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Comparison Of The Human Optimized Bacterial Luciferase Gene Cassette With Firefly Luciferase And Green Fluorescent Protein And Development Into A Real-Time Biosentinel For Toxic Chemicals

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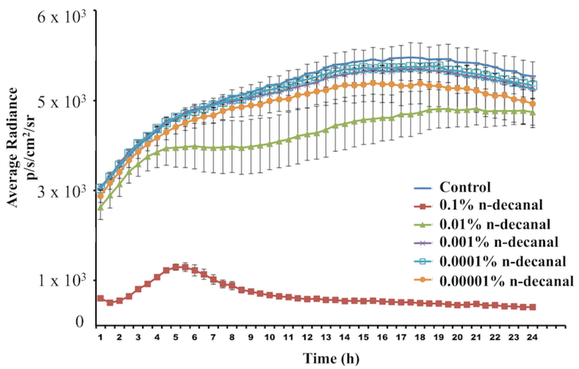
Abstract

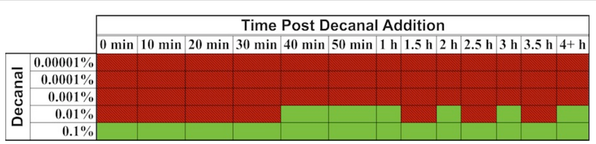
Our group has previously demonstrated the ability to elicit autonomous bioluminescent production from the bacterial luciferase gene cassette (*lux*) from within the mammalian cellular environment. With an emission spectra that is virtually background-free and the ability to generate light autonomously, the sensitivity and real-time imaging capacity of bacterial luciferase far surpasses that of green fluorescent protein (GFP) and firefly luciferase (Luc).

To provide a frame of reference for use of this new technology, we have performed side-by-side comparisons of the *lux*, Luc, and GFP reporter systems under both culture and small animal imaging conditions. A mammalian cell line was transfected with either the human optimized (ho) *lux* genes, ho*luc* gene, or ho*gfp* gene. These cell lines were then interrogated either in culture or following injection into a nude mouse model. Comparisons of the resulting signal strengths and dynamics were compared to evaluate their ability to function as reporters under these conditions. To validate the utility of the *lux* system as an autonomous reporter, it was challenged with the known toxicant n-decanal and evaluated for its ability to report on bioavailability of the compound in real time.

At equivalent cellular population sizes the *lux* system was shown to be more sensitive than GFP (a minimum of 1.5×10^4 *lux* transfected cells visible compared to 5.0×10^5 GFP transfected cells visible) due to the reduction of detectable background signal during bioluminescent imaging. Larger cell populations are required to detect *lux* transfected cells than are required for the detection of Luc in mouse models, however, this can be overcome at population sizes above 2.5×10^4 by increasing integration time to 1 min. Additionally, the *lux* system retains the benefit of not requiring exogenous substrate input, allowing for repeated imaging and consistent correlation of the luminescent signal with cell population size. It is further demonstrated that the *lux* system is capable of detecting cytotoxicity quickly and in the µM concentration range for the known toxicant n-decanal.

Real-Time Cytotoxicity Monitoring Upon Aldehyde Challenge





Constitutively autobioluminescent cells were exposed to increasing concentrations of the cytotoxic aldehyde n-decanal and changes in bioluminescent production were monitored over time. At levels of 0.01% and above cells showed decreased bioluminescent output (green boxes), while lower treatment levels did not significantly ($p = 0.05$) differ from untreated control cells (red boxes).

Acknowledgments

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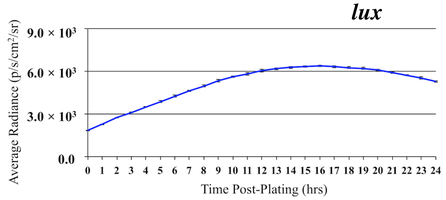
Detection in Culture

lux

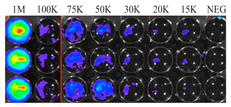


- Light is produced continuously without substrate addition
- Very low variability in luminescent output
- Detection possible down to population sizes of 15,000 cells/well
- Integration times of up to 10 min can be required for pseudocolor detection of low population sizes

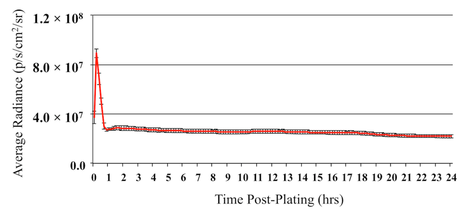
Average Radiance
6.4 (±0.1) × 10³ p/s/cm²/sr

Peak Radiance
16 h

Average Error
58 (±4) p/s/cm²/sr



Luc

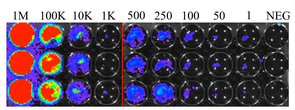


- High rate of luminescent flux
- Light signal dependent on available concentration of substrate
- Detection possible below 100 cells/well
- High photon counts can saturate detection equipment
- Relatively high variability in luminescent output
- High revolving cost of use

