Alternative reporters for paper-based cell-free biosensing

Jennifer Lee[1,2], Steve Blum[2], Stephanie Cole[2], Matthew Lux[2], Aleksandr Miklos[2]
[1]Defense Threat Reduction Agency, Fort Belvoir, VA, [2]U.S. Army Combat Capabilities Development Command Chemical Biological Center, Aberdeen Proving Ground, MD

Abstract

Lyophilized cell-free (CF) systems on paper can be rapidly developed as sensors for emerging biological threats. In these sensors, genetic material from biological threats (trigger mRNA) binds to RNA toehold switches encoding the LacZ gene to produce β-galactosidase (β-gal) (Figure 1). The reaction of β-gal with CPRG produces a yellow to purple colorimetric output in ~30 minutes. Because β-gal is a large protein, a potential strategy to reduce the time-to-answer is to use smaller reporter proteins. Here we present the use of two small-reporter strategies, LacZα complementation and luminescent output via NanoLuc® luciferase for lyophilized CF biosensor output (Figure 2). LacZα is a small peptide of the full LacZ product. Expression of LacZα leads to binding with the complementary LacZω peptide in the CF reaction, forming an enzymatically active protein that acts upon CPRG. NanoLuc® luciferase is a small enzyme that catalyzes intense light emission from its substrates. In this work we characterize the activity of each reporter strategy in CF reactions.

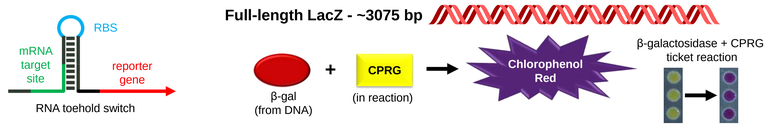


Figure 1. RNA toehold switch structure and full-length LacZ reporter system

Approach

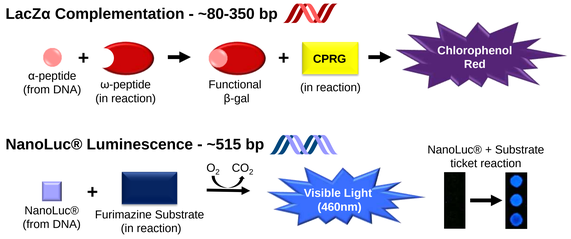


Figure 2. Small-reporter strategies for CF sensors. Expression of LacZα leads to binding with the LacZω peptide already present in the CF reaction for a yellow to purple color change. Enzymatic NanoLuc® reactions produce eye-readable blue light for an alternative output that expands the operational capacity of CF sensors.

DEVCOM CBC @ DTRA CBD S&T Conference
Scan the QR Code to view all of CBC's
2022 DTRA CBD S&T Conference materials
https://cbc.devcom.army.mil/cbdst-conference/

NanoLuc® Luminescence

Substrate Selection

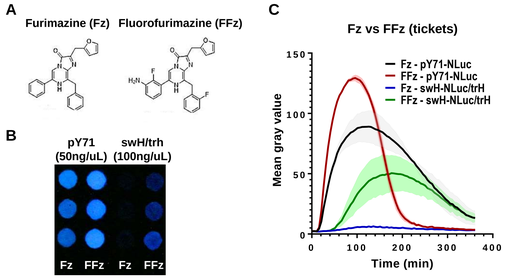
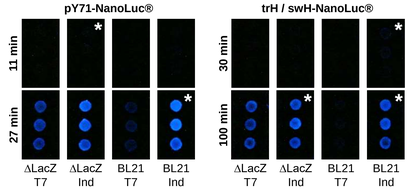


Figure 3. Comparison of NanoLuc® substrates, furimazine (Fz) and fluorofurimazine (FFz) on paper tickets. Fz is the standard substrate for NanoLuc® while FFz is an analog substrate made for *in vivo* use. **A)** Fz and FFz structures. **B)** SLR camera image of Fz and FFz reaction outputs. **C)** Quantification of luminescence over time (mean gray value ± stdev). The FFz substrate resulted in faster reactions with greater signal outputs. Constitutive (pY71) and RNA toehold switch expression (swH/trH) of NanoLuc® was evaluated.

