATGGATTAG
SisLac	GTTTGTATCCAGGAAGATATTGATAGGACATTTGGGGGACACTGATAATACGGATTAT-ATAAGAAGATAGCAGATAAAGGATCCTTTATCGGATTAG
SacPox	GTGTAGACCCAGGGAGAGTGCTAATAGGTCACCTTGGGGGACACTGATAACGTGGATTAC-ATAAGAAGATAGCCGATAAAGGCTCCTTTGTAGGTCTAG
VmoLac	GGGTGGATTGGCTCGTACCGTGATTGGCCATGTTGGCGATACAGACGACATCTCCTTC-ATTGAACAGATTCTGCGCGAAGGAGCCTTTATCGGACTTG
GKL	GCGCCGATCCGAAAAAAATGTGATCGGCCATATGTGCGGCAACACGGACCCGGACTATCATC-GAAAGACGCTTGCTTACGGCGTTTACATTGCGGTTTG
Dr0930	GCGCGGACCGCGCGCATCATGATCGGGCAGATGGAGCGCAACCCGCGCTACCACC-GCGAGACGCTGCGCCACGGCGTGAGCATGCCTTTTG
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SsoPox3Mut	ATAGATATGGTTTA---GATTTATT---CTTACCTGTTGATA---AGAGAAATGAAACGACCTTAAGACTAATCAAAGATGGTTATTACAGATAAGATAAT
SisLac	ATAGATATGGTTTA---GATTTGTT---CTTACCACTGTTGATA---AGAGAAATGAAACAACATTTGAACTAATCAAGGATGGTTACTCTGATAGGATAAT
SacPox	ATAGATACGGTCTA---GATCTATT---CTTACCTATAGATA---AAAGGAACGAGGTGTTGTTGAAATTAATTAAGATGGATACTTGGACAGGATTAT
VmoLac	ATCCGCTTTGGCGCTG---GACATTTA---TCTGCGCTGAGATA---AACCGGTGAAACCTGCGATTGAGCTGATCAACACGTGGTTGGATTGACAGCTTAT
GKL	ACCGCTTTCGGCATCCAAAGGATGTGTCGGCGCGCAGGATGAGGAGCGGGTCGCGAGCGCTCTTGTCTGCTCCGCGATGGGTACGAAACAAATTTAT
Dr0930	ACCCATCTGGCTTTCAGGGCATGGTGGGCACCCACCGACGCGGAGCGGCTAAGCGTGCTGACCACTGCTGCTGCGGAGGGGCTACGCGACCGGCTGCT
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SsoPox3Mut	GATCTCTCAGCATATTATATGACATTCGACGCTGG-AAC---TGCAAAACAGAAATATAAACCTAAGCTTGC-TCCAAGA-TGGAGTATAACTCTAAT
SisLac	GATTTGCATGACTATTGTTGCACAATTGACTGGG-GAC---TGCTAGACAGAGCTTAAACCTAAACTCGC-TCCAAGA-TGGAGTATGGCACTAAT
SacPox	GGTGTCACAAGATTACTGTTGCACAATTGACTGGG-GAT---AGCAAAAGCCGAGTACAAACCTAAACTAGC-TCCAAGA-TGGAGTATGAGTTTAAT
VmoLac	GTTGTGCGATGACTATTGCCCGACAATTGATTGGTA-TCC-----TCCGGAAGTTGTACGTTCAACCGT-CCCGGAT-TGGACAATGAGCGCTGAT
GKL	GCTGTGCGATGACAC-TG-TCAACGTTTGCGCTCGTCCGTTTACGCTGCCGGAACCGTTTGCGGAAATGA--TGAAAAATTGGCATGTGACGACATTT
Dr0930	GCTCTCGACGACAG-CATCTGGC-ACTGGCTGGGACGCGCGCGGCGCATCCCGGAAGCC--GCCTTGCGCGCGCTCAAGGACTGGCACCTCTCCACAT
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SsoPox3Mut	ATTTGAGGATACGATACCGTTCTTAAAGAGAAATGGAGTGAATGAAGAGTTATAGCTACAATATTTAAGGAAAAATCCGAAAAAGTTCTTC-----AG-
SisLac	ATTTGAGGATACCATACCGTTCTTAAAGAAAAATGGAGTGAATGAAGAGTTATAGATATAAATATTCAGGAGAAATCCGAAAAAATCTTC-----AG-
SacPox	ATTTACAGACGTTATACCTCAATTAAGAGAGCAGGAGTAACGTGATGAGCAGTTGCATGTAATCTTCGTAAGAAATCCAGCTAGACTATTT-----AG-
VmoLac	CTTCGAGAAAGTATCTCTCGCATGCGGAGTGAAGTATTACCGAGGAACAGATTAAACCGGGTTCTCATCGATAACCCACGCGCTGTGTTTACTGGCCG-
GKL	GTTTGTGAACATCATCCCGCGCTGAAAAATGAAGGAATCCGCGACGAAGTGCTTGAGCAAAATGTTTCATCGGCAATCCGGCGGCGCTGTTCC--TC--GGC
Dr0930	CTCCGACGACATCTTGCTGATCTCGGGCGCGGGGCATCACCGAGGAGCAGTGGGGCAGATGACGGTGGCAACCGGCGCGGCTGTTC-----GG-
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SsoPox3Mut	CTAA
SisLac	CTAA
SacPox	TTAG
VmoLac	TTAA
GKL	TTGA
Dr0930	GTAA
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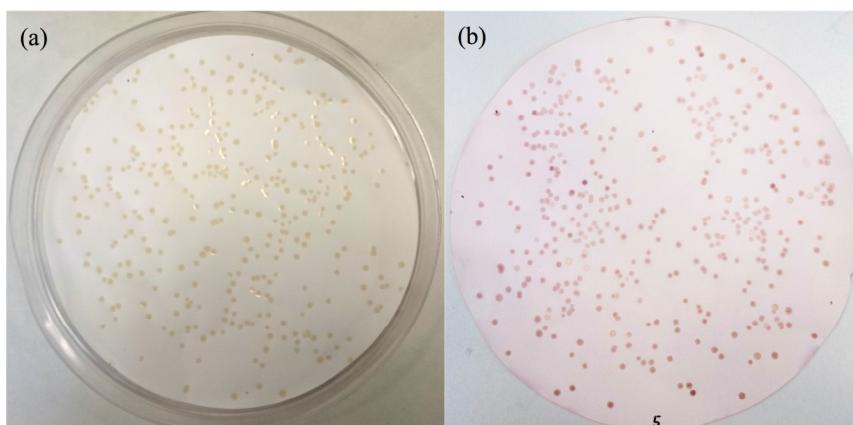
**Figure S1.** Multiple sequence alignment of 6 homologous PLL genes utilized in this study by CLUSTAL W 1.83. Conserved nucleotides are marked by asterisk. The gene encoding *vmolac* was optimized for the expression in *E. coli* and cloned into vector pEX-A2 by Eurofins Genomics, Germany.

