Luciferase in der Neurowissenschaft

Description

Trimmer, C., Snyder, L. L., & Mainland, J. D.. (2014). High-throughput analysis of mammalian olfactory receptors: Measurement of receptor activation via luciferase activity. Journal of Visualized Experiments

Plain numerical DOI: 10.3791/51640
DOI URL
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“Odorants create unique and overlapping patterns of olfactory receptor activation, allowing a family of approximately 1, 000 murine and 400 human receptors to recognize thousands of odorants. odorant ligands have been published for fewer than 6% of human receptors1-11. this lack of data is due in part to difficulties functionally expressing these receptors in heterologous systems. here, we describe a method for expressing the majority of the olfactory receptor family in hana3a cells, followed by high-throughput assessment of olfactory receptor activation using a luciferase reporter assay. this assay can be used to (1) screen panels of odorants against panels of olfactory receptors; (2) confirm odorant/receptor interaction via dose response curves; and (3) compare receptor activation levels among receptor variants. in our sample data, 328 olfactory receptors were screened against 26 odorants. odorant/receptor pairs with varying response scores were selected and tested in dose response. these data indicate that a screen is an effective method to enrich for odorant/receptor pairs that will pass a dose response experiment, i.e. receptors that have a bona fide response to an odorant. therefore, this high-throughput luciferase assay is an effective method to characterize olfactory receptors-an essential step toward a model of odor coding in the mammalian olfactory system.”
Crespo, E. L., Bjorefeldt, A., Prakash, M., & Hochgeschwender, U.. (2021). Bioluminescent Optogenetics 2.0: Harnessing Bioluminescence to Activate Photosensory Proteins *In Vitro* and *In Vivo*. Journal of Visualized Experiments

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“Bioluminescence – light emitted by a luciferase enzyme oxidizing a small molecule substrate, a luciferin – has been used in vitro and in vivo to activate light-gated ion channels and pumps in neurons. while this bioluminescent optogenetics (bl-og) approach confers a chemogenetic component to optogenetic tools, it is not limited to use in neuroscience. rather, bioluminescence can be harnessed to activate any photosensory protein, thus enabling the manipulation of a multitude of light-mediated functions in cells. a variety of luciferase-luciferin pairs can be matched with photosensory proteins requiring different wavelengths of light and light intensities. depending on the specific application,

efficient light delivery can be achieved by using luciferase-photoreceptor fusion proteins or by simple co-transfection. photosensory proteins based on light-dependent dimerization or conformational changes can be driven by bioluminescence to effect cellular processes from protein localization, regulation of intracellular signaling pathways to transcription. the protocol below details the experimental execution of bioluminescence activation in cells and organisms and describes the results using bioluminescence-driven recombinases and transcription factors. the protocol provides investigators with the basic procedures for carrying out bioluminescent optogenetics in vitro and in vivo. the described approaches can be further extended and individualized to a multitude of different experimental paradigms."
Srinivasan, P., Griffin, N. M., Joshi, P., Thakur, D., Nguyen-Le, A., McCotter, S., … Theogarajan, L.. (2019). An Autonomous Molecular Bioluminescent Reporter (AMBER) for voltage imaging in freely moving animals. BioRxiv

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"Genetically encoded reporters have greatly increased our understanding of biology, especially in neuroscience. while fluorescent reporters have been widely used, light delivery and phototoxicity have hindered their utility. bioluminescence overcomes some of these challenges but requires the addition of an exogenous luciferin limiting its use. using a modular approach we have engineered autonomous molecular bioluminescent reporter (amber), an indicator of membrane potential. unlike other luciferase-luciferin bioluminescent systems amber encodes the genes to express both the luciferase and luciferin. amber is a voltage-gated luciferase coupling the functionalities of the ciona voltage sensing domain (vsd) and bacterial luciferase, luxab. when amber is co-expressed with the luciferin producing genes it reversibly switches the bioluminescent intensity as a function of membrane potential. utilizing both biophysical and biochemical methods we show that unlike other voltage indicators amber modulates its enzymatic activity as a function of the membrane potential. amber shows a several fold increase in the luminescent (?l/l) output upon switching from off to on state when the cell is depolarized. in vivo expression of amber in c. elegans allowed detecting pharyngeal pumping action and mechanosensory neural activity from multiple worms simultaneously. since we are able to report neural activity of multiple animals at the same time, we believe amber can be used in social behavior assays to elucidate the role of membrane potential underlying the behaviors."
Carullo, N. V. N., Hinds, J. E., Revanna, J. S., Tuscher, J. J., Bauman, A. J., & Day, J. J.. (2021). A cre-dependent crispr/dcas9 system for gene expression regulation in neurons. ENeuro

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"Site-specific genetic and epigenetic targeting of distinct cell populations is a central goal in molecular neuroscience and is crucial to understand the gene regulatory mechanisms that underlie complex phenotypes and behaviors. while recent technological advances have enabled unprecedented control

over gene expression, many of these approaches are focused on selected model organisms and/or require labor-intensive customization for different applications. the simplicity and modularity of clustered regularly interspaced short palindromic repeats (crispr)-based systems have transformed genome editing and expanded the gene regulatory toolbox. however, there are few available tools for cell-selective crispr regulation in neurons. we designed, validated, and optimized crispr activation (crispra) and crispr interference (crispri) systems for cre recombinase-dependent gene regulation. unexpectedly, crispra systems based on a traditional double-floxed inverted open reading frame (dio) strategy exhibited leaky target gene induction even without cre. therefore, we developed an intron-containing cre-dependent crispra system (svi-dio-dcas9-vpr) that alleviated leaky gene induction and outperformed the traditional dio system at endogenous genes in hek293t cells and rat primary neuron cultures. using gene-specific crispr sgrnas, we demonstrate that svi-dio-dcas9-vpr can activate numerous rat or human genes (grm2, tent5b, fos, sstr2, andgadd45b) in a cre-specific manner. to illustrate the versatility of this tool, we created a parallel crispri construct that successfully inhibited expression from a luciferase reporter in hek293t cells only in the presence of cre. these results provide a robust framework for cre-dependent crispr-dcas9 approaches across different model systems, and enable cell-specific targeting when combined with common cre driver lines or cre delivery via viral vectors.”