

Evaluating the Cytotoxic Effects of Cellulose Nanocrystals (CNCs) Using Autobioluminescent Yeast and Human Cells

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INTRODUCTION

Cellulose nanocrystals (CNCs) are widely used in different industries, including pharmaceutical and cosmetic production due to their adept physical and biological properties. Because CNCs are becoming a more prevalent material and have a high potential of being redistributed in the environment, it is important to understand their toxic potentials in biological systems, including organisms of various trophic levels. This study evaluated the cytotoxic effects of CNCs in the lower eukaryotic organism *Saccharomyces cerevisiae* and human embryonic kidney (HEK293) cells using autobioluminescent yeast and human cell reporters, respectively. The *S. cerevisiae* and HEK293 reporter cells were engineered to express a synthetic bacterial luciferase operon (*luxCDABEfrp*) that self-generate all the required substrates for bioluminescent production. As a result, these reporter cells allow for continuous monitoring of the same cell population throughout the period of toxicant exposure, providing a facile means for tracking the temporal dynamics of toxic effects on living cells.

THE BACTERIAL BIOLUMINESCENCE (*lux*) REPORTER SYSTEM

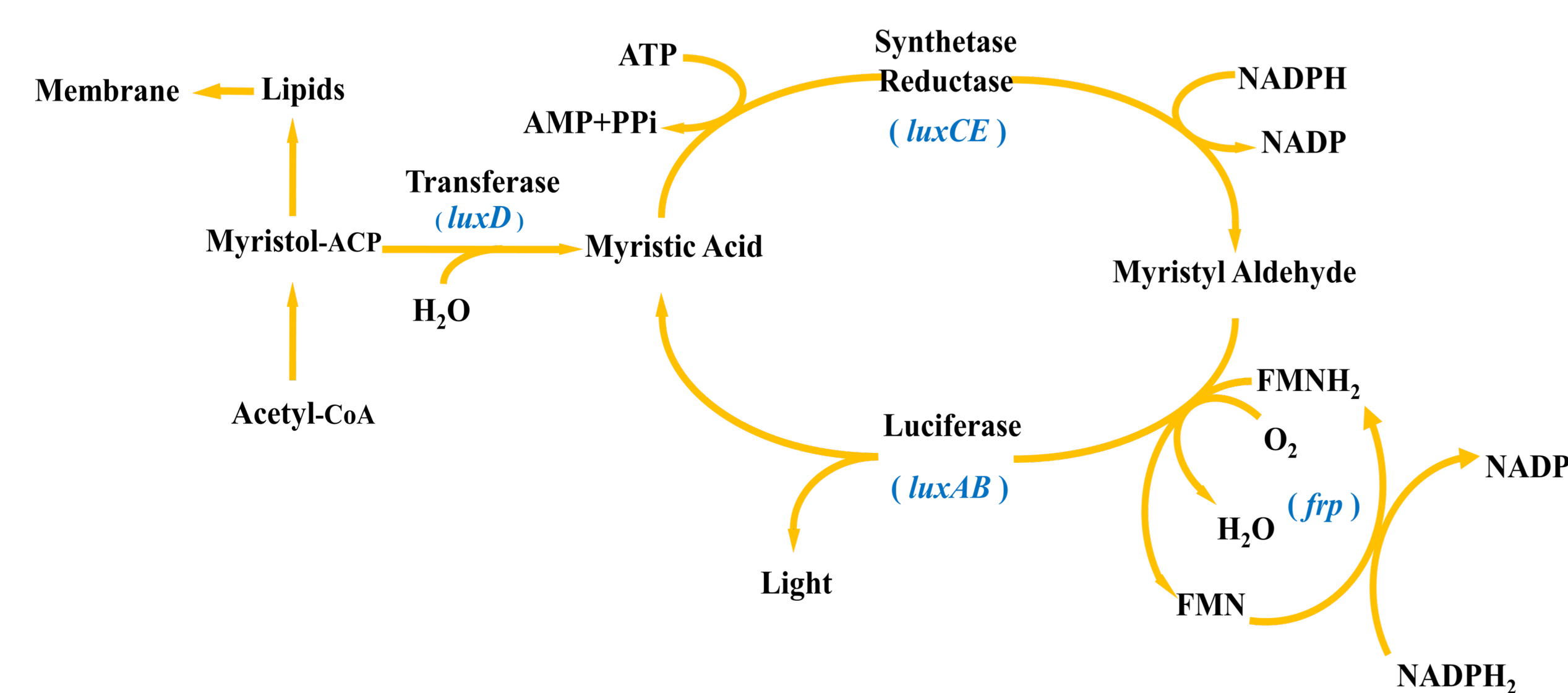


Figure 1. The bacterial bioluminescence (*lux*) reporter system consists of six genes to facilitate autonomous bioluminescence expression without the addition of an exogenous substrate. The LuxCDE and Frp proteins are responsible for generating and recycling the aldehyde and reduced flavin mononucleotide (FMN₂) substrate respectively, which are catalyzed by the LuxAB luciferase dimer to produce light in the presence of oxygen.