<i>SsoPox3Mut</i>	1	--MRIPLVGKDSIESKDIGFTLIHEHLRVFSEAVRQQWPHLY-NEDEEFNAVNEVKRAMQFGVKTI
<i>SisLac</i>	1	--MRIPLVGKEPIEAEDMGFTLIHEHLRVFSEAVRYQWPHLY-NEDEELNAVNEVKRAMQFGVKTI
<i>SacPox</i>	1	-MTKIPLVGKGEISPGEIMGFTLIHEHLRVFSEPVRYQWPHLY-NEDEELKNAVNEVKTIMSYGVKTI
<i>VmoLac</i>	1	-MVRISIAAGNEIDPGSMGLTLFHEHLRLITEVVRWNWPHLY-NEDEELKRAIDAVNAKKYGVKTI
<i>GKL</i>	1	MAEMVETVC-GPVPEQLGKTLIHEHFLFGYPGFQGDVTRGTFREDESLRVAVEAAEKMKRHGIQTV
<i>Dr0930</i>	1	--MTAQTVT-GAVAAQLGATLPEHVIFGYPGYAGDVTLGPFDAHAALASCTETARALLARGIQTV
		. * * * * *
<i>SsoPox3Mut</i>	65	VDPTVMGLGRDIRFMEKVVKATGINLVAGTGIYIYI-DLPFYFLNRS-----IDEIADLFIHDIK
<i>SisLac</i>	65	VDPTVMGLGRDIRFMEKVVKTTGINLVAGTGIYIYV-DLPFYFLNRS-----IDEIADLFIHDIK
<i>SacPox</i>	66	VDPTVMGLGRDIRFSEKVVKETGINVIAATGLYTYT-DLPFFFNGRS-----LEEIAELLIHDIK
<i>VmoLac</i>	66	IDLTVAGIGCDVRFNEKVAKATGVNIIMGTGFYTYTE-IPFYFKNRG-----IDSLVDAFVHDIT
<i>GKL</i>	67	VDPTPNDCGRNPAFLRRVAEETGLNIICATGYYYEGEGAPPYFQFRRLGTAEDDIYDMFMAELT
<i>Dr0930</i>	65	VDATPNDCGRNPAFLREVSEATGLQILCATGFYYEGEGATTYFKFRASLGDAESEIYEMMRTEVT
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<i>SsoPox3Mut</i>	124	EGIQGTLNKAGFVKIAADEPGITKDVEKVIIRAAAIANKETKVPIITHSNAHNNTGLEQQRIILTEE
<i>SisLac</i>	124	EGIQATSNKAGFVKIAADEPGITKDVEKVIIRAAAIHKEAKVPIITHSNAHNNTGLEEQRIILMEE
<i>SacPox</i>	125	KGIQGTNNRAGFIKVAADEPGITRDVERAIRAAAIQKETNPVIITHSNAHNNTGLEQQRIILMEE
<i>VmoLac</i>	125	IGIQGTNTRAAFVKAVIDSSGLTKDVEMAIRAAAKAHIKTDVPIITHSFVGNKSSLDLIRIFKEE
<i>GKL</i>	132	EGIADTGKAGVIKLASSKGRITEYEKMFRAAARAQKETGAVIITHTQEGT-MGPEQAAYLLEH
