Functional and phylogenetic analysis of TetX variants to design a new classification system

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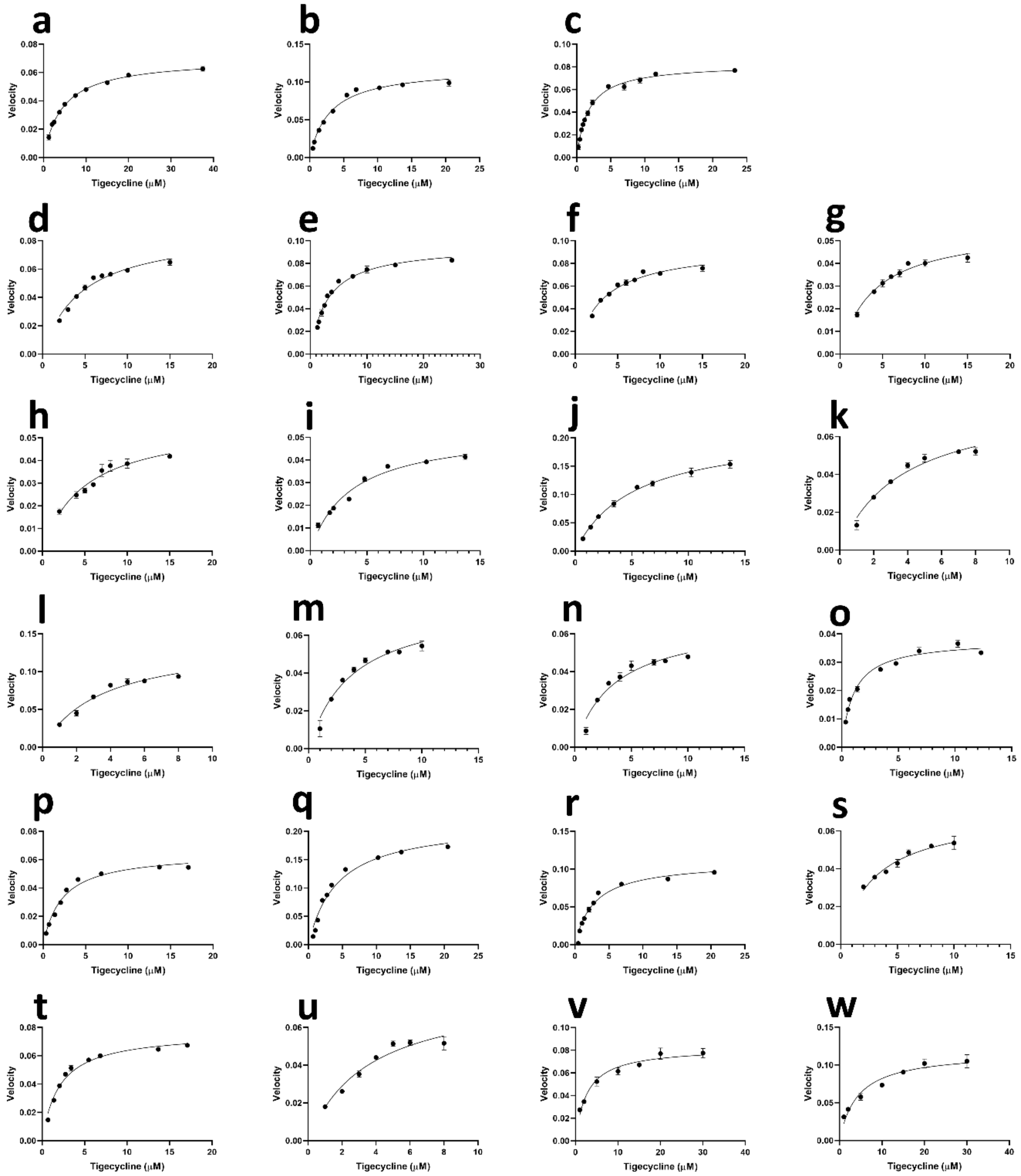
Supplementary Table 1. Primers used in this study.

