Using Continuous Bioluminescent Production to Perform Real-Time Metabolic Activity/Cytotoxicity Screening

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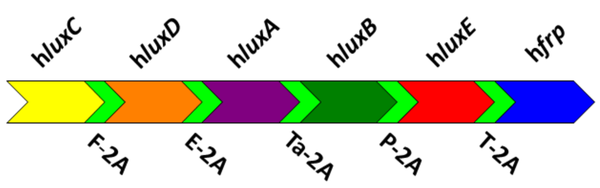
Introduction

- A continuously active synthetic luciferase that does not require luciferin treatment was evaluated for its ability to track real-time metabolic responses to therapeutic compound exposure
- The functionality of the synthetic luciferase was correlated with existing assays to validate its performance
- Autonomously bioluminescent cell models were exposed to compound either directly, or following biotransformation by an alternate cell type
- Metabolic kinetics were evaluated to determine the toxicity of various compounds and their associated metabolic breakdown products

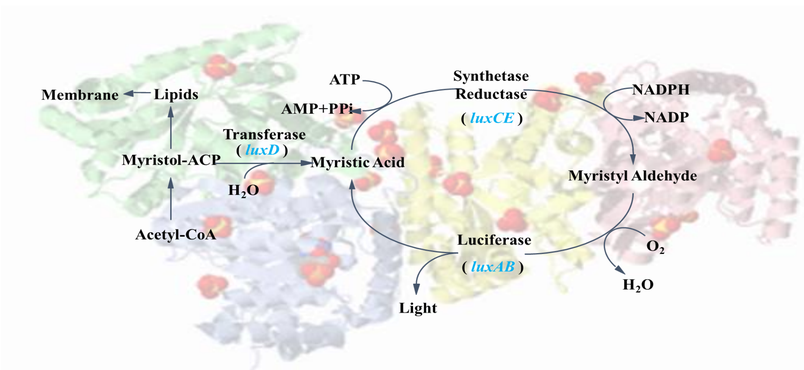
Basics of Autobioluminescence

Autonomous six gene (*luxCDABEfrp*) bioluminescent reporter system



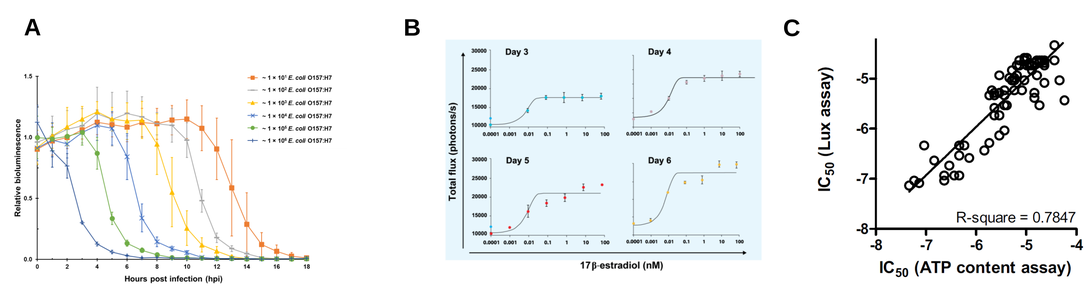
Eukaryotic expression optimized genetic cassette based on bacterial luciferase genes.

Custom promoters and expression options possible.



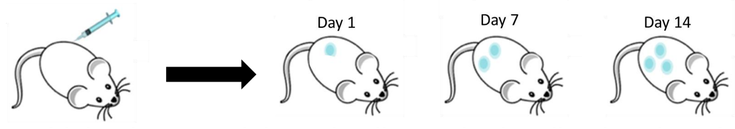
Gene cassette enables endogenous synthesis and regeneration of all necessary substrates to produce light. Eliminates the need for exogenous substrate addition. **No luciferin required!**

Reagent-Free Continuous Monitoring

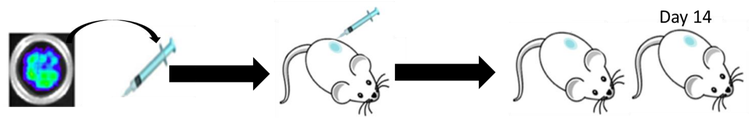


(A) Real-time tracking of the metabolic activity in autobioluminescent HEK293 cells during the course of infection with pathogenic *E. coli* O157:H7[2]. (B) Population growth of constitutively autobioluminescent T-47D breast cancer cells exposed to 17β-estradiol was non-invasively tracked by monitoring autobioluminescent dynamics. EC_{50} values were similar to other common destructive tests (10 pM)[3]. (C) Autobioluminescent HEK293 cells showed comparable IC_{50} predictive capability compared to ATP content assay when used to screen the Library of Pharmacologically Active Compounds (LOPAC)[4].