<i>Dr0930</i>	130	EGIAGTGIRAGVIKLASSRDAITPYEQLFRAAARVQRETGVPIITHTQEGQ-QGPQQAELLTSL
		* * * . * . . . * . * . . . . * . * . . .
<i>SsoPox3Mut</i>	189	GVDPGKILIGHLGDTDNIDYIKKIADKGSFIGLDRYGLD----LFLPVDKRNETTLRLIKDGYSD
<i>SisLac</i>	189	GVDPGKILIGHLGDTDNIDYIKKIADKGSFIGLDRYGLD----LFLPVDKRNETTLRLIKDGYSD
<i>SacPox</i>	190	GVDPGKILIGHLGDTDNIDYIKKIADKGSFIGLDRYGLD----LFLPIDKRNEVLLKLKIDGYLD
<i>VmoLac</i>	190	GVDLARTVIGHVGDTHDISFIEQILREGAFIGLDRFGLD----IYPLDKRVKTAIELIKRGWID
<i>GKL</i>	196	GADPKKIVIGHMCGNTDPDYHRKTLAYGVYIAFDRFGIQGMVG-APTDEERVRTLALLRDGYEK
<i>Dr0930</i>	194	GADPARIMIGHMDGNTDPAYHRETLRHGVSIAFDRIGLQGMVG-TPTDAERLSVLTTLLEGYAD
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<i>SsoPox3Mut</i>	250	KIMISHDYLCFTD-AGTAKPEYKPKLAPRWSITLIFEDTIPFLKRNNGVNEEVIAITIFKENPKKFFS-
<i>SisLac</i>	250	RIMISHDYCCTID-WGTARPELKPPLAPRWSMALIFEDTIPFLKKNVSEEVIDIIFKENPKKFFS-
<i>SacPox</i>	251	RIMVSQDYCCTID-WGIAKPEYKPKLAPKWSMSLIFTDVIPSIKRAAGTDEQLHVIFVKNPARLFS-
<i>VmoLac</i>	251	QLLLSHDYCPTID-WYP--PEVVRSTVPDWTMTLIFEKVIIPMRSEGITEEQINRVLIDNPRRLFTGR-
<i>GKL</i>	260	QIMLSHDTVNVWLGRPFITPEPFAEMMNWVHVEHLFVNIIPALKNEGIRDEVLEQMFIGNPAALFSA-
<i>Dr0930</i>	258	RLLLSHDSIWHWLGRPPAIPAAALPAVKDWHPLHISDDILPDLRRRGITEEQVGQMTVGNPARLFG-
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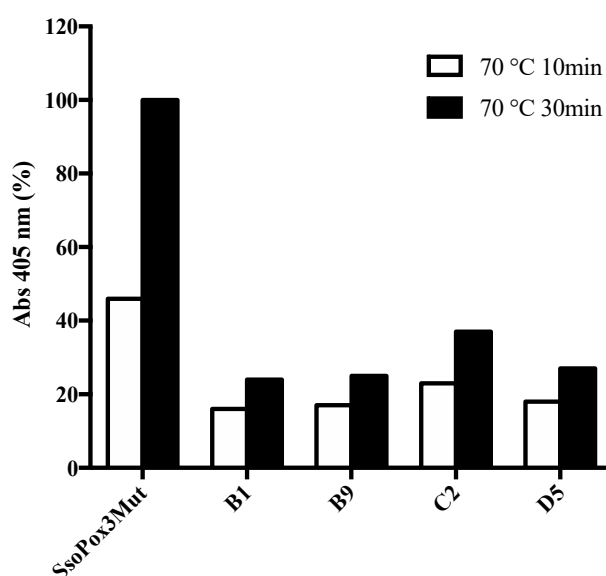
**Figure S2.** Structure-based protein sequence alignment of 6 homologous PLL enzymes utilized in this study. Protein sequence alignment was performed using Swiss-pdb viewer 4.1.0, with manual improvement.