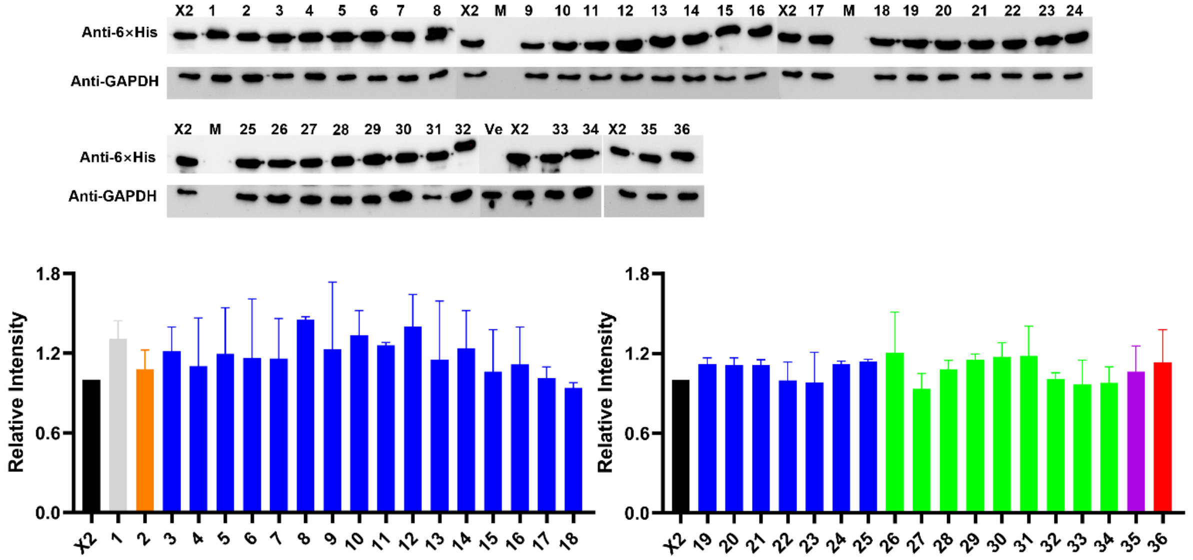
Primer	Sequence
tet(X2)-F[a]	CGCCATATGATGACAATGCGAATAGATACAG
tet(X2)-R[b]	CGCGGATCCTTATACATTTAACAATTGCTGAAAC
tet(X3)-R	CGCGGATCCTTATAGATTCAATAATTTTTGAAACGA
tet(X4)-F	CGCCATATGATGAGCAATAAAGAAAAACAAATG
H^{196}L-F	ATACAAGCCGATATTCTGCAACCAGAGATAAAC
H^{196}L-R	GTTTATCTCTGGTTGCAGAATATCGGCTTGTAT
Q^{197}H-F	CAAGCCGATATTCATCATCCAGAGATAAACTGT
Q^{197}H-R	ACAGTTTATCTCTGGATGATGAATATCGGCTTG
S^{217}A-F	AACCGGCTAATGGCAGCACACCAAGGTAATTTA
S^{217}A-R	TAAATTACCTTGGTGTGCTGCCATTAGCCGGTT
S^{217}G-F	AACCGGCTAATGGCAGGTCACCAAGGTAATTTA
S^{217}G-R	TAAATTACCTTGGTGACCTGCCATTAGCCGGTT
H^{279}R-F	TACAAAGAATTGATTCGTACGACGTTGTCATTT
H^{279}R-R	AAATGACAACGTCGTACGAATCAATTCTTTGTA
T^{280}S-F	AAAGAATTGATTCATAGCACGTTGTCATTTGTA
T^{280}S-R	TACAAATGACAACGTGCTATGAATCAATTCTTT
T^{280}V-F	AAAGAATTGATTCATGTGACGTTGTCATTTGTA
T^{280}V-R	TACAAATGACAACGTCACATGAATCAATTCTTT
T^{280}L-F	AAAGAATTGATTCATCTGACGTTGTCATTTGTA
T^{280}L-R	TACAAATGACAACGTCAGATGAATCAATTCTTT
L^{282}S-F	TTGATTCATACGACGAGCTCATTTGTAGGATTG
L^{282}S-R	CAATCCTACAAATGAGCTCGTCGTATGAATCAA