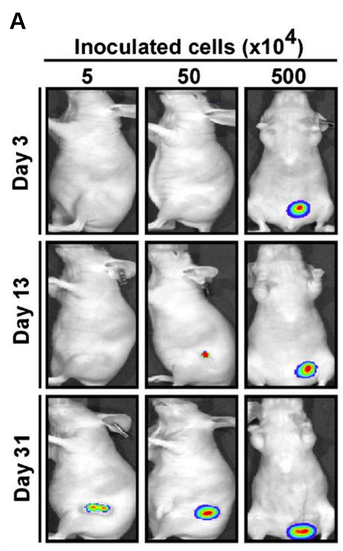
In Vivo Bioluminescent Imaging Without Repeated Needle Sticks

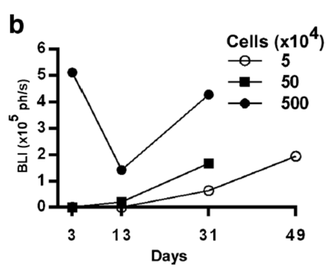


Continuously autobioluminescent injected cells can be localized and tracked across any relevant timescale.



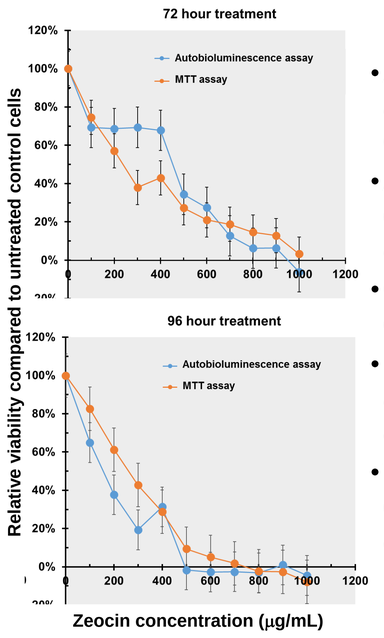
Cells can be seeded into a scaffold and implanted at specific locations to increase retention and monitor cellular health at ROI.





Autobioluminescent cassette used to track tumor formation *in vivo*. (A) Human J82 cells expressing H-Ras(V12) and the autobioluminescent gene cassette were injected subcutaneously at the indicated cell density and tracked over 31 days. (B) Quantification of BLI signal observed in A[1].

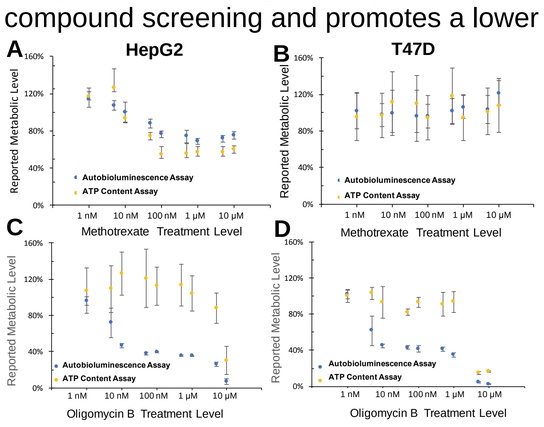
Strong Correlation With Existing Assay Formats



- Autobioluminescent HEK293 cells were treated with the antibiotic Zeocin and repeatedly evaluated for bioluminescent output intensity every 24 hours for 4 days
- Autobioluminescence was repeatedly measured without sample destruction or investigator interaction, demonstrating extreme amenability to workflow automation
- Concurrent MTT assays required individual sample sets for each time point and significant hands-on processing time
- Exposure to 1000 µg Zeocin/ml reduced signal output to 11.9% and 13.9% after 2 days of exposure in the autobioluminescence and MTT assays, respectively
- The minimum concentration of Zeocin to induce 100% cell death within 4 days was determined to be 500 µg/ml in both assays