**Figure S3.** Scheme for the screening for PLL mutant library generated by DNA StEP using 6 homologous PLL genes. First, colonies expression mutant PLL enzymes were transferred to nitrocellulose membrane for *in situ* esterase assay. Secondly, cells were lysed by repeated incubations at  $-20^{\circ}\text{C}$  and room temperature. Esterases derived from mesophilic host were inactivated by incubation at  $70^{\circ}\text{C}$ . Subsequently, esterase activity assay was performed by incubating nitrocellulose membrane in the reaction solution prepared by mixing 10 mg  $\beta$ -naphthyl acetate in 500  $\mu\text{l}$  MeOH and 50 mg Fast Blue RR in 50 ml of 50 mM Tris-HCl (pH 8.5). After incubation for 10 min under gentle shaking, nitrocellulose membrane was washed with distilled water and air-dried. Significantly positive colonies were selected for secondary phosphotriesterase activity screening using paraoxon as substrate.



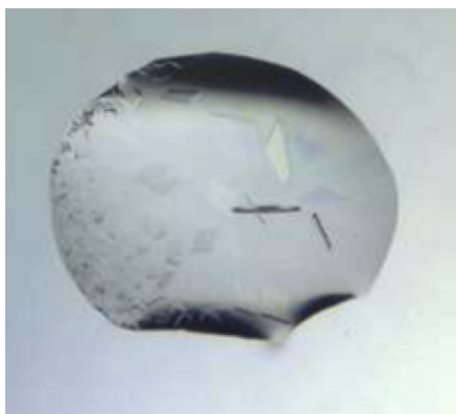
**Figure S4.** *In situ* esterase activity assay. (a) colonies transferred on nitrocellulose membrane for in situ assay. (b) shows nitrocellulose membrane after esterase activity assay. The intensity of violet color of each colony varies according to the esterase activity of the mutant enzyme expressed.



