

Supplementary materials

A Luciferase Mutant with Improved Brightness and Stability for Whole-Cell Bioluminescent Biosensors and *In Vitro* Bio-sensing

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1                               82
PpyLuc MEDAKNIKKGPAPFYPLEDGTAGEQLHKAMKRYALVPGTIAFTDAHIEVNITYAEYFEMSVRLAEAMKRYGLNTNHRIVVCS
BgLuc  MEDAKNIKKGPAPRYPLEDGTAGEQLHKAMKRYALVPGTIAFTDAHIEVDITYAEYFEMSVRLAEAMKRYGLNTNHRIVVCS
.....

83                               164
PpyLuc ENSLQFFMPVLGALFIGVAVAPANDIYNERELLNSMNI SQPTVVVFSKKG LQKILNVQKLP I IQK I IIMDSKTDYQGFQSM
BgLuc  ENSLQFFMPVLGALFIGVAVAPANDIYNERELLNSMNI SQPTVVVFSKKG LQKILNVQKLP I IQK I IIMDSKTDYQGFQSM
.....

165                              246
PpyLuc YTFVTSHLPPGFNEYDFVPESFDRDKTIALIMNSSGSTGLPKGVALPHRTACVRFSHARDPIFGNQIIPDTAILSVVPFHHG
BgLuc  YTFVTSHLPPGFNEYDFKPEFSDRDKTIALIMNSSGSTGLPKGVALPHRALCVRFSHARDPIFGNQIKPDTAILSVVPFHHA
.....

247                              328
PpyLuc FGMFTTLGYLICGFRVVLMYRFEEELFLRSLQDYKIQSALLVPTLFSFFAKSTLIDKYDLSNLHEIASGGAPLSKEVGEAVA
BgLuc  FGMSTTLGYLICGFRVVLMYRFEEELFLRSLQDYKIQSALLVPTLFSFIAKSTLIDKYDLSNLHEIASGGAPLSKEVGEAVA
.....

329                              410
PpyLuc KRFHLPGIRQGYGLTETTSAILITPEGDDKPGAVGKVVPFPEAKVVDLDTGKTLGVNQRGELCVRGPMIMSGYVNNPEATNA
BgLuc  KRFHLPGIRQGYGLTETTSAILITPKGDDKPGAVGKVVPFPEAKVVDLDTGKTLGVNQRGELCVRGPMIMSGYVNNPEATNA
.....

411                              492
PpyLuc LIDKDGWLHSGDIAYWDEDEHFFIVDR LKSLIKYKGYQVAPAELESILLQHPNIFDAGVAGLPDDDAGELPAAVVVLEHGKT
BgLuc  LIDKDGWLHSGDIAYWDEDEHFFIVDR LKSLIKYKGYQVAPAELESILLQHPNIRDAGVAGLPDDDAGELPAAVVVLEHGKT
.....

493                              550
PpyLuc MTEKEIVDYVASQVTTAKKLRGGVVFVDEVPKGLTGKLDARKIREILI KAKKGGKSKL
BgLuc  MTEKEIVDYVASQVTTAKKLRGGVVFVDEVPKGLTGKLDARKIREILI KAKKGGKI--
.....

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Figure S1. Amino acid alignment of the BgLuc and PpyLuc luciferases. The red highlighted residues indicate the mutated amino acids.

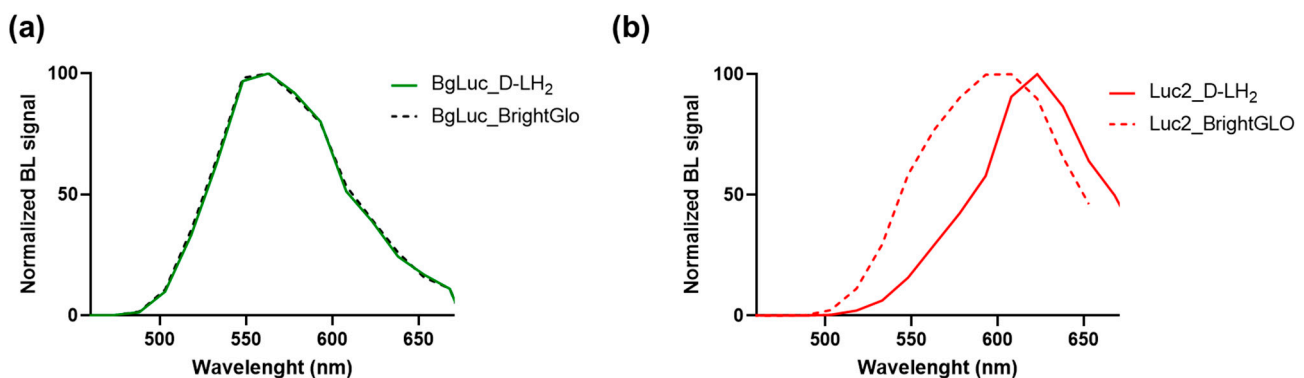
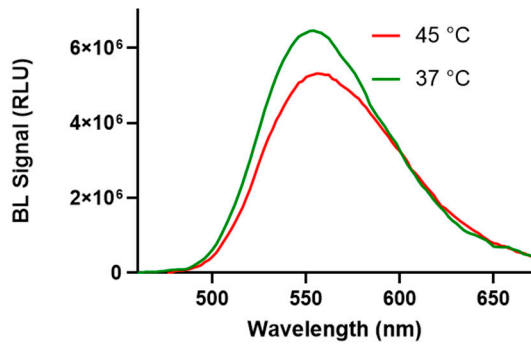


Figure S2: Emission spectra of (a) BgLuc and (b) Luc2 luciferase in HEK293T cells obtained with D-LH₂ substrate in citrate buffer (1.0 mM, pH 5.0) (solid line) and BrightGlo substrate (dotted line).

(a)



(b)

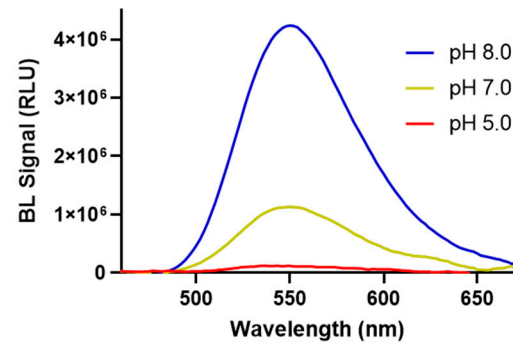


Figure S3: BgLuc mutant emission spectra obtained (a) at different temperatures and (b) at different pH with the commercial BrightGlo substrate.