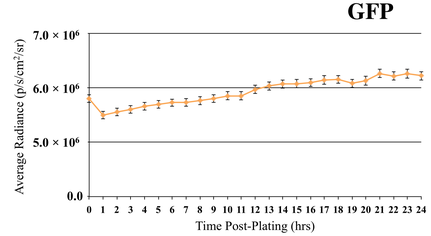
Average Radiance
8.8 (±0.4) × 10⁷ p/s/cm²/sr

Peak Radiance
6 min

Average Error
2.9 (±0.6) × 10⁶ p/s/cm²/sr



GFP

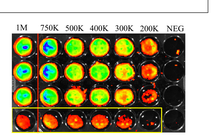


- High background due to autofluorescence
- Significant detection not possible below 500,000 cells/well
- Moderate variability of fluorescent signal production
- Large number of wavelength variants available to tailor expression to experimental design

Average Radiance
6.6 (±0.1) × 10⁶ p/s/cm²/sr

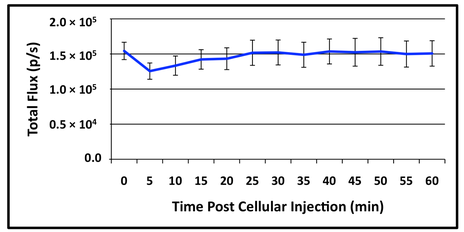
Peak Radiance
22 h

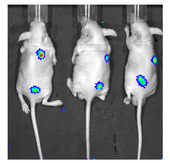
Average Error
7.3 (0.3) × 10⁴ p/s/cm²/sr

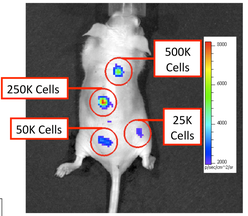


Bioluminescent Detection in Small Animal Models

lux



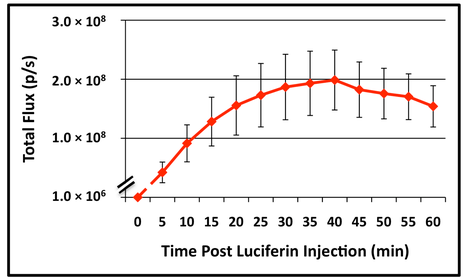


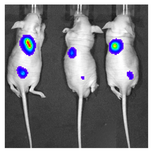


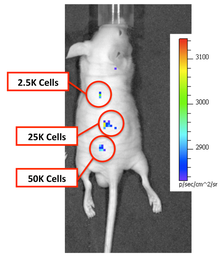
Average Radiance
1.5 (±0.2) ×10⁵ p/s
Average Error
1.6 (±0.3) × 10⁴ p/s
Integration Time
60 sec
Cells Injected
5 × 10⁶

- Detection down to 25,000 cells
- Requires longer integration time than Luc
- Detection is stable over relatively prolonged time periods
- Can be used in conjunction with other fluorescent and bioluminescent reporters

Luc



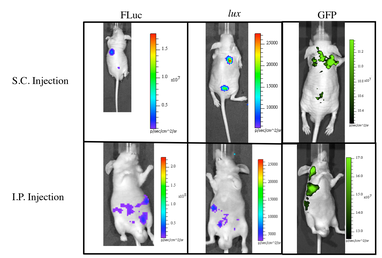




Average Radiance
2.0 (±0.2) × 10⁸ p/s
Average Error
4.0 (±0.5) × 10⁷ p/s
Integration Time
1 sec
Cells Injected
5 × 10⁵

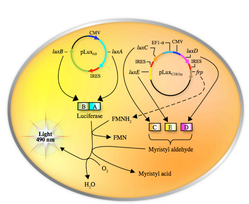
- 10-fold lower detection level than *lux*
- Bioluminescent signal is highly dependent on substrate availability
- Injection route can alter bioluminescent output kinetics

Side By Side Comparison

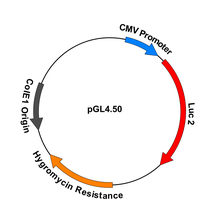


- Pseudocolor detection patterns are similar between the *lux* and Luc bioluminescent reporters
- Luc can be detected with lower integration times than *lux*
- GFP can not be significantly detected immediately following injection at population sizes below 1 x 10^7 cells
- *lux* detection requires only a single injection
- Luc is more easily detected than *lux* at increased tissue depths because of its higher flux
- All reporters can be used simultaneously to image multiple targets

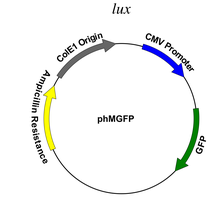
Differences in Genetic Expression



lux



Luc



GFP

- Luc and GFP systems require expression of only a single gene
- *lux* system is composed of 6 genes that work together to produce light from endogenously available components