**Figure S5.** Comparison of paraoxonase activity with 3Mut and 4 variants. Generation of *p*-nitrophenol, the hydrolysis product of paraoxon, was detected by measuring absorbance at 405 nm. Data collection was performed at two time points (10 min and 30 min) after 70 °C incubation. The absorbance obtained at each measurement was converted in percentage by considering the value in SsoPox3Mut after 30 min incubation at 70 °C as 100%.

B1	1	--MRIPLVGKEPIEAEDMGFTLIHEHLRVFSEAVRYQWPHLY-NEDEELRNAVNEVKRAMQFGVKTI
B9	1	--MRIPLVGKEPIEAEDMGFTLIHEHLRVFSEAVRQWPHLY-NEDEEFRNAVNEVKRAMQFGVKTI
C2	1	--MRIPLVGKDSIESKDIGFTLIHEHLRVFSEAVRQWPHLY-NEDEEFRNAVNEVKRAMQFGVKTI
D5	1	--MRIPLVGKEPIEAEDMGFTLIHEHLRVFSEAVRYQWPHLY-NEDEELRNAVNEVKRAMQFGVKTI
<i>SsoPox3Mut</i>	1	--MRIPLVGKDSIESKDIGFTLIHEHLRVFSEAVRQWPHLY-NEDEEFRNAVNEVKRAMQFGVKTI
<i>SisLac</i>	1	--MRIPLVGKEPIEAEDMGFTLIHEHLRVFSEAVRYQWPHLY-NEDEELRNAVNEVKRAMQFGVKTI
<i>SacPox</i>	1	-MTKIPLVGKEISPGEIMGFTLIHEHLRVFSEPVRYQWPHLY-NEDEELKNAVNEVKTIMSYGVKTI
<i>VmoLac</i>	1	-MVRISIAGGNEIDPGSMGLTLFHEHLRLITEVVRWNWPHLY-NEDEELKRAIDAVNAAKKYGVKTI
GKL	1	MAEMVETVC-GPVPVEQLGKTLIHEHFLFGYPGFQGDVTRGTFREDESRLVAVEAAEKMKRHIQTV
<i>Dr0930</i>	1	--MTAQTVT-GAVAAAQLGATLPHEHVIFGYPGYAGDVTLGPFDHAAALASCTETARALLARGIQTV
		. * * * * *
B1	65	VDPTVMGLGRDIRFMEKVVKTGTGINLVAGTGIYIYID-LPFYFLNRS-----IDEIADLFIHDIK
B9	65	VDPTVMGLGRDIRFMEKVVKTGTGINLVAGTGIYIYID-LPFYFLNRS-----IDEIADLFIHDIK
C2	65	VDPTVMGLGRDIRFMEKVVKTGTGINLVAGTGIYIYID-LPFYFLNRS-----IDEIADLFIHDIK
D5	65	VDPTVMGLGRDIRFMEKVVKTGTGINLVAGTGIYIYVD-LPFYFLNRS-----IDEIADLFIHDIK
<i>SsoPox3Mut</i>	65	VDPTVMGLGRDIRFMEKVVKTGTGINLVAGTGIYIYI-DLPFYFLNRS-----IDEIADLFIHDIK
<i>SisLac</i>	65	VDPTVMGLGRDIRFMEKVVKTGTGINLVAGTGIYIYV-DLPFYFLNRS-----IDEIADLFIHDIK
<i>SacPox</i>	66	VDPTVMGLGRDIRFSEKVVKETGINVIAATGLTYTYT-DLPFFFNRS-----LEEIAELLIHDIK
<i>VmoLac</i>	66	IDLTVAGIGCDVRFNEKVAATGVNIIMGTGFYTYTE-IPFYFKNRG-----IDSLVDAFVHDIT
GKL	67	VDPTPNDGCRNPAFLRRVAEETGLNIIICATGYEYEGEGAPPYFQFRLLGTAEDDIYDMFMAELT
<i>Dr0930</i>	65	VDATPNDGCRNPAFLREVSEATGLQILCATGFYEGEGATYFVKFRASLGDAESEIYEMMRTEVT
		. * * * . * * * * . * . . . .
B1	124	EGIQGTNLKAGFVKIAADEPGITKDVEKVIIRAAAIANKETKVPIITHSNAHNNTGLEEQQRILTEE
B9	124	EGIQGTNLKAGFVKIAADEPGITKDVEKVIIRAAAIANKETKVPIITHSNAHNNTGLEEQQRILTEE
C2	124	EGIQGTNLKAGFVKIAADEPGITKDVEKVIIRAAAIANKETKVPIITHSNAHNNTGLEEQQRILTEE