ACKNOWLEDGEMENTS

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BIOLUMINESCENT YEAST REPORTER (BLYR)/CNC TOXICITY ASSAY

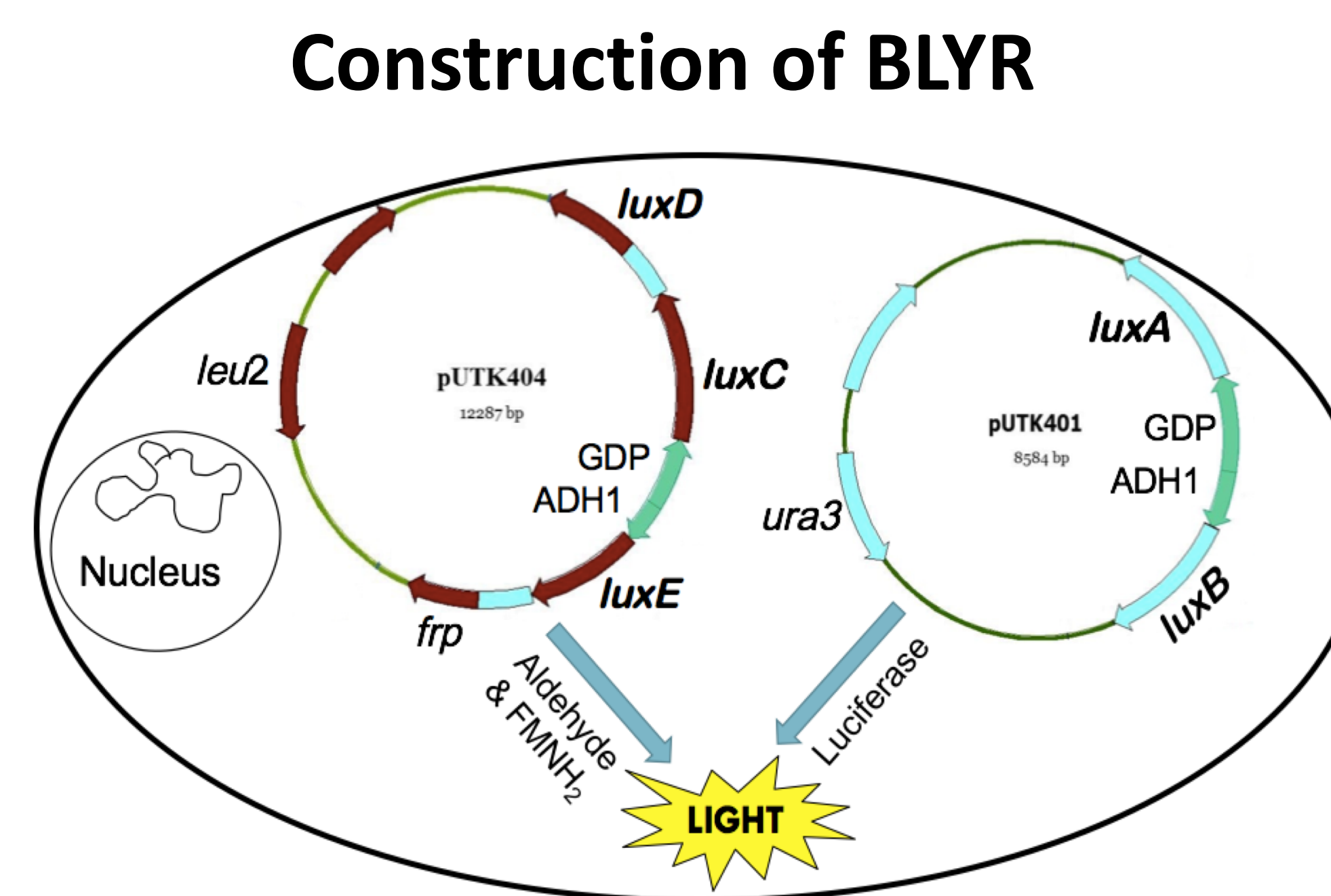


Figure 2. The bioluminescent yeast reporter (BLYR) is created by introducing two plasmids, pUTK404 and pUTK401, into a *S. cerevisiae* host strain. The pUTK401 plasmid contains the luciferase genes *luxA* and *luxB*, whereas the pUTK404 plasmid provides the *luxCDEfrp* genes of the *lux* pathway. Co-expression of the two reporter plasmids provide all necessary components to generate bioluminescence autonomously.