E^{295}N-F	CGGATATTTCCTTTAAACAAGCCTTGGAAAAGC
E^{295}N-R	GCTTTTCCAAGGCTTGTTTAAAGGAAATATCCG
E^{295}G-F	CGGATATTTCCTTTAGGAAAGCCTTGGAAAAGC
E^{295}G-R	GCTTTTCCAAGGCTTTCCTAAAGGAAATATCCG
E^{295}D-F	CGGATATTTCCTTTAGATAAGCCTTGGAAAAGC
E^{295}D-R	GCTTTTCCAAGGCTTATCTAAAGGAAATATCCG
K^{296}N-F	ATATTTCCTTTAGAAAACCCTTGGAAAAGCAAG
K^{296}N-R	CTTGCTTTTCCAAGGGTTTTCTAAAGGAAATAT
P^{297}D-F	TTTCCTTTAGAAAAGGATTGGAAAAGCAAGCGC
P^{297}D-R	GCGCTTGCTTTTCCAATCCTTTTCTAAAGGAAA
P^{297}S-F	TTTCCTTTAGAAAAGAGTTGGAAAAGCAAGCGC
P^{297}S-R	GCGCTTGCTTTTCCAACTCTTTTCTAAAGGAAA
V^{329}L-F	GTAAATAGTGGGTTGCTGGATGCCTTGATATTG
V^{329}L-R	CAATATCAAGGCATCCAGCAACCCACTATTTAC
V^{329}M-F	GTAAATAGTGGGTTGATGGATGCCTTGATATTG
V^{329}M-R	CAATATCAAGGCATCCATCAACCCACTATTTAC
A^{339}T-F	TTGTCTGATAATCTAACCGATGGAAAATTTAAT
A^{339}T-R	ATTAAATTTTCCATCGGTTAGATTATCAGACAA
D^{340}N-F	TCTGATAATCTAGCCAATGGAAAATTTAATAGC
D^{340}N-R	GCTATTAAATTTTCCATTGGCTAGATTATCAGA
V^{350}I-F	AGCATTGAAGAGGCTATTAAAAATTATGAACAG
V^{350}I-R	CTGTTCATAATTTTTAATAGCCTCTTCAATGCT
K^{351}E-F	ATTGAAGAGGCTGTTGAAAATTATGAACAGCAA
K^{351}E-R	TTGCTGTTCATAATTTTCAACAGCCTCTTCAAT