Reaction Optimization

Figure 4. *E. coli* extract optimization in cell-free reactions expressing NanoLuc® in paper tickets. Extracts made from BL21 (DE3) LacZ knockout (ΔLacZ) and BL21 (DE3) Rosetta 2 (BL21) strains were compared. Strains were either supplemented with T7 RNA polymerase (T7) or induced during culture to contain T7 (Ind). The induced BL21 (BL21 Ind) extract proved to have the best balance of speed and maximum output for all reactions. (* indicates brightest reactions at the indicated time point)



Viewing Capabilities

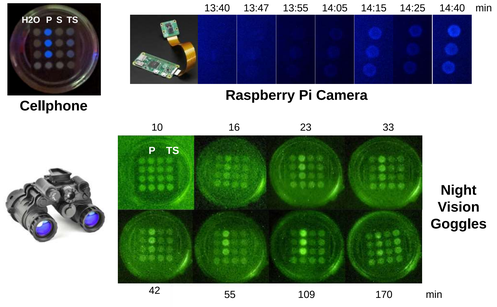


Figure 5. Methods to view NanoLuc® sensors. NanoLuc® reactions can be seen by eye, but detection can be quicker using fieldable devices. Cellphones can be used to take images of reactions. Raspberry Pi cameras are an inexpensive option to wirelessly image reactions over time. Night vision goggles offer an operationally relevant method to evaluate reaction outcomes.

LacZα Complementation

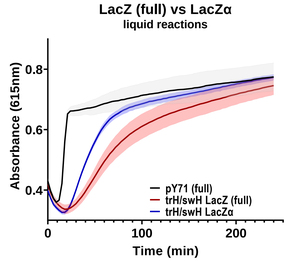


Figure 6. Comparison of full-length LacZ and LacZα plasmid reporters in liquid reactions. Constitutive (pY71, 25nM) expression of the full length LacZ gene was compared to RNA toehold switch reactions with the full LacZ (swH LacZ(full), 50nM) or alpha fragment (swH LacZα, 50nM). The same trigger DNA (trH, ~100nM) was used for each swH/trH reaction. Use of the LacZα reporter resulted in a faster reaction compared to the full-length LacZ.

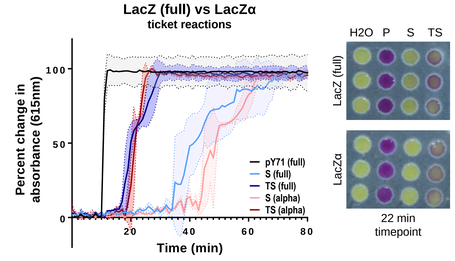


Figure 7. Comparison of full length LacZ and LacZα plasmid reporters in paper tickets. Constitutive (pY71 (P), 125nM) expression of the full length LacZ gene was compared to RNA toehold switch reactions with the full LacZ (S(full), 125nM) or alpha fragment (S(alpha), 125nM). The same trigger DNA (T, ~250nM) was used for each TS reaction. Representative images of tickets at 22 minutes following rehydration are depicted.
While there was no significant difference between the TS reactions for the full-length and alpha fragment of LacZ, the LacZα reporter had less background activity overall (S(alpha) reaction only).

Conclusions and Future Directions

Conclusions

- NanoLuc® luminescence provides an alternative reporter for potential night operations - time to detection depends on viewing technique
- LacZα complementation reduces time to detection in liquid reactions, however more optimization is needed to finalize behavior in ticket reactions
- The use of the LacZα reporter reduces background signal from leaky switch expression

Future Directions

- Optimize a fieldable protocol for implementation of NanoLuc® based sensors
- Transition from the full-length LacZ to LacZα for paper-based, cell free sensors
- Transition from plasmid DNA to linear DNA sequences in cell-free reactions
- Evaluate limits of detection

Acknowledgements: We would like to thank DTRA-JSTO for providing funding for the presented work. We would also like to acknowledge Dr. Keith Pardee (University of Toronto) and Dr. Alex Green (Boston University) for their support implementing the RNA toehold switch sensing technology. The views expressed in this abstract are those of the authors and do not necessarily reflect the official policy or position of the Department of Defense or the U.S. Government.