# Activity improvement and vital amino acid identification on the marine-derived quorum quenching enzyme MomL by protein engineering

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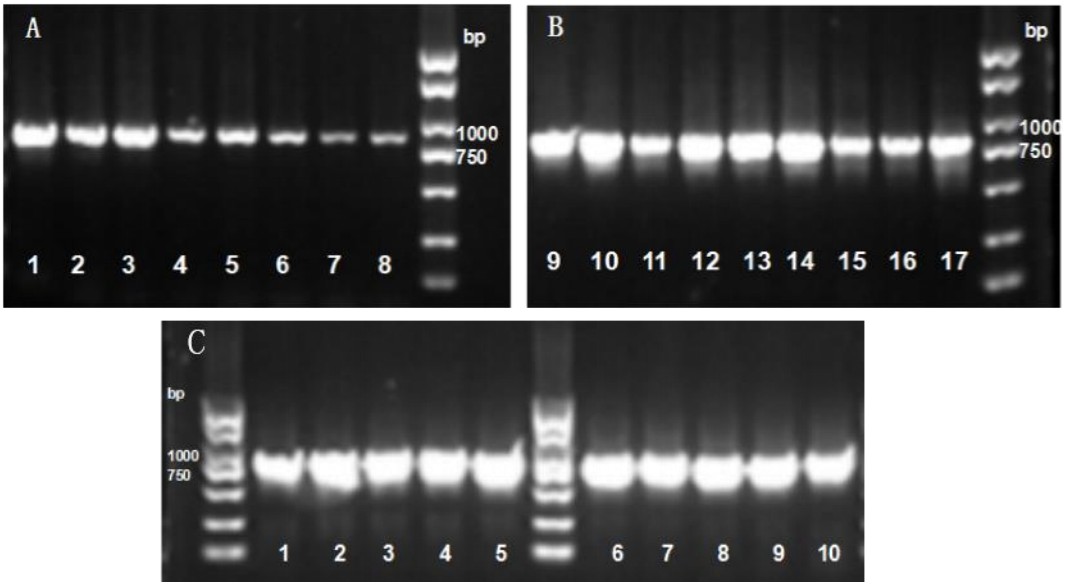


**Figure S1.** The detection of gel electrophoresis of *momL* fragment with series of epPCR condition. (A) The concentration gradient of $Mg^{2+}$. 1. 1mM; 2. 2mM; 3. 3mM; 4. 4mM; 5. 5mM; 6. 6mM; 7. 7mM; 8. 8mM. (B) The concentration gradient of $Mn^{2+}$. 9. 0mM; 10. 0.05mM; 11. 0.10mM; 12. 0.15mM; 13. 0.20mM; 14. 0.30mM; 15. 0.4mM; 16. 0.5mM; 17. 0.6mM. (C) The different concentration gradient test of $Mn^{2+}$ and $Mg^{2+}$. 1. $Mg^{2+}$ 1mM, $Mn^{2+}$ 0.00mM; 2. $Mg^{2+}$ 1mM, $Mn^{2+}$ 0.05mM; 3. $Mg^{2+}$ 1mM, $Mn^{2+}$ 0.10mM; 4. $Mg^{2+}$ 1mM, $Mn^{2+}$ 0.15mM; 5. $Mg^{2+}$ 1mM, $Mn^{2+}$ 0.20mM; 6. $Mg^{2+}$ 2mM, $Mn^{2+}$ 0.00mM; 7. $Mg^{2+}$ 2mM, $Mn^{2+}$ 0.05mM; 8. $Mg^{2+}$ 2mM, $Mn^{2+}$ 0.10mM; 9. $Mg^{2+}$ 2mM, $Mn^{2+}$ 0.15mM; 10. $Mg^{2+}$ 2mM, $Mn^{2+}$ 0.20mM.