I^{359}V-F	GAACAGCAAATGTTTGTTTATGGCAAAGAAGCA
I^{359}V-R	TGCTTCTTTGCCATAAACAAACATTTGCTGTTC
I^{359}M-F	GAACAGCAAATGTTTATGTATGGCAAAGAAGCA
I^{359}M-R	TGCTTCTTTGCCATACATAAACATTTGCTGTTC
tet(X3)-L^{329}V-F	GTAAATACGGGATTAGTGGATGCTTTGATATTG
tet(X3)-L^{329}V-R	CAATATCAAAGCATCCACTAATCCCGTATTTAC

tet(X3)-T^{339}A/N^{340}D -F	TTGTCTGAAAACCTTGCCGATGGAGAATTTACAAGT
tet(X3)-T^{339}A/N^{340}D -R	ACTTGTAAATTCTCCATCGGCAAGGTTTTCAGACAA
tet(X3)-I^{350}V/E^{351}K -F	AGTATTGAAAATGCCGTTAAAAACTACGAACAACAA
tet(X3)-I^{350}V/E^{351}K -R	TTGTTGTTCGTAGTTTTTAACGGCATTTTCAATACT
tet(X4)-M^{329}V-F	GTAAACAGCGGGTTGGTGGATGCCTTGATATTG
tet(X4)-M^{329}V-R	CAATATCAAGGCATCCACCAACCCGCTGTTTAC
tet(X4)-T^{339}A/N^{340}D -F	TTGTCGGATAATCTGGCCGATGGGAAATTTAACAGC
tet(X4)-T^{339}A/N^{340}D -R	GCTGTTAAATTTCCCATCGGCCAGATTATCCGACAA
tet(X4)-I^{350}V/E^{351}K -F	AGCATTGAAGAGGCTGTTAAAAATTATGAACAGCAA
tet(X4)-I^{350}V/E^{351}K -R	TTGCTGTTCATAATTTTTAACAGCCTCTTCAATGCT

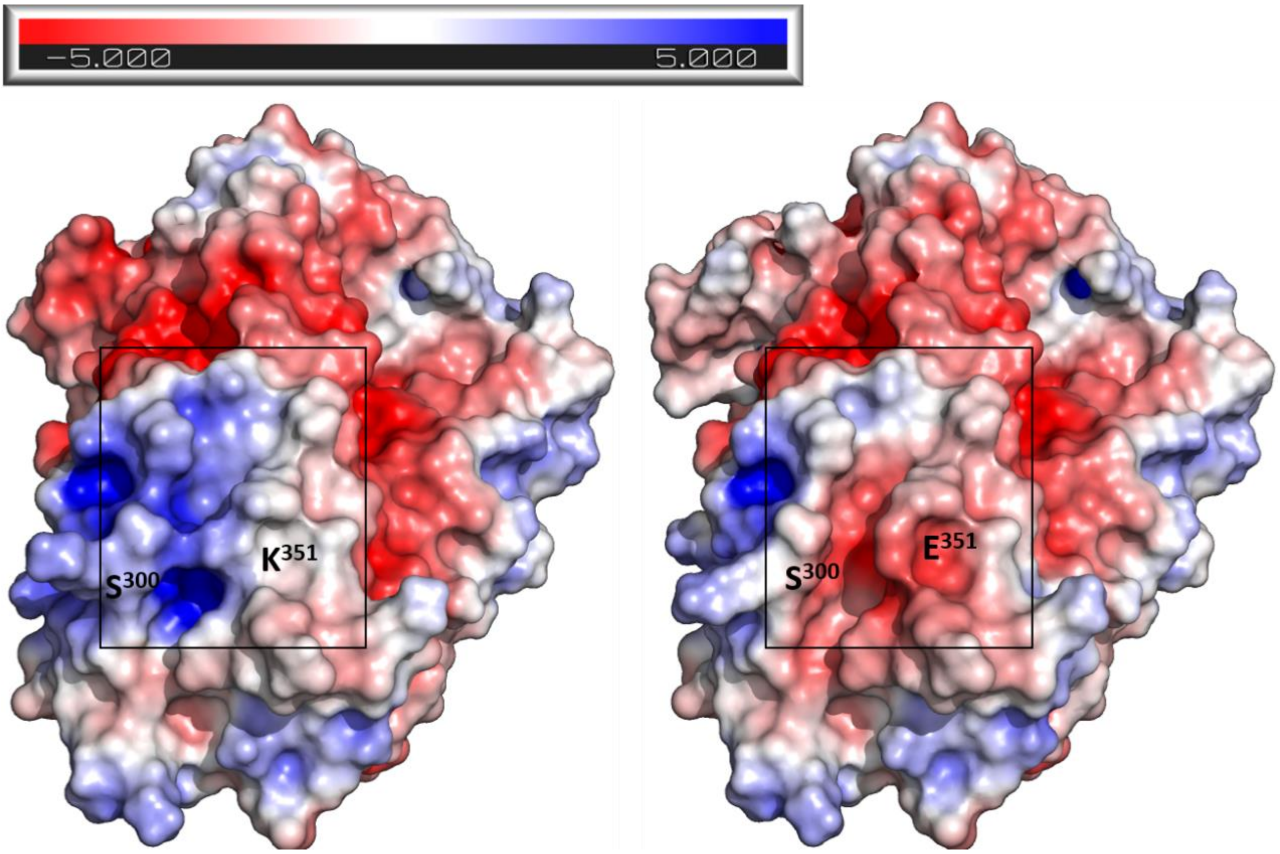
Note: [a] *tet*(X2)-F used for *tet*(X3) forward primer; [b] *tet*(X2)-R used for *tet*(X4) reverse primer.