D5	124	EGIQATSNKAGFVKIAADEPGITKDVEKVIIRAAAIHKEAKVPIITHSNAHNNTGLEEQQRILTEE
<i>SsoPox3Mut</i>	124	EGIQGTNLKAGFVKIAADEPGITKDVEKVIIRAAAIANKETKVPIITHSNAHNNTGLEEQQRILTEE
<i>SisLac</i>	124	EGIQATSNKAGFVKIAADEPGITKDVEKVIIRAAAIHKEAKVPIITHSNAHNNTGLEEQQRILTEE
<i>SacPox</i>	125	KGIQGTNNRAGFIKVAADEPGITRDVERAIRAAAIQKETNVPIITHSNAHNNTGLEEQQRILTEE
<i>VmoLac</i>	125	IGIQGTNTRAAFVKAVIDSSGLTKDVEMAIRAAAKAHIKTDVPIITHSFVGNKSSDLIRIFKEE
GKL	132	EGIADTGIKAGVIKLASSKGRITEYEKMFRAAARAQKETGAVIITHTQEGT-MGPEQAAYLLEH
<i>Dr0930</i>	130	EGIAGTGIRAGVIKLASSRDAITPYEQLFRAAARVQRETGVPIITHTQEGQ-QGPPQAELLTSL
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B1	189	GVDPGKILIGHLGDTDNIDYIKKIADKGSFIGLDRYGLD----LFLPVDKRNETTTLKLIKDGYS
B9	189	GVDPGKILIGHLGDTDNIDYIKKIADKGSFIGLDRYGLD----LFLPVDKRNETTTLKLIKDGYS
C2	189	GVDPGKILIGHLGDTDNIDYIKKIADKGSFIGLDRYGLD----LFLPVDKRNETTTLKLIKDGYS
D5	189	GVDPGKILIGHLGDTDNIDYIKKIADKGSFIGLDRYGLD----LFLPVDKRNETTTLKLIKDGYS
<i>SsoPox3Mut</i>	189	GVDPGKILIGHLGDTDNIDYIKKIADKGSFIGLDRYGLD----LFLPVDKRNETTTLRLIKDGYS
<i>SisLac</i>	189	GVDPGKILIGHLGDTDNIDYIKKIADKGSFIGLDRYGLD----LFLPVDKRNETTTLKLIKDGYS
<i>SacPox</i>	190	GVDPGRVLIGHLGDTDNVDYIKKIADKGSFVGLDRYGLD----LFLPIDKRNEVLLKLIKDGYS
<i>VmoLac</i>	190	GVDLARTVIGHVGDITDIDISFIEQILREGAFIGLDRFGLD----IYLPVDKRVKTAIELIKRGWID
GKL	196	GADPKKIVIGHMCGNTDPDYHRKTLAYGVYIAFDRFGIQGMVG-APTDEERVTTLLALLRDGYEK
<i>Dr0930</i>	194	GADPARIMIGHMCGNTDPAYHRETLRHGVSIAFDRIGLQGMVG-TPTDAERLSVLTLLGEGYAD
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B1	250	RIMISHDYCCTID-WGTARPELKPKLAPRWSMALIFEDTIPFLKKNVSEEVIDIN----PKKFFS
B9	250	RIMISHDYCCTID-WGTARPELKPKLAPRWSMALIFEDTIPFLKKNVSEEVIDI----PKKFFS
C2	250	RIMISHDYCCTID-WGTARPELKPKLAPRWSMALIFEDTIPFLKKNVSEEVIDIKFIEIPKKFFS
D5	250	RIMISHDYCCTID-WGTARPELKPKLAPRWSMALIFEDTIPFLKKNVSEEVIDINARRIPKKFFS
<i>SsoPox3Mut</i>	250	KIMISHDYLCFTD-AGTAKPEYKPKLAPRWSITLIFEDTIPFLKKNVNEEVIAITFKENPKKFFS-
<i>SisLac</i>	250	RIMISHDYCCTID-WGTARPELKPKLAPRWSMALIFEDTIPFLKKNVSEEVIDIIFKENPKKFFS-
<i>SacPox</i>	251	RIMVSQDYCCTID-WGIAKPEYKPKLAPKWSMISLIFTDVIPSIKRAGVTDEQLHVIFVKNPARLFS-
<i>VmoLac</i>	251	QLLLSHDYCPTID-WYP--PEVVRSTVPDWTMTLIFEKVIPRMRSEGITEEQINRVLIDNPRRLFTGR-
GKL	260	QIMLSHDTVNVWGRPFITLPEPFAEMMKNWVHEHLFVNIIIPALKNEGIVQMFIGNPAALFSA-
<i>Dr0930</i>	258	RLLLSHDSIHWHLGRPPAIPAALPAVKDWHPLHISDDILPDLRRRGITEEQVGMQTVGNPARLFG-
		. . . * . * . . * . * . . * . *