Bio-transformed Compound Toxicology Assay

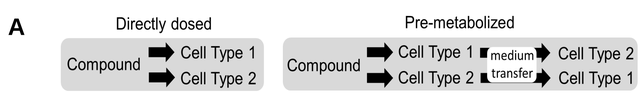
Autobioluminescent cellular models provide an improved screening method for simultaneously assessing metabolic and toxicological impacts of chemicals in both their pre- and post-biotransformed states. The ability of this assay to address both pre- and post-metabolism compound effects significantly alleviates a major hurdle endemic to existing tier 1 therapeutic compound screening and promotes a lower cost, higher throughput drug discovery process.



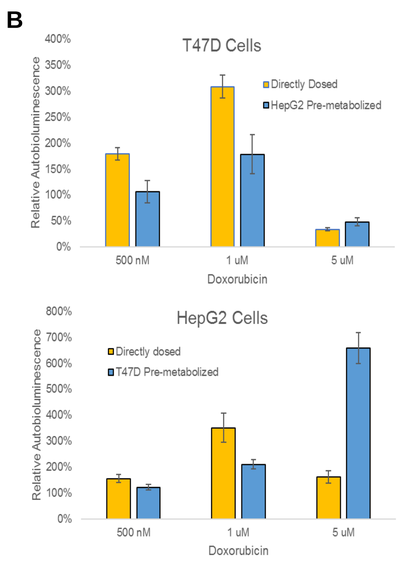
(A-B) Treatment with Methotrexate, which alters the ATP content levels of HepG2 cells, but not T47D cells, showed similar responses between the autobioluminescence assay and ATP content assay. (C-D) Regardless of the host cell type, the autobioluminescent phenotype was more sensitive to ATP depletion than was the CellTiter-Glo assay.

Single Assay Bio-transformed Compound Toxicology Screening

Drug metabolism can produce compounds with unique toxicities compared to the original. Here, we show how the autobioluminescent cassette, simultaneously expressed in paired cell lines, can be harnessed to recapitulate in vivo relationships and provide a fast, efficient toxicity screening platform to assess the effects of multi-organellular or multicellular drug breakdown toxicity.



(A) Direct and pre-metabolized assay format. Direct dose consists of adding compound directly to cell type 1 and measuring autobioluminescence at the desired interval(s). Pre-metabolized monitoring consists of incubating the compound first with one cell type and then transferring the medium to a different cell type. Requisite controls are not shown but should be included (i.e., media blank control, autologous media addition). (B) Autobioluminescent cassette expressing HepG2 and T47D cell lines were exposed directly to doxorubicin or doxorubicin metabolized by the paired cell line at the indicated concentration.



Conclusions

- Continuous luminescent tracking is a viable method for high-throughput metabolic activity/cytotoxicity screening
- Autobioluminescent output kinetics correlate well with the discrete imaging outputs of alternative monitoring systems
- Differential cellular backgrounds display significantly different metabolic responses to therapeutic compound treatment
- Real-time metabolic activity kinetics reveal important transitory response data that may be missed by intermittent time point studies

References

[1]John, B.A., et al., A Real-Time Non-invasive Auto-bioluminescent Urinary Bladder Cancer Xenograft Model. Mol Imaging Biol, 2017. 19(1): p. 10-14

[2]Xu, T., et al., Real-time toxicity and metabolic activity tracking of human cells exposed to Escherichia coli O157:H7 in a mixed consortia. Ecotoxicology, 2015. 24(10): p. 2133-40

[3]Xu, T., et al., Expression of a humanized viral 2A-mediated lux operon efficiently generates autonomous bioluminescence in human cells. PLoS One, 2014. 9(5): p. e96347

[4]Class, B., et al., High-throughput viability assay using an autonomously bioluminescent cell line with a bacterial Lux reporter. J Lab Autom, 2015. 20(2): p. 164-74

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