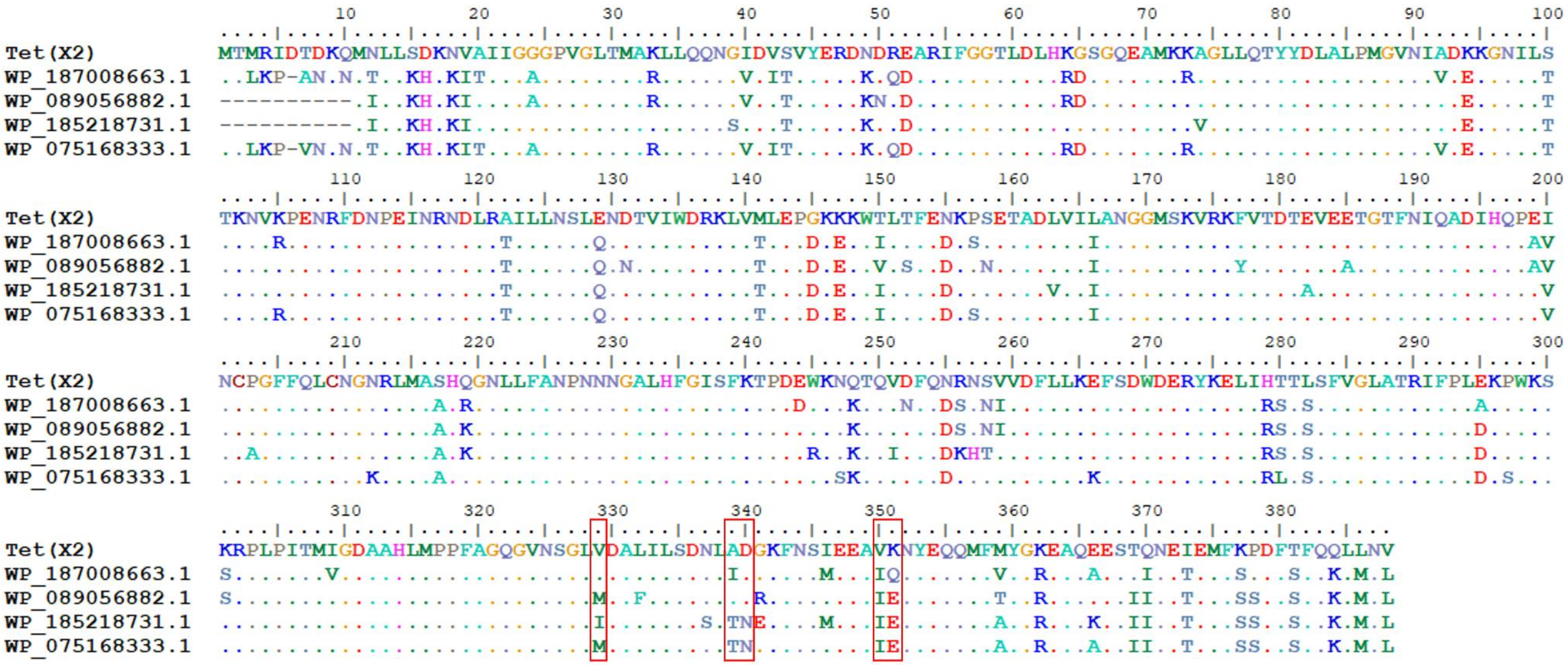
Supplementary Figure 1. Michaelis–Menten plot of Tet(X) variants and mutants. a Tet(X2), **b** Tet(X3),**c** Tet(X4), **d** S^{217}A, **e** T^{280}V, **f** T^{280}S, **g** L^{282}S, **h** E^{295}N, **i** V^{329}L, **j** V^{329}M, **k** A^{339}T, **l** K^{351}E, **m** A^{339}T/D^{340}N, **n** V^{350}K/I^{351}E, **o** V^{329}L/A^{339}T/D^{340}N, **p** V^{329}L/V^{350}I/K^{351}E, **q** V^{329}M/A^{339}T/D^{340}N, **r** V^{329}M/V^{350}I/K^{351}E, **s** A^{339}T/D^{340}N/V^{350}I/K^{351}E, **t** V^{329}L/A^{339}T/D^{340}N/V^{350}I/K^{351}E, **u** V^{329}M/A^{339}T/D^{340}N/V^{350}I/K^{351}E, **v** Tet(X3)-L^{329}V/T^{339}A/N^{340}D/I^{350}V/E^{351}K, **w** Tet(X4)-L^{329}V/T^{339}A/N^{340}D/I^{350}V/E^{351}K.



Supplementary Figure 2 Protein expression levels of TetX variant and mutant enzymes. **M** is the protein ladder, **Ve** is vector control, **X2** is Tet(X2) as a positive control and shown in black. **1** is Tet(X3) and shown in grey, **2** is Tet(X4) and shown in orange, **3** is H^{196}L, **4** is Q^{197}H, **5** is S^{217}A, **6** is S^{217}G, **7** is H^{279}R, **8** is T^{280}V, **9** is T^{280}L, **10** is T^{280}S, **11** is L^{282}S, **12** is E^{295}G, **13** is E^{295}N, **14** is E^{295}D, **15** is K^{296}N, **16** is P^{297}D, **17** is P^{297}S, **18** is I^{359}M, **19** is I^{359}V, **20** is V^{329}L, **21** is V^{329}M, **22** is A^{339}T, **23** is D^{340}N, **24** is V^{350}I, **25** is K^{351}E , **26** is A^{339T}/D^{340}N, **27** is V^{350I}/K^{351}E, **28** is V^{329}L/A^{339T}/D^{340}N, **29** is V^{329}L/V^{350I}/K^{351}E, **30** is V^{329}M/A^{339T}/D^{340}N, **31** is V^{329}M/V^{350I}/K^{351}E, **32** is A^{339T}/D^{340}N/V^{350I}/K^{351}E, **33** is V^{329}L/A^{339}T/D^{340}N/V^{350}I/K^{351}E, **34** is V^{329}M/A^{339}T/D^{340}N/V^{350}I/K^{351}E, **35** is Tet(X3)-L^{329}V/T^{339}A/N^{340}D/I^{350}V/E^{351}K and shown in purple, **36** is Tet(X4)-M^{329}V/T^{339}A/N^{340}D/I^{350}V/E^{351}K and shown in red. Single mutants are in blue and multiple mutants are in green. Band intensities from two independent experiments were used to plot the bar graph.



