Basic and Applied Aspects for Color Tuning of Bioluminescence Systems

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Bioluminescence in beetles is characterized by a wide range of colors: green-yellow in fireflies green-orange in click beetles, and green-red in railroad worms. This variety of bioluminescence colors was attributed to the luciferase structures, since luciferin is the same in all forms. On the other hand, reporter assay systems using luciferases have been widely used to study promoter activity, interactions of promoter and transcription factors, signal transduction, other cell activities, and for drug screening, because this system is highly sensitive and strictly quantitative. Recently, we have developed a novel reporter assay system, a tricolor reporter in vitro assay system, in which three gene expressions can be monitored simultaneously by splitting the emissions from areen-, orange-, and red-emitting luciferases (green, orange, and red luciferases) with an optical filter. We have successfully measured simultaneously the activities of the green, orange, and red luciferases in a mixture by splitting their emissions with optical filters. Then, this system allows us to simply and rapidly monitors simultaneously three gene expressions (two are test reporters and one is an internal control) by using a single luminescent substrate in one tube. Here we present a basic aspect for color tuning in beetle bioluminescence and applied aspects of beetle luciferase based on the color differences.