Expanding the Optogenetic Toolkit through the Development of Light-inducible Phenotype Switches for Multiplexed Control of Gene Expression

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Site-specific recombinases are powerful genetic engineering tools that can recognize short, specific nucleic acid sequences and can excise, invert, or insert other DNA sequences. Engineering these recombinases with light-inducible domains will confer better spatiotemporal control of gene expression than previous chemical-inducible systems. Utilizing these concepts, we designed photoactivatable genetic circuits that activate an apoptotic gene, HBax, upon blue (450nm) or red (660nm) light illumination. We molecularly cloned 20 plasmid constructs (16 split recombinases and 4 HBax-containing targets). Natively, these engineered light-inducible recombinases are split in half: the N-terminus half is attached to one domain of the light-inducible system and the C-terminus half is attached to the other half of the light-inducible system. In the presence of light, the two halves recombine, reconstituting the catalytic abilities of the recombinase that allow it to recognize and activate the expression of HBax. We transfected this system into plates of human embryonic kidney cells (HEK293), illuminated these plates with micro-Arduino-controlled LED lights, and measured the amount of infrared fluorescent protein, a marker of cell survivability, using flow cytometry. We then analyzed this flow cytometry data using FlowJo and Excel. We observed that some genetic circuits reduced cell survivability by over 50%. Future work can improve these systems by reducing the basal activity of split recombinases, optimizing temperature pulsing for the blue-light system, and increasing the applicability and translational impact of this system by testing in other mammalian cell lines, potentially in conjunction with immunotherapy technologies, like CAR-T.