Bioluminescent Output of BLYR Over the Course of CNC Treatment

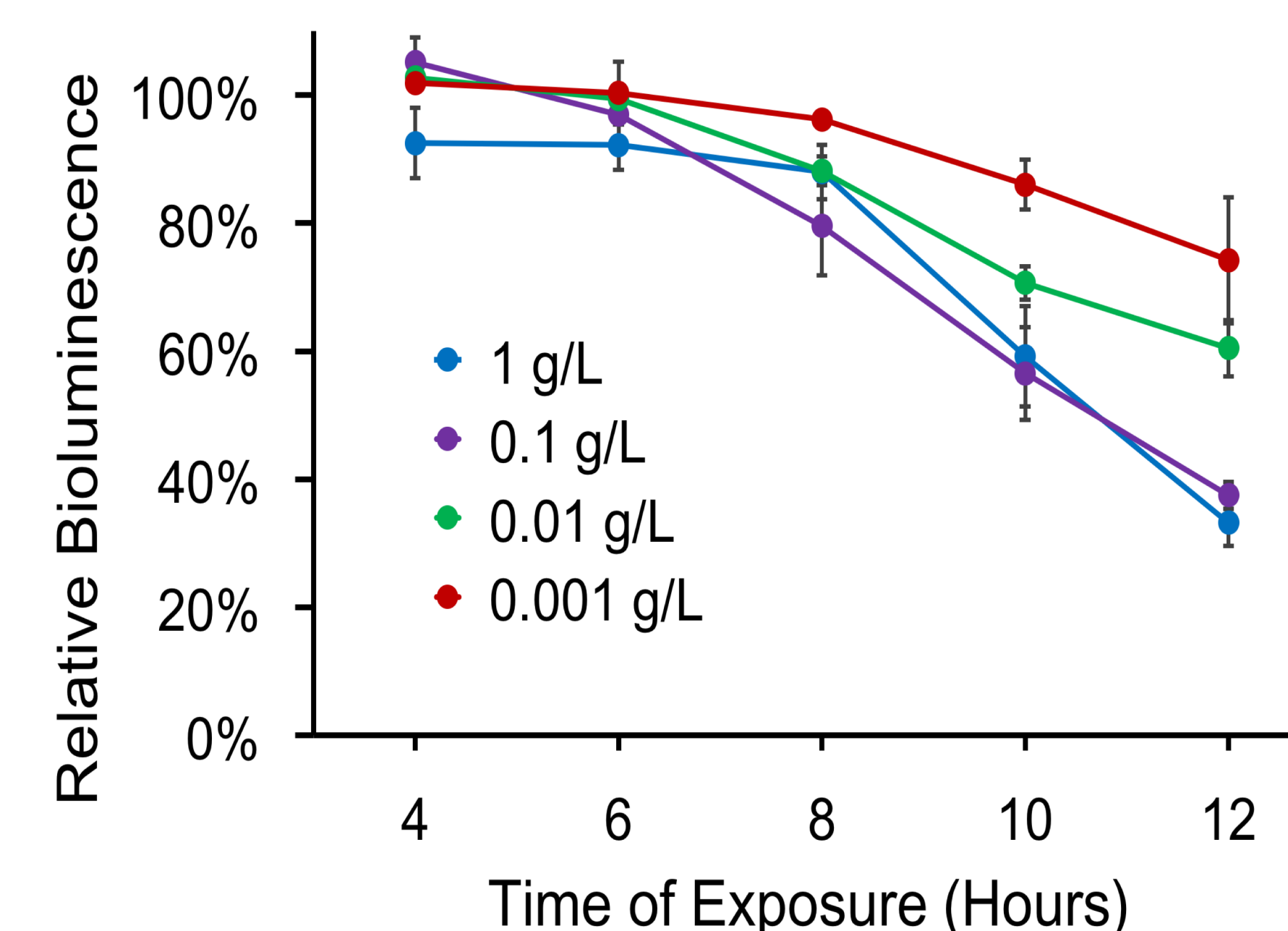


Figure 3. Exposure to CNCs at concentrations 0.001 g/L to 1 g/L induced time and dose-dependent responses. The bioluminescent output at 1 g/L CNC 12 hours post-exposure was decreased 70% compared to untreated control wells. During the same time point, the lowest concentration (0.001 g/L) decreased the bioluminescent output by 25%.