**Figure S6.** Structure-based sequence alignment between 4 variants and 6 template PLL genes. Protein sequence alignment was performed using Swiss-Pdb viewer 4.1.0, with manual improvement.



**Figure S7.** A representative crystal of SsoPox4Mut from which the structure was obtained.

**Table S1.** Sequence identities at DNA levels between 6 homologous PLL genes utilized in this study.

(%)	<i>SsoPox3Mut</i>	<i>SisLac</i>	<i>SacPox</i>	<i>VmoLac</i>	GKL	Dr0930
<i>SsoPox3Mut</i>	100	86,8	70,7	55,7	46,7	42,6
<i>SisLac</i>	86,8	100	72,3	55,3	48,4	42,6
<i>SacPox</i>	70,7	72,3	100	55,5	47,2	44,3
<i>VmoLac</i>	55,7	55,3	55,5	100	47,4	47,2
GKL	46,7	48,4	47,2	47,4	100	62,9
Dr0930	42,6	42,6	44,3	47,2	62,9	100

**Table S2.** Sequence identities at protein levels between 6 homologous PLL enzymes utilized in this study

(%)	<i>SsoPox3Mut</i>	<i>SisLac</i>	<i>SacPox</i>	<i>VmoLac</i>	GKL	Dr0930
<i>SsoPox3Mut</i>	100	90,5	75,2	51,9	35,8	29,9
<i>SisLac</i>	90,5	100	76,1	52,2	35,8	28,9
<i>SacPox</i>	75,2	76,1	100	52,7	35,0	32,7
<i>VmoLac</i>	51,9	52,2	52,7	100	31,6	31,3
GKL	35,8	35,8	35,0	31,6	100	59,1
Dr0930	29,9	28,9	32,7	31,3	59,1	100

**Table S3.** Sequence identities between 4 variants and template PLL genes used in DNA StEP at protein level.

(%)	B1	B9	C2	D5
B1	100,0	98,7	96,1	97,1
B9	98,7	100,0	97,4	96,5
C2	96,1	97,4	100,0	94,0
D5	97,1	96,5	94,0	100,0
<i>SsoPox3Mut</i>	93,2	93,9	94,0	89,2
<i>SisLac</i>	97,1	95,8	94,6	98,1
<i>SacPox</i>	77,4	76,5	75,5	75,2
<i>VmoLac</i>	53,9	53,6	52,7	52,2
GKL	36,7	36,0	35,3	34,9
Dr0930	30,9	30,6	29,3	28,9