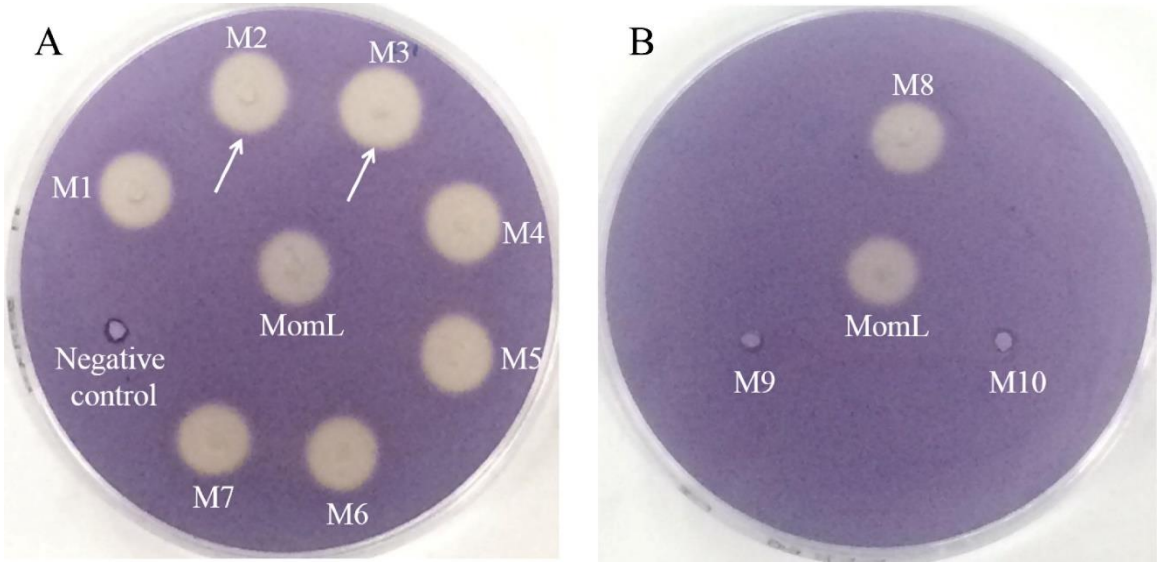


**Figure S2.** The protein activity test. In second round screening step, IPIG was not added to the CV026-loaded screening plate. White halos indicate that MomL and its mutants degraded C6-HSL. QQ ability of MomL and its mutants were determined due to halo diameter.

**Table S1.** Mutation sites and mutation primers of MomL. Primers with mutant sites were designed using the Exsite™ method. Mutagenic bases are underlined.

| protein | primer name | sequence | mutation site |
|---|---|---|---|
| $MomL_{N51Y}$ | N51Y-F | 5-GGAGGTACCGTA<u>T</u>ATGCCAATATGT-3 | Asn51→Tyr |
| | N51Y-R | 5- GCTAAAAGCATAGAGCTTTATCTCG-3 | |
| $MomL_{N179S}$ | N179S-F | 5-CCGGACATCTACA<u>G</u>TTCCATTAAAG-3 | Asn179→Ser |
| | N179S-R | 5-ATTGCTCTTTTGGTTGTCCTCGCTT-3 | |
| $MomL_{M228V}$ | M228V-F | 5-CCGTTG<u>G</u>TGCTTTCTGGGGAC-3 | Met228→Val |
| | M228V-R | 5-TCCGTGCTCAACCATATCCAA-3 | |
| $MomL_{K205E}$ | K205E-F | 5- GTAAT<u>G</u>GAATTTATGCCAGGC-3 | Lys205→Glu |
| | K205E-R | 5- CACACTTCCATCCCCGAACAC-3 | |
| $MomL_{E238G}$ | E238G-F | 5-TACCATTTTTACG<u>G</u>GAACCGGGAGT-3 | Glu238→Gly |
| | E238G-R | 5-CATGTCCCCAGAAAGCATCAACGGT-3 | |
| $MomL_{L254R}$ | L254R-F | 5-AATTACGATGTGGCCC<u>G</u>CACCAAGA-3 | Leu254→Arg |
| | L254R-R | 5-AAAAATGGGCACTCTTCGGAACTCC-3 | |
| $MomL_{T84A}$ | T84A-F | 5-GTTCACCCCAGGGGC<u>A</u>CTTTGATGT-3 | Thr84→Ala |
| | T84A-R | 5-GATGACGTAAAAAGCATCGGCAAAT-3 | |
| $MomL_{K82R}$ | K82R-F | 5-GTTCACCCCA<u>G</u>GGGCACTTTGATGT-3 | Lys82→Arg |
| | K82R-R | 5-GATGACGTAAAAAGCATCGGCAAAT-3 | |

**Table S2.** The mutation rate comparison of ep-PCR products with different conditions.

| PCR conditions | | no mutation | premature termination | number of mutated bases | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $Mg^{2+}$ (mM) | $Mn^{2+}$ (mM) | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
| 1.0 | 0.2 | 23% | 8% | 31% | 16% | 22% | - | - | - | - | - | - | - | - |
| 2.0 | 0.1 | 0 | 20% | 10% | 40% | - | 10% | 20% | - | - | - | - | - | - |
| 2.0 | 0.15 | 0 | - | - | 25% | 25% | - | 33% | 8% | 9% | - | - | - | - |
| 2.0 | 0.20 | 0 | 30% | - | - | 30% | - | - | - | 10% | 10% | 10% | - | 10% |