Sigmoidal Dose-Response

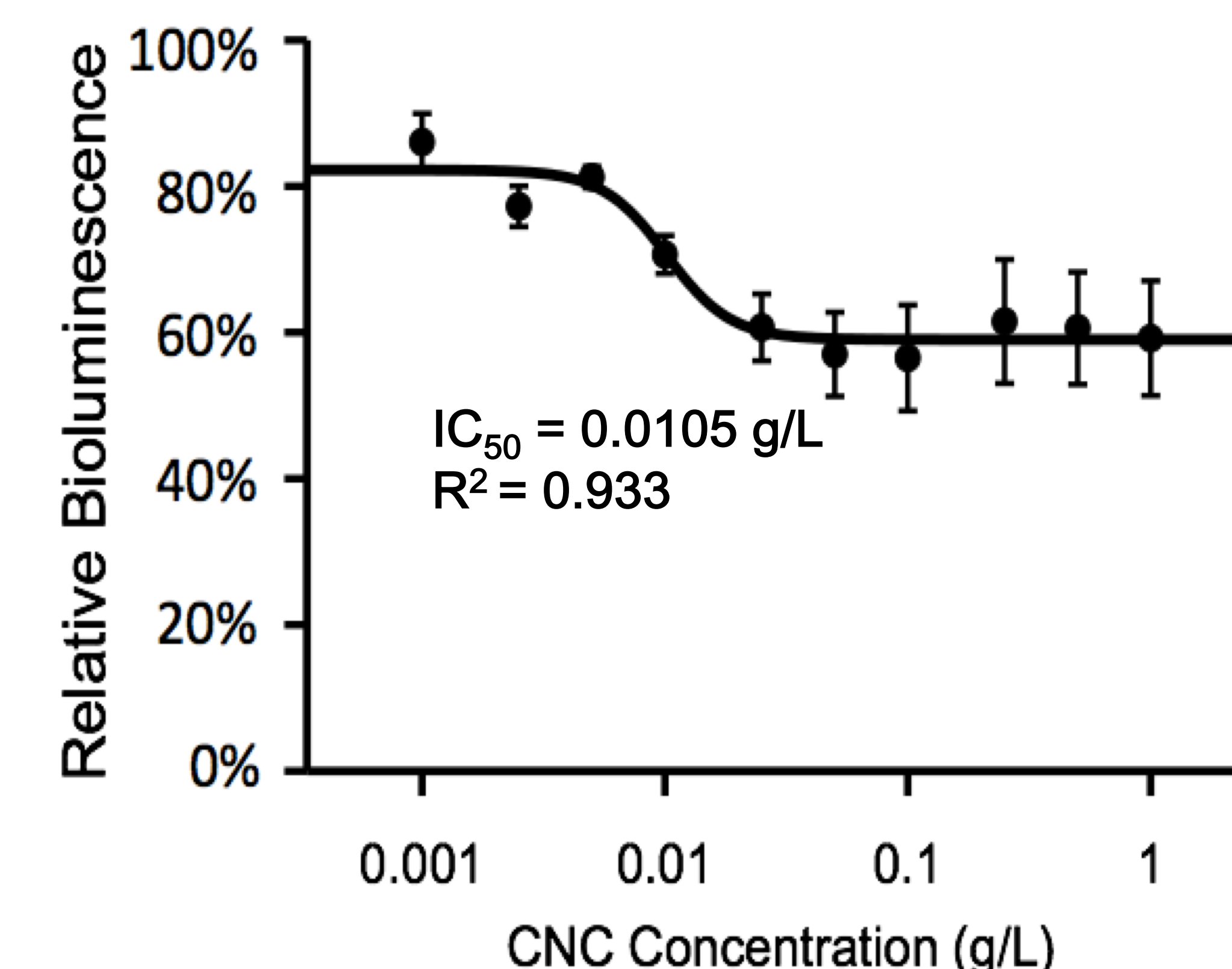


Figure 4. Exposure to CNC induced a sigmoidal dose-dependent response in the bioluminescence from BLYR. The IC₅₀ value was determined to be 0.0105 g/L after 10 hours of exposure.