Supplementary Figure 3. Electrostatic potential map of Tet(X2) and the corresponsing mutants carrying the V^{329}M/A^{339}T/D^{340}N/V^{350}I/K^{351}E amino acid changes. The area between Loop 5 and C terminal α helix is marked by a black rectangle. The color change from red to blue reflects a shift of electrostatic potential from negative to positive.



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                           10        20        30        40        50        60        70        80        90       100
                   ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Tet(X2)            MTMRIDTDKQMNLLSDKNVAIIGGGPVGLTMAKLLQQNGIDVSVYERDNDREARIFGGTLDLHKGSGQEAMKKAGLLQTYYDLALPMGVNIADKKGNILS
WP_187008663.1     ..LKP-AN.N.T..KH.KIT...A........R......V.IT.....K.QD...........RD.......R..................V.E.....T
WP_089056882.1     ----------.I..KH.KI....A........R......V..T.....KN.D...........RD............................E.....T
WP_185218731.1     ----------.I..KH.KI...................S...T.....K..D.....................V...................E.....T
WP_075168333.1     ..LKP-VN.N.T..KH.KIT...A........R......V.IT.....K.QD...........RD.......R..................V.E.....T
                          110       120       130       140       150       160       170       180       190       200
                   ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Tet(X2)            TKNVKPENRFDNPEINRNDLRAILLNSLENDTVIWDRKLVMLEPGKKKWTLTFENKPSETADLVILANGGMSKVRKFVTDTEVEETGTFNIQADIHQPEI
WP_187008663.1     ....R................T......Q...........T...D.E..I....D.S........I................................AV
WP_089056882.1     .....................T......Q.N.........T...D.E..V.S..D..N.......I..........Y.......A.............AV
WP_185218731.1     .....................T......Q...........T...D.E..I....D.......V..I...............A.................V
WP_075168333.1     ....R................T......Q...........T...D.E..I....D.S........I.................................V
                          210       220       230       240       250       260       270       280       290       300
                   ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Tet(X2)            NCPGFFQLCNGNRLMASHQGNLLFANPNNNGALHFGISFKTPDEWKNQTQVDFQNRNSVVDFLLKEFSDWDERYKELIHTTLSFVGLATRIFPLEKPWKS
WP_187008663.1     ................A.R........................D...K...N..DS.NI...................RS.S............A.....
WP_089056882.1     ................A.K............................K......DS.NI...................RS.S............D.....
WP_185218731.1     ..A.............A.K.........................R..K..I...DKHT....................RS.S............D.....
WP_075168333.1     ...........K....A.............................SK......D..........K............RL.S............D.S...
                          310       320       330       340       350       360       370       380
                   ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|...
