

Supporting Information

Firefly Luciferase Mutant with Enhanced Activity and Thermostability

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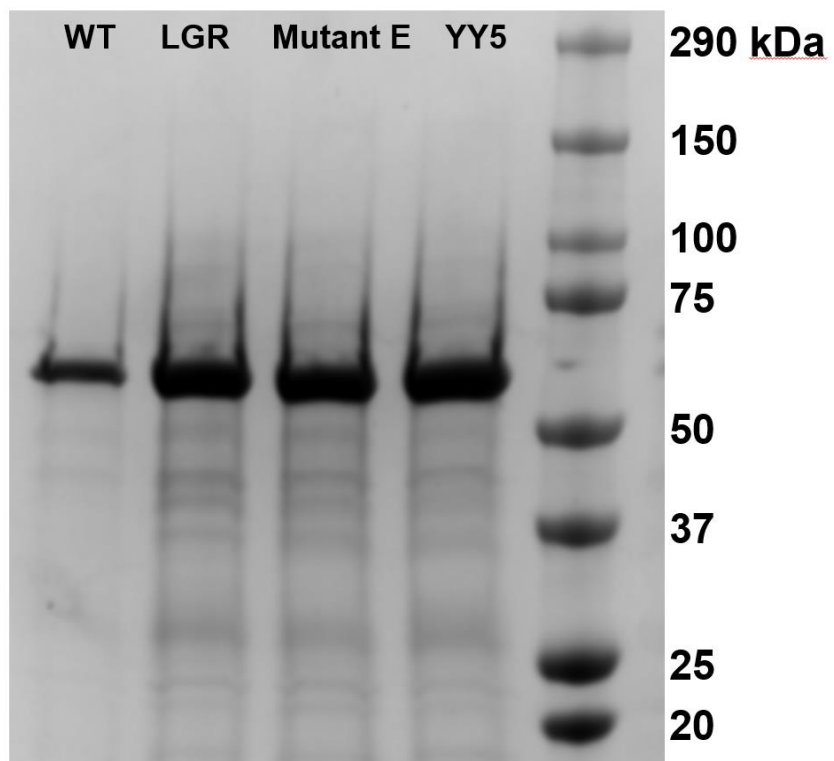


Figure S1. SDS-PAGE image of the purified luciferase variants. All purified protein samples were assessed for purity prior to dialysis. All four enzymes displayed the expected molecular weight of approximately 62 kDa.

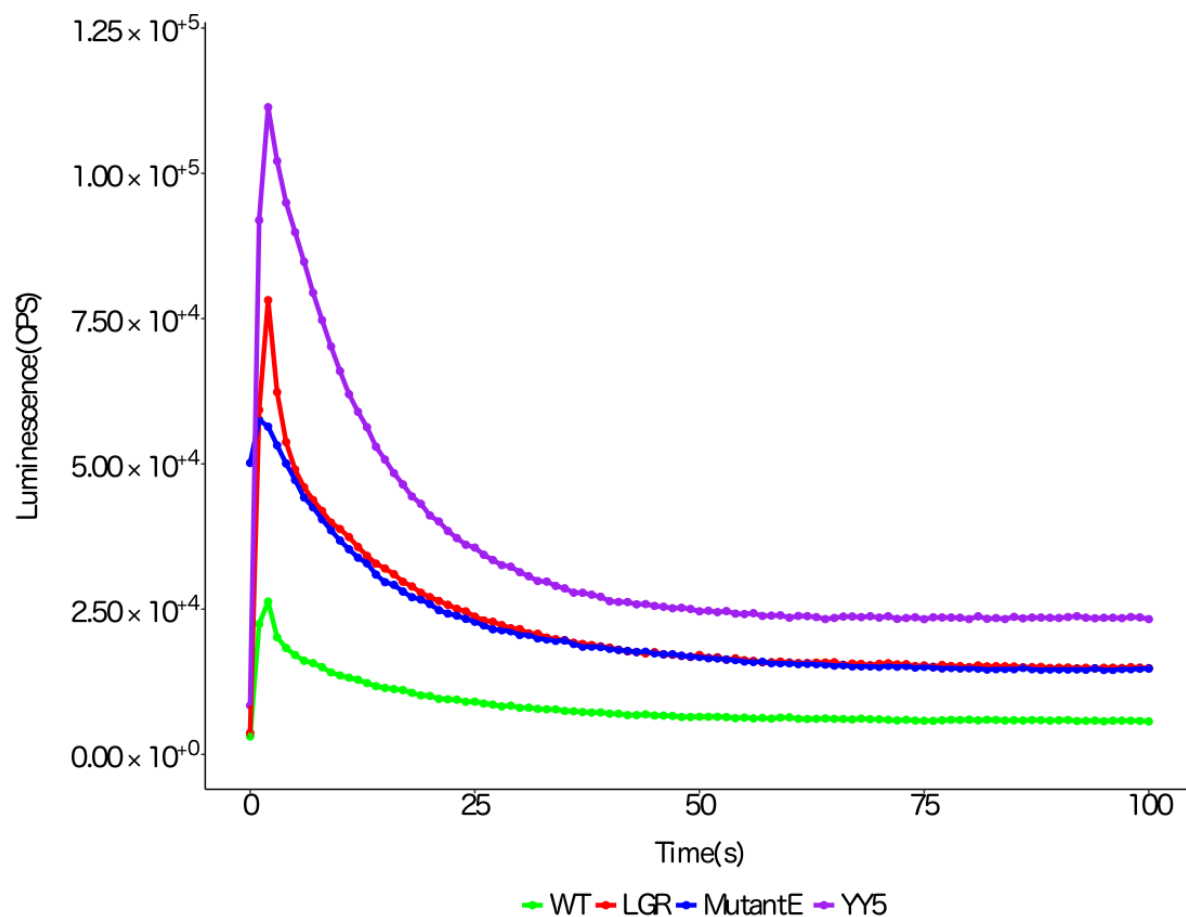


Figure S2. Luminescence time course of the luciferase variants. Each reaction contained 50 μ M D-Luciferin, 2 mM ATP, and 1.6 μ g/mL enzyme. The initial burst (flash) was observed within the first 5 s of the reaction followed by the steady glow phase. The measurements were recorded using Fluorolog-3 spectrofluorometer (HORIBA).

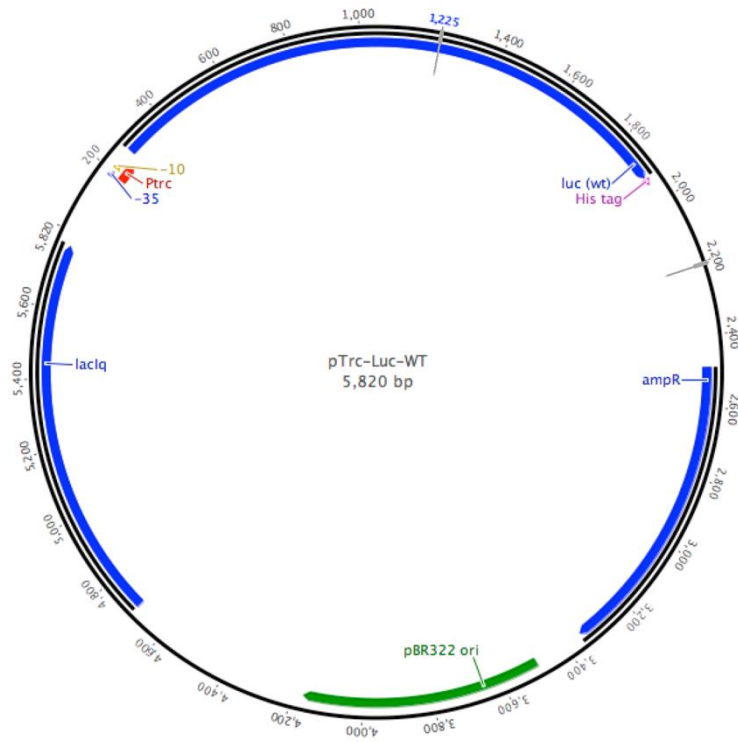


Figure S3. Map of the luciferase expression plasmids. Ptrc: trc promoter, luc (wt): luciferase coding gene, His tag: 6x histidine tag, ampR: ampicillin resistance marker (β -lactamase), pBR322 ori: origin of replication, lacIq: Lac repressor.

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CTGTTGACAA TTAATCATCC GGCTCGTATA ATGTGTGGAA TTGTGAGCGG ATAACAATTG 60
AATTAAGCTT TCAGGAGGTA TGAGATGGAA GACGCCAAAA ACATAAAGAA AGGCCCGGCG 120
CCATTCTATC CTCTAGAGGA TGAACCGCT GGAGAGCAAC TGCATAAGGC TATGAAGAGA 180
TACGCCCTGG TTCCTGGAAC AATTGCTTTT ACAGATGCAC ATATCGAGGT GAACATCACG 240
TACGCGGAAT ACTTCGAAAT GTCCGTTTCG TTGGCAGAAG CTATGAAACG ATATGGGCTG 300
AATACAAATC ACAGAATCGT CGTATGCAGT GAAAACCTCT TCAATTCTT TATGCCGGTG 360
TTGGGCGCGT TATTTATCGG AGTTGCAGTT GCGCCCGCGA ACGACATTTA TAATGAACGT 420
GAATTGCTCA ACAGTATGAA CATTTCGCAG CCTACCGTAG TGTTTGTTC CAAAAAGGGG 480
TTGCAAAAAA TTTTGAACGT GCAAAAAAAA TTACCAATAA TCCAGAAAAT TATTATCATG 540
GATTCTAAAA CGGATTACCA GGGATTTTCAG TCGATGTACA CGTTCGTCAC ATCTCATCTA 600
CCTCCCGGTT TTAATGAATA CGATTTTGTG CCAGATCCCT TTGATCGTGA CAAAACAATT 660
GCACTGATAA TGAATTCCTC TGGATCTACT GGGTTACCTA AGGGTGTGGC CCTTCCGCAT 720
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CTCGGATATT TGATATGTGG ATTTTCGAGTC GTCTTAATGT ATAGATTTGA AGAAGAGCTG 900
TTTTTACGAT CCCTTCAGGA TTACAAAATT CAAAGTGCCT TGCTAGTACC AACCCATTTT 960
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CCCGACGATG ACGCCGGTGA ACTTCCCGCC GCCGTTGTTG TTTTGGAGCA CGGAAAGACG 1560
ATGACGGAAA AAGAGATCGT GGATTACGTC GCCAGTCAAG TAACAACCGC GAAAAAGTTG 1620
CGCGGAGGAG TTGTGTTTGT GGACGAAGTA CCGAAAGGTC TTACCGGAAA ACTCGACGCA 1680
AGAAAAATCA GAGAATCCT CATAAAGGCC AAGAAGGGCG GAAAGTCAA ATTGCTCGAG 1740
CATCATCATC ATCATCATTG AGTTTAAACG GTCTCCAGCT TGGCTGTTTT GCGGGATGAG 1800
AGAAGATTTT CAGCCTGATA CAGATTAAAT CAGAACGCAG AAGCGGTCTG ATAAAACAGA 1860
ATTTGCCTGG CGGCAGTAGC GCGGTGGTCC CACCTGACCC CATGCCGAAC TCAGAAGTGA 1920
AACGCCGTAG CGCCGATGGT AGTGTGGGGT CTCCCCATGC GAGAGTAGGG AACTGCCAGG 1980
CATCAAATAA AACGAAAGGC TCAGTCGAAA GACTGGGCCT TTCGTTTTAT CTGTTGTTTG 2040

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Figure S4. DNA sequence of the luciferase expression cassette. Red: trc promoter, blue: luciferase (WT) coding sequence, pink: 6x histidine tag, green: transcription terminator. The locations of the eight point mutations are highlighted. The codon changes made for the mutations are as follows: T214A (ACT>GCC), A215L (GCC>CTG), I232A (ATT>GCC), F295L(TTC>CTG), E345K(GAG>AAA), I423L (ATA>CTG), D436G (GAC>GGC), L530R (CTC>CGC).

WT :

MEDAKNIKKGPAPFYPLEDGTAGEQLHKAMKRYALVPGTIAFTDAHIEVNIITYAEYFEMSVRLAEAMKRYGLNTNHR
IVVCSENSLQFFMPVLGALFIGVAVAPANDIYNERELLNSMNISQPTVVVFSKKGGLQKILNVQKKLP I IQK I IIMDS
KTDYQGFQSMYTFVTSHLPPGFNEYDFVPESFDRDKTIALIMNSSGSTGLPKGVALPHR**T**ACVRFSHARDPIFGNQI
IPDTAILSVVPFHHGFGMFTTLGYLICGFRVVLMYRFEEELFLRSLQDYKIQSALLVPTLFS**F**AKSTLIDKYDLSN
LHEIASGGAPLSKEVGEAVAKRFHLPGIRQGYGLTETTSAILITP**E**GDDKPGAVGKVVPF**F**EAKVVDLDTGKTLGVN
QRGELCVRGPMIMSGYVNNPEATNALIDKDGWLHSGD**I**AYWDEDEHFFIV**D**RLKSLIKYKGYQVAPAELESILLQHP
NIFDAGVAGLPDDDAGELPAAVVVLEHGKTMTEKEIVDYVASQVTTAKKLRGGVVVDEVPKGLTGK**L**DARKIREIL
IKAKKGGKSKLHHHHHH

YY5 :

MEDAKNIKKGPAPFYPLEDGTAGEQLHKAMKRYALVPGTIAFTDAHIEVNIITYAEYFEMSVRLAEAMKRYGLNTNHR
IVVCSENSLQFFMPVLGALFIGVAVAPANDIYNERELLNSMNISQPTVVVFSKKGGLQKILNVQKKLP I IQK I IIMDS
KTDYQGFQSMYTFVTSHLPPGFNEYDFVPESFDRDKTIALIMNSSGSTGLPKGVALPHR**A**LCVRFSHARDPIFGNQI
APDTAILSVVPFHHGFGMFTTLGYLICGFRVVLMYRFEEELFLRSLQDYKIQSALLVPTLFS**L**AKSTLIDKYDLSN
LHEIASGGAPLSKEVGEAVAKRFHLPGIRQGYGLTETTSAILITP**K**GDDKPGAVGKVVPF**F**EAKVVDLDTGKTLGVN
QRGELCVRGPMIMSGYVNNPEATNALIDKDGWLHSGD**L**AYWDEDEHFFIV**G**RLKSLIKYKGYQVAPAELESILLQHP
NIFDAGVAGLPDDDAGELPAAVVVLEHGKTMTEKEIVDYVASQVTTAKKLRGGVVVDEVPKGLTGK**R**DARKIREIL
IKAKKGGKSKLHHHHHH

Figure S5. Amino acid sequences of the WT luciferase and the YY5 mutant. The highlighted residues indicate the mutated amino acids. Yellow residues indicate mutations derived from Mutant E and green residues were derived from the LGR mutant.