AUTOBIOLUMINESCENT HUMAN CELLS/CNC TOXICITY ASSAY

The 'Humanized' *lux* Reporter Cassette

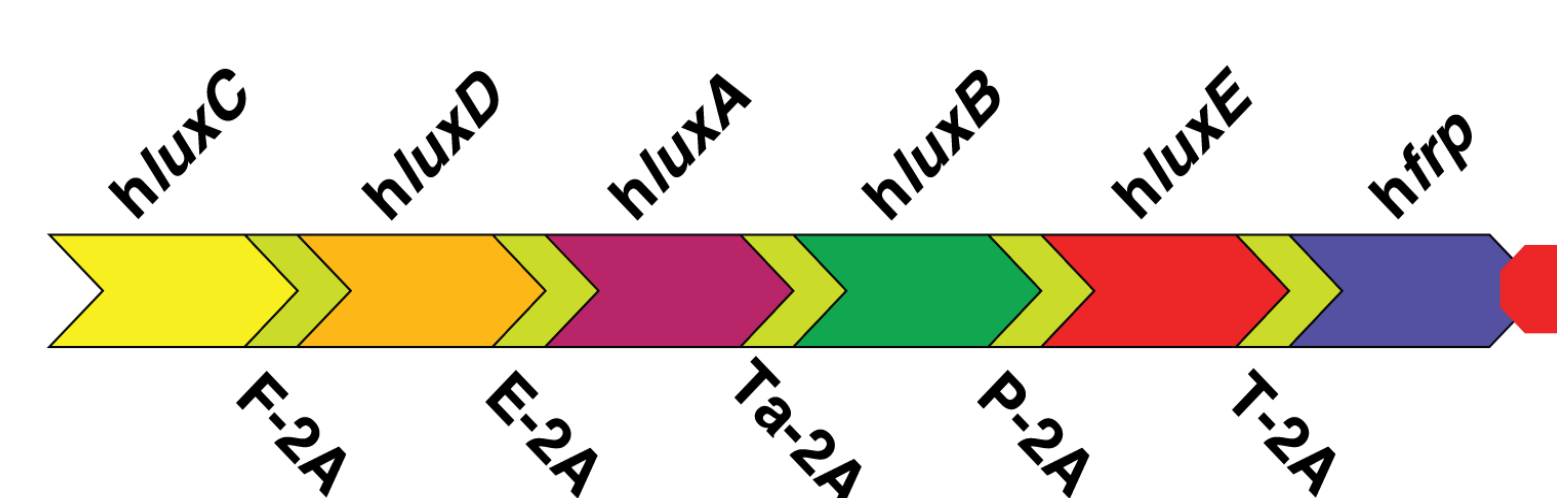
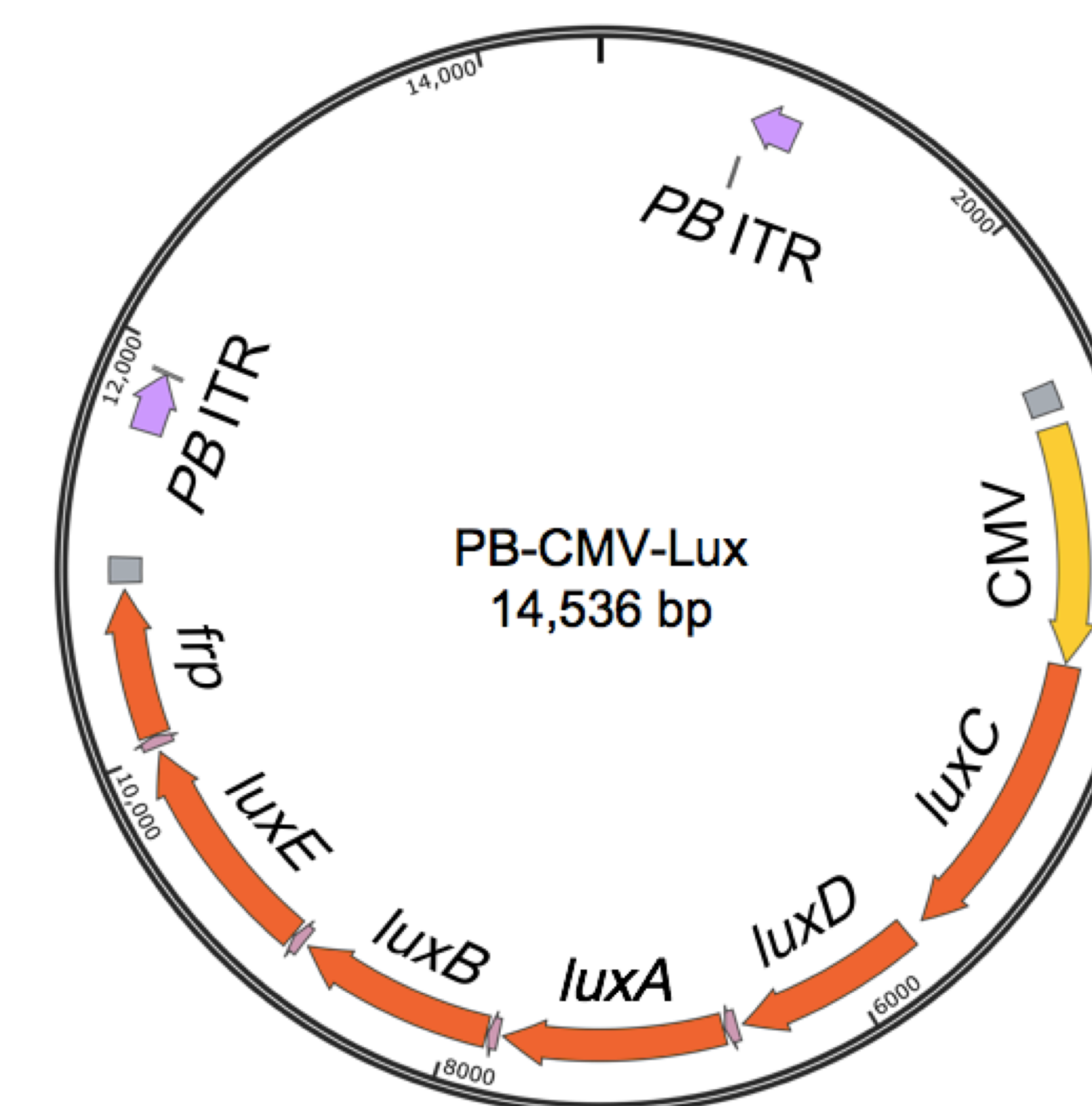


Figure 5. In order to efficiently express the multigene *lux* cassette in human cells, each gene was codon-optimized for human expression and linked using viral 2A elements to mimic polycistronic expression from a single promoter.

Autobioluminescent Human Embryonic Kidney (HEK293) Cells

Figure 6. Autobioluminescent HEK293 cells were generated by integrating the CMV promoter-driven *luxCDABEfrp* reporter cassette into HEK293 cells. A *piggyBac* transposon was employed to facilitate efficient chromosomal integration of the *lux* cassette. During transfection, a second plasmid containing the *piggyBac* transposase gene was co-introduced with the PB-CMV-Lux vector. The transposase will integrate the DNA fragment between the *piggyBac* inverted terminal repeat (PB-ITR) sequence into the chromosomal in a 'cut-and-paste' manner.