Tet(X2)            KRPLPITMIGDAAHLMPPFAGQGVNSGLVDALILSDNLADGKFNSIEEAVKNYEQQMFMYGKEAQEESTQNEIEMFKPDFTFQQLLNV
WP_187008663.1     S.......V.............................I......M...IQ.......V..R...A...I..T...S...S..K.M.L
WP_089056882.1     S...........................M..F........R........IE.......T..R......II..T...SS..S..K.M.L
WP_185218731.1     ............................I.......S.TNE....M...IE.......A..R..K..II..T...SS..S..K.M.L
WP_075168333.1     ............................M.........TN.........IE.......A..R...A..II..T...SS..S..K.M.L
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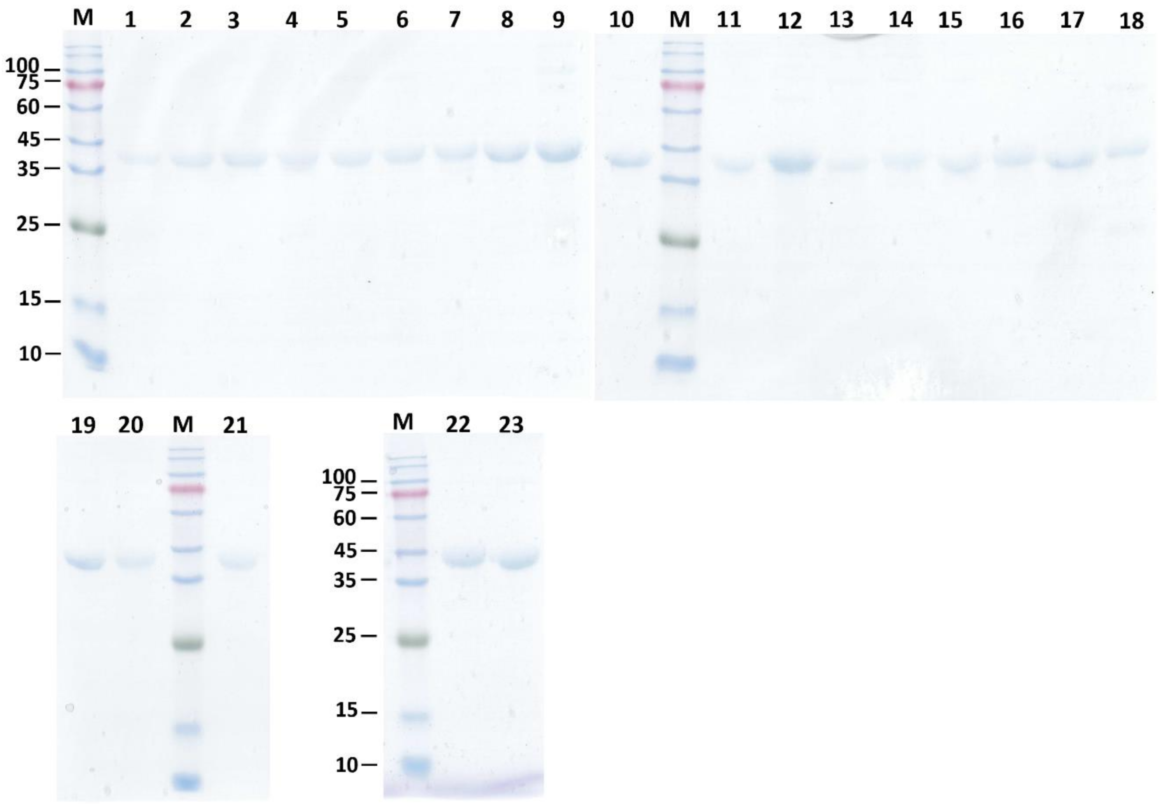
Supplementary Figure 4. Multiple alignment for WP_187008663.1, WP_089056882.1, and WP_185218731.1 with the variant (WP_075168333.1) of the Tet(X4) class in the same branch.



Supplementary Figure 5. SDS-PAGE analyses of recombinant TetX variants and mutants. **M** protein ladder**, 1** Tet(X2), **2** Tet(X3), **3** Tet(X4), **4** S^{217}A, **5** T^{280}V, **6** T^{280}S, **7** L^{282}S, **8** E^{295}N, **9** V^{329}L, **10** V^{329}M, **11** A^{339}T, **12** K^{351}E, **13** A^{339}T/D^{340}N, **14** V^{350}K/I^{351}E, **15** V^{329}L/A^{339}T/D^{340}N, **16** V^{329}L/V^{350}I/K^{351}E, **17** V^{329}M/A^{339}T/D^{340}N, **18** V^{329}M/V^{350}I/K^{351}E, **19** A^{339}T/D^{340}N/V^{350}I/K^{351}E, **20** V^{329}L/A^{339}T/D^{340}N/V^{350}I/K^{351}E, **21** V^{329}M/A^{339}T/D^{340}N/V^{350}I/K^{351}E, **22** Tet(X3)-L^{329}V/T^{339}A/N^{340}D/I^{350}V/E^{351}K, **23** Tet(X4)-L^{329}V/T^{339}A/N^{340}D/I^{350}V/E^{351}K**.**