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## Determination of BRET-pair fluorescent proteins for the luciferase from bioluminescent fungus *Neonothopanus nambi*

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The fungus *Neonothopanus nambi* is the first eukaryotic bioluminescent organism with fully discovered enzymes, involved in luciferin biosynthesis. Also the luciferase and structure of the fungal luciferin molecule are both identified as well. Bioluminescent genetically encodable sensors that rely on BRET, bioluminescence resonance energy transfer, are widely used in biomedical research and are typically composed of luciferase and a fluorescent protein that serves as a light acceptor. The efficiency of BRET and the dynamic range of a sensor are largely determined by the spectral match between luciferase and a fluorescent protein, as well as by the structure and the length of amino acid linker that separates them. Water solubility, small size and membrane permeability of the luciferin (e.g., fungal luciferin) can also broaden the range of applications of a particular BRET system. In this study, we explored the potential of the recently discovered *Neonothopanus nambi* luciferase (nnLuz) for BRET-based application by analyzing the efficiency of energy transfer to several red fluorescent proteins. We assembled 16 constructs containing various combinations of nnLuz, acceptor proteins TagRFP, tdTomato or mRuby2, and 8 different linkers and analyzed light emission by spectrally resolved imaging of transiently transfected HEK293T cells. This allowed us to determine promising acceptors and linkers for further construction of BRET-sensors based on fungal luciferase. The study was funded by the Ministry of Science and Higher Education of the Russian Federation, project identifier RFMEFI61317X0062.

### Recent Publications

1. Schultz DT, Kotlobay AA, Ziganshin R, Bannikov A, Markina NM, Chepurnyh TV, Shakhova ES, Palkina K, Haddock SHD, Yampolsky IV, Oba Y (2018). Corrigendum to "Luciferase of the Japanese syllid polychaete *Odontosyllis undecimdongata*" [Biochem. Biophys. Res. Commun. 2018 Jul 20; 502(3):318–323]. Biochem Biophys Res Commun 503 (2), 1179
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