Bioluminescent Response to CNC Treatment in Autobioluminescent HEK293 Cells

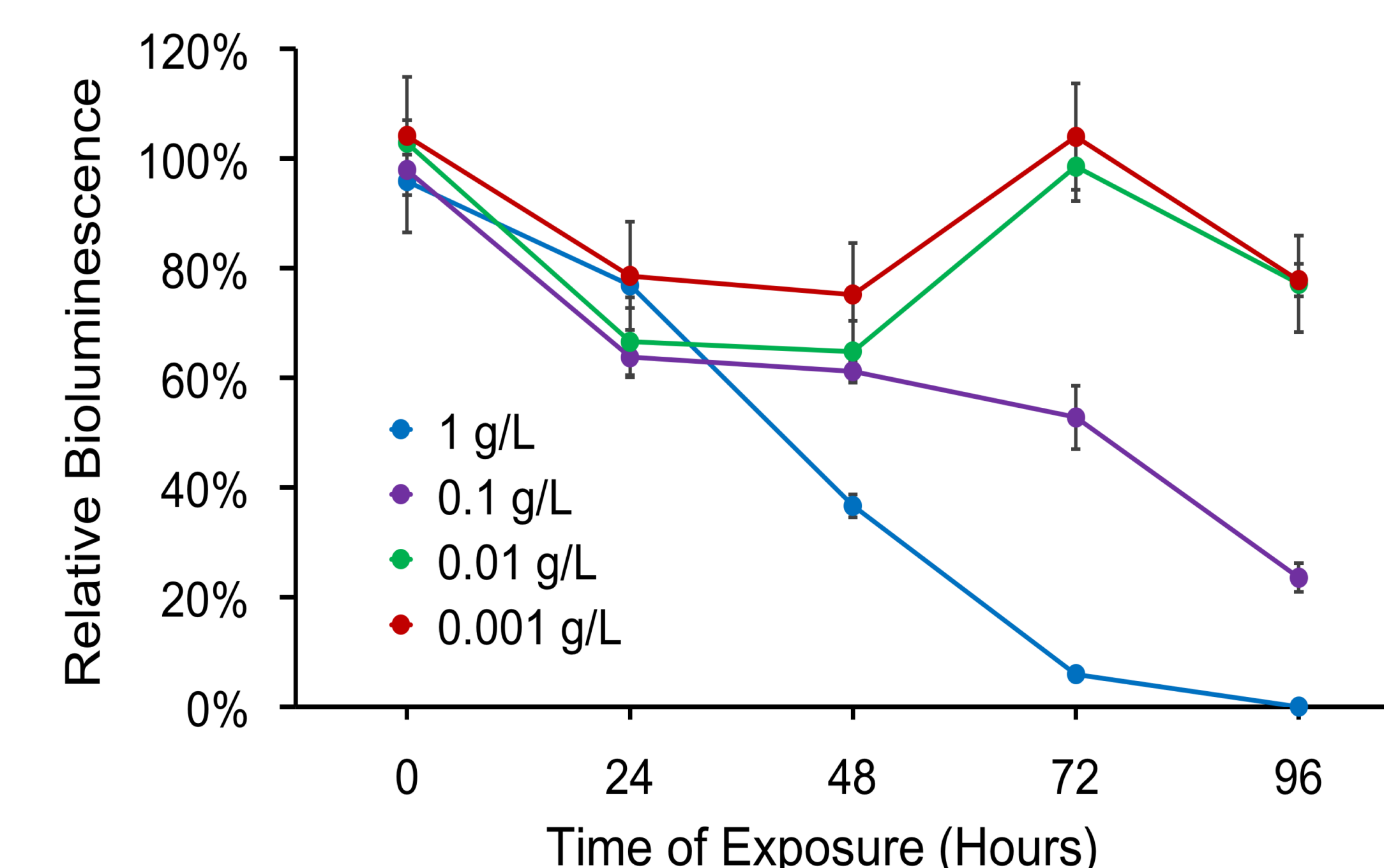


Figure 7. Exposure to CNCs induced time and dose-dependent responses in autobioluminescent HEK293 cells. Exposure to CNC at 1 g/L decreased bioluminescence to a undetectable level after 96 hours. During the same time period, the 0.01 g/L CNC concentration reduced bioluminescence by 80% compared to untreated control cells. Lower CNC concentrations (0.01 g/L and 0.001 g/L) appeared to be less toxic, only reducing light production by 20